Biology of *Salmonella* – Prof David Holden

- **the disease and model systems**
- the paths to systemic infection
- virulence genes and pathogenicity islands
- virulence protein delivery by type III secretion systems
- vaccines

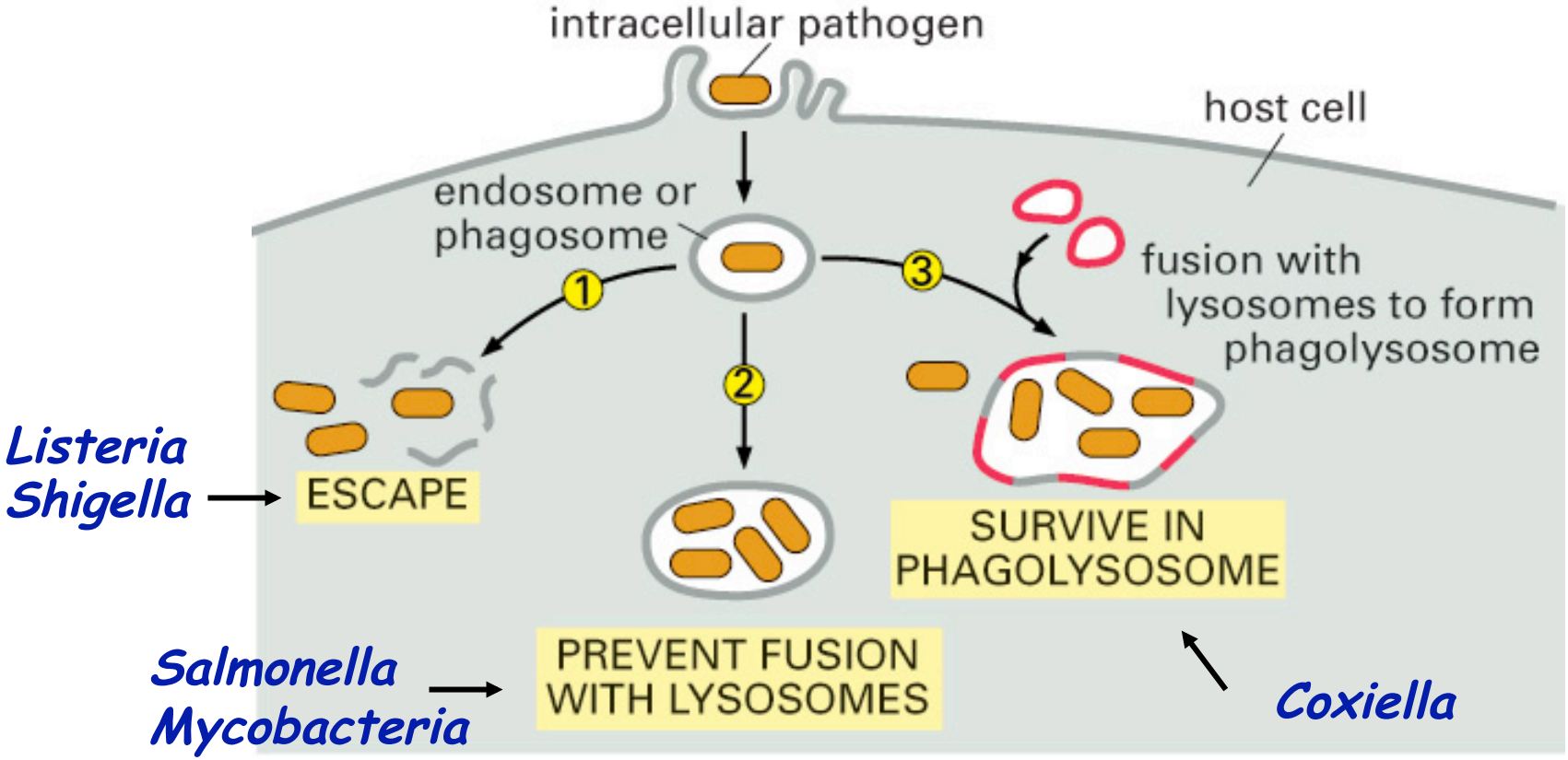


Figure 25–28. Molecular Biology of the Cell, 4th Edition.

Salmonella pathogenesis

human diseases

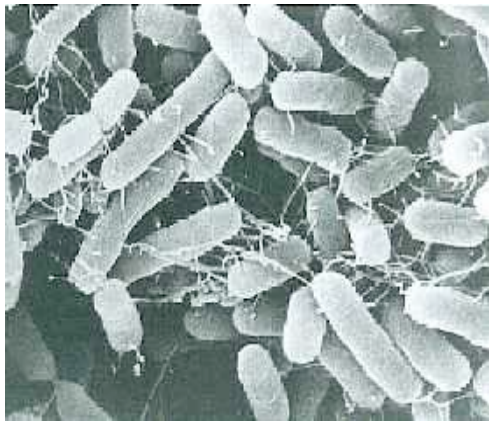
non-typhoidal (e.g *S. enterica* Typhimurium): 1.3 billion cases and 3 million deaths/yr (major problem in sub-Sarahan Africa)

typhoid (*S. enterica* Typhi and *S.* Paratyphi)*:* 17 million cases and 600,000 deaths/yr - increasing multiple drug resistance

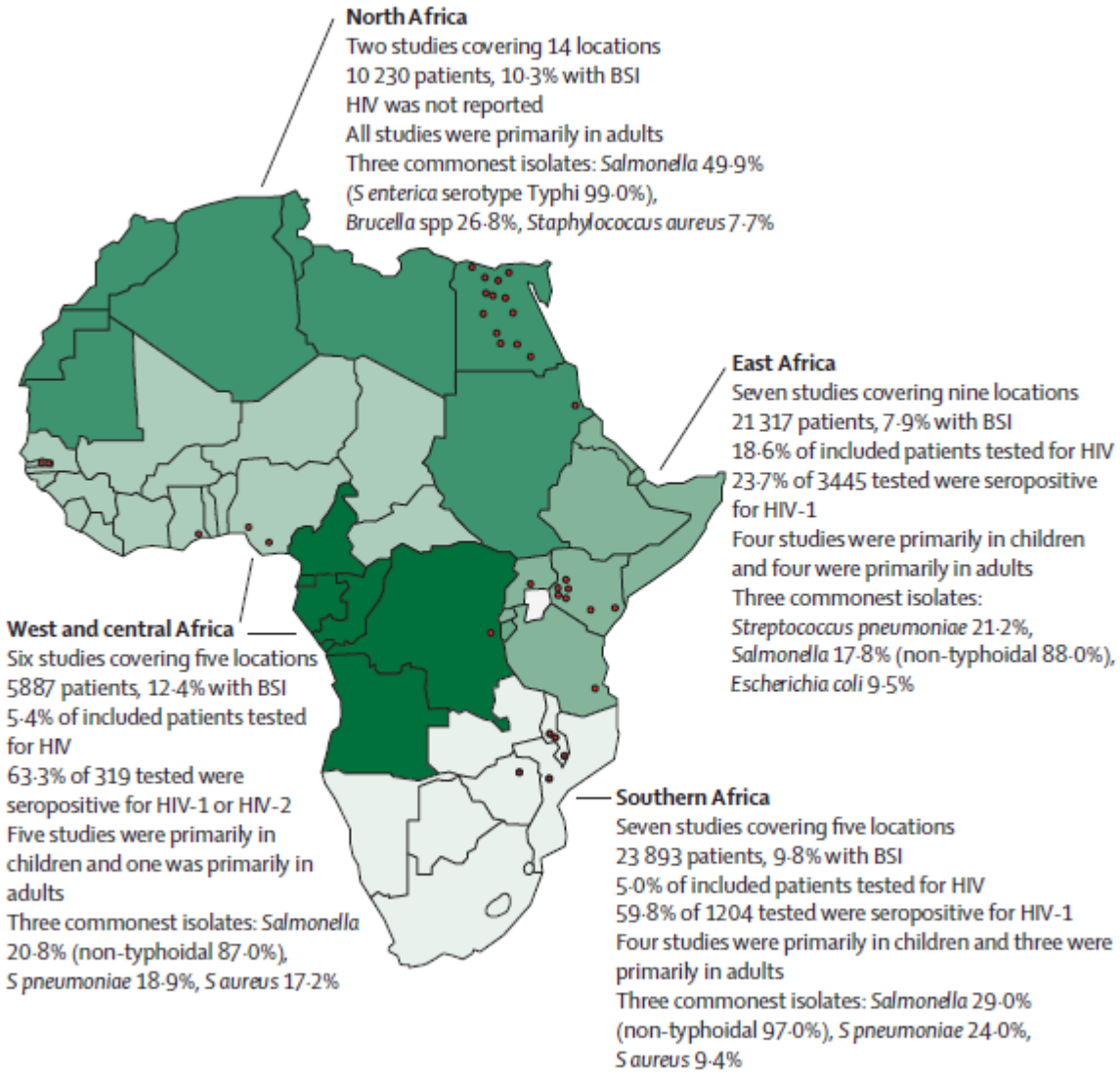
molecular basis of virulence: *S.*Typhimurium

excellent molecular genetics

good model of typhoid fever in mice (replicates intracellularly in spleen and liver, up to 10^9 bacterial cells/organ)

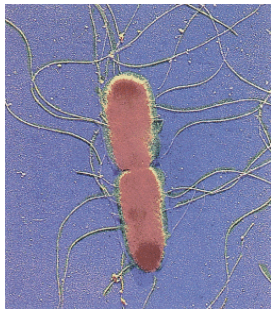


Salmonellae are the commonest cause of bacterial bloodstream infection in Africa



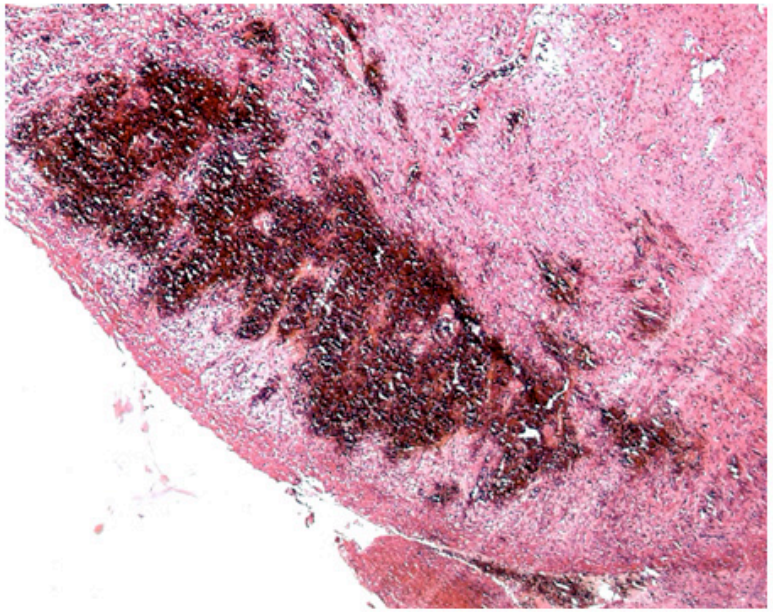
(Reddy EA, et al. **Lancet Infect Dis** 2010)

Salmonella typhi and human typhoid

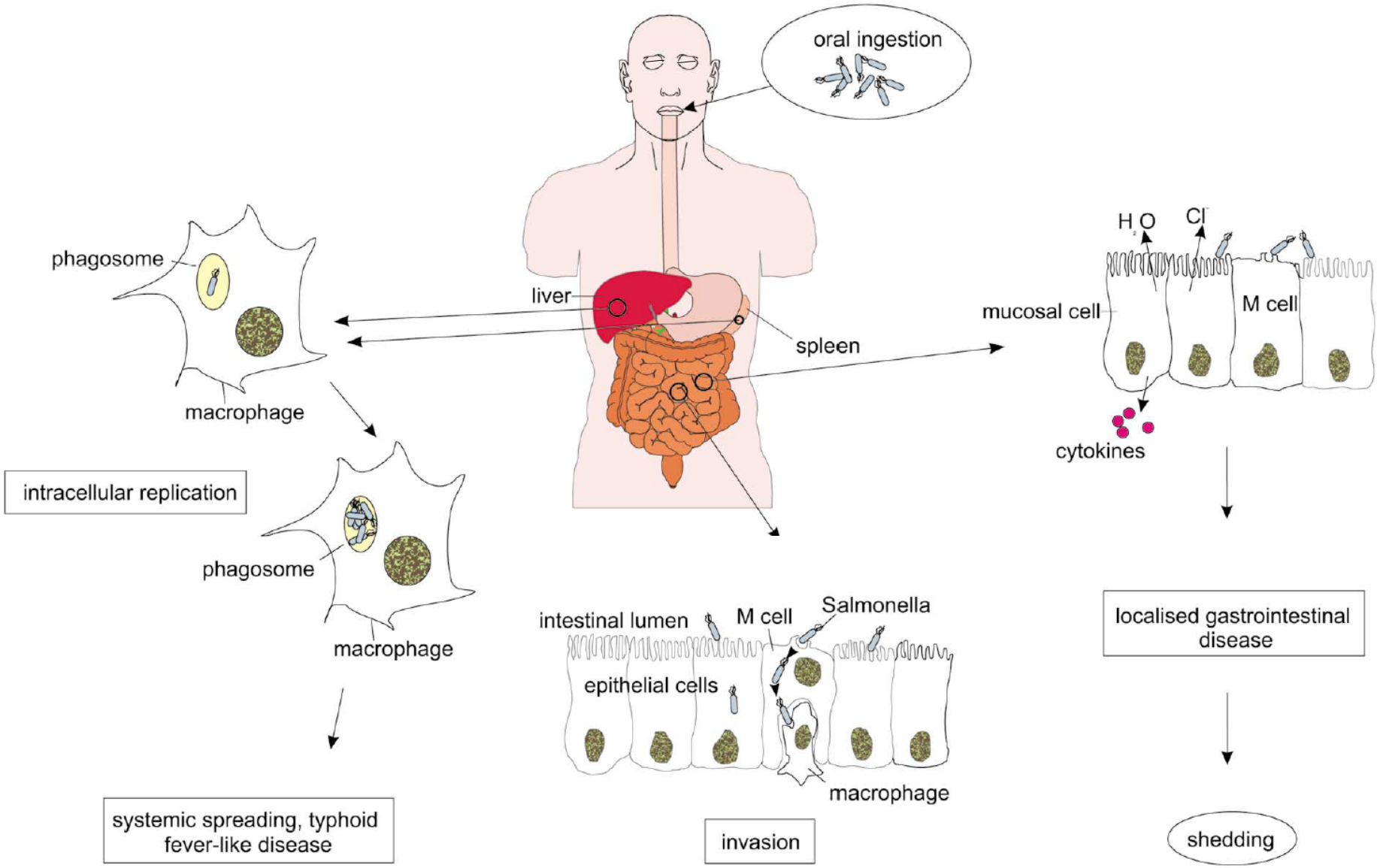


Transmission (faecal-oral)

Infection (10^5- 10^9 cells)



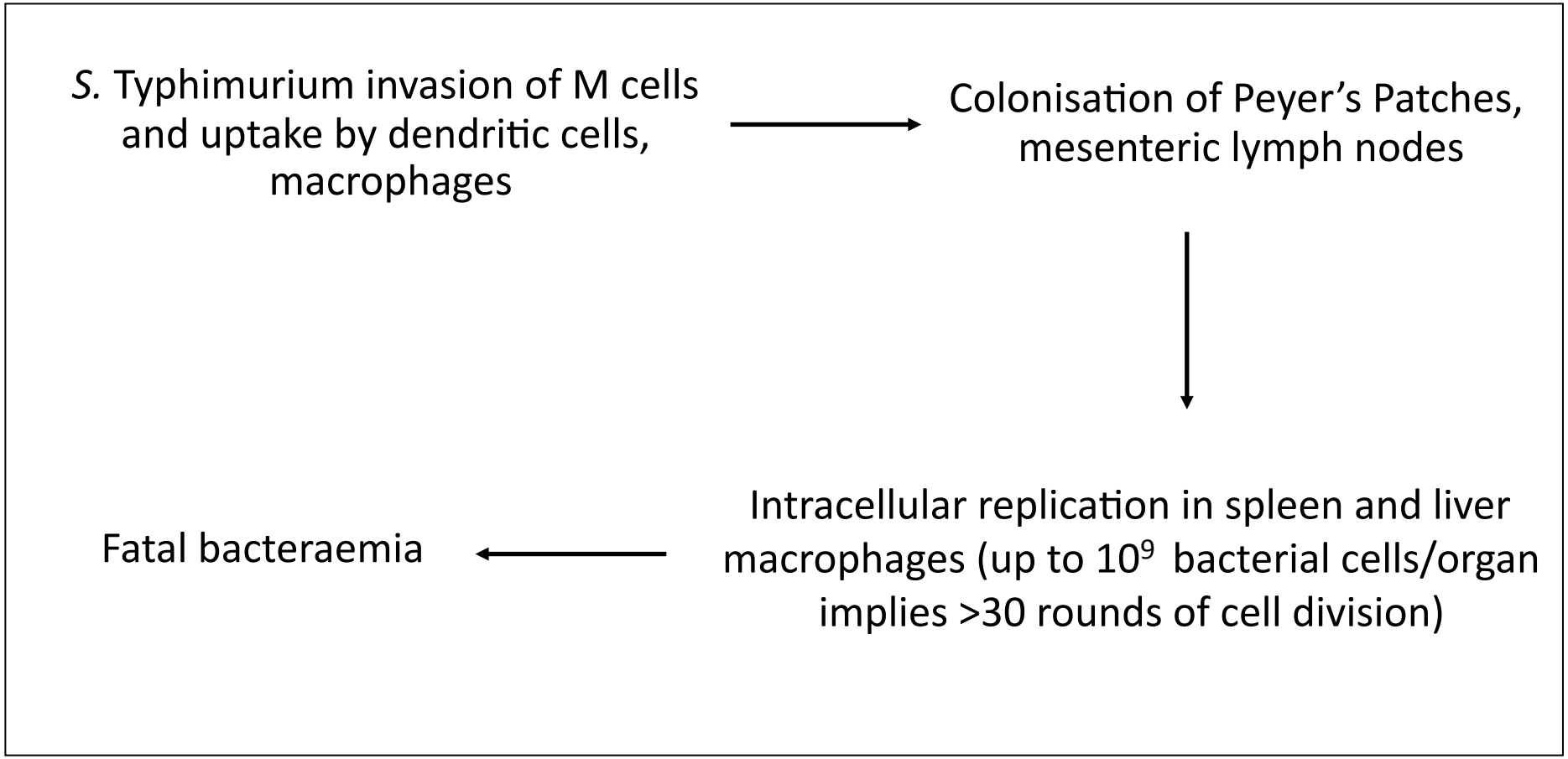
Intestinal perforation with bleeding into tissues



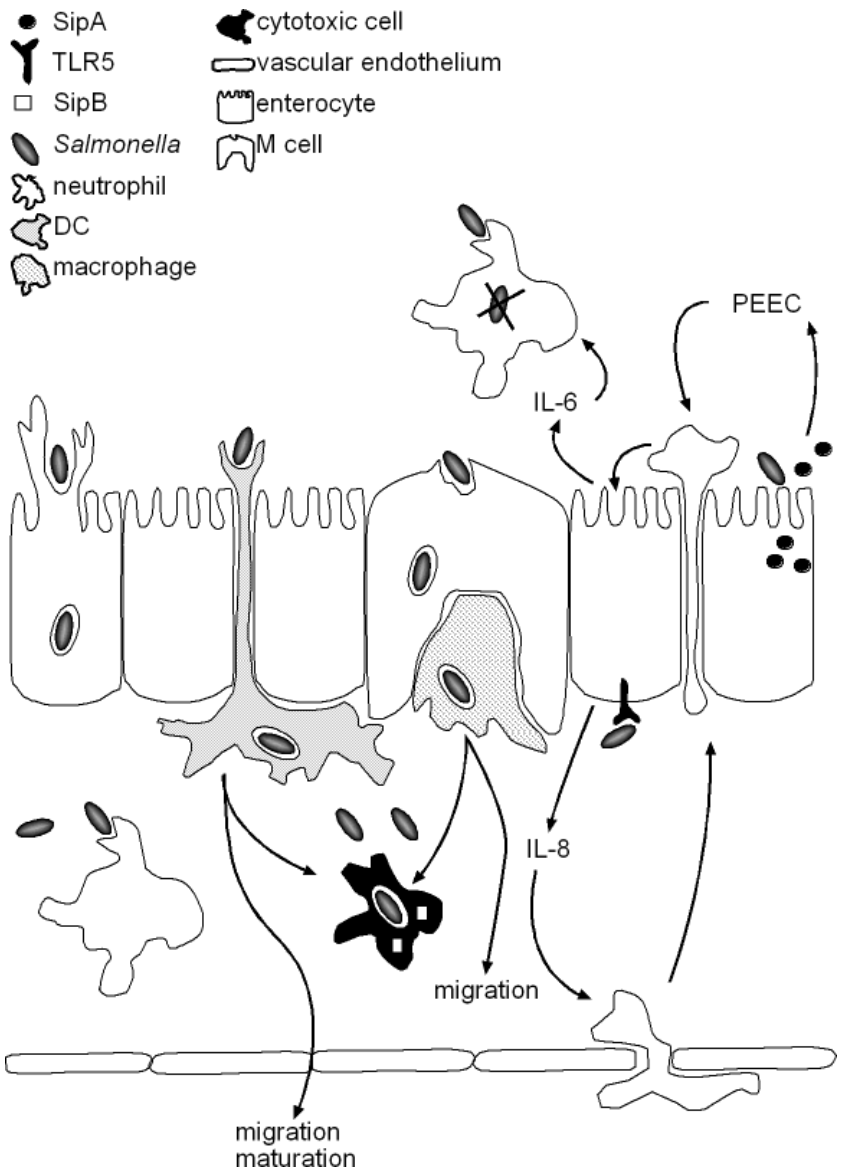
S. Typhi

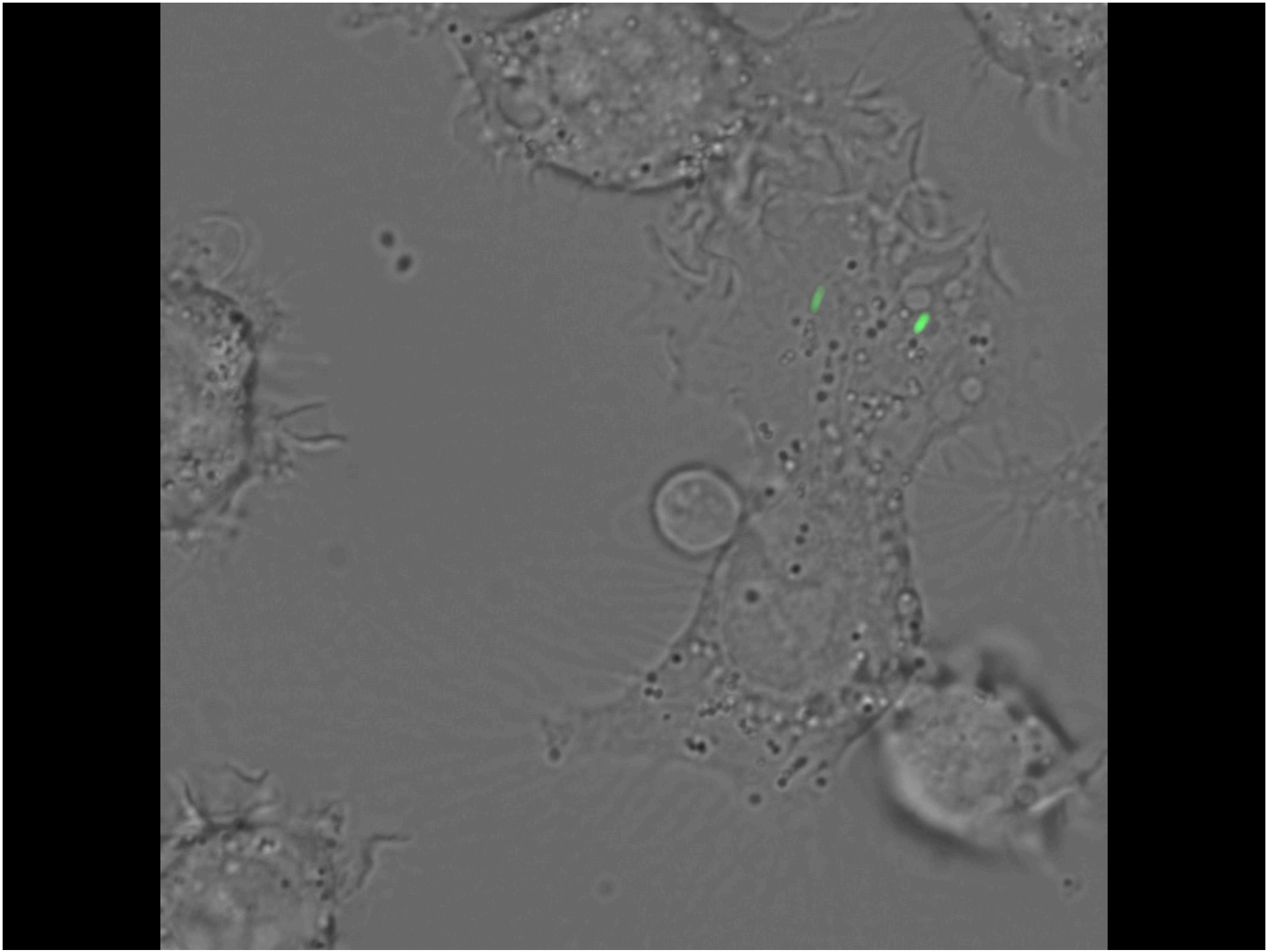
S. Typhimurium

Mouse model of typhoid fever

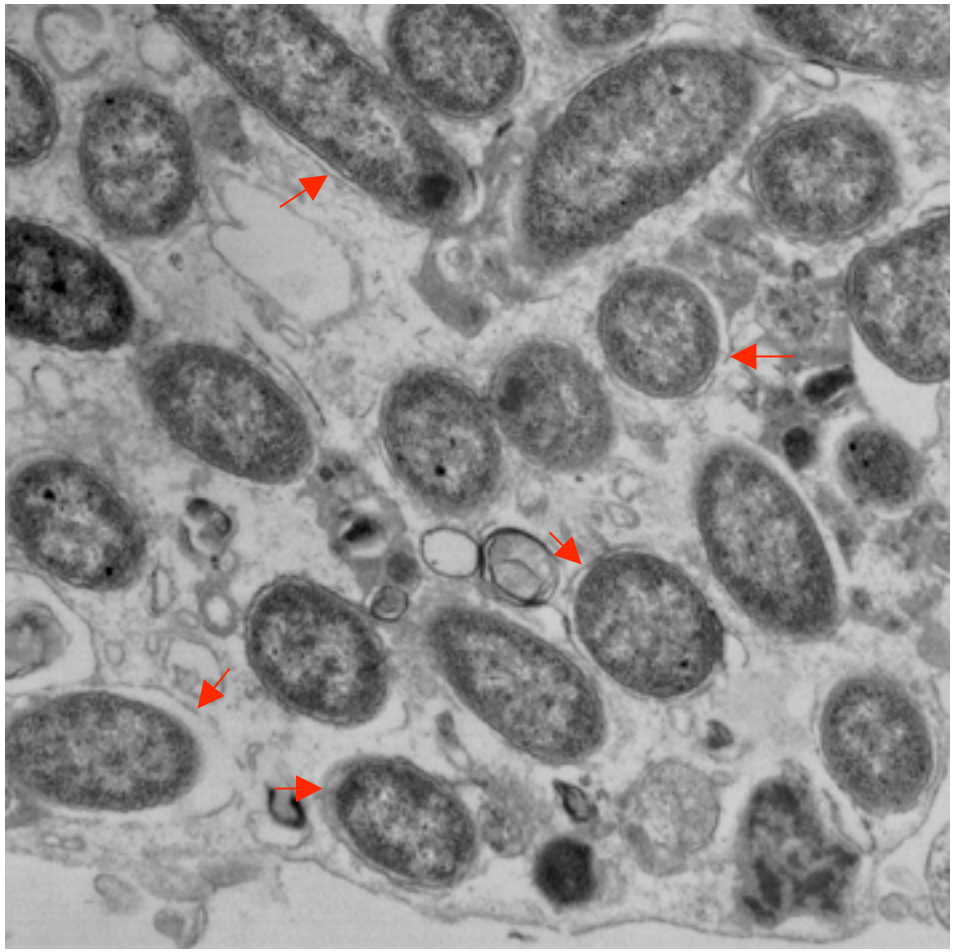


3 ways to cross the gut epithelium..



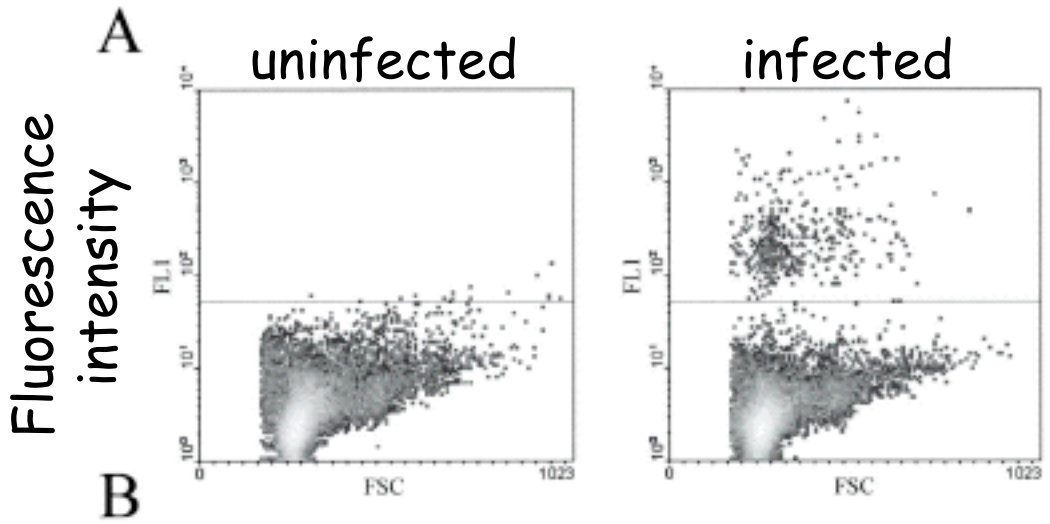


Salmonella-containing vacuoles in spleen cells



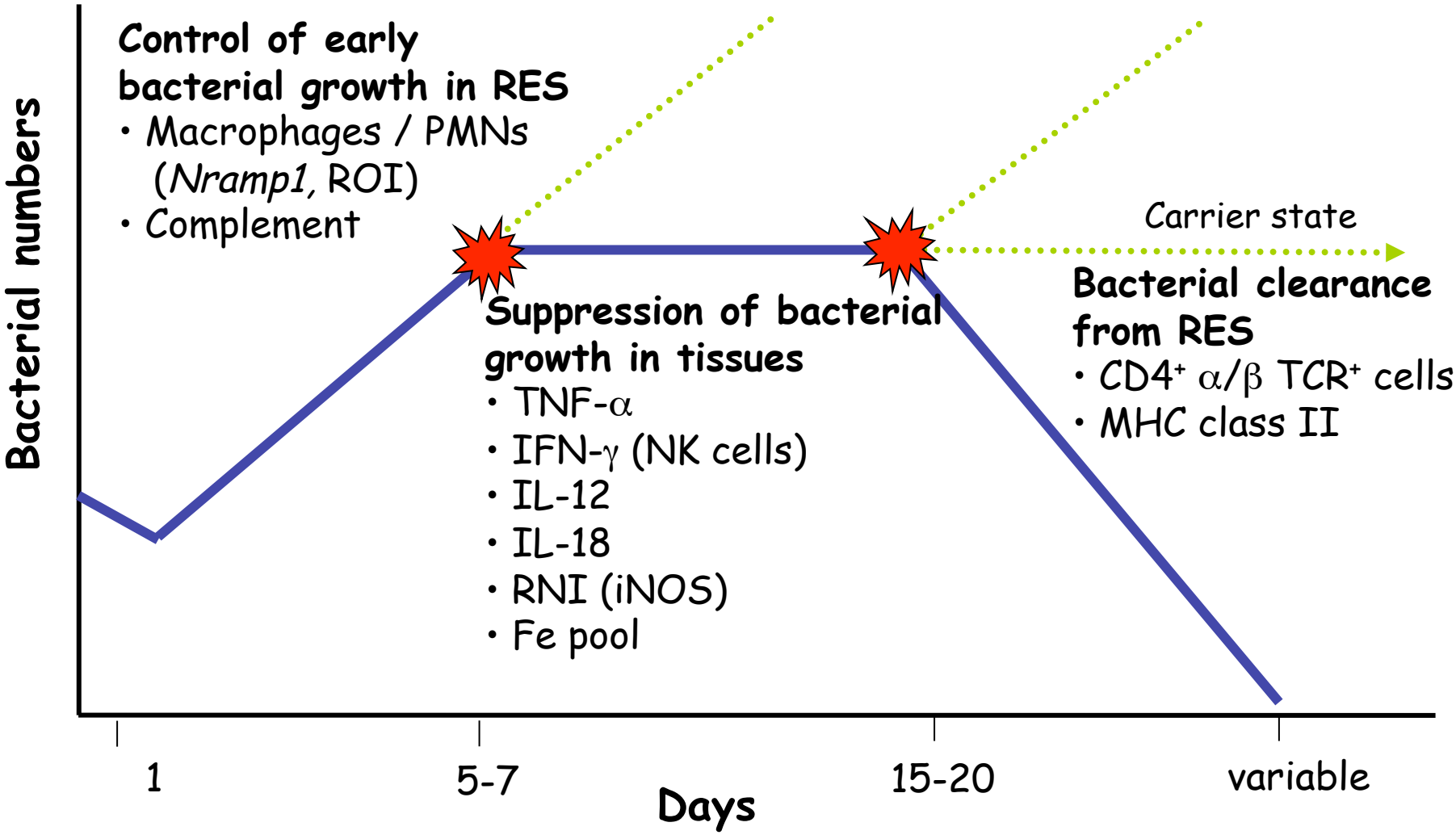
Flow cytometry and confocal microscopy of *GFP-S.typhimurium* in spleen macrophages

(Salcedo et al., Cell Microbiol 3: 587-597 [2001])



Z-stack projection (stained for F-actin (red)

Effector mechanisms in primary sublethal infection of *S. typhimurium* in the mouse



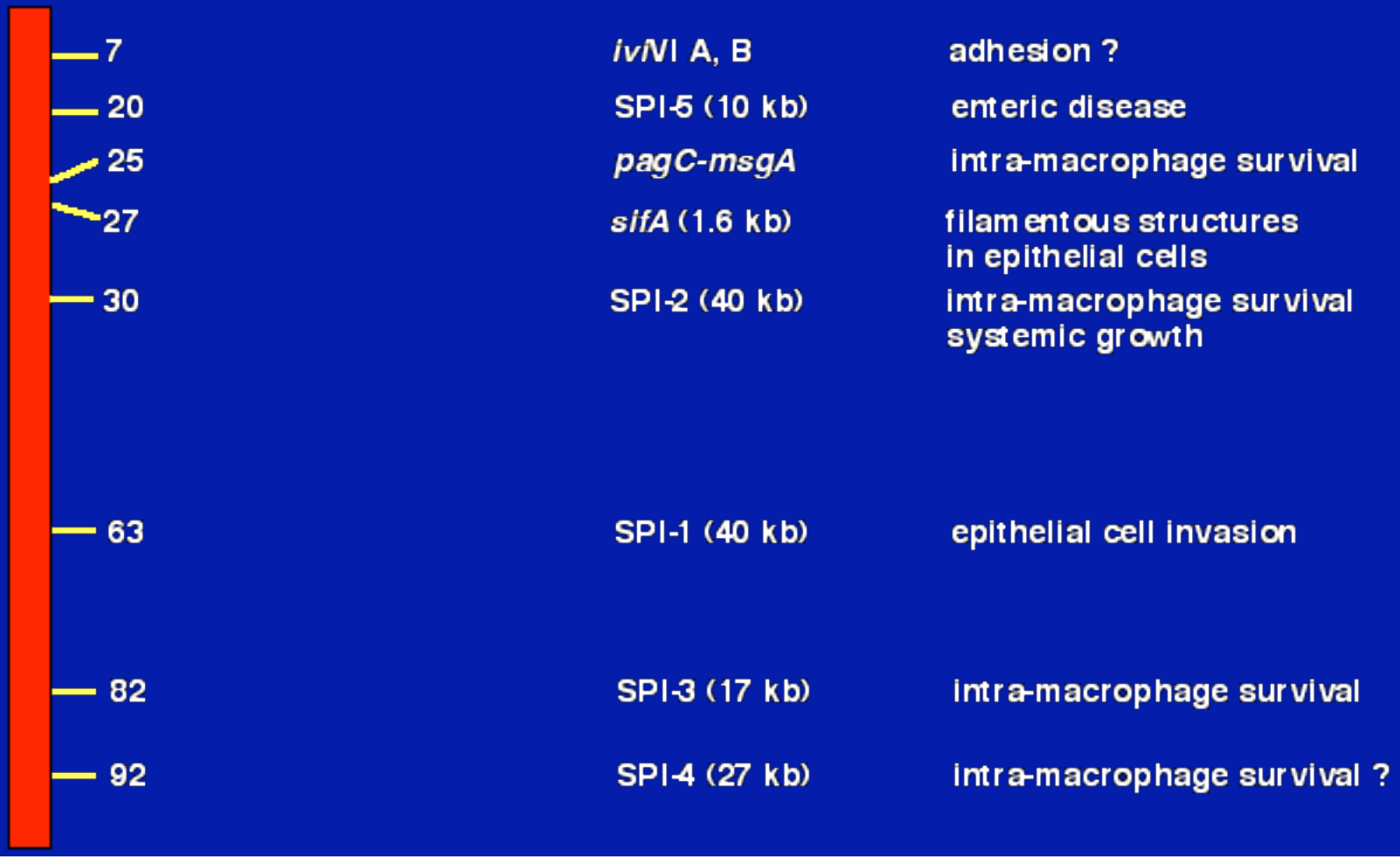
Different genes act at different stages of *S.* Typhimurium virulence

stage of infection	environmental stimulus	response genes	virulence
stomach	low pH	acid tolerance	*fur, atr*
small intestine	osmotic stress anaerobiosis	motility adhesion	*flg, lpf and pef*
epithelium penetration	anaerobiosis cell contact	invasion	many (e.g.*inv/spa*)
blood-stream	complement antimicrobial peptides	serum resistance peptide resistance	*rfb, rfc, rck* *sap, htr*
macrophage	low pH, low Mg^{++}	complex	many (*pag,* SPI-2, *spv*)

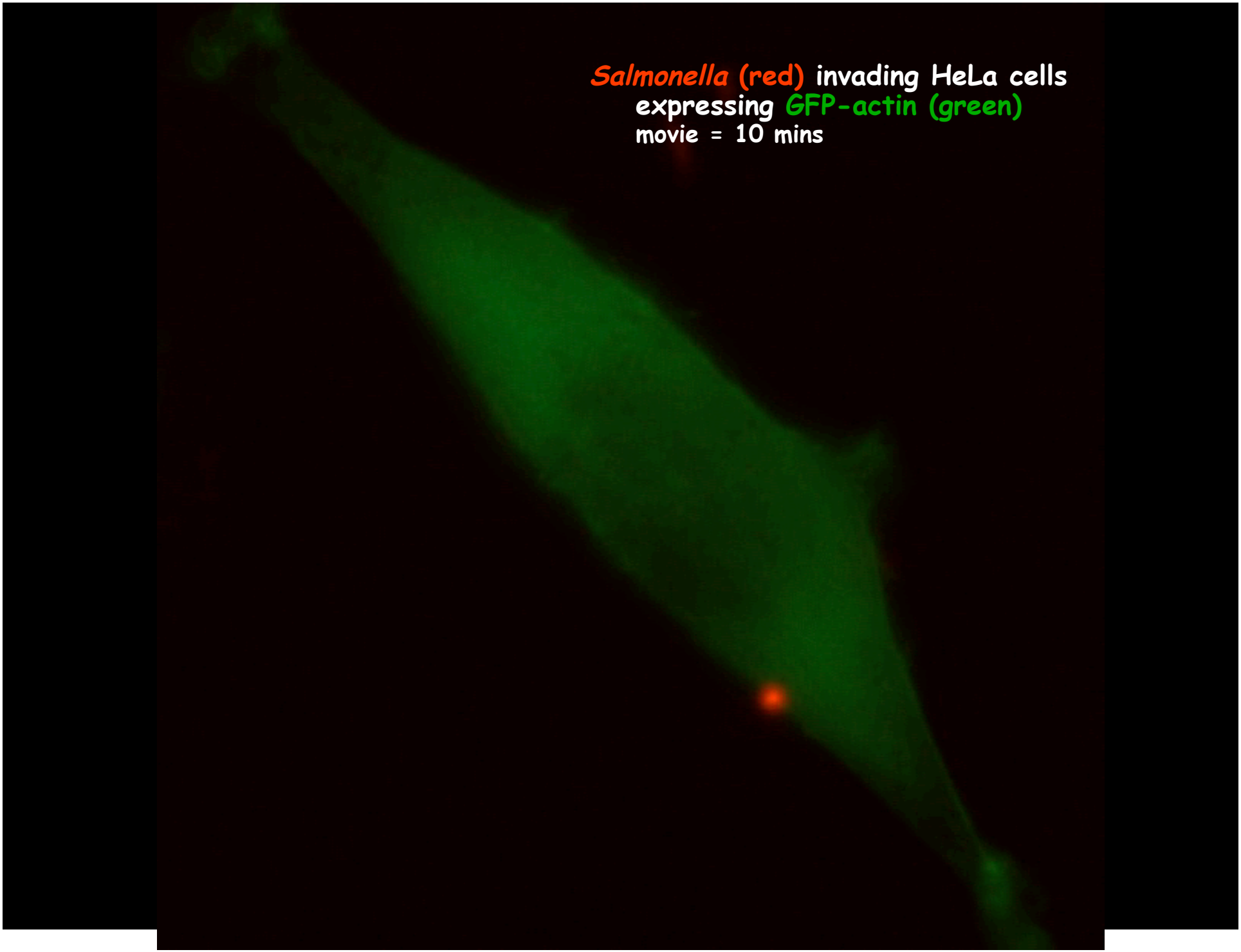
Total number of virulence genes...

- Estimates suggest approx. 3.5% of *S. typhimurium* genome required for virulence
- *S.* Typhimurium has 4600 ORFs => approx. 160 virulence genes
- Approx. 140 genes identified to date
- Most are present on Pathogenicity Islands/islets (> 60).
- But, biochemical and physiological functions of many remain unknown

62 pathogenicity islands in the *S. typhimurium* genome; many fimbrial, flagellar and phage genes

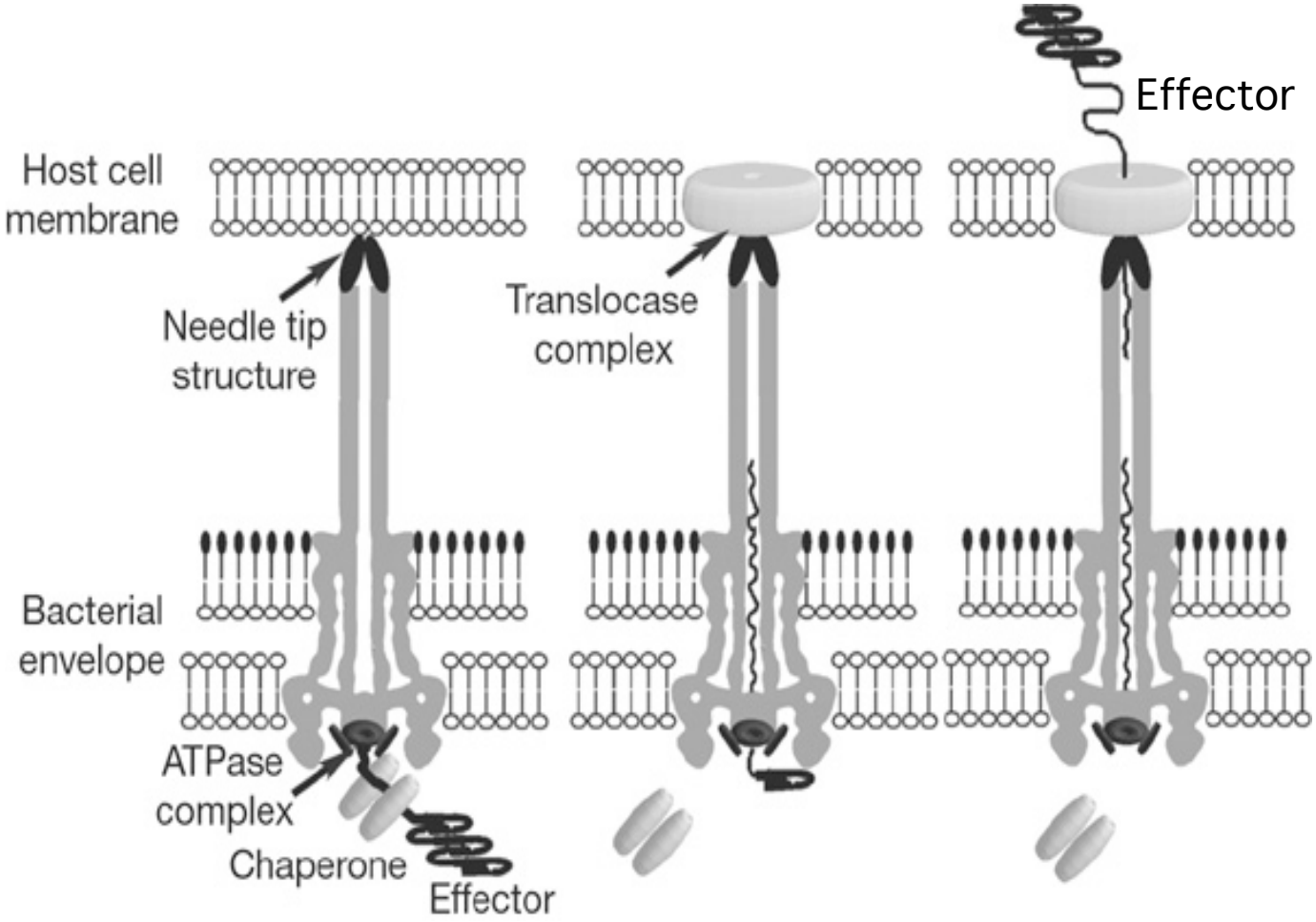


Salmonella SPI-1 pathogenicity island encodes the machinery for a Type III secretion system

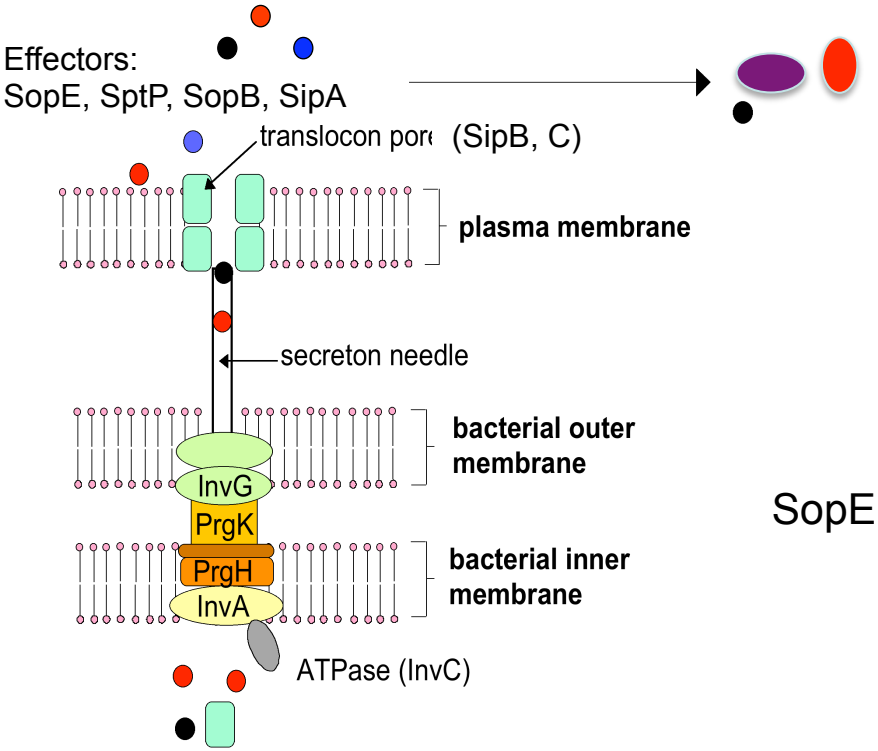

Salmonella (red) invading HeLa cells
expressing GFP-actin (green)
movie = 10 mins

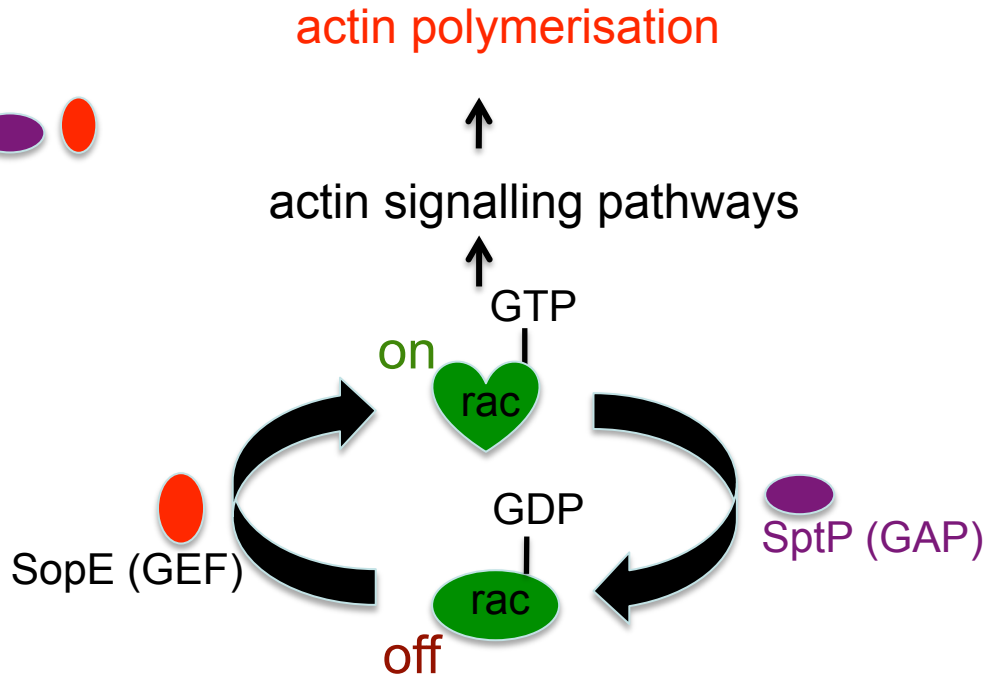
Salmonella invasion of epithelial cells occurs via the SPI-1 type III secretion system

effectors induce actin polymerisation, membrane ruffling and bacterial internalisation



SPI-1 secretion and translocation

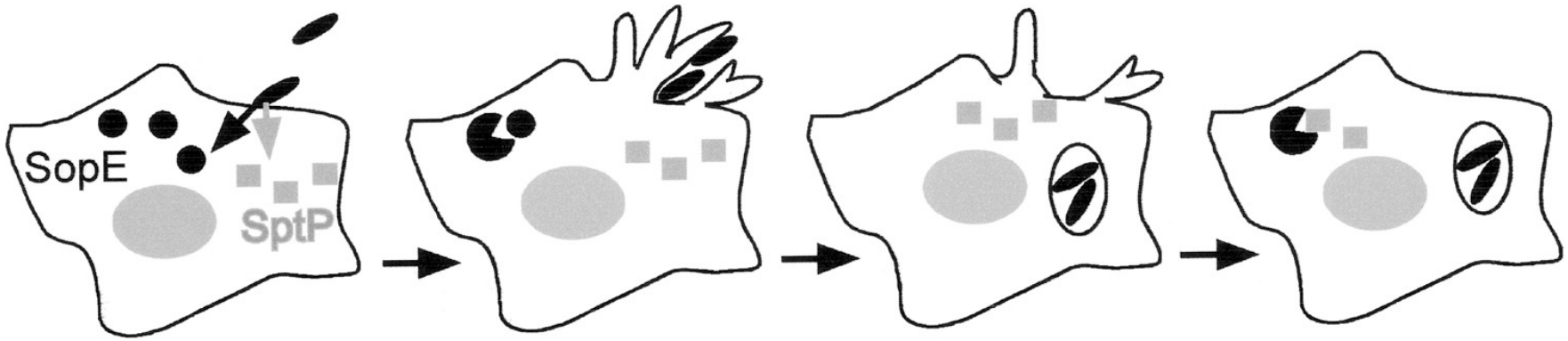




Cell, Vol. 115, 333–342, October 31, 2003, Copyright ©2003 by Cell Press

Temporal Regulation of *Salmonella* Virulence Effector Function by Proteasome-Dependent Protein Degradation

Tomoko Kubori and Jorge E. Galán*
Section of Microbial Pathogenesis
Yale University School of Medicine
New Haven, Connecticut 06536

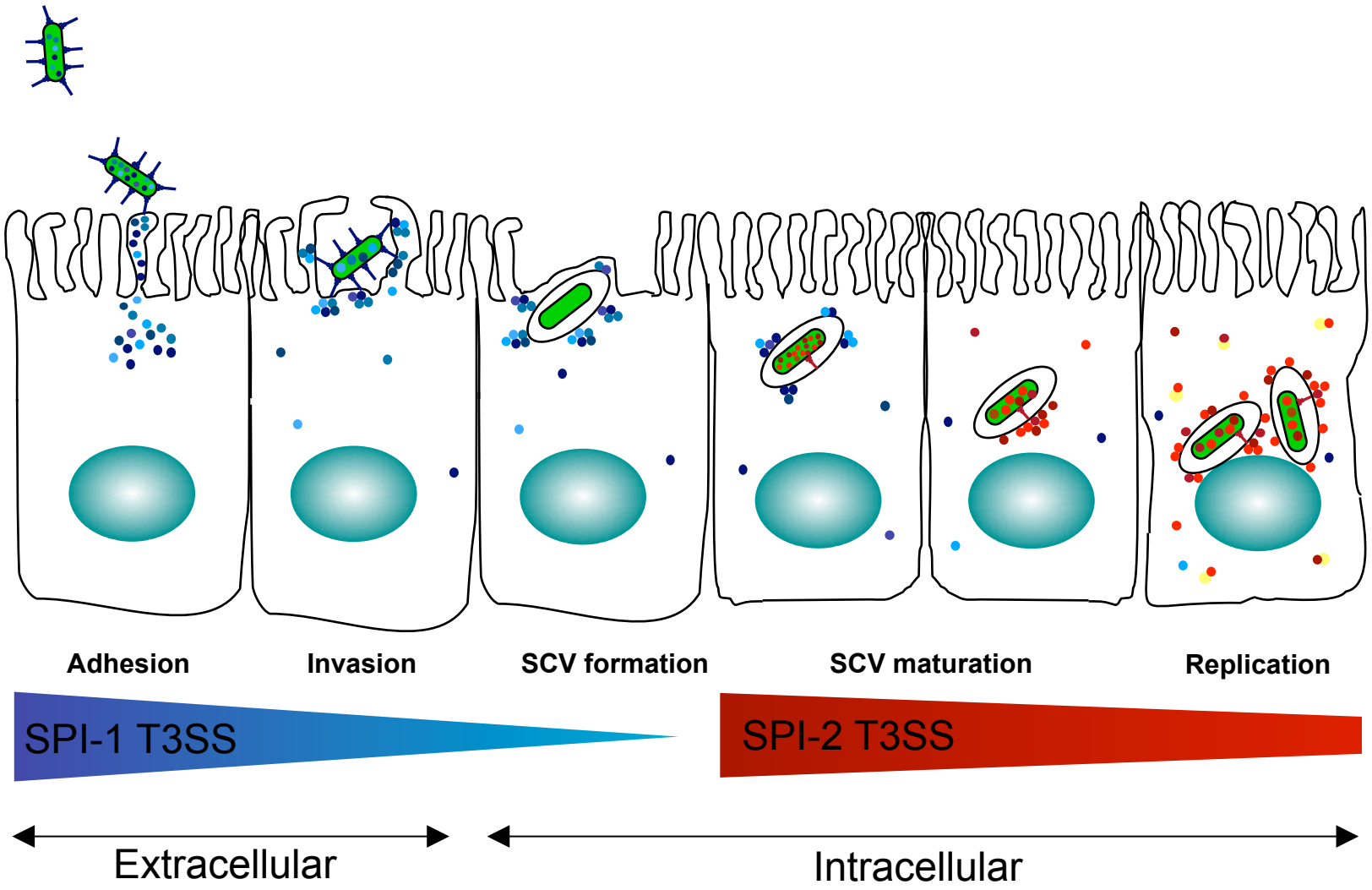


Salmonella delivers roughly equivalent amounts of SopE and SptP. Shortly after infection (15–20 min), SopE is rapidly degraded by the host-cell proteasome via the amino terminal secretion and translocation domain of the bacterial protein. SptP is eventually also degraded by the proteasome, but with a half-life much longer than that of SopE.

Salmonella has two type III secretion systems, both encoded by pathogenicity islands

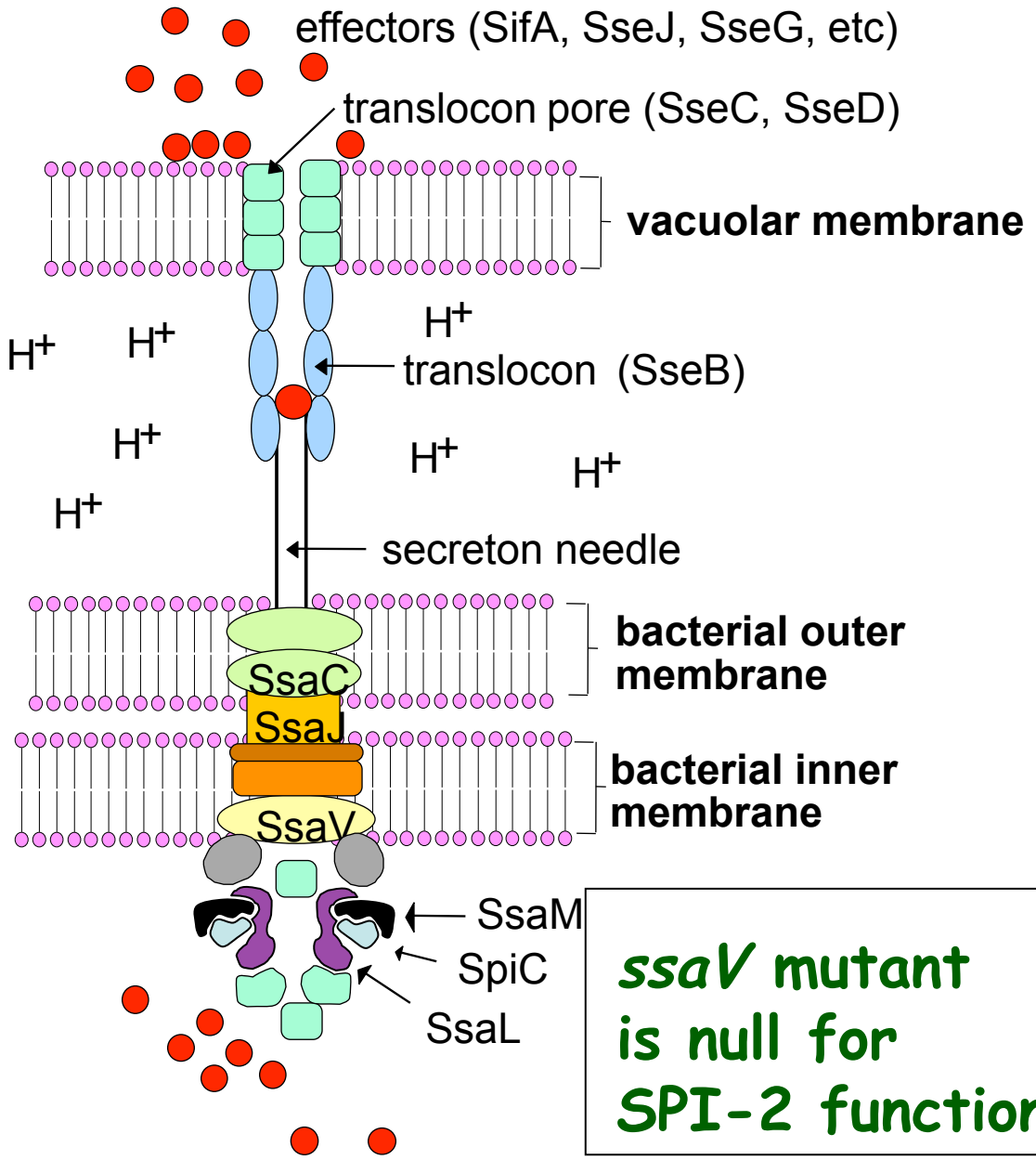
SPI-1 mediates:
(i) invasion of host cells (stimulates actin polymerisation; effectors act as a GEF and a GAP for Rac and RhoG)
(ii) intestinal secretory and inflammatory responses

SPI-2 is required for bacterial replication and immune modulation (translocates effectors across the vacuolar membrane)

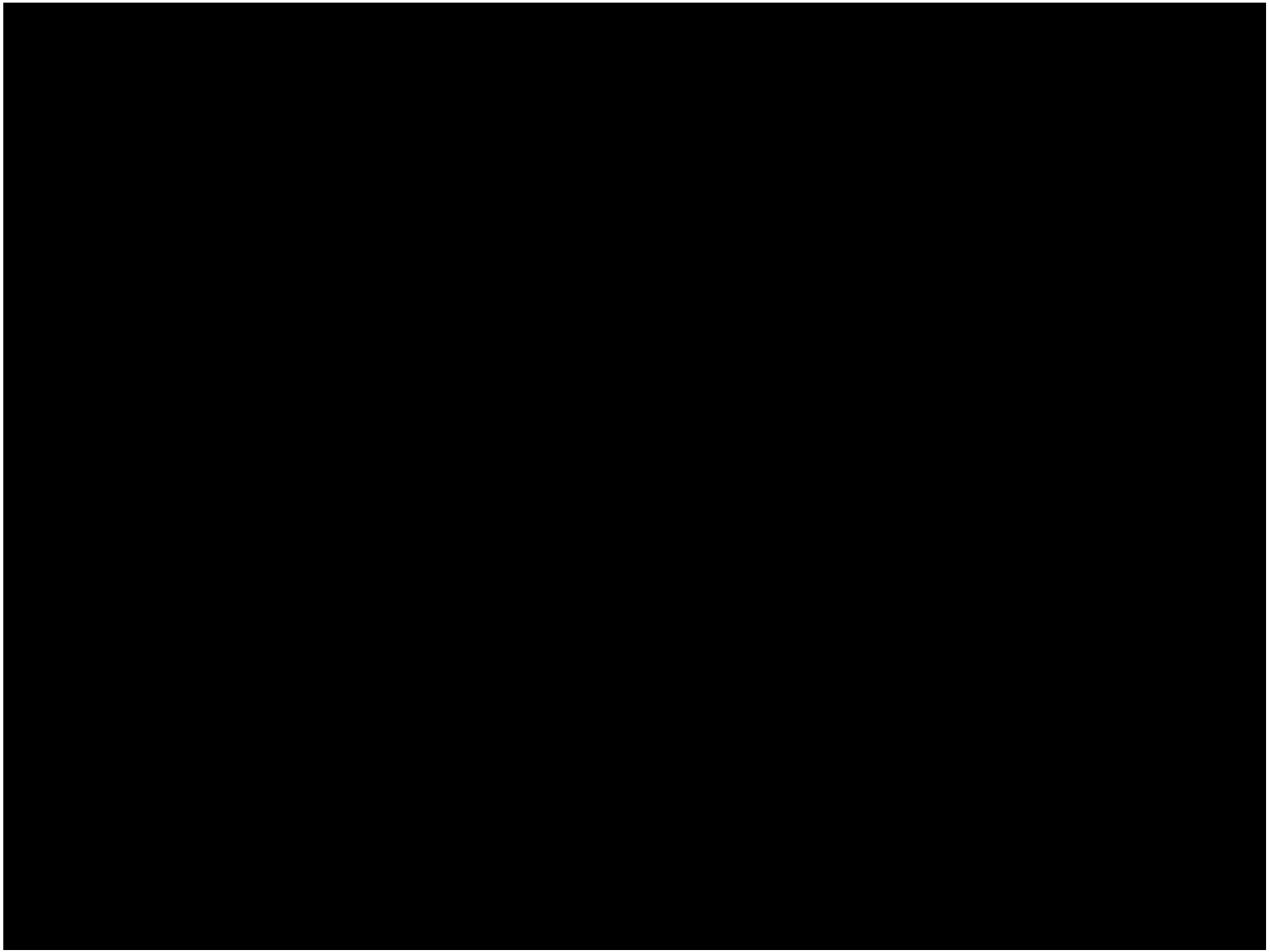

Adhesion
Invasion
SCV formation
SCV maturation
Replication
SPI-1 T3SS
SPI-2 T3SS
Extracellular
Intracellular

SPI-2 Type III secretion system

acidic pH triggers secretion



ssaV mutant is null for SPI-2 function

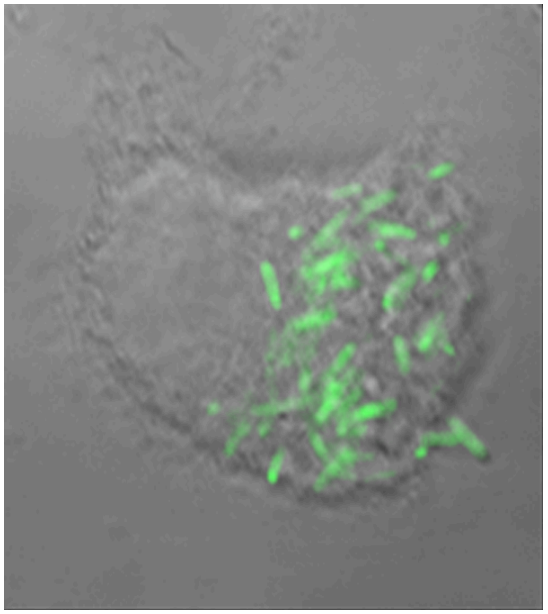


Effectors of the SPI-2 T3SS

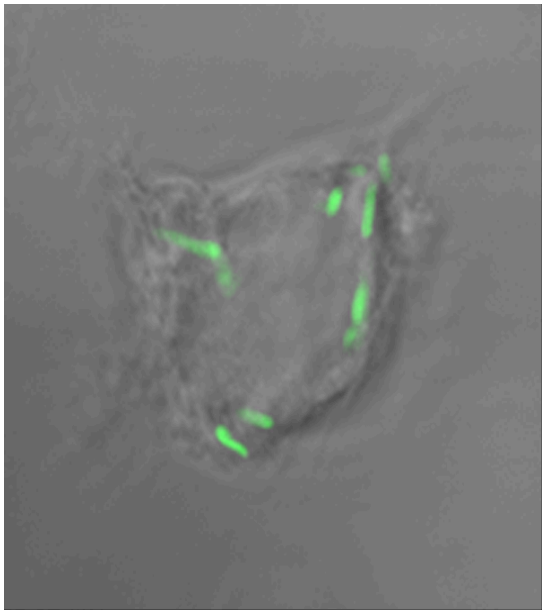
Protein	Function
SifA	Interacts with SKIP (kinesin regulator) Maintains vacuolar membrane Induces tubular extensions from SCV derived from late endosomes
SseJ	Acyltransferase; vacuolar membrane dynamics
PipB2	Kinesin-1 linker
SifB	Similarity to SifA
SopD2	Antagonizes SifA
SseF, SseG	Localise SCVs to Golgi/MTOC in epithelial cells
SseI/SrfH	Induces motility of macrophages
SspH-1	E3 ligase
SspH-2	E3 ligase
SlrP	E3 ligase
SseL	Deubiquitinase
SteC	Kinase
SpvC	Phosphothreonine lyase
SrfJ	Glucosyl ceramidase ?
PipB	unknown
SseK1, SseK2	unknown
GogB	unknown
SteA	unknown
SteB	unknown

Intracellular replication defect of a SPI-2 null mutant in RAW macrophages (16 h)

wild-type



SPI-2 mutant



SpvC is a *Salmonella* effector with phosphothreonine lyase activity on host mitogen-activated protein kinases

Piotr Mazurkiewicz,[1] Jerry Thomas,[2] Jessica A. Thompson,[1] Mei Liu,[1] Laurence Arbibe,[3] Philippe Sansonetti[3] and David W. Holden[1]*

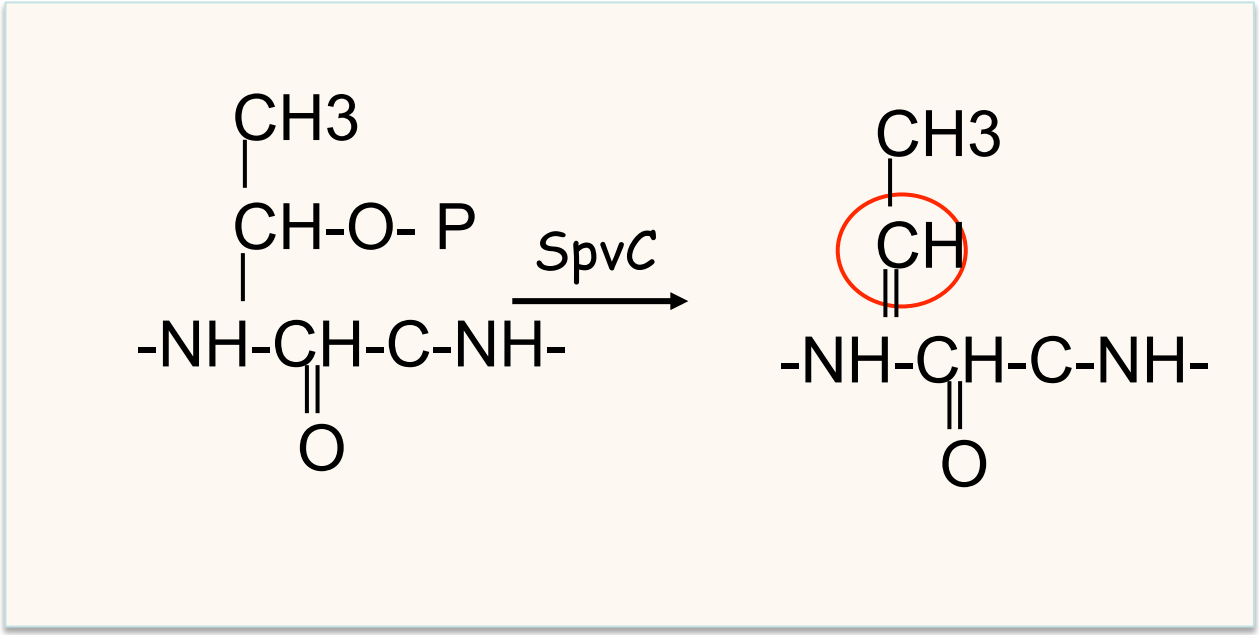
[1]*Centre for Molecular Microbiology and Infection, Imperial College London, Armstrong Road, London SW7 2AZ, UK.*

[2]*Technology Facility, Department of Biology, University of York, Heslington, York, UK.*

[3]*Unité de Pathogénie Microbienne Moléculaire, INSERM U786, Institut Pasteur, 28 Rue du Docteur Roux, 75724 Paris Cedex 15, France.*

S. Typhimurium pathogenesis depends upon a large number of virulence proteins. Some of these, called effectors, are injected into the host cell by two specialized protein translocation machineries – type three secretion systems (T3SS). The T3SS encoded within *Salmonella* pathogenicity island 1 (SPI-1) is produced by extracellular bacteria and delivers effectors across the host cell plasma membrane; these are necessary for bacterial internalization and the early stages of *Salmonella*-containing vacuole (SCV) formation (Zhou and Galan, 2001; Schlumberger and Hardt, 2006). The SPI-2-encoded T3SS is activated by internalized *Salmonella* and translocates

phosphothreonine lyase activity of SpvC



leads to irreversible dephosphorylation (eliminylation)…!

Typhoid vaccines

1. Vi capsule injectable single dose. 50-70% efficacy
2. Ty21a live attenuated (genetic basis unknown) 3 dose 33-70% efficacy

1995

2005

MAJOR ARTICLE

The Novel Oral Typhoid Vaccine M01ZH09 Is Well Tolerated and Highly Immunogenic in 2 Vaccine Presentations

Beth D. Kirkpatrick,[1] Katherine M. Tenney,[1] Catherine J. Larsson,[1] J. Patrick O'Neill,[2] Cassandra Ventrone,[1] Matthew Bentley,[3] Anthony Upton,[3] Zoë Hindle,[3] Christine Fidler,[3] Deborah Kutzko,[1] Regan Holdridge,[1] Casey LaPointe,[1] Sandra Hamlet,[1] and Steven N. Chatfield[3]

[1]Department of Medicine, Unit of Infectious Diseases, and [2]Department of Pediatrics, University of Vermont College of Medicine, Burlington; [3]Microscience Limited, Wokingham, Berkshire, United Kingdom

(See the editorial commentary by von Seidlein, on pages 357–9.)

Background. M01ZH09 (*Salmonella enterica* serovar Typhi [Ty2 *aroC*$^{-}$ *ssaV*$^{-}$] ZH9) is a live oral-dose typhoid vaccine candidate. M01ZH09 was rationally modified with 2 independently attenuating mutations, including a novel mutation in *Salmonella* pathogenicity island (SPI)–2. We demonstrate that M01ZH09, in a single oral dose, is well tolerated and prompts broad immune responses, regardless of whether prevaccination with a bicarbonate buffer is given.

summary

- *Salmonella enterica* – different diseases, typhoid and non-typhoidal
- Invades the small intestine by different routes
- Replicates in macrophages in membrane-bound vacuoles
- Most of c.160 virulence genes present on PAIs (> 60).
- SPI-1 and SPI-2 Type III secretion systems are important virulence components: SPI-1 for invasion and SPI-2 for replication.

More reading…

Salmonella takes control: effector driven manipulation of the host. McGhie et al., Curr Opinion Microbiol 12: 117 (2009)

Salmonella interplay with host cells: Haraga et al., Nature Reviews Microbiol 6: 53 (2008)