Salmonella entry mechanisms known and to be discovered

Treball Final de Grau - Grau en Microbiologia

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1. INTRODUCTION

This review collects different mechanisms used by Salmonella to enter cells. For many years, only type III secretion system (T3SS-1) was known, but other, T3SS-1-independent systems have been recently described -such as Rck and PagN-. Nevertheless, new T3SS-1-independent systems like the T6SS are continuously being discovered and are yet to be described [1,2,3].

2. OBJECTIVES

- Differentiate the two main pathways of entry into the host cell: type III secretion system dependent entry and type III secretion system independent entry.
- Relate this pathways to the mechanisms "trigger" and "zipper".
- Paying special attention to the mechanisms which are still under study.

3. RESULTS

Mechanisms used by *Salmonella* to enter cells

1. T3SS-1-dependent entry

The T3SS-1 apparatus (T3SS-1) is a needle-like structure, encoded by *Salmonella* pathogenicity island 1 (SPI-1), which directly injects bacterial effector proteins into the host cytoplasm to manipulate the cell cytoskeleton, allowing bacterial internalization[2]. The T3SS-1 promotes a Trigger entry mechanism, which is characterized by the apparition of large membrane ruffles at the bacterial entry site [1] **(fig.1A).**

Salmonella is the first bacterium to be described as able to induce both Zipper and Trigger mechanisms for host cell invasion[1].

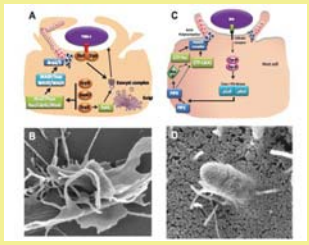


Figure 1. Trigger and Zipper mechanisms used by *Salmonella* to enter cells. A) Schematic representation of the Trigger mechanism. B) Scanning electron microscopy of Salmonella entering into cells via a Trigger mechanism. C) Schematic representation of the Zipper Mechanism. D) Scanning electron microscopy of Samonella entering into cells Via a Zipper mechanism. (Fig.1. Taken from [1]).

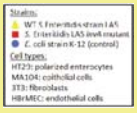
2. T3SS-1-independent entry

Role of the outer membrane proteins (OMP), as a RcK and PagN have been clearly identified as *Salmonella* invasins. **Rck** alone is able to promote adhesion and internalization[1,4]. RcK mediates a Zipper-like entry mechanism, which is characterized by weak membrane rearrangements[1] **(fig.1B)**. The function of **PagN** as an invasin is supported by the fact that PagN overexpressed in a noninvasive *E. coli* strain induced cell invasion. However, PagN-defective bacteria displayed a consistent two- to fivefold reduction in cell invasion when compared to the wild-type strain[1,5].

In vivo evidences for a non-essential rofe of T3SS-1

Although numerous studies demonstrate a role of T3SS-1 in host infection, recent evidences suggests that *S.* Typhimurium and other serotypes can also cause infection in a SPI-1-independent manner[1].

Salmonella T3SS-1-independent entry plays a more important role in fibroblasts and some epithelial cells compared with enterocytes and endothelial cells[1,2] (fig.1).



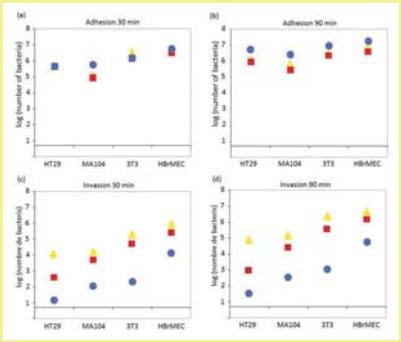


Fig.1. Quantification of *Salmonella* T3SS-1-independent entry mechanisms into cells. The T3SS-1-dependent entry corresponds to the difference in invasion between the wild-type LA5 strain and the *invA* mutant, whereas the T3SS-1-independent entry corresponds to the difference in invasion between the *invA* mutant and the *E.coli* strain. (Fig.1.Modified by [2]).

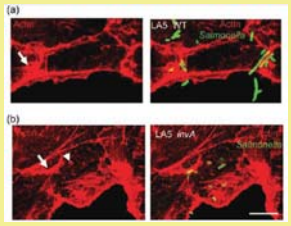


Fig.3. Actin cytoskeletal rearrangements induced by *Salmonella* T3SS-1-dependent and -independent invasion mechanisms. F-actin was labelled red with phalloidin conjugated to rhodamine, and *Salmonella* cells were localized with an anti-09 antigen antibody labelled green. (Fig.3. Taken from [2]).

These experimental studies support clinical observations. *Salmonella* isolates carrying a deletion encompassing a vast segment of SPI-1 have been responsible for food-borne disease outbreaks[1].

Internalization of all the wild-type LA5 *S.* Enteritidis bacteria into epithelial cells was characterized by a massive actin rearrangement at the bacterial entry site, typical trigger mechanism[1] **(Fig.3a)**.

The entry process of the *invA* mutant induced either a massive or a local actin accumulation at the engulfment site[2] (fig.3b).

Table 2. Effect of *rck* deletion on invasion levels of the wild-type and *invA* mutant strains in HT29 and MA104 cell lines.

Cell line	*Salmonella* invasion (%)*			
	LA5 wild-type	LA5 Δ*rck*	LA5 *invA*	LA5 *invA*Δ*rck*
MA104	2.63 ± 0.66	2.8 ± 0.30	0.16 ± 0.02	0.25 ± 0.08
HT29	4.16 ± 0.56	4.4 ± 1.34	0.02 ± 0.20	0.02 ± 0.02

Invasion levels were obtained after 90 min of infection (m.o.o 50:1). The percentage fo the ratio between viable bacteria surviving gentamicin treatment and the original inoculum. (Table 2.Taken from [2]).

Rck is not involved in cell invasion of the *invA* mutant. **These results demonstrate that there are new mechanisms T3SS-1-independent entry.**

New mechanism to entry

• Type VI Secretion System (T6SS)

1. systemic dissemination during in vivo infections of mice

Mice infected with *sciG* and *sciS mutant,* genes which have been established as essential for T6SS assembly and functional secretion in other systems, reached an endpoint 2 days later than those infected with wild-type (wt) bacteria **(fig. 4A)**. The *sciG* and *sciS* mutants were less fit in the liver and spleen than wild-type wt bacteria, a defect that could be resolved by plasmid-based complementaion of *sciG* **(fig. 4B)**[3].

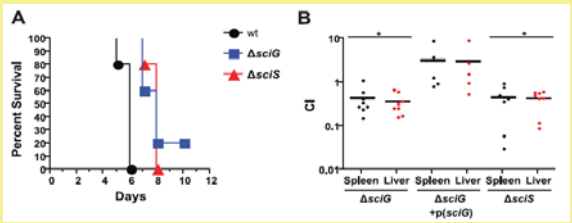


Fig.4. Contribution of the SPI-6 T6SS to *S.* Typhimurium pathogenesis in mice. (A) Survival curve of SPI-1 T6SS mutant bacteria in C57BL/6 mice. (B) CI experiments with WT and SPI-6 T6SS mutant bacteria and C67BL/6 mice orally infected with 10^6 CFU. (Fig.4.Taken from [3])

2. Possible invasion factors

- Adhesins
- Outer membrane vesicles (OMV)
- Cell receptors: cystic fibrosis transmembrane (CFTR)[1]

2. Contribute to intracellular replication in macrophages

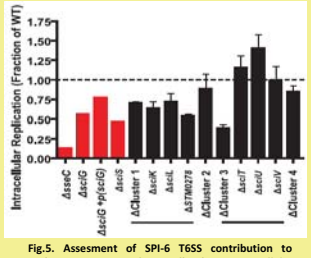


Fig.5. Assesment of SPI-6 T6SS contribution to replication in macrophage cell culture. Intracellular replication of SPI-6 T6SS mutants in RAW 264.7 murine macrophage-like cells expressed as a fraction of that of the WT at 24h postinfection compared with that of a Δ*sseC* SPI-2 T3SS translocon mutant. **(T6SS core gene. Non-core gene).** (Fig.5. Taken from [3]).

It has identified that the disruption of noncore T6SS **clusters 2 and 4** caused significant defects in **systemic dissemination in mice[3].** And that disruption of **noncore gene clusters 1 and 3** resulted in a significant **intracellular replication defect in macrophages[3] (fig.5)**.

SPI-6 T6SS has been integrated into the *S.* Typhimurium virulence[3].

4. CONCLUSIONS

- Unlike the classical knowledge, salmonella is able to enter the host cell without the T3SS-1.
- *Salmonella* is the first bacterium to be described as able to inducte both Zipper and Trigger mechanisms for host cell invasion.
- *Salmonella* T3SS-1-independent entry plays a more important role in fibroblasts and some epithelial

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