



Massachusetts
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Technology



Under the Radar Screen: How Bugs Trick Our Immune Defenses

Session 2: Phagocytosis

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Salmonella

- Gram negative bacteria that can cause diseases such as typhoid fever or gastroenteritis in humans or other animals.
- Salmonella are facultative intracellular bacteria.
- Their survival is linked to their ability to “hide” within a variety of cell type.
- They penetrate cells through a mechanism similar to phagocytosis but can also penetrate non-phagocytic cells.
- Their genes encode for several proteins that are necessary to assemble a complex apparatus termed the “type III secretion system” (TTSS).
- The TTSS allows Salmonella to inject effectors into the cell and promote its uptake by phagocytic and non phagocytic cells.

Phagocytosis vs Macropinocytosis

Salmonella triggers uptake through membrane ruffling similar to macropinocytosis.

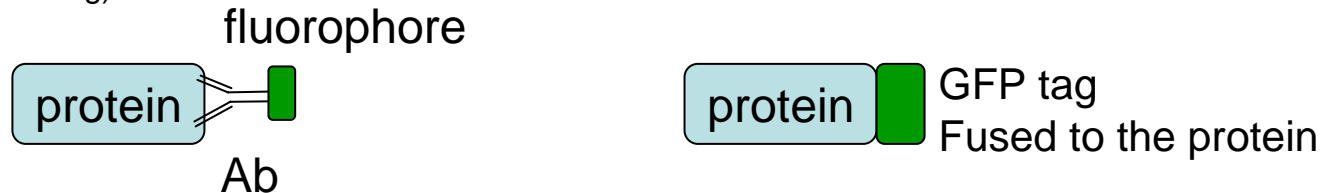
Macropinocytosis allows to pick up smaller particles and large droplets of liquid. There is no specific recognition involved in macropinocytosis.

Phagocytosis is exclusively performed by certain cells such as macrophages or neutrophils. It is usually mediated by cell surface receptors

The micropinosome tends to be larger than the phagosome but they both eventually mature and fuse with the endocytic compartment.

The papers

- Shared question: How does *Salmonella* modify the phagosome so that it can survive in it ?
- Shared approaches: Both use microscopy
 - 1st paper is from 1994 and uses light microscopy to observe the phagosome maturation
 - 2nd paper is from 2004 and uses confocal microscopy to observe similar phenomenon (try to get a color print out)
 - 2nd paper uses fluorescence to detect the proteins of interest. Fluorescence can be “added” to a protein through a fluorescently labeled antibody that recognizes the protein or a fluorescent tag (such as the green fluorescent protein or GFP) can be fused to the protein of interest through protein engineering).



- They also use FRAP=fluorescence recovery after photobleaching to measure the ability of molecules to move inside the cell over time. <http://www.bio.davidson.edu/Courses/Molbio/FRAPx/FRAP.html>
- Nomenclature in both papers is slightly different
 - 1st paper calls the *Salmonella* phagosomes “spacious phagosomes” because they appear larger than usual phagosomes
 - 2nd paper calls the *Salmonella* phagosomes “*Salmonella* Containing Vacuoles” (SCV)*

*abbreviations used in the papers are summarized at the bottom of the first page

Biological concepts discussed

- **Endosome:** Membrane bound compartments within the cell that are responsible for sorting of proteins en route to the lysosome or to the plasma membrane. Lamp1 is a protein known to be found in the endosome and is used as an endosomal marker to locate this organelle within the cell.
- **Lysosome:** Membrane bound organelles/vacuoles containing enzymes and chemicals able to destroy proteins/pathogens. They can fuse with the endosome or with the phagosome.
- **Microtubules:** Elongated protein structures inside the cell (filaments). Part of the cytoskeleton, they are responsible for segregation of chromosomes during cell division as well as vesicular transport.
- **Intracellular protein transport**
 - Rab7: small GTPase involved in the transport of proteins/vesicles from the cell surface to the lysosome. Rab7 becomes active upon binding of GTP (versus GDP)
 - RILP: **R**ab7 interacting **l**ysosomal **p**rotein. Binds to both Rab7 containing vesicles and molecular motors involved in the transport of these vesicles to the lysosome.
 - Dynein: molecular motor that uses energy from ATP and transforms it into movement. It is involved in carrying proteins/vesicles along microtubules.
 - Dynamitin: protein involved in the disassembly of Dynein from its cargo.
 - Kinesin: Molecular motor involves in the transport of proteins/vesicles along microtubules.
- **Opsonization:** coating of a pathogen with specific molecules (antibodies/complement) that will enhance the efficiency of phagocytosis.
- **LPS:** Lipopolysaccharide, major component of the outer membrane of gram negative bacteria such as Salmonella.

Paper 1

Alpuchearanda, C. M., Racoosin, E. L., Swanson, J. A.,
and Miller, S. I. "*Salmonella* Stimulate Macrophage
Macropinocytosis and Persist within Spacious
Phagosomes." *Journal of Experimental Medicine* 179
(1994): 601-608.

Meaning of all the words in the title ?

After reading the paper, does the title convey right idea about the paper?

Introduction to Paper 1

- Bacterial genes essential to pathogenesis:
 - PhoQ
 - PhoF
 - Pho activated genes, induced by PhoQ and F (PAGs). PAGs induce virulence. PAGs are essential.
 - Pho repressed genes (PRGs) (delay acidity of the phagosomes)
- Time scale of PAGs induction
 - 3-6h post infection, which is the time at which phagosomes get acidified (pH 4.9)
 - Acidification (environment) triggers PAG expression
 - But Salmonella delays and attenuates acidification through PRGs... > ??
- In summary, we know:
 - That the phagosomal environment (acidity) sends signals inducing PAG expression
 - That Salmonella must survive inside the phagosome until PAGs are expressed

What are the questions this paper is asking ?

Paper 1 Questions

- Did the technology of confocal microscopy really help to answer the question or was light microscopy sufficient ?
- Why compare opsonized versus non-opsonized bacteria ?

Questions/hypothesis of the paper

- How does *Salmonella* survive inside the phagosome?
- Hypothesis:
 - Nature of the phagosome is important for survival of *Salmonella*
 - The larger size of the *Salmonella* phagosome might be critical

Discussion of Paper 1

- All experiments use macrophages but Salmonella also infects epithelial cells.
- Formation of SP seems to be linked to entry by macropinocytosis but even when bacteria enter through phagocytosis (opsonization), fusion with macropinosomes occurs.
- How are SP maintained ? (SP creation and maintenance is required for virulence)
 - Continuous creation of new SP
 - Salmonella may express proteins that inhibit shrinkage of SP or fusion with lysosome
- What is the significance of the PhoPc data ?
- What is the advantage of large phagosomes for survival ?
 - Reduces concentration of toxins
 - Induces gradual exposure to acidity
- Did this paper answer the question asked in the beginning ?

Paper 2

Harrison, R. E., Brumell, J. H., Khandani, A., Bucci, C., Scott, C. C., Jiang, X. J., Finlay, B. B., and Grinstein, S. L. "*Salmonella* Impairs RILP Recruitment to Rab7 during Maturation of Invasion Vacuoles." *Molecular Biology of the Cell* 15 (2004): 3146-3154.

Title ? Too many acronyms ?

Introduction to Paper 2

- Type III secretion system (TTSS)
 - Injects effectors that lead to macropinocytosis of the bacteria and the formation of a Salmonella Containing Vacuole (SCV = spacious phagosome from previous paper)
- SCVs mature and acquire filaments called Sifs (Salmonella induced filaments).
 - Sif genes (like SifA) are important for the formation of these filaments.
- Salmonella survive inside the SCVs
- SCVs don't fuse with lysosomes
- We know:
 - That Rab7 exclusion from phagosomes is not the cause for not fusing with lysosomes
 - RILP (Rab7 interacting lysosomal protein) is involved in the movement of vesicles such as lysosomes towards MTOCs.

What question are they asking ?

Question/Hypothesis

- What keeps Salmonella alive inside the SCV and what prevents fusion of SCV with lysosomes ?
- Hypothesis:
 - Something downstream of Rab7, involved in vesicle trafficking, blocks phago-lysosomal fusion
 - RILP, Rab7 interacting protein that brings lysosomes to MTOCs, is targeted by Salmonella to prevent the fusion of SCVs with lysosomes
 - In the absence of RILP, Sifs can extend away from MTOCs and avoid lysosomal degradation of Salmonella

Paper 2 Questions

- Did the technology of confocal microscopy really help to answer the question or was light microscopy sufficient ?
- Is hiding in the phagosome a good way to hide from the immune system ?
- How can the immune system “see” Salmonella ?
- Was it necessary to make fusion proteins with GFP to image the proteins ? What might be the disadvantage ?
- Why are they using anti-LPS antibodies to detect Salmonella ?

Discussion of Paper 2

- Did the paper answer the question asked at the beginning ?
- Did the data support the conclusions ? (that Rab7 fails to recruit RILP and that SifA is responsible for that)
- Is SifA likely to be the only bacterial factor involved in keeping Salmonella in the SCVs ?
 - SifA deficient bacteria escape in the cytosol