

Legions of Doom part 2

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Professor

2-2-17

Outline and Questions

- History of Legionella infection at UIHC
- What are the novel aspects of *Legionella pneumophila* infection of macrophages?
 - Coiling phagocytosis
 - Generation of an ER-derived compartment
 - Harnessing the proteasome for nutrient acquisition and growth
- Antibiotics – macrolides vs. fluoroquinolones?

Legionella pneumophila – causative agent of Legionnaire's disease

- 1976 – Philadelphia hosted the convention of the Pennsylvania American Legion
- within 6 days 182 attendees were sick → 29 deaths
 - pneumonia caused by unknown unrecognized bacterium
 - air conditioning and water handling system implicated

Legionella pneumophila

- 1-40% of nosocomial pneumonias
- spread by inhalation of contaminated water
- natural host of *Legionella pneumophila* in the environment is fresh water amoebae

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LEGIONNAIRES' DISEASE

Description of an Epidemic of Pneumonia

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Abstract An explosive, common-source outbreak of pneumonia caused by a previously unrecognized bacterium affected primarily persons attending an American Legion convention in Philadelphia in July, 1976. Twenty-nine of 182 cases were fatal. Spread of the bacterium appeared to be air borne. The source of the bacterium was not found, but epidemiologic analysis suggested that exposure

may have occurred in the lobby of the headquarters hotel or in the area immediately surrounding the hotel. Person-to-person spread seemed not to have occurred. Many hotel employees appeared to be immune, suggesting that the agent may have been present in the vicinity, perhaps intermittently, for two or more years. (N Engl J Med 297:1189-1197, 1977)

PERSISTENCE OF *LEGIONELLA PNEUMOPHILA* IN A HOSPITAL'S WATER SYSTEM: A 13-YEAR SURVEY

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ABSTRACT

OBJECTIVE: To describe the molecular epidemiology of *Legionella pneumophila* infections in the University of Iowa Hospitals and Clinics (UIHC).

DESIGN: Molecular epidemiological study using pulsed-field gel electrophoresis (PFGE).

SETTING: A large university teaching hospital.

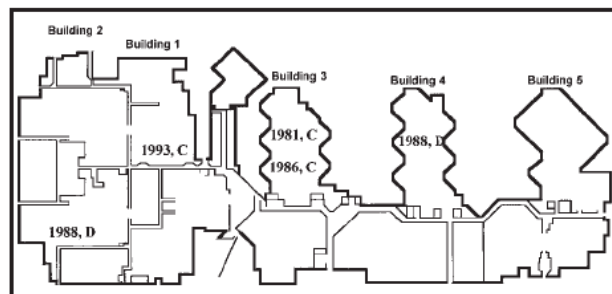
ISOLATES: All surviving isolates obtained from culture-proven nosocomial *L. pneumophila* infections and all surviving isolates obtained from the University of Iowa Hospital and Clinics' water supply between 1981 and 1993.

RESULTS: Thirty-three isolates from culture-proven nosocomial cases of *L. pneumophila* pneumonia were available for typing. PFGE of genomic DNA from the clinical isolates identified six

different strains. However, only strain C (16 cases) and strain D (13 cases) caused more than 1 case. Strain C caused clusters of nosocomial infection in 1981, 1986, and 1993 and also caused 4 sporadic cases. Strain D caused a cluster in 1987 and 1988 plus 4 sporadic cases. Of the six strains causing clinical infections, only strains C and D were identified in water samples. PFGE identified three strains in the water supply, of which strains C and D caused clinical disease and also persisted in the water supply during most of the study period.

CONCLUSION: Specific strains of *L. pneumophila* can colonize hospital water supplies and cause nosocomial infections over long periods of time (*Infect Control Hosp Epidemiol* 1999;20:793-797).

UIHC



2015 – outbreaks
In New York City

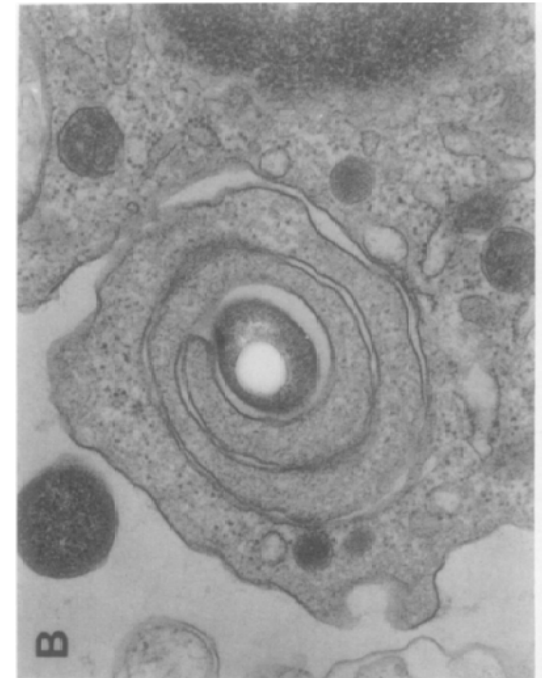
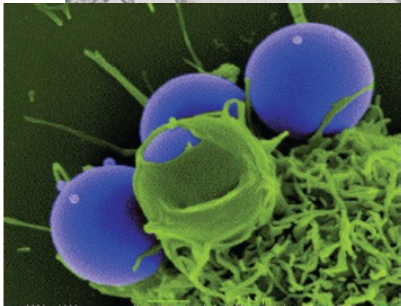
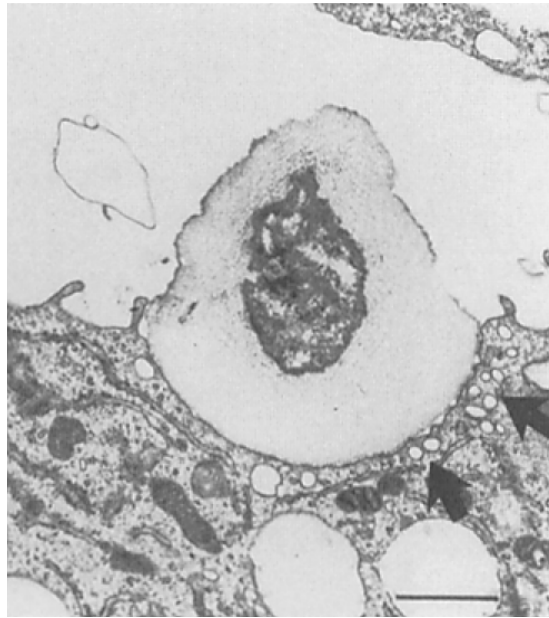
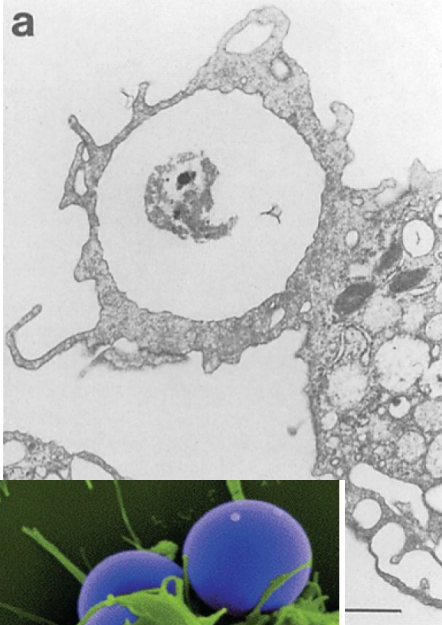
FIGURE 2. Schematic drawing of the University of Iowa Hospitals and Clinics illustrating the location of the four outbreaks and the *Legionella pneumophila* strains causing each outbreak.

Unusual “coiling phagocytosis”

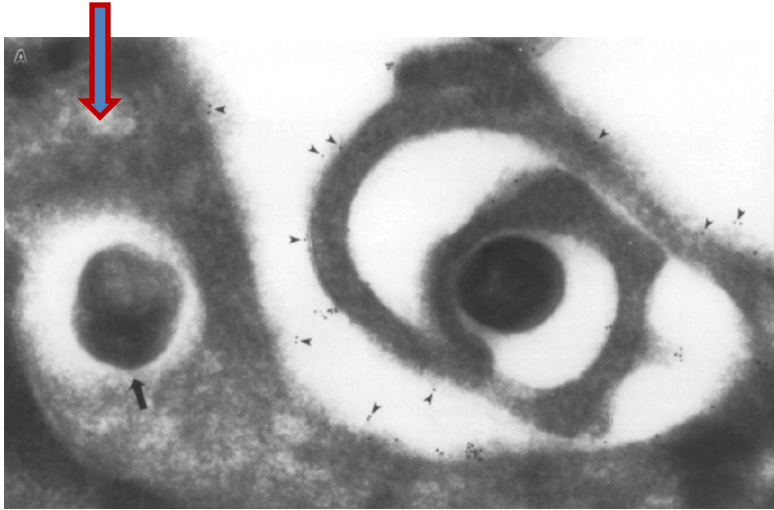


Phagocytosis = receptor and actin-mediated mechanism macrophages use to engulf bacteria, fungi, and parasites.

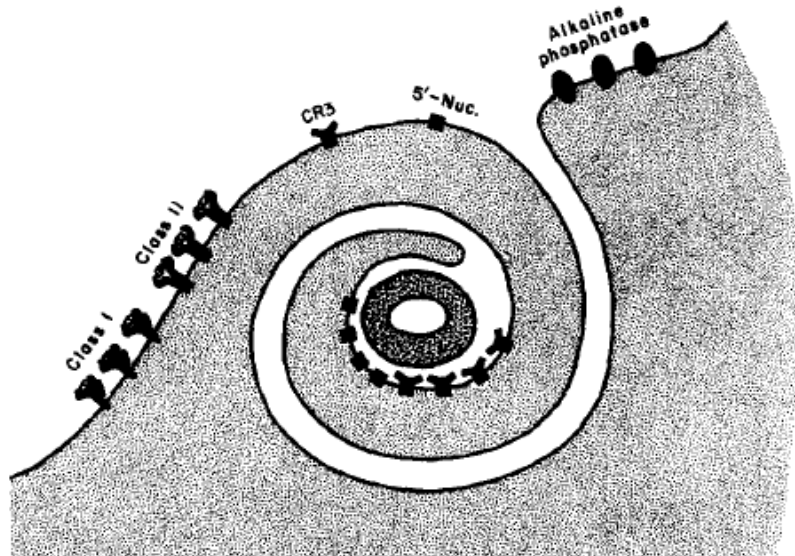
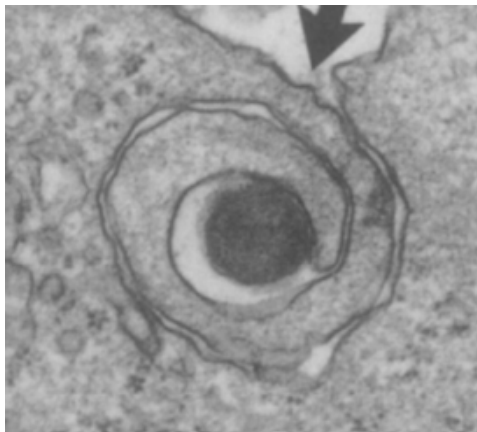
Typical forming phagosome morphologies:
'pseudopod extension' or 'sinking':



Coils rapidly resolve – mechanism unknown



Gold → Beta2 microglobulin



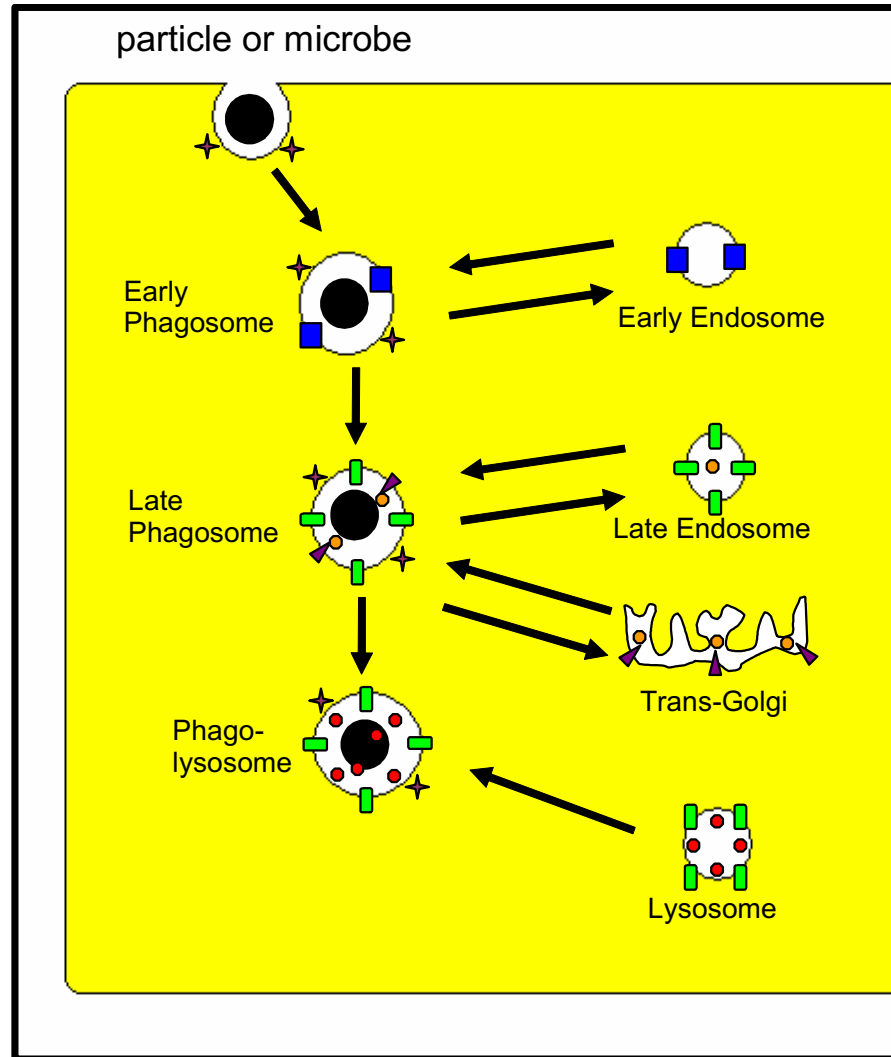
CR3 enriched vs. exclusion of HLA-DR, B2M and other antigen presentation molecules from the inner coil

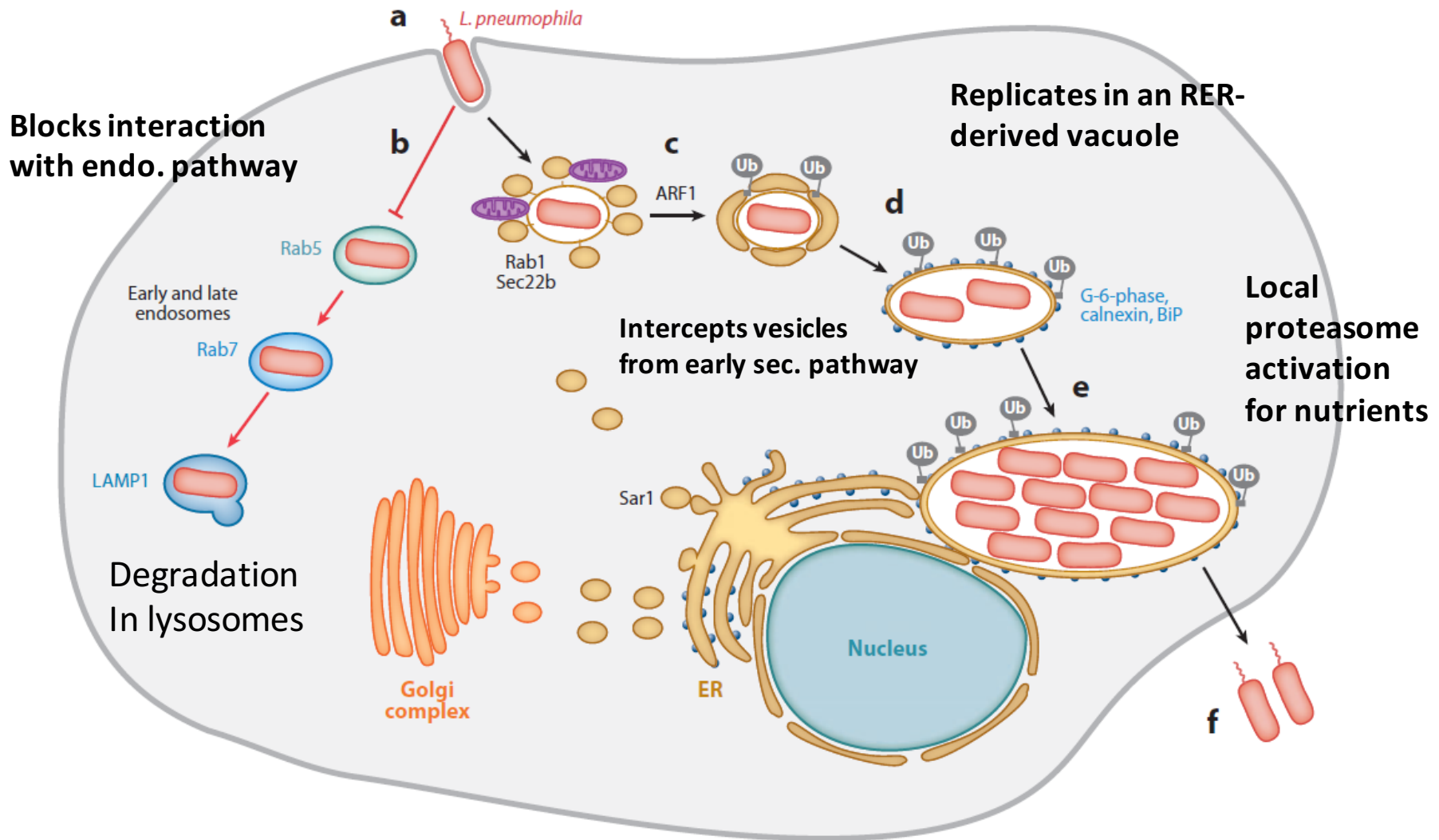
Phagosome maturation

pH 6.5

Sequential modification of phagosome composition by fusion with early endosomes, late endosomes and lysosomes.

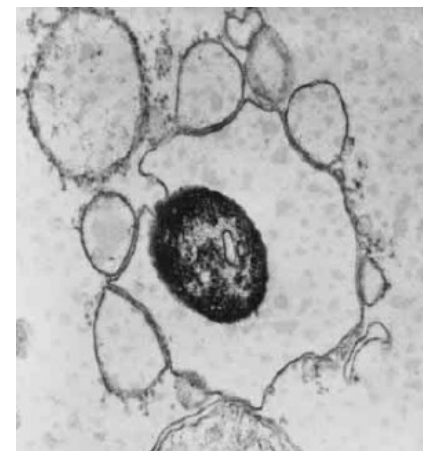
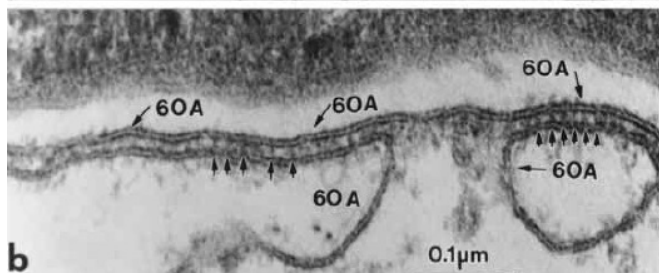
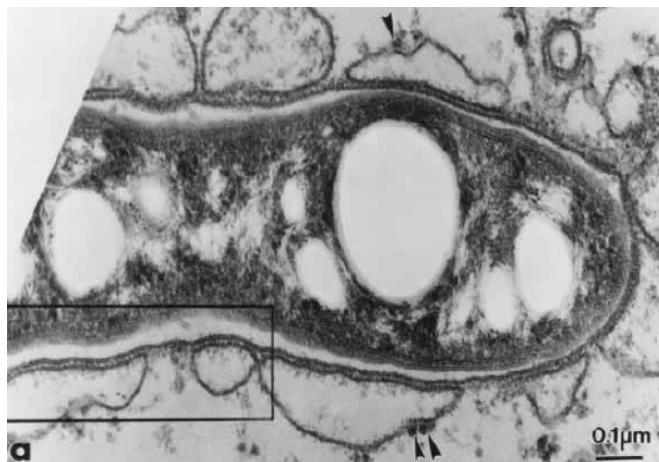
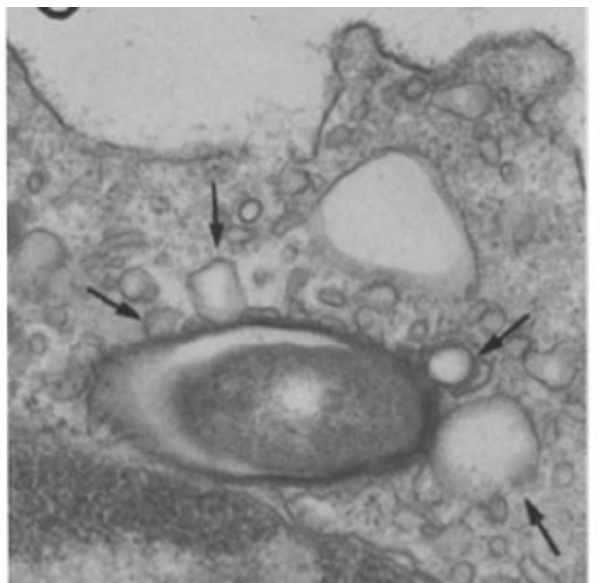
pH 5.0





Dot/ICM effectors allow avoidance of lysosomes and promote association with ER-derived vesicles by manipulation of GTPases including Rab1 and Arf

Smooth vesicles surround *Legionella* compartments within 15 min of infection



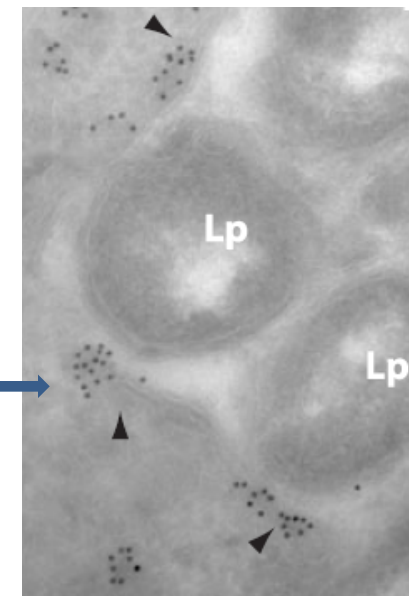
Additional studies revealed that the vesicles were derived from the **smooth ER**

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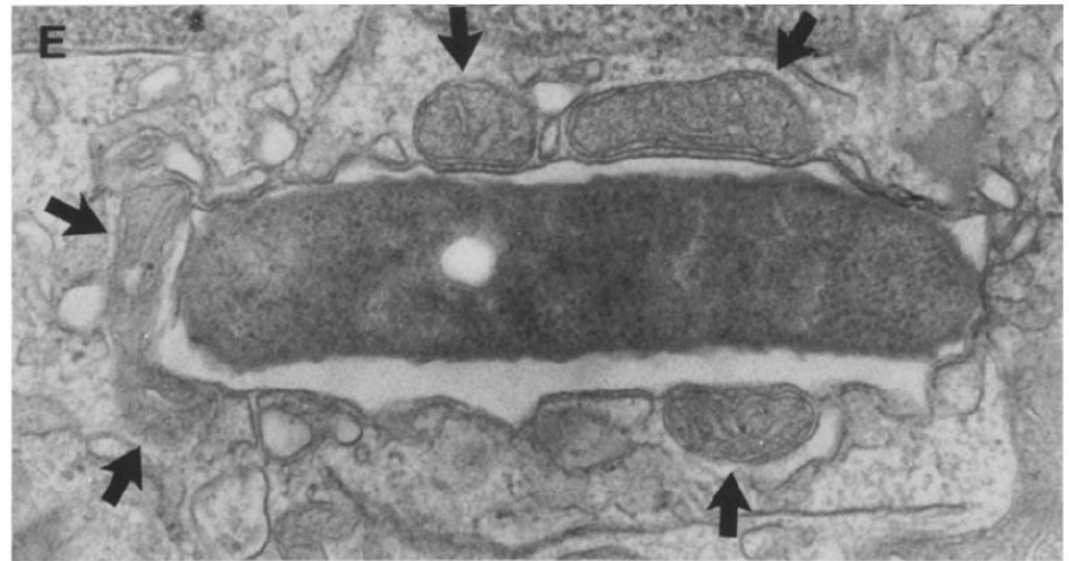
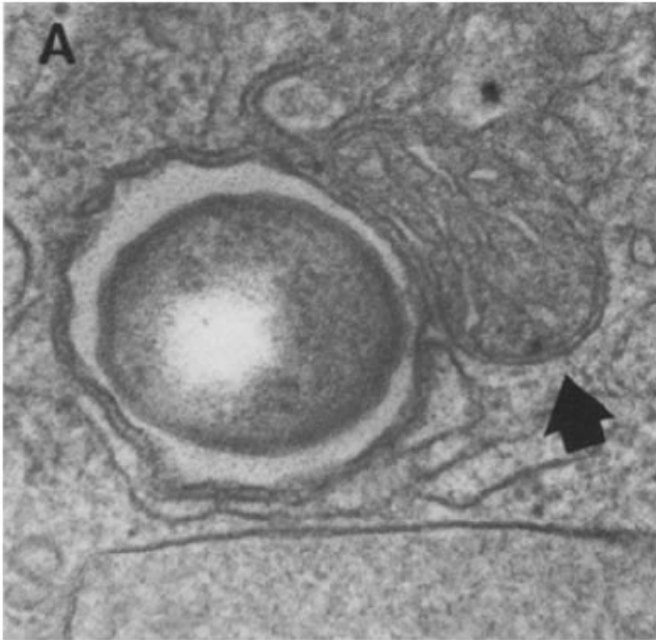
Volume 158 October 1983 1319-1331

Journal of Cell Science 114, 4637-4650 (2001)

Vesicles contain ER proteins by immuno-EM



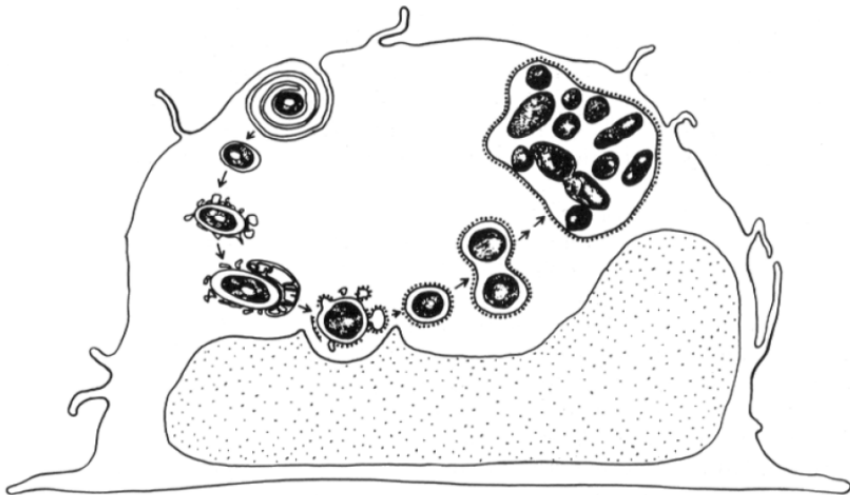
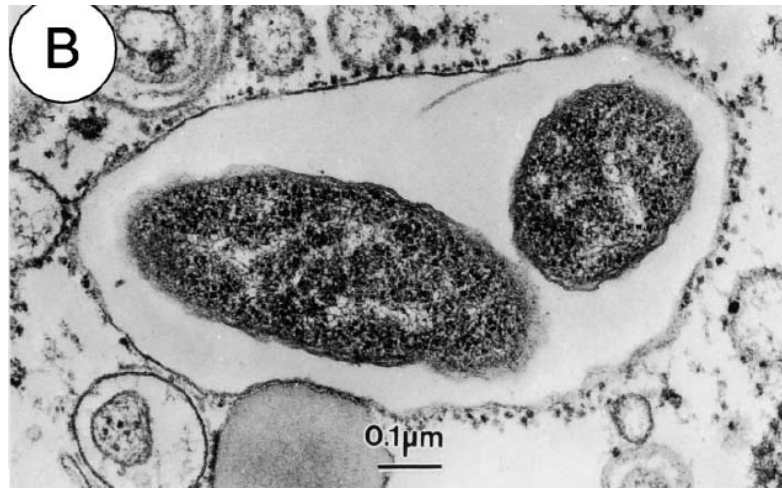
After 1-2 hours, Legionella compartments are also surrounded by **mitochondria**



The significance of proximity to smooth vesicles and mitochondria unclear

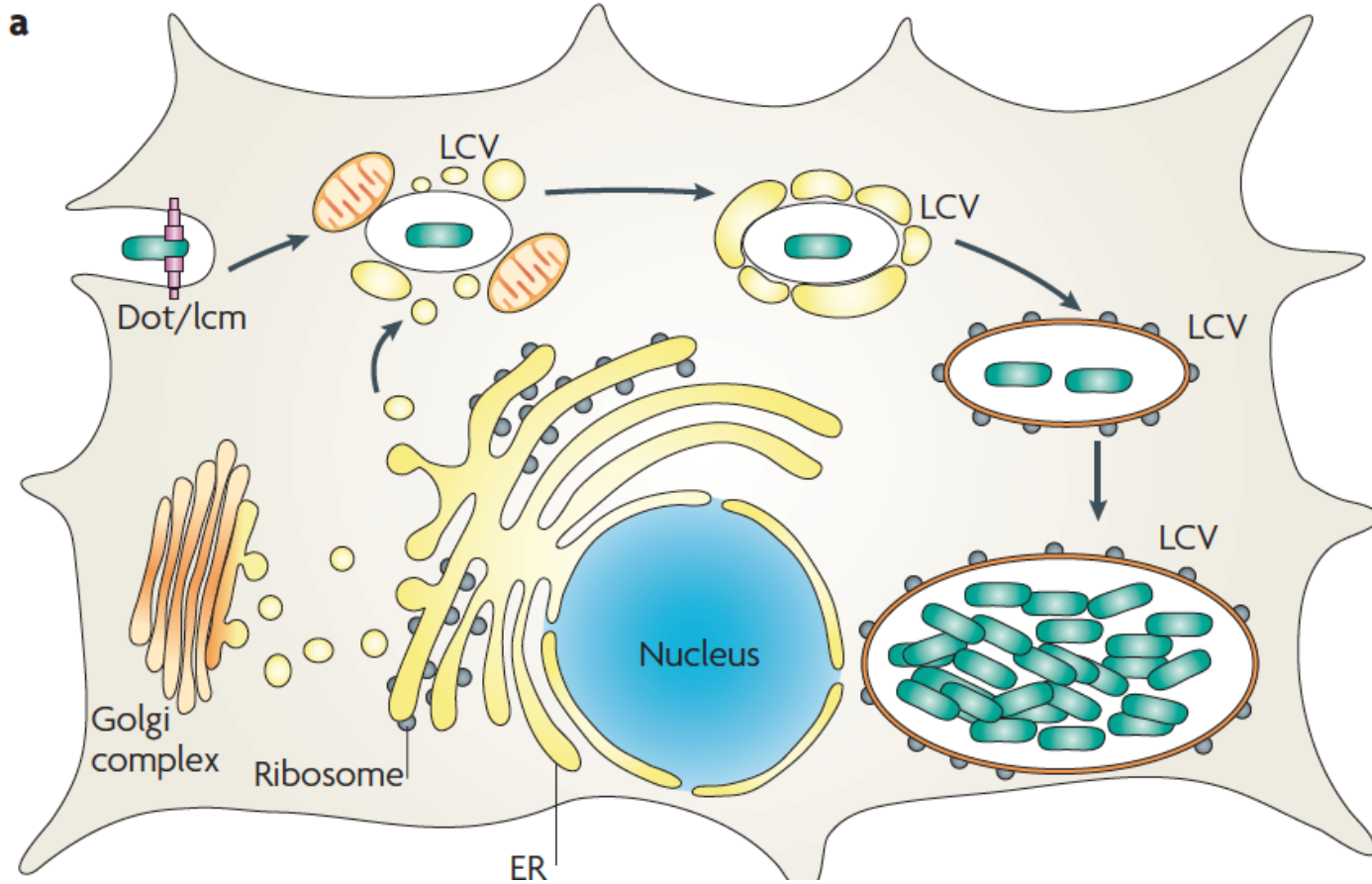
By 8 hours, *Legionella* compartments become studded with ribosomes, **Vacuole membrane resembles the Rough ER (RER)**.

The RER-derived vacuole supports Legionella replication

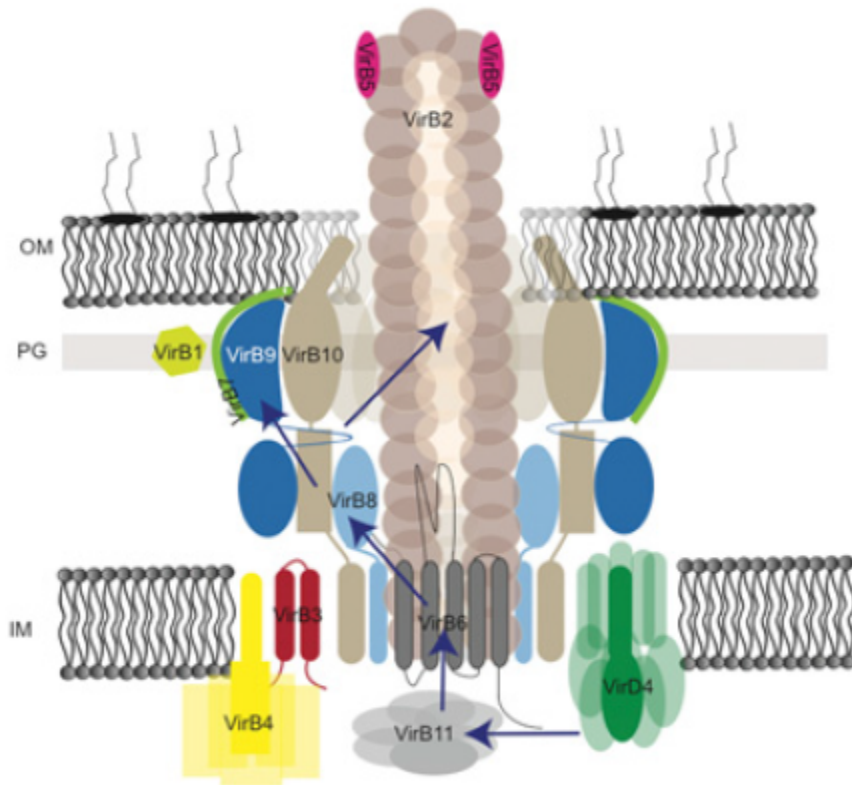


Model

What have we learned about the underlying mechanism since 1983?



Legionella – Type IVB Secretion System – **More than 300 injected effector proteins!!**



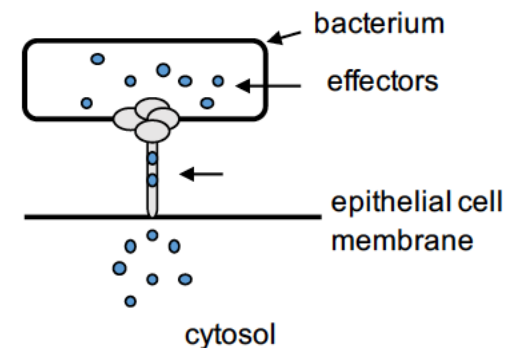
Effectors are also called 'Dot/Icm' substrates

Some effectors alter membrane trafficking

Others facilitate bacterial nutrient acquisition

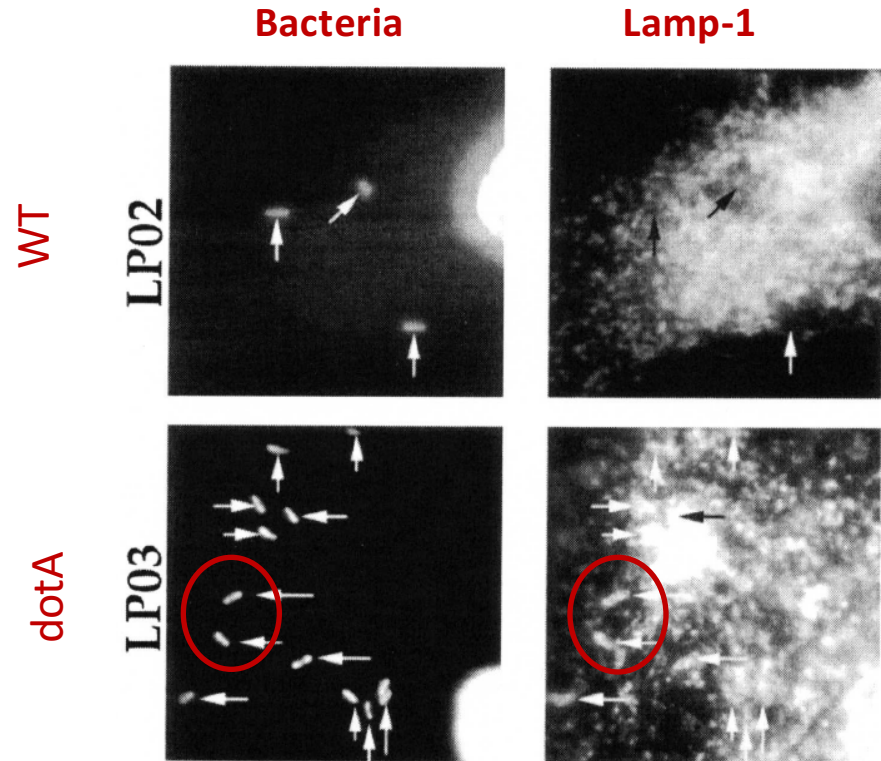
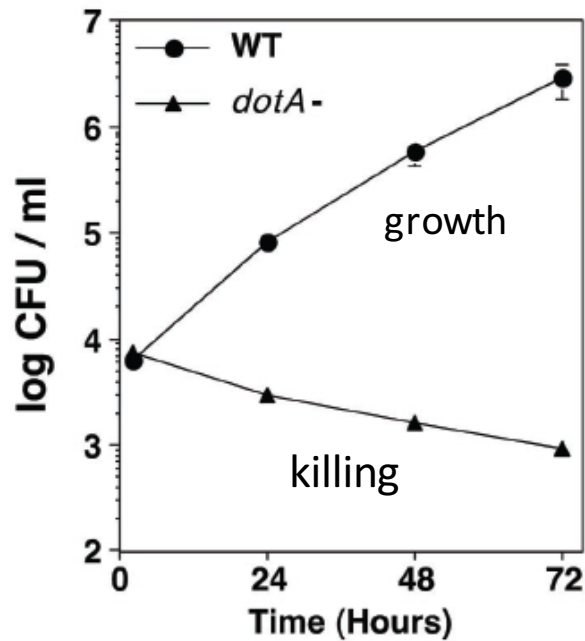
Required for Legionella growth and survival

Effectors redundancy complicates studies of effector function.

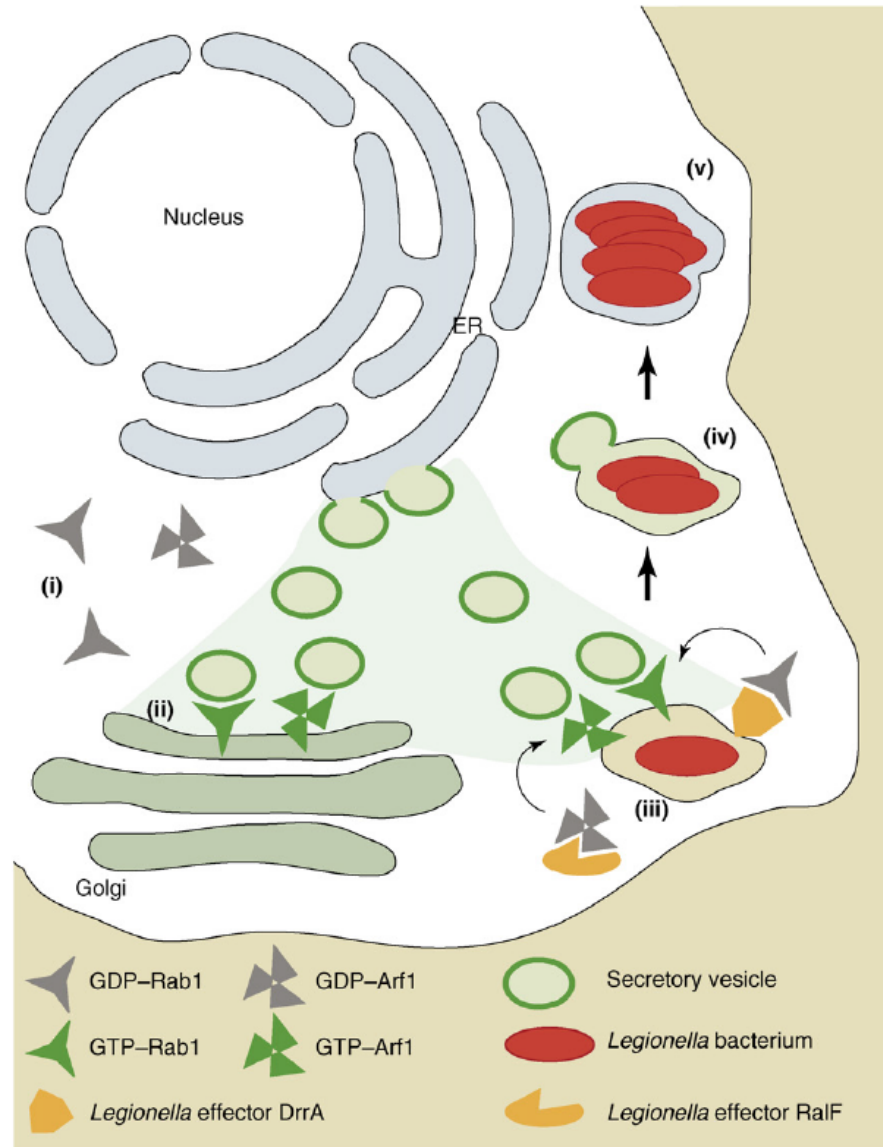


DotA allows Legionella to avoid trafficking to lysosomes

dotA mutants colocalize with LE/lysosome markers



Other Effectors intercept vesicles from ER or ERGIC to modify the *Legionella* vacuole

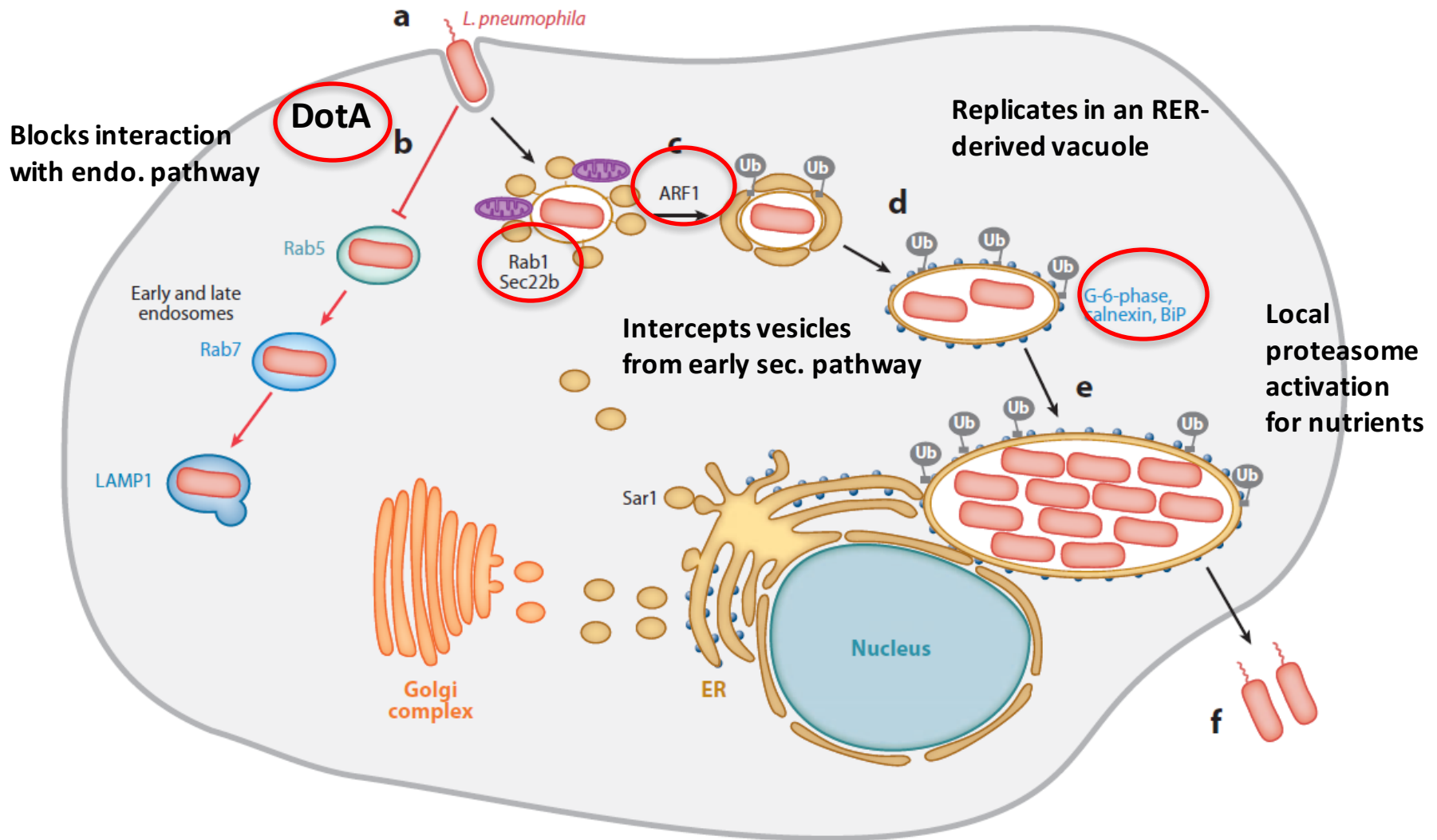


Small GTPases of the Rab and Arf families regulate membrane trafficking.

RaIF and **SidM/DrrA** are effectors that activate Arf and Rab1

Normal role in ER → Golgi traffic,
But co-opted by *Legionella*

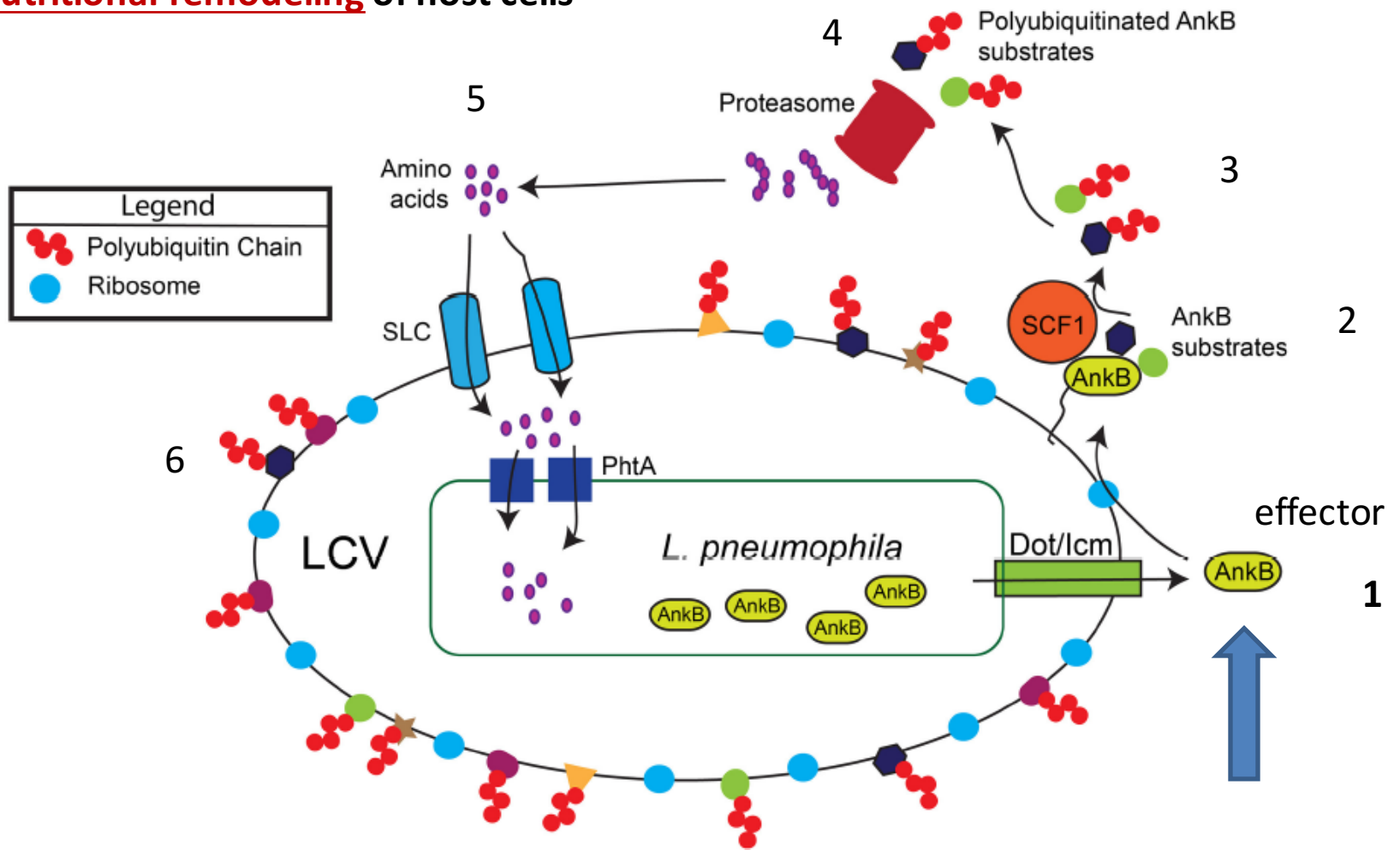
Allow modification of the *Legionella* compartment



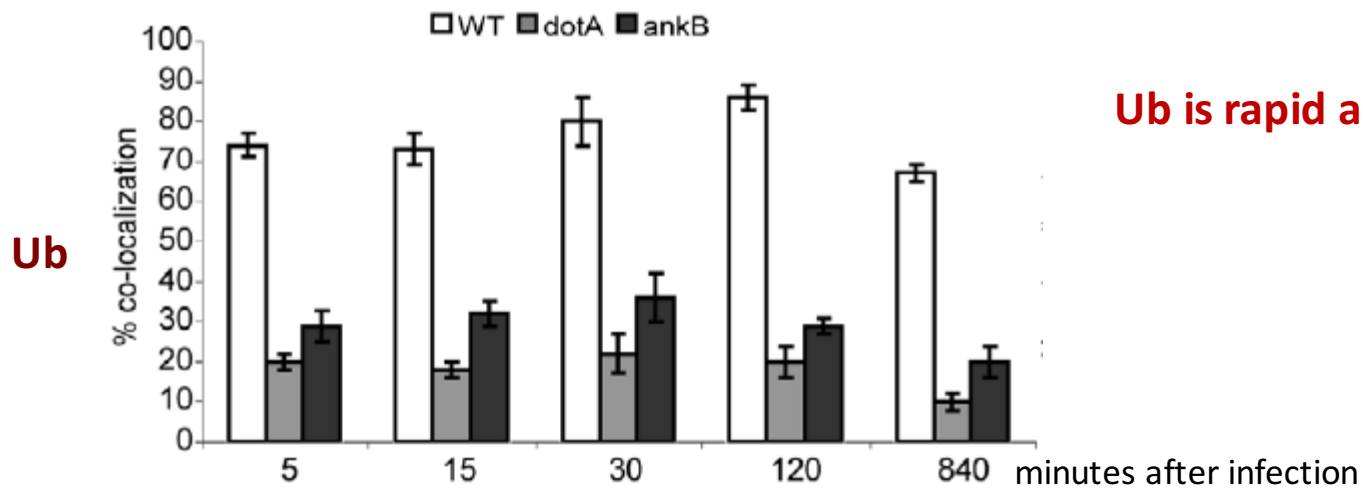
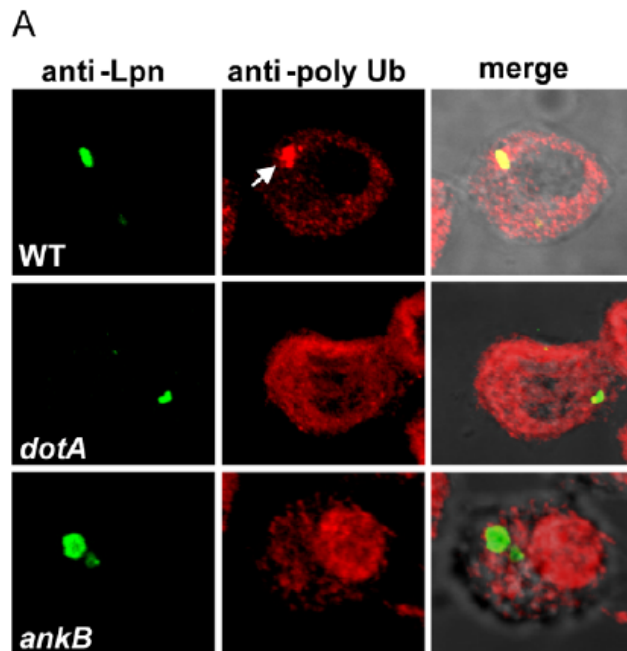
Dot/ICM effectors allow avoidance of lysosomes and promote association with ER-derived vesicles by manipulation of GTPases including Rab1 and Arf

Legionella also harnesses the proteasome to obtain nutrients for growth –

- nutritional remodeling of host cells -

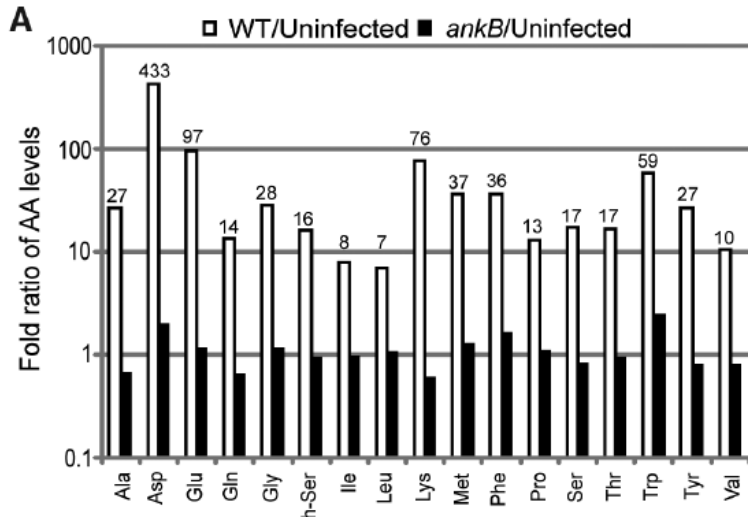


Poly-Ubiquitinated proteins accumulate on the LCV – requires AnkB and DotA

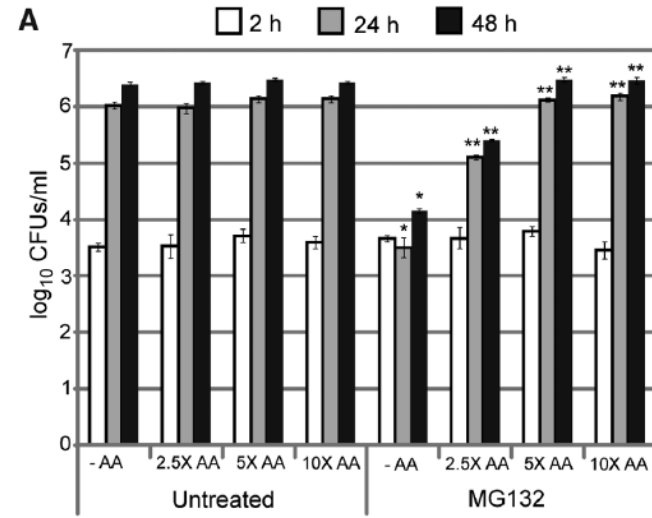


Ub is rapid and sustained

AA accumulate with Legionella growth

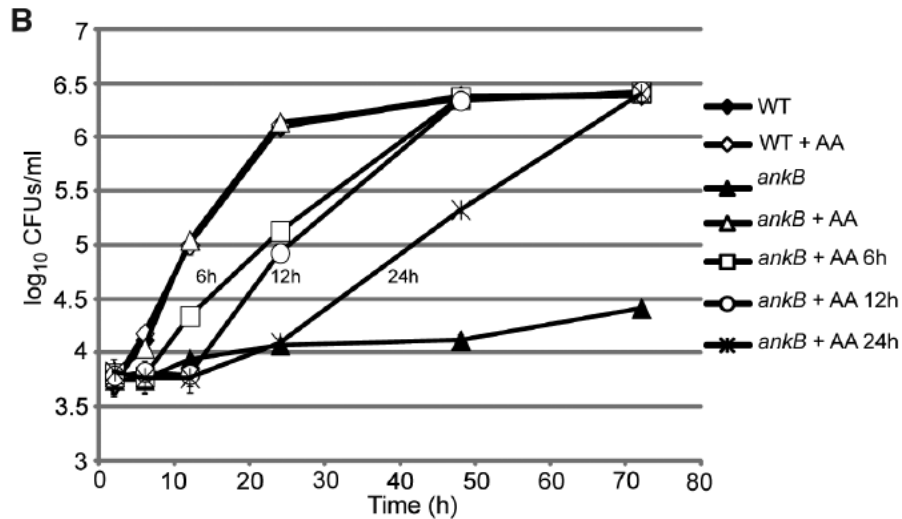


Proteasome inhibitors impair growth -rescue with exogenous amino acids



Exogenous AA rescue growth of AnkB mutant

Proteasome inhibitor



Summary

- Like T3SS, type IV secretion systems (T4SS) allow pathogenic bacteria to introduce effector proteins into host cells that alter cell function and are required for bacterial growth and survival.
- *Legionella* is engulfed via an unusual coiling mechanism, and the new LCV avoids interactions with the endo/lyso pathway in a T4SS-dependent manner that requires the effector DotA.
- Additional effectors allow the LCV to intercept vesicles from the early secretory pathway via effects on Rab1, Sec22 and ARF, and bacteria replicate in an RER-like vacuole.
- Still other effectors allow *Legionella* to harness the proteasome to acquire amino acids that support bacterial growth.
- Now back to Dan....