Salmonella

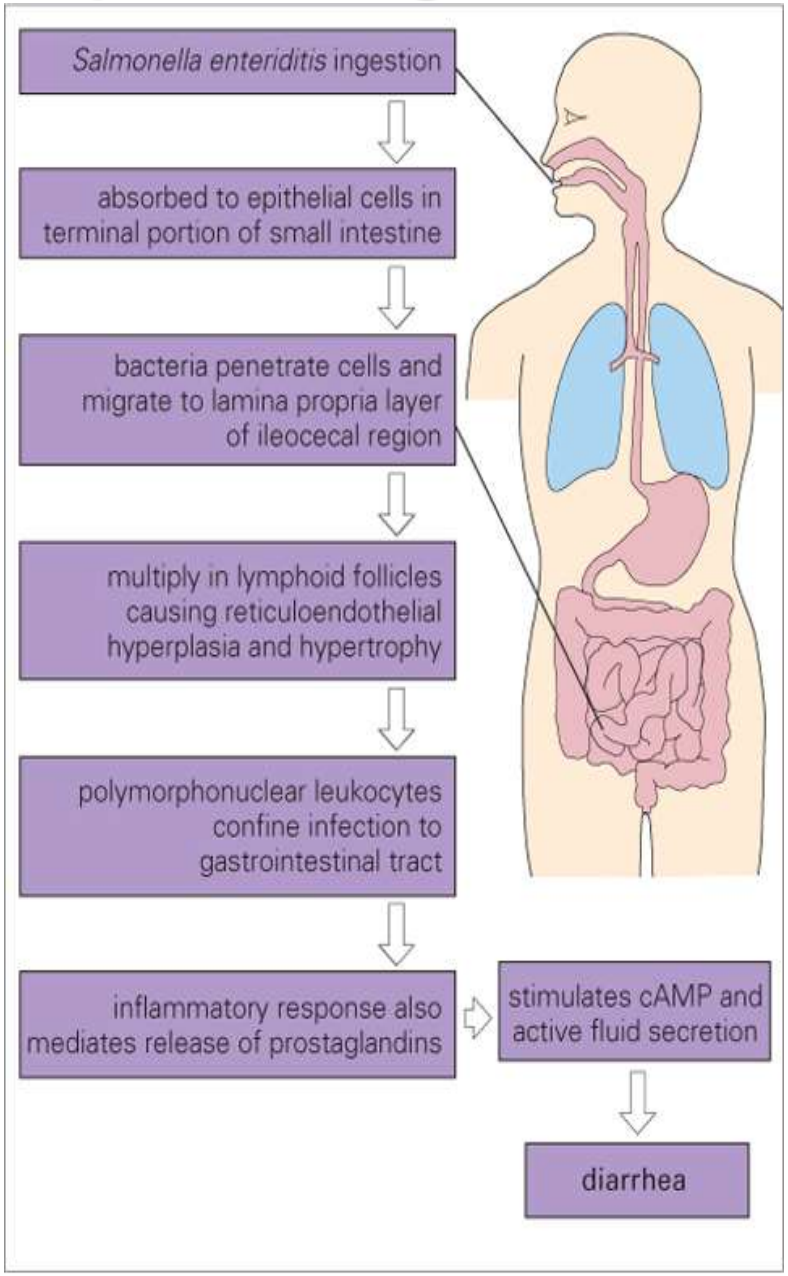
the ‘real Gram-negative pathogen’ capable to grow in in egg salad in the fridge and in your gallbladder, as well…

Salmonella is a champion bug!

How much of adaptability, regulation/sensing

Capacity must it have…:

- grows in egg salad with mayonnaise in oxic environment of high osmolarity at 8-10 ^{0}C
- Is ingested and has to resist acidic pH 3 in stomach..
- Has to resist alkaline pH of duodenum, protease
- and lipase action and detergent effect of bile salts…
- Has to strive in microaerophilic environment of small intestine, drill through mucus, adhere and compete with commensal flora
- Has to resist killing by phagocytes in Payers patches
- Has to survive in SCVacuole at low osmolarity, low oxygen,
- low Mg^{2+}, oxidative burst, cationic peptides etc…
- Has to escape phagocytes, multiply in he GALT and migrate through lymphatics to luver and gallbladder / for shedding in bile…., or cause systemic disease in case of *S. typhi*…
- Settle down in heptocytes and colonize gallbladder, resisting to detergents
- Resist inflammatory response and provoke gastritis and diarrhoea to spread to a new host…



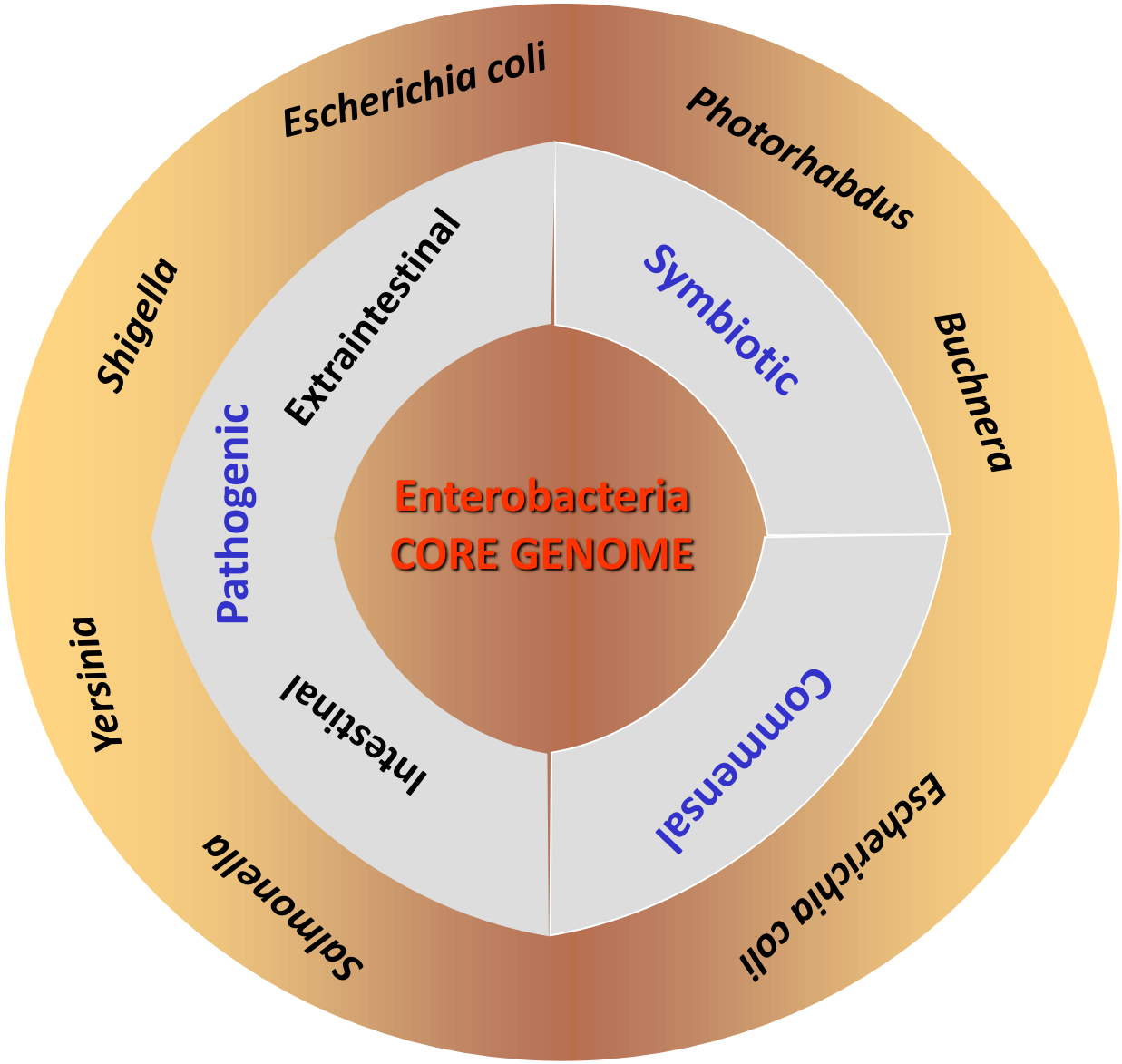
Confusion: fitness or virulence factor???

- *Salmonella has only about 200 genes specifically required for virulence?*
- What should be considered as a virulence factor?
  - traits and products enabling adherence, toxic protein prduction, directly linked to infection process???
  - traits like energy from sugar fermentation – housekeeping functions, but still essential for infection???
  - virulence factors can be used as a target of a vaccine or therapeutic strategy

Core *E. coli* genome is about 3500 genes, additional 3500 *E. coli* genes are in mobile genomic islands, serving for adaptation to a given niche, some involved in pathogenicity…

To me, a virulence factor is a bacterial product or strategy that contributes to virulence or pathogenicity and is REALLY absent in the commensal variants of the same organism…

Enterobacteria have many faces



How Salmonella became a pathogen

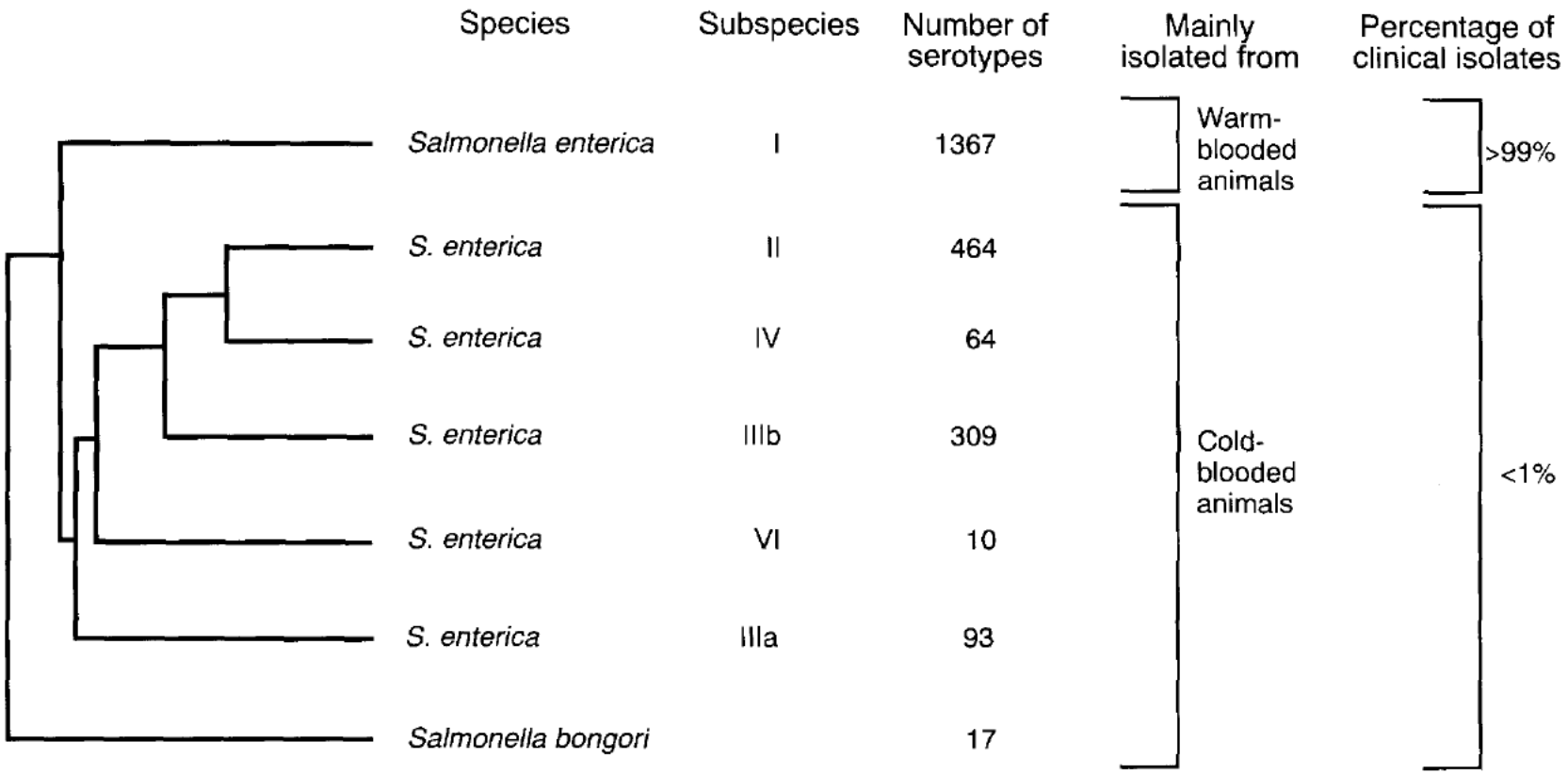


Fig. 1. Characteristics of phylogenetic groups of the genus *Salmonella*. The dendrogram shows the phylogenetic relatedness between the different species and subspecies contained in the genus *Salmonella*, as determined by Reeves *et al.*[20] The number of serotypes in each species and subspecies[18] and the frequency of clinical isolation of *Salmonella* serotypes[32] have been reported previously.

How *Salmonella* became a pathogen

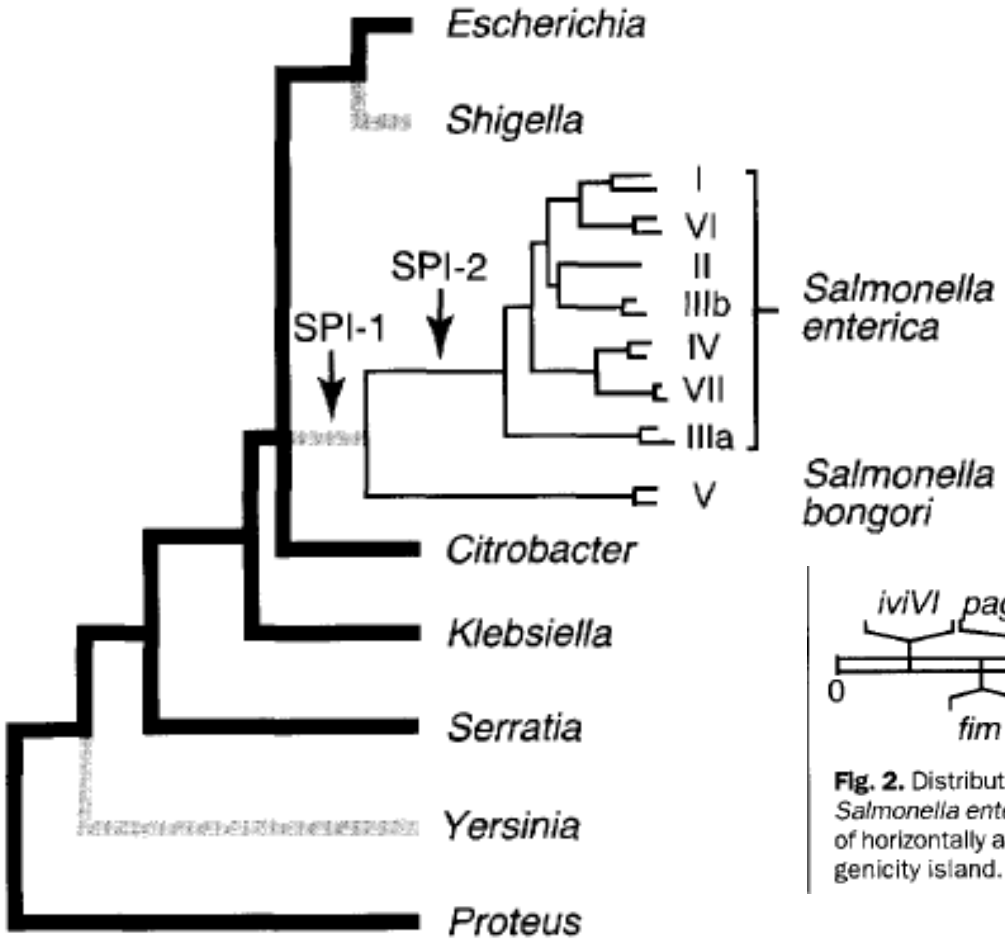


Fig. 3. Phylogenetic relationships among enteric bacteria. The branches shown in gray denote taxa that are typically capable of invading eukaryotic cells. Within *Salmonella enterica*, the acquisition of two pathogenicity islands (SPI-1 and SPI-2) is based on the phylogenetic distribution of island-specific virulence genes among strains representing the eight subspecific groups (I-VII) of this species.

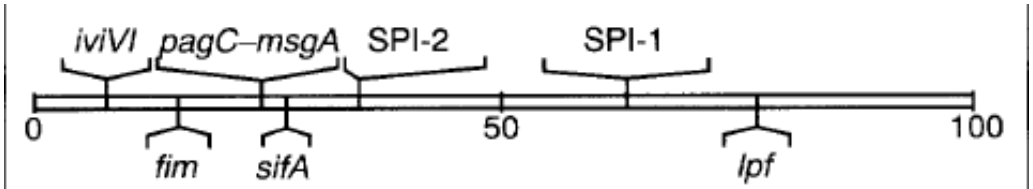


Fig. 2. Distribution of pathogenicity islands and islets in *Salmonella*. The chromosome of *Salmonella enterica* sv. Typhimurium is portrayed linearly, and the relative map positions of horizontally acquired virulence regions are shown. Abbreviation: SPI, *Salmonella* pathogenicity island.

How Salmonella became a pathogen - version 1997…

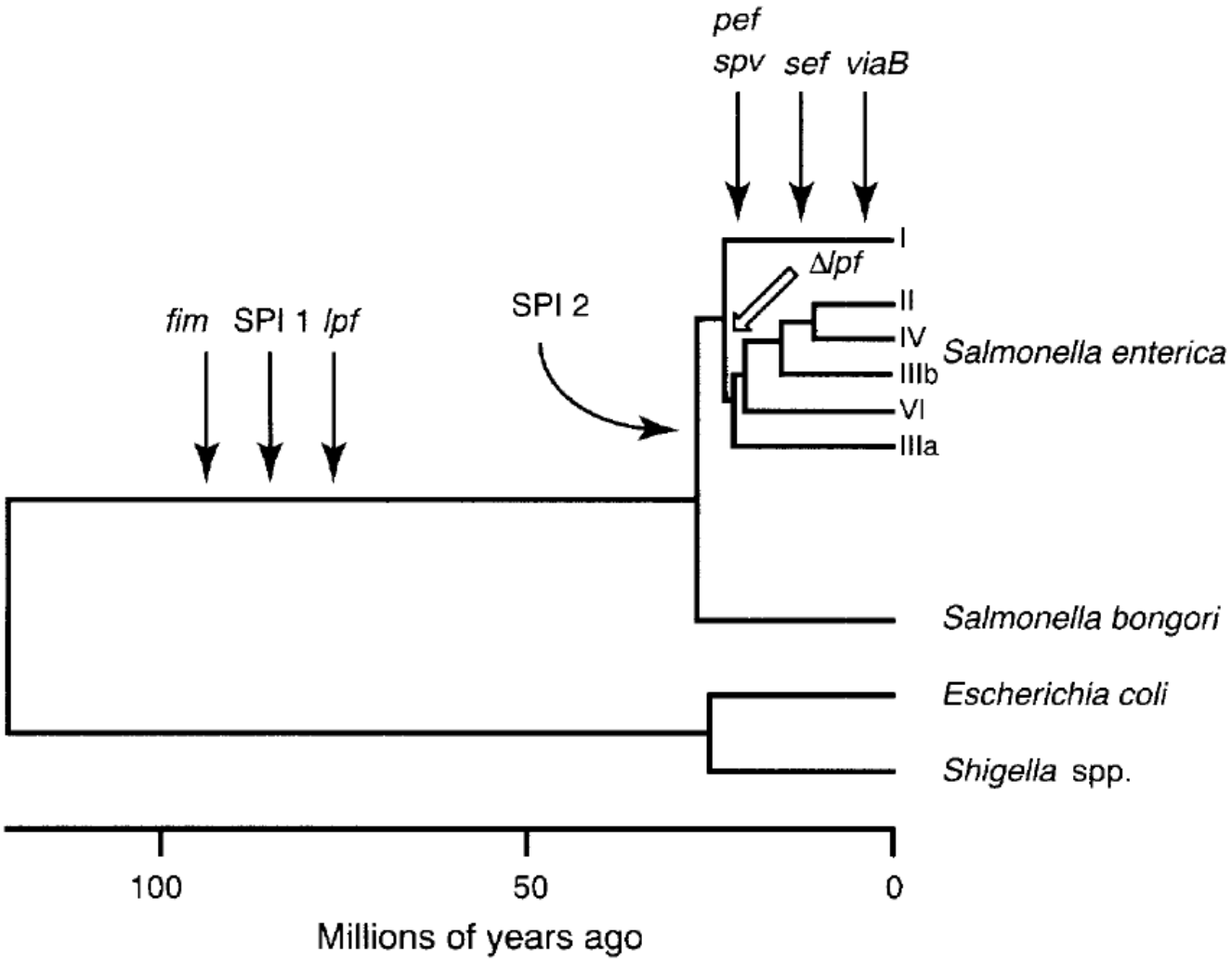


Fig. 2. The record of horizontal transfer of virulence genes within the genus *Salmonella*. Deletion of the *lpf* operon is indicated by an open arrow. Filled arrows mark acquisition of genes by horizontal transfer. Abbreviations: *fim*, type I fimbriae; *lpf*, long polar fimbriae; *pef*, plasmid-encoded fimbriae; *sef*, *Salmonella enterica* serotype Enteritidis fimbriae; SPI 1, *Salmonella* pathogenicity island 1; SPI 2, *Salmonella* pathogenicity island 2; *spv*, *Salmonella* plasmid virulence; *viaB*, Vi capsular antigen.

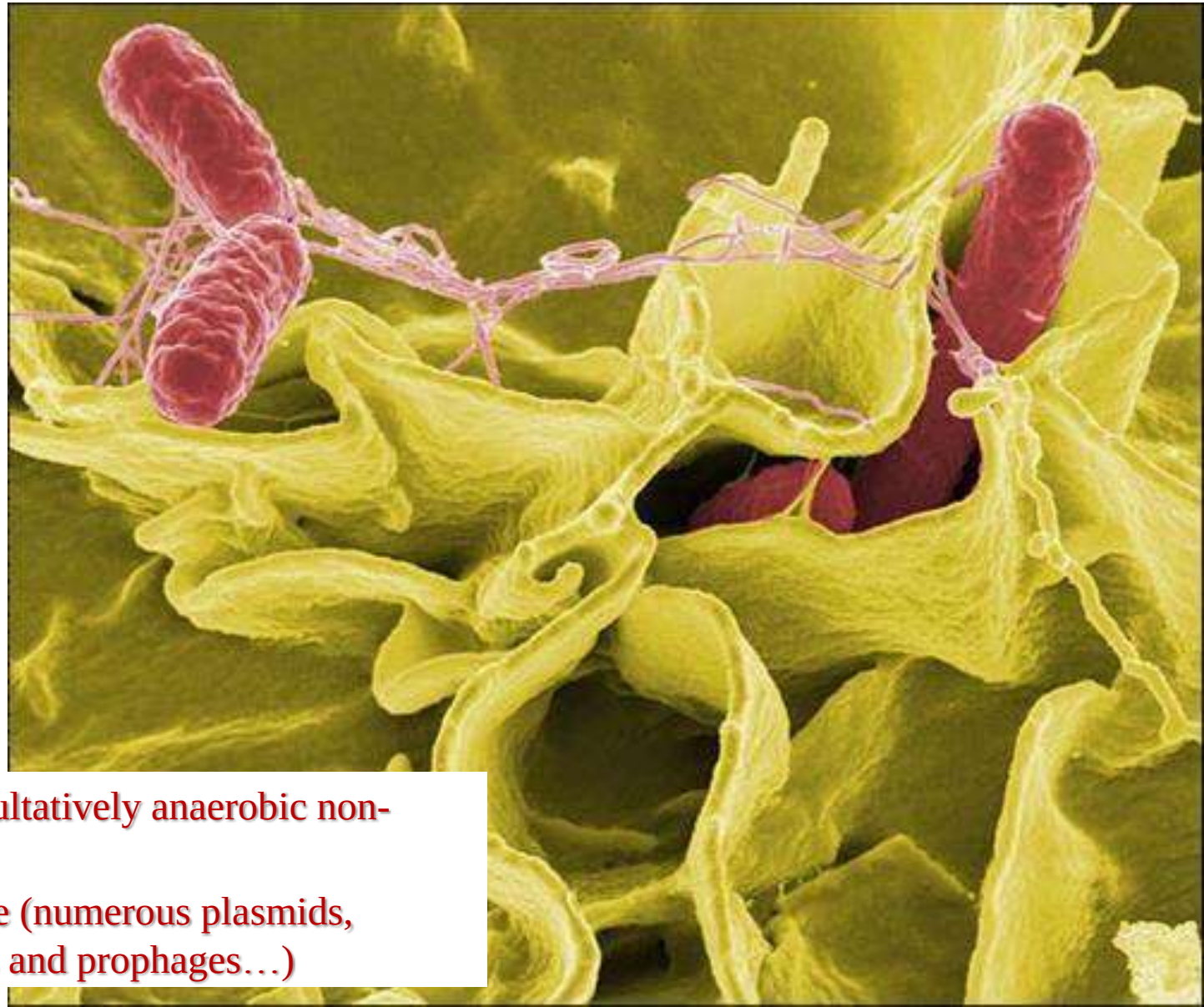
How *Salmonella* became a pathogen

Table 1. Systemic infections caused by host-adapted *S. enterica* subspecies I serotypes

Serotype	Host species	Disease	Putative host range factor[a]
Typhi	Humans	Typhoid fever	*viaB*
Paratyphi A	Humans	Paratyphoid fever	Unknown
Paratyphi B	Humans	Paratyphoid fever	Unknown
Paratyphi C	Humans	Paratyphoid fever	*viaB*
Gallinarum[b]	Poultry	Fowl typhoid	*sef*
Pullorum[b]	Poultry	Pullorum disease	*sef*
Enteritidis[c]	Rodents	Murine typhoid	*pef, spv*
Typhimurium[c]	Rodents	Murine typhoid	*pef, spv*
	Cattle	Bacteremia	
Dublin	Cattle	Bacteremia	*spv*
Choleraesuis	Swine	Bacteremia	*pef, spv*

[a]Abbreviations: *pef*, plasmid-encoded fimbriae; *sef*, *Salmonella enterica* serotype Enteritidis fimbriae; *spv*, *Salmonella* plasmid virulence; *viaB*, Vi capsular antigen.
[b]Pullorum and Gallinarum are considered as biotypes that belong to the same serotype.
[c]Other serotypes that are host adapted to rodents are reported by Roudier *et al.*[39]

Salmonella



- Gram-negative, fakultatively anaerobic non-sporulating rods
- Very plastic genome (numerous plasmids, transposons, phages and prophages…)

Virulence factors

- Adhesion
- Invasion
- Intracellular survival - resistance
- Resistence to acid, bile, antimicrobial peptides, complement action (long LPS chains)
- 5 specific SPI pathogenicity islands
- 2 dedicated T3SS ‘injectosome’ systems

Bile salt resistance

High level resistance due to long LPS and efflux pumps

- *marRAB* a *acrAB* detergent efflux system..
- Bile is an important signal for gene regulation:
  - Reduced expression of flagelin (environmental mobility?)
  - PhoP-PhoQ reduces PagC protein expressionand upregulates YciF

Complement resistance

- *rck* gene encodes OMP conferring serum resistance
- Very long polysaccharide O antigen activates MAC of complement far from cells membrane – very smooth colonies…

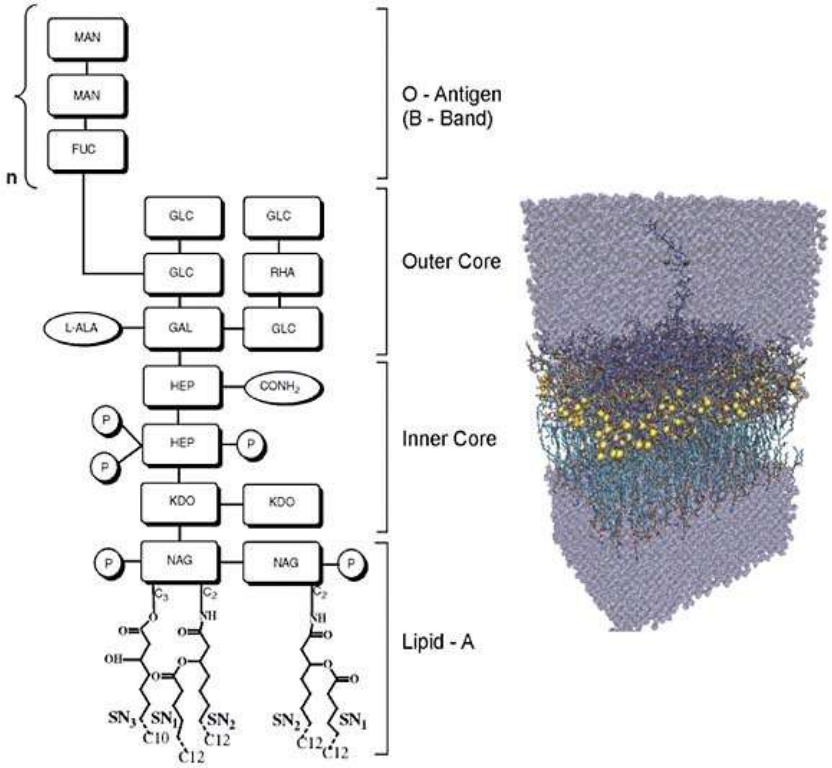


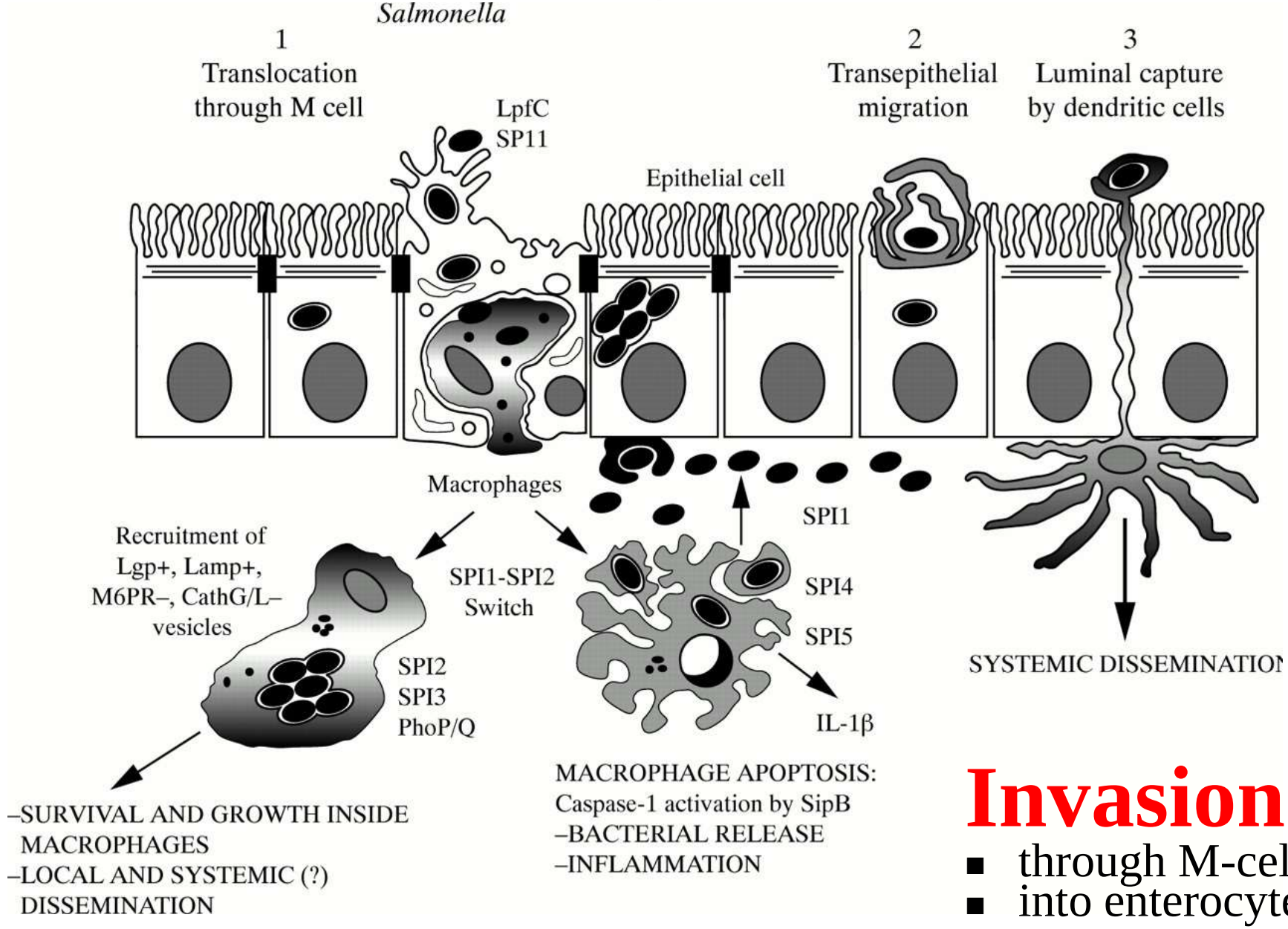
Figure 1. Schematic representation of a LPS unit (NAG: N-acetyl-D-glucosamine; P: phosphatidyl group; KDO: 3-Deoxy-D-manno-octulosonic Acid; HEP: heptose; GAL: D-galactose; GLC: D-glucose; L-ALA: L-alanine; RHA: D-rhamnose; FUC: D-fucose; MAN: D-manose). Acyl lipid chains SN_1,SN_2 and SN_3 are labeled (right). Atomistic model of the A-B+ LPS membrane of *Pseudomonas aeruginosa* (left). Membrane atoms are represented in "sticks", Ca^{++} ions in filled yellow CPK and water in transparent blue CPK model.

Infect Immun. 1996 Jun;64(6):2019-23
Identification of a domain in Rck, a product of the Salmonella typhimurium virulence plasmid, required for both serum resistance and cell invasion

Salmonella in general

- Localized gastroenteritidis, up to typhoid fever disease like disease in humans
  - *S. enterica* subsp. enterica serotype *typhimurium* (diarrhoeal enteritidis)
  - *S. enterica* subsp. enterica serotype *typhi* (exclusively human disease / typhoid fever / systemic disease)
  - *S. enterica* subsp. enterica serotype *paratyphi* milder forms of typhoid disease
- alimentary infection
- Symptoms in 6-48 hours
- With *S. enterica* subsp. *typhimurium* it ranges from asymptomatic, to diarhoea, through life-threatening typhoid disease, depending on the equipment of the bug and of the host defense capabilities
- Problem with multidrugresistant *S.* enterica subsp. *typhimurium* DT104 strains from zoonotic infections

Life according to *Salmonella*….



Invasion

- through M-cells
- into enterocytes

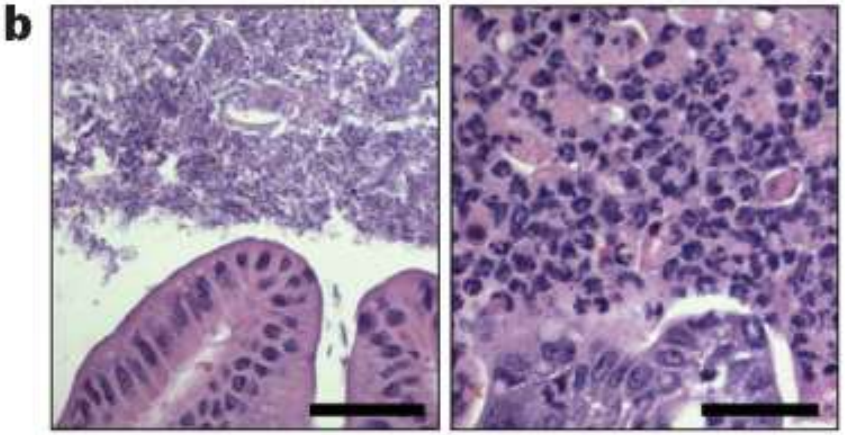
Host–microbe interaction: Inflammation for growth

Winter et al. Nature, 467, 426-9: comment S. I. Miller online 22 September 2010

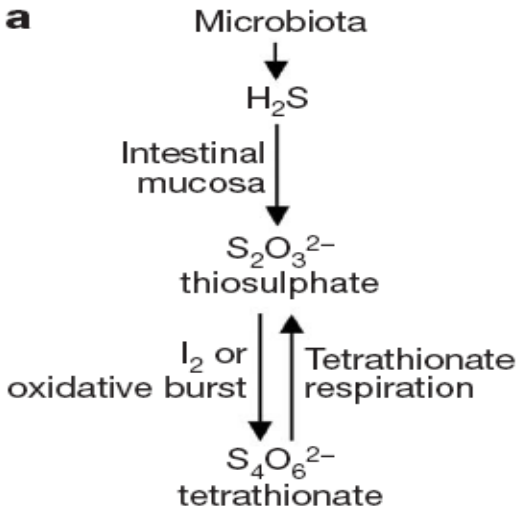
- Infection often leads to inflammatory immune responses
- Intestinal inflammatory responses might favour pathogen growth by nutrient release ?
- In 1923 *Salmonella* was observed to use **sulphur-containing tetrathionate as electron acceptor in the microaerobic environment of limited oxygen availability**
  - growth medium containing tetrathionate used to enrich salmonellae from stool
- Winter *et al.* show that tetrathionate could be generated in the intestinal tract from sulphur-containing thiosulphates generated from hydrogen sulphide (produced from food by microbiota)
- The thiosulphates can be oxidized to tetrathionate by reactive oxygen species produced by phagocytes at sites of *Salmonella* invasion .
- The authors show that:
  - ability to metabolize tetrathionate promotes *S*. Typhimurium colonization of the host
  - this compound is formed *in vivo* by an inflammatory response that generates oxygen radicals.
- Winter *et al*. find that salmonellae require the T3SS to exploit the tetrathionate pathway
  - T3SS itself is known to lead to activation of a pro-inflammatory pathway
  - Genes encoding components of both the T3SS and the tetrathionate-respiration pathway in *Salmonella* have a different nucleotide content from each other and from the core, evolutionarily conserved, genome content.
  - T3SS and tetrathionate respiration were likely acquired through horizontal gene transfer, after *Salmonella* differentiated from other intestinal bacteria such as the commensal *Escherichia coli*.
- Evolutionary driving force for the inflammatory periods, called disease, may be dissemination and transmission.

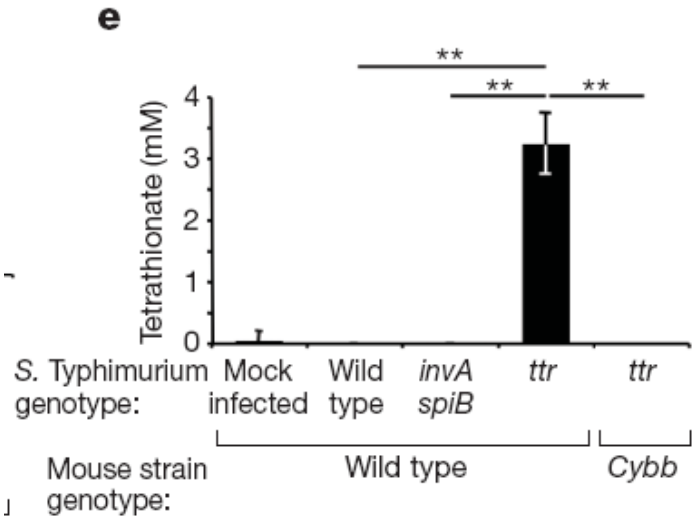
Gut inflammation provides a respiratory electron acceptor for *Salmonella*

- $S_4O_6^{2-}$ availability in the gut
- $S_4O_6^{2-}$ promotes growth in the gut
- Oxygen radicals generate $S_4O_6^{2-}$ in vivo
- Outgrowth by $S_4O_6^{2-}$ respiration



Haematoxylin and eosin-stained caecal sections





Tetrathionate detected in caecal contents

Adhesins

Fimbrial and molecular:

- type 1 fimbriae (*fim*) unknown specificity
- Plasmid-encoded *pef* genes on virulence plasmid pSLT – specific for microvili in small intestine (typhimurium)
- long polar fimbriae (*lpf*) specific for Peyer`s patch cells
- thin aggregative fimbriae called curli (*agf*), specific for enterocyte vili and/or involved in biofilm formation
- Functional redundancy: all 4 need to be mutated to lose virulence
- protein Rck (on pSLT), adhesion and complement resistance

Infect Immun. 1998 Jun;66(6):2803-8.
Multiple fimbrial adhesins are required for full virulence of Salmonella typhimurium in mice
Infect Immun. 1996 Jun;64(6):2019-23.
Identification of a domain in Rck, a product of the Salmonella typhimurium virulence plasmid, required for both serum resistance and cell invasion

Invasion

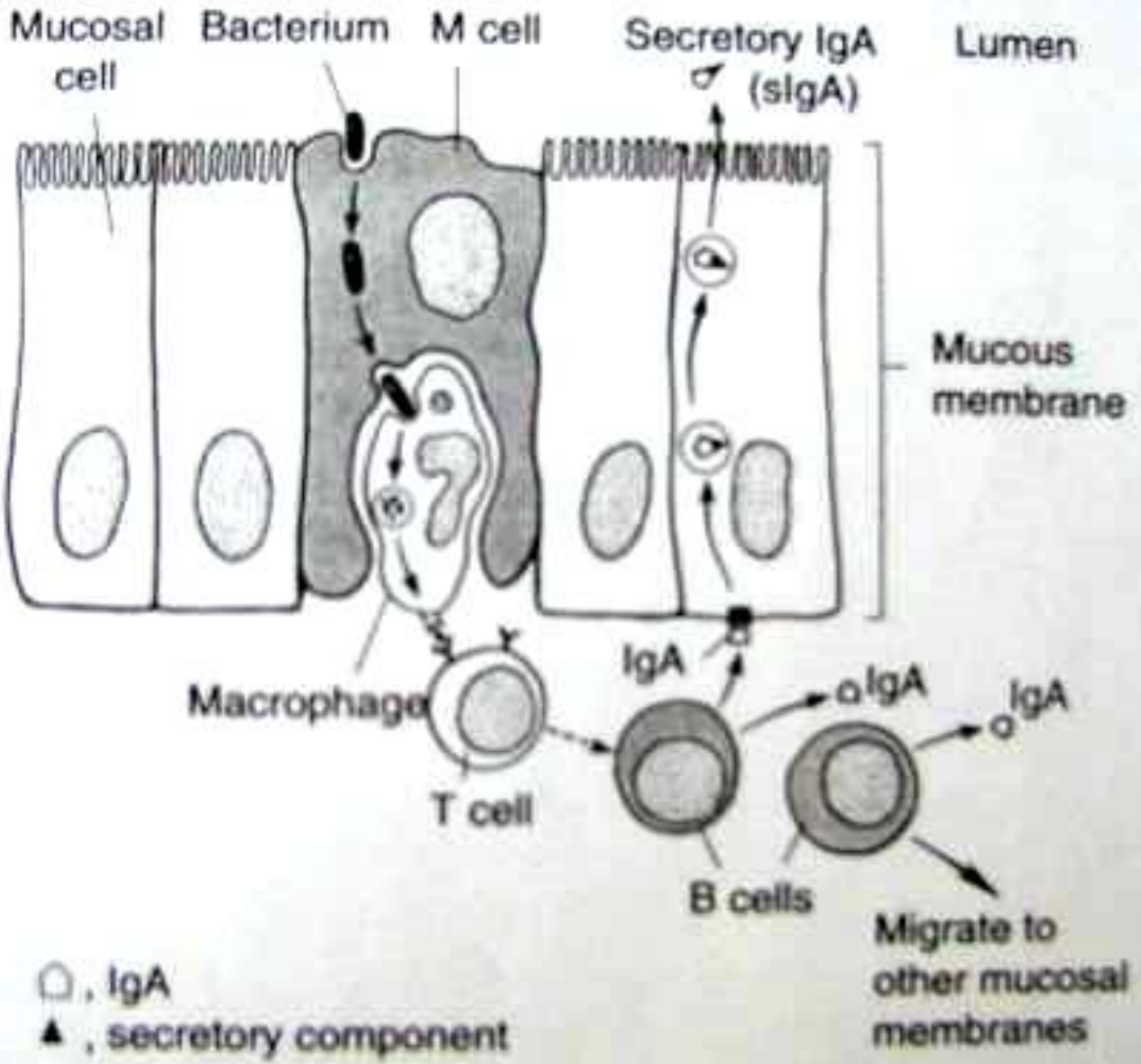
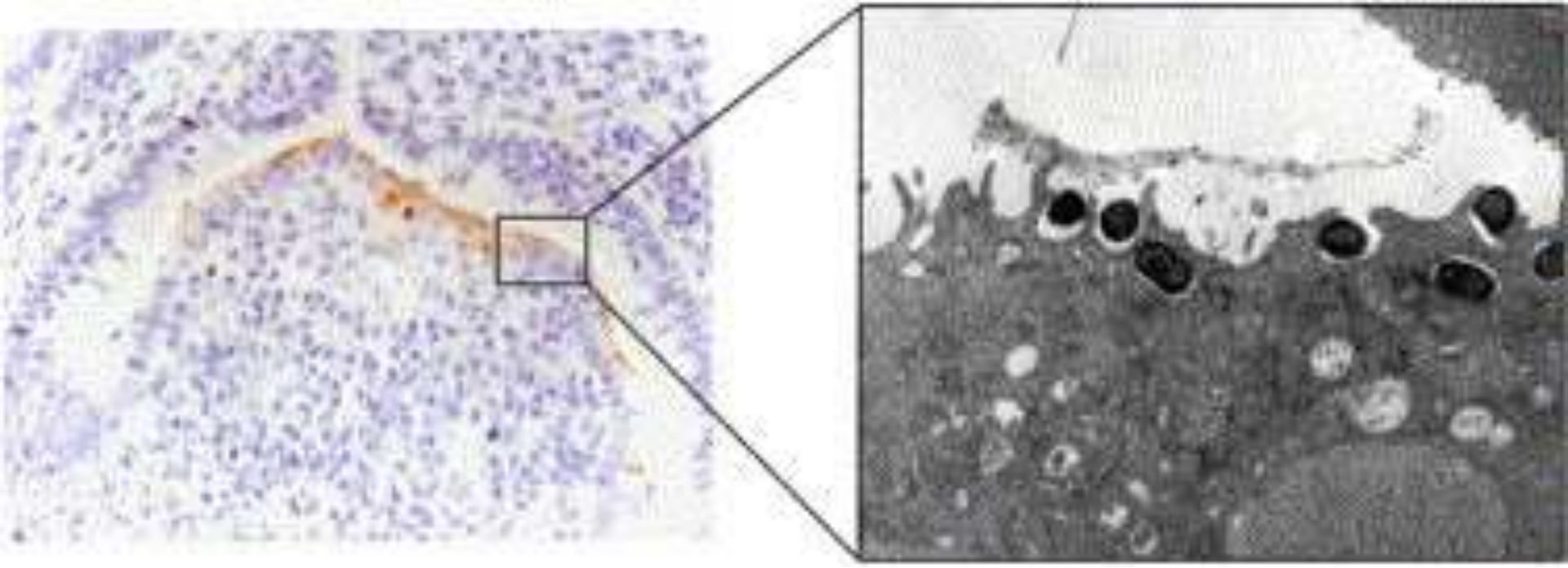
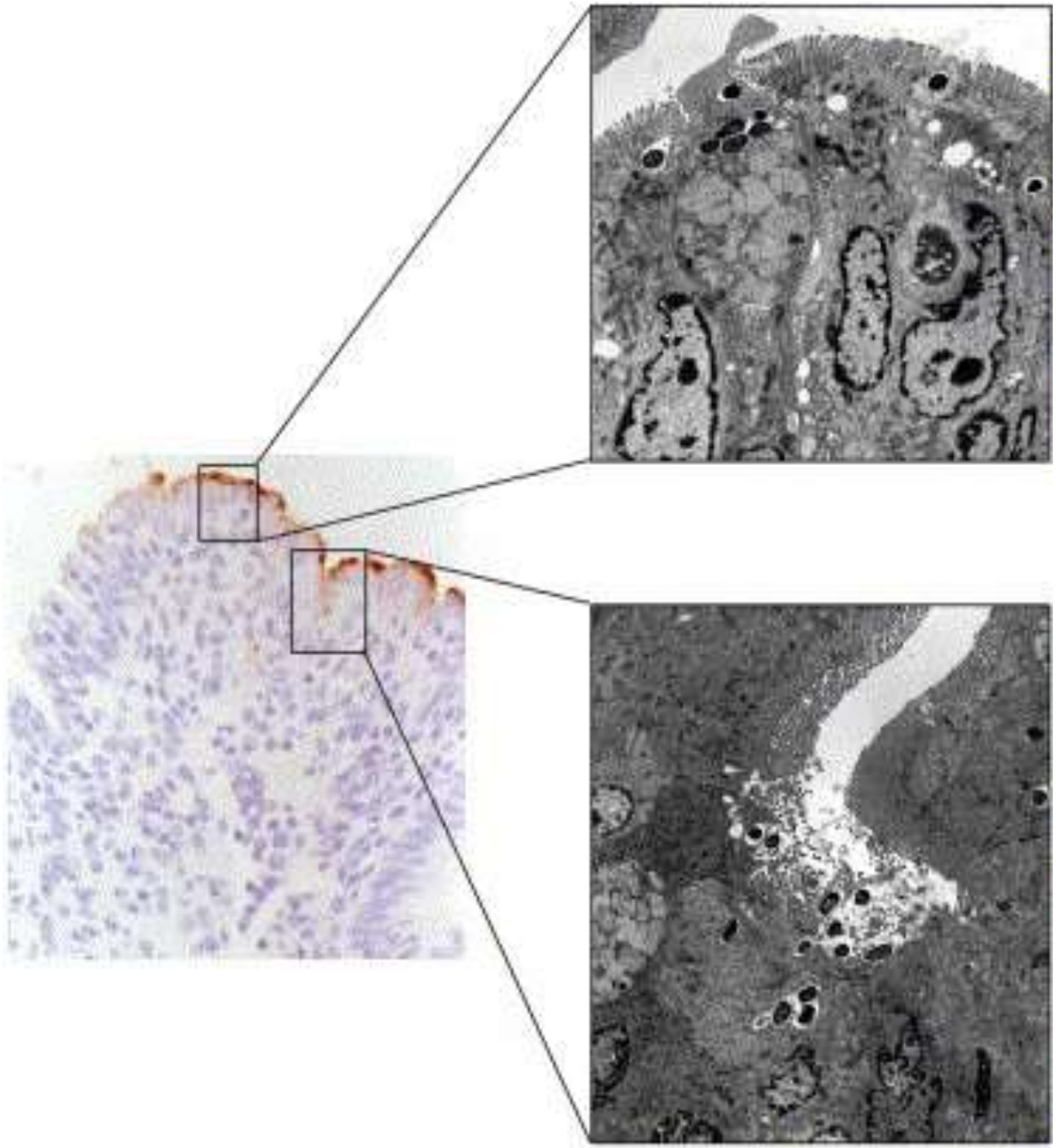


Figure 1-1 Cells of the GALT. M cells and their associated lymphoid cells (T and B cells) are sometimes called follicles. Collections of follicles are called Peyer's patches.



S. typhimurium invasion of the follicle-associated epithelium in calves. Detection of S. typhimurium by immunohistochemistry (brown precipitate) in sections of the bovine ileal mucosa collected 15 min after infection of ligated ileal loops is shown on the left. The section shows a domed villus of a Peyer's patch lymphoid follicle flanked by two absorptive villi. Note the tropism of *S. typhimurium* for the follicle-associated epithelium of the domed villus while the epithelium of the adjacent base of each absorptive villus is not colonized.

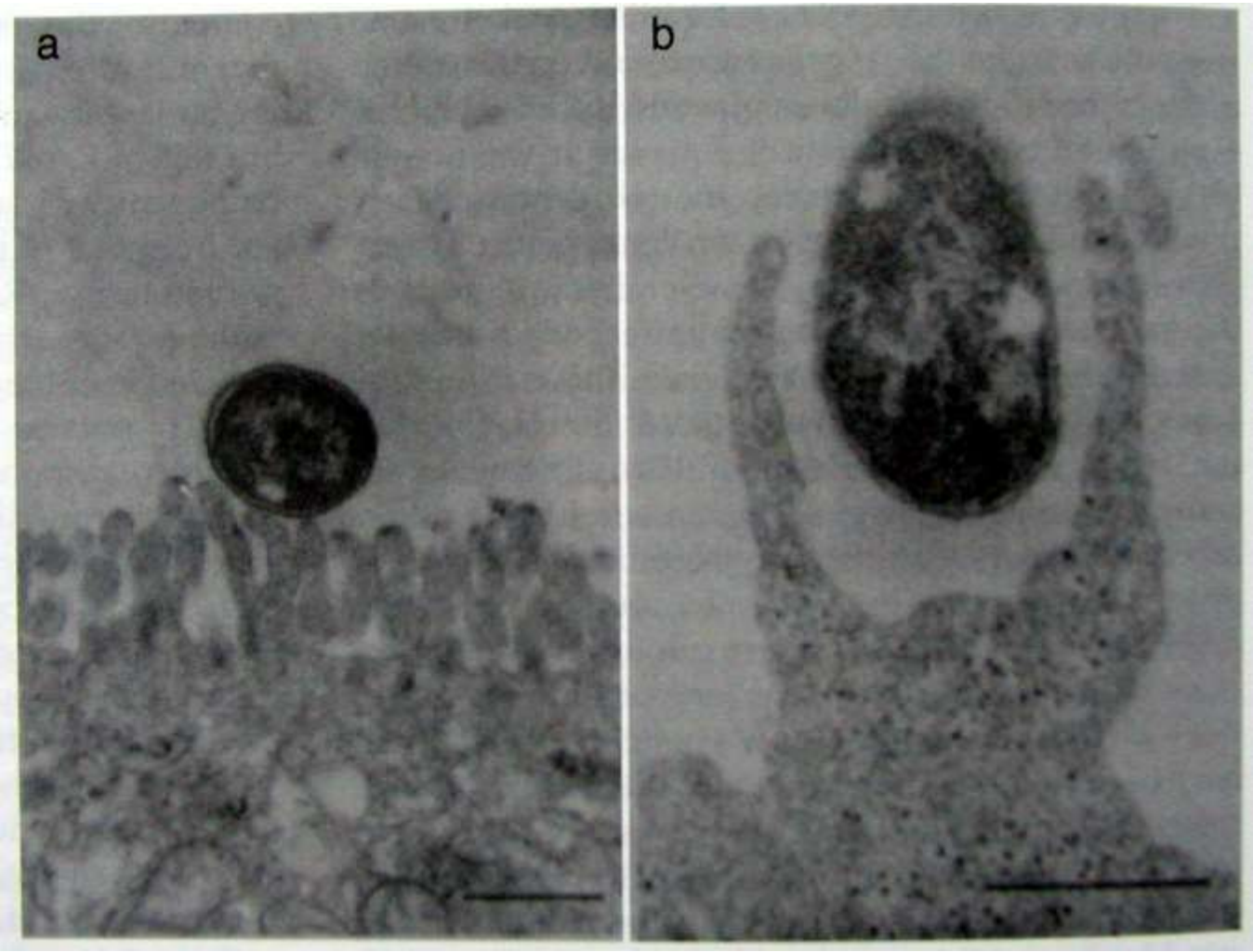
A transmission electron micrograph of the *S.* Typhimurium invasion of M-cells in the bovine follicle-associated epithelium is shown on the right. In this host species, the follicle-associated epithelium is entirely composed of M-cells.



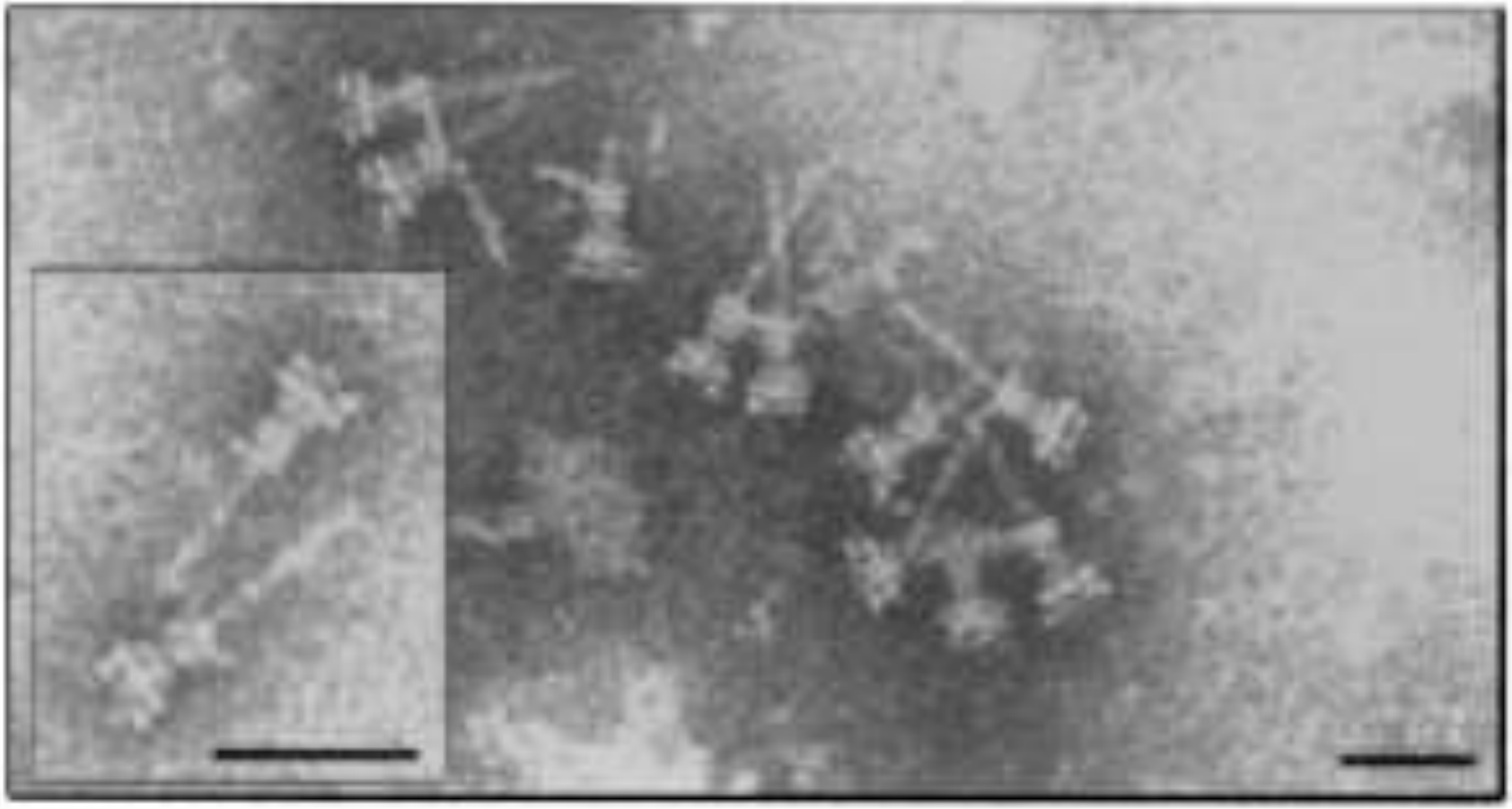
S. typhimurium invasion of the tip of an absorptive villus in calves.

Detection of *S.* Typhimurium by immunohistochemistry (brown precipitate) in sections of the bovine ileal mucosa collected 1 h after infection of ligated ileal loops is shown on the left. Transmission electron micrographs of the *S.* Typhimurium invasion of a goblet cell (top) and enterocytes (bottom) are shown on the right

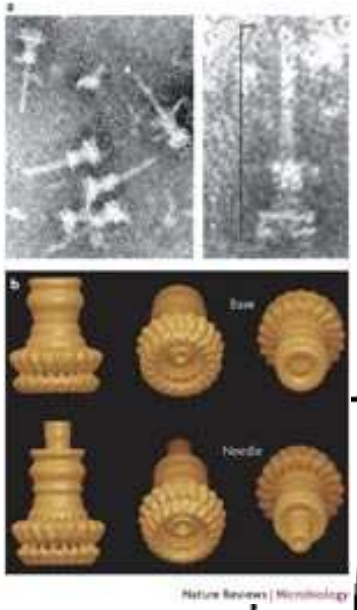
Membrane ruffling



Salmonella Pathogenicity Island 1-T3SS

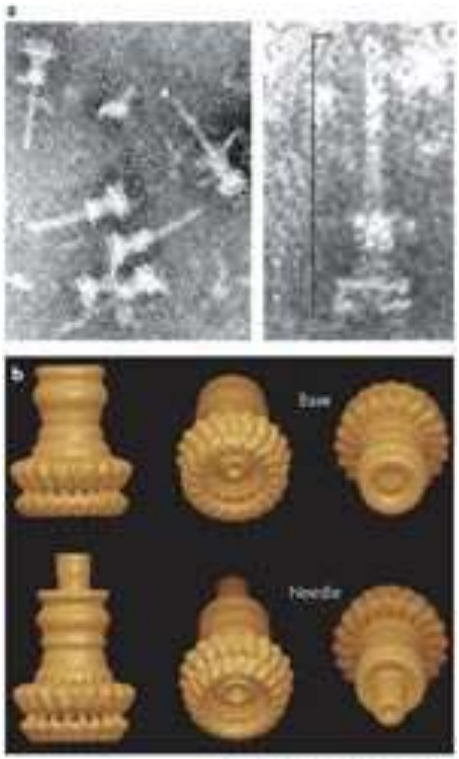


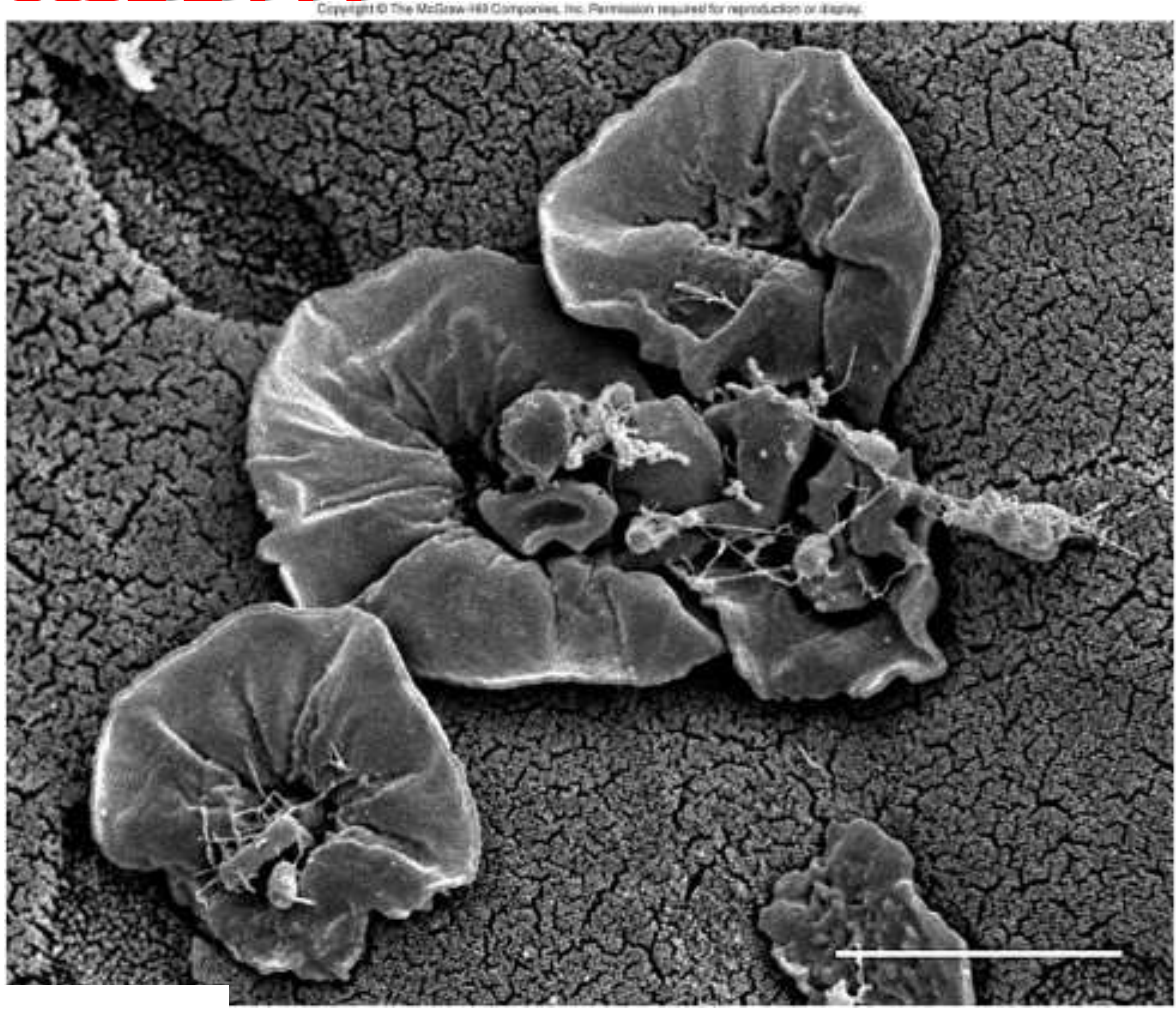
Sensing, regulating and adapting = surviving and disseminating



Model for the regulation of *Salmonella* invasion genes during intestinal infection. *Salmonella* cell is depicted in association with an intestinal epithelial cell in the small bowel. Exposure to environmental cues present in the bowel lumen, including low O2 concentrations, relatively high osmolarity, and alkaline pH, acts to induce expression of the *hilA gene located on SPI1. HilA in turn induces operons* in SPI1 encoding the components of a type III protein secretion system as well as the secreted proteins (Sips). Contact between the bacterium and the epithelial cell stimulates Sip secretion by the type III system located at the bacterial cell surface. The Sips interact with the epithelial cell membrane and certain secreted proteins enter the cytoplasm. The Sips promote uptake of the acterium into a membrane-bound intracellular vacuole. The virulence plasmid *spv genes* are not required in this stage of pathogenesis.

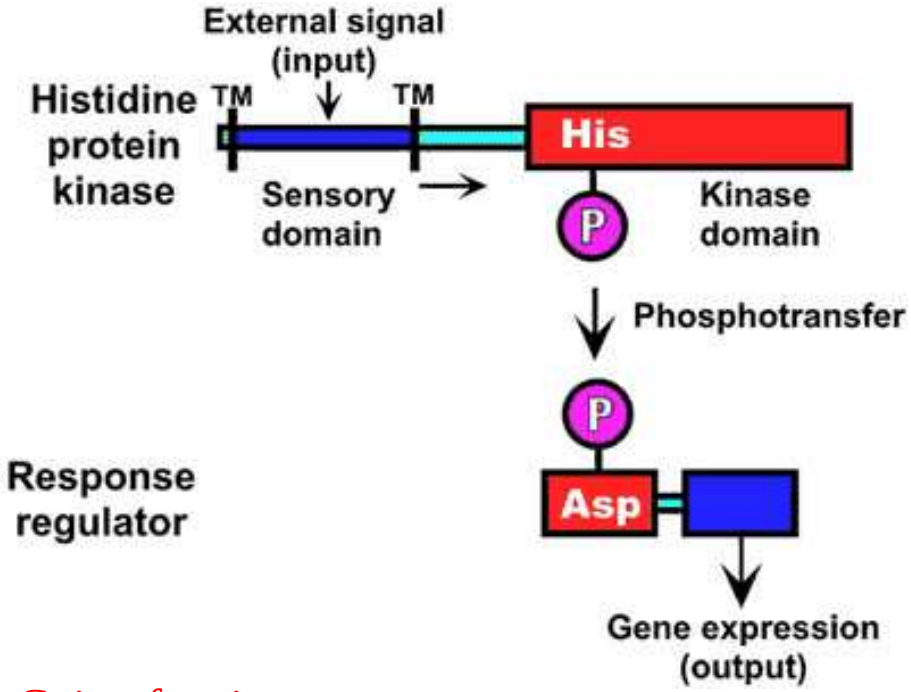
The kiss…





Use of T3SS makes entrocytes to phagocytes…

SPI-1 T3SS – 13 effector13 proteins:
SopB, SopE a SopE2activate Rho GTPases
SipA a SipC modulate actin filament assembly
for promoting ruffling (RcsB-RcsC regulated)
SptP restores actin homeostasis



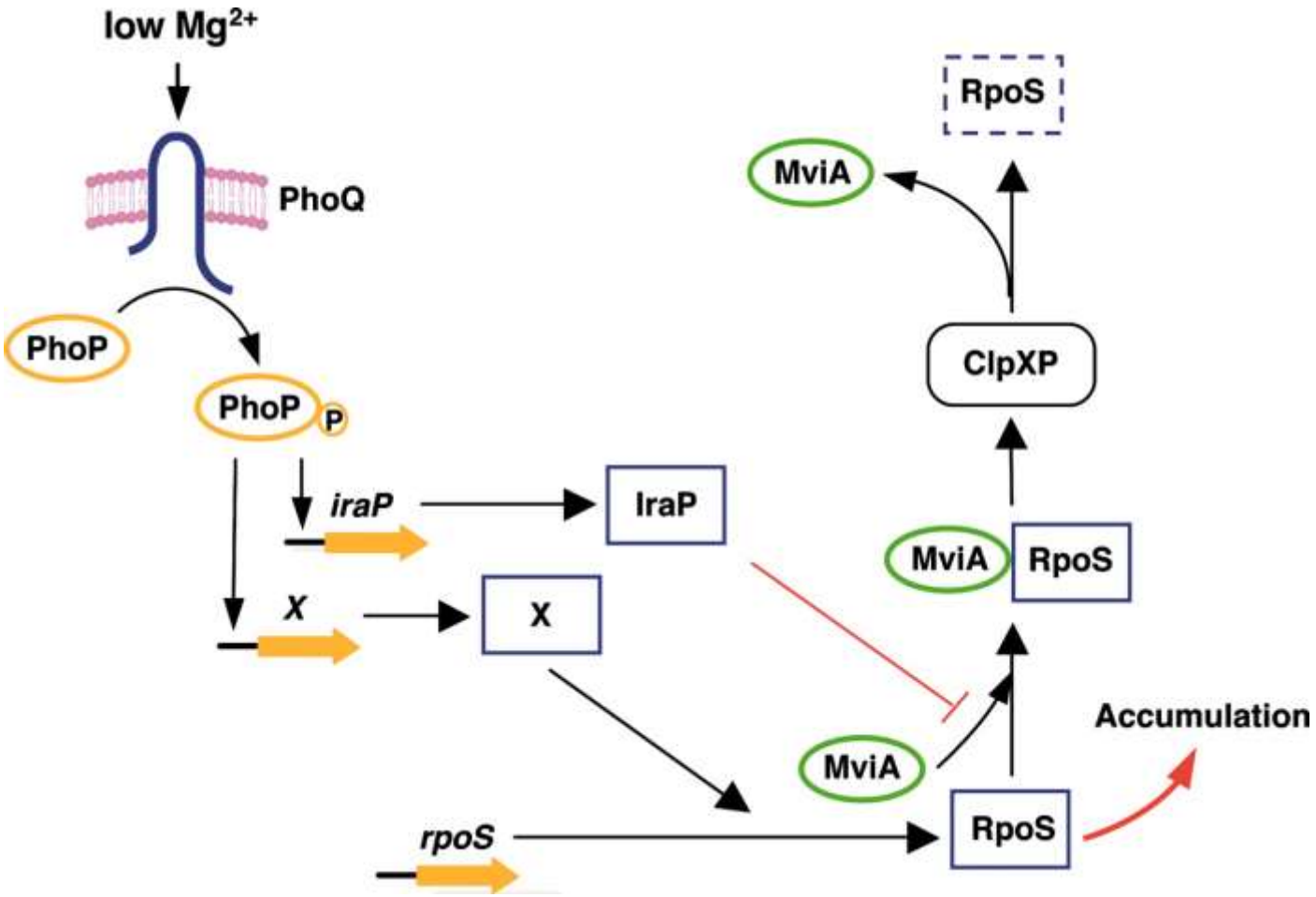
Gain of resistance to antimicrobial peptides
PhoP-PhoQ, PmrA-PmrB a RpoS regulation (alternativní sigma faktor)

Downregulation of SPI-1 genes and flagellin

The PhoQ/PhoP two-component regulatory system monitors environment and responds to internalization and CONTROLS

the adaptation to low Mg^{2+} environments by governing the expression and/or activity of Mg2+ transporters and of enzymes modifying the Mg2+-binding sites on the bacterial cell surface. The regulator PhoP modifies expression of ~3% of the Salmonella genes in response to the periplasmic Mg2+ concentration detected by the PhoQ protein. Genes that are directly controlled by the PhoP protein often differ in their promoter structures, resulting in distinct expression levels and kinetics in response to the low Mg2+ inducing signal. PhoP regulates a large number of genes indirectly: via other transcription factors and two-component systems that form a panoply of regulatory architectures including transcriptional cascades, feedforward loops and the use of connector proteins that modify the activity of response regulators. These architectures confer distinct expression properties that may be important contributors to Salmonella's lifestyle.

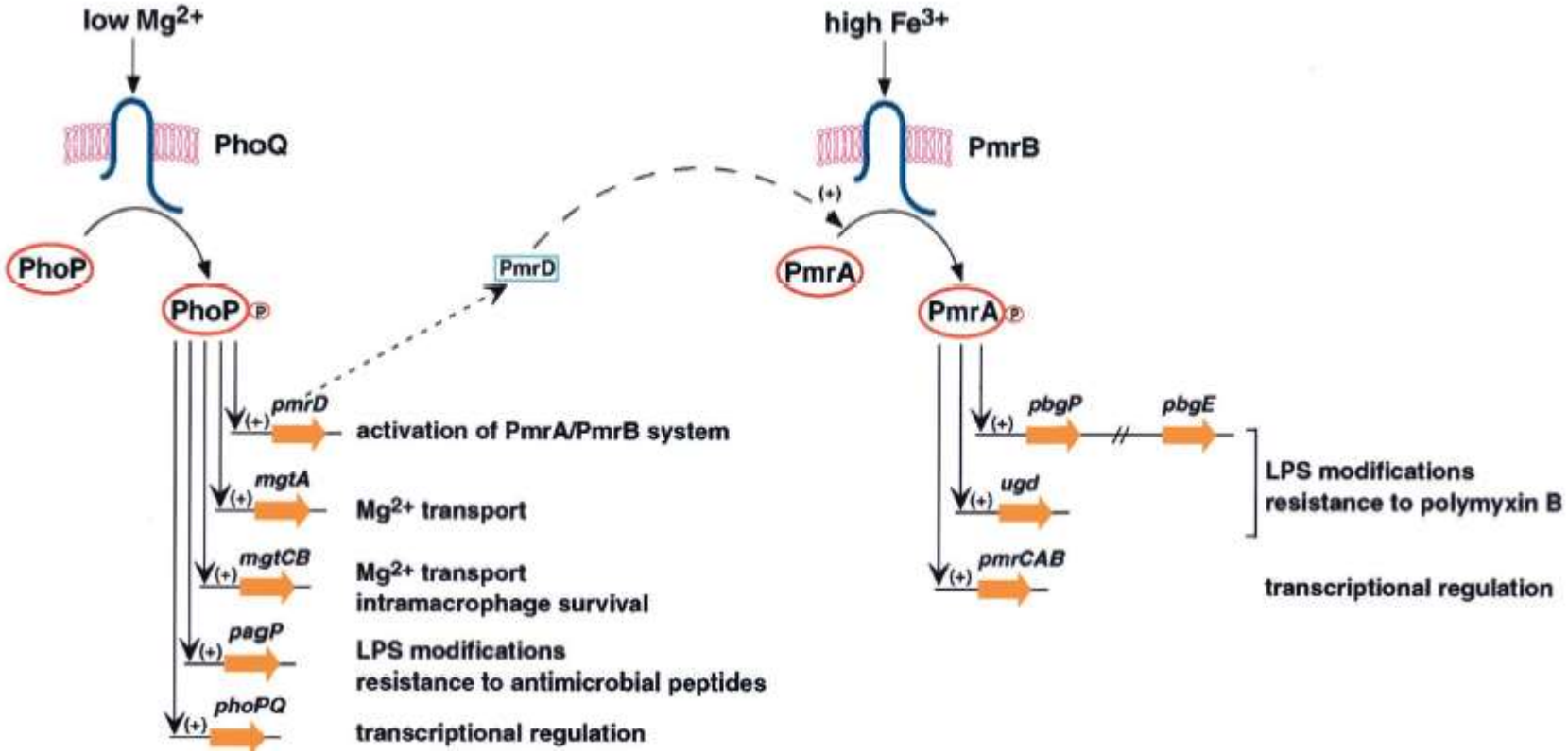
PhoP/PhoQ signaling results in stabilization and accumulation of the RpoS protein when Salmonella experiences low Mg2+.



IraP blocks of pre-made MviA association to pre-made RpoS and blocks its committment for degradation = extremely fast adaptation to phagocytosis

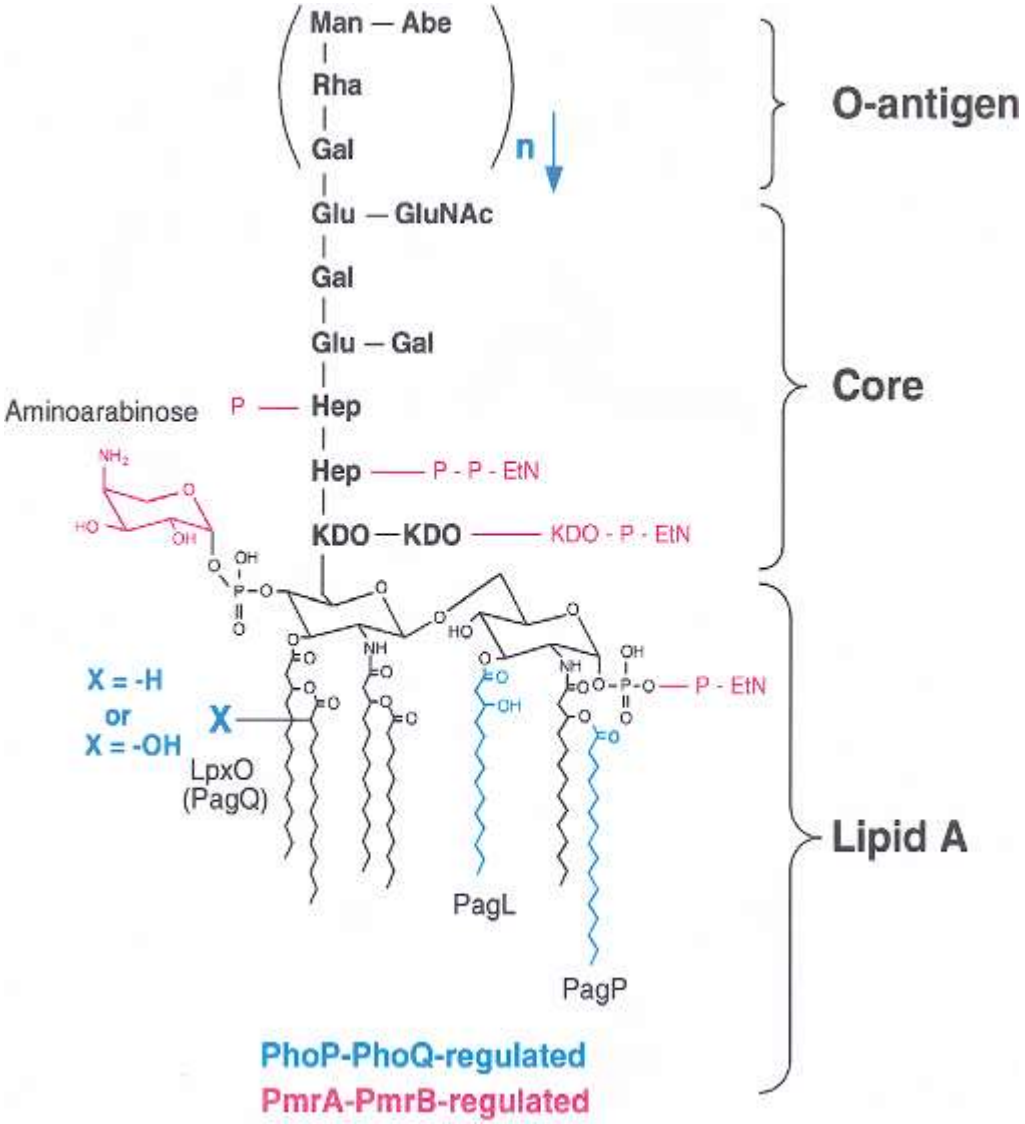
Tu X et al. PNAS 2006;103:13503-13508

PhoP-PhoQ system



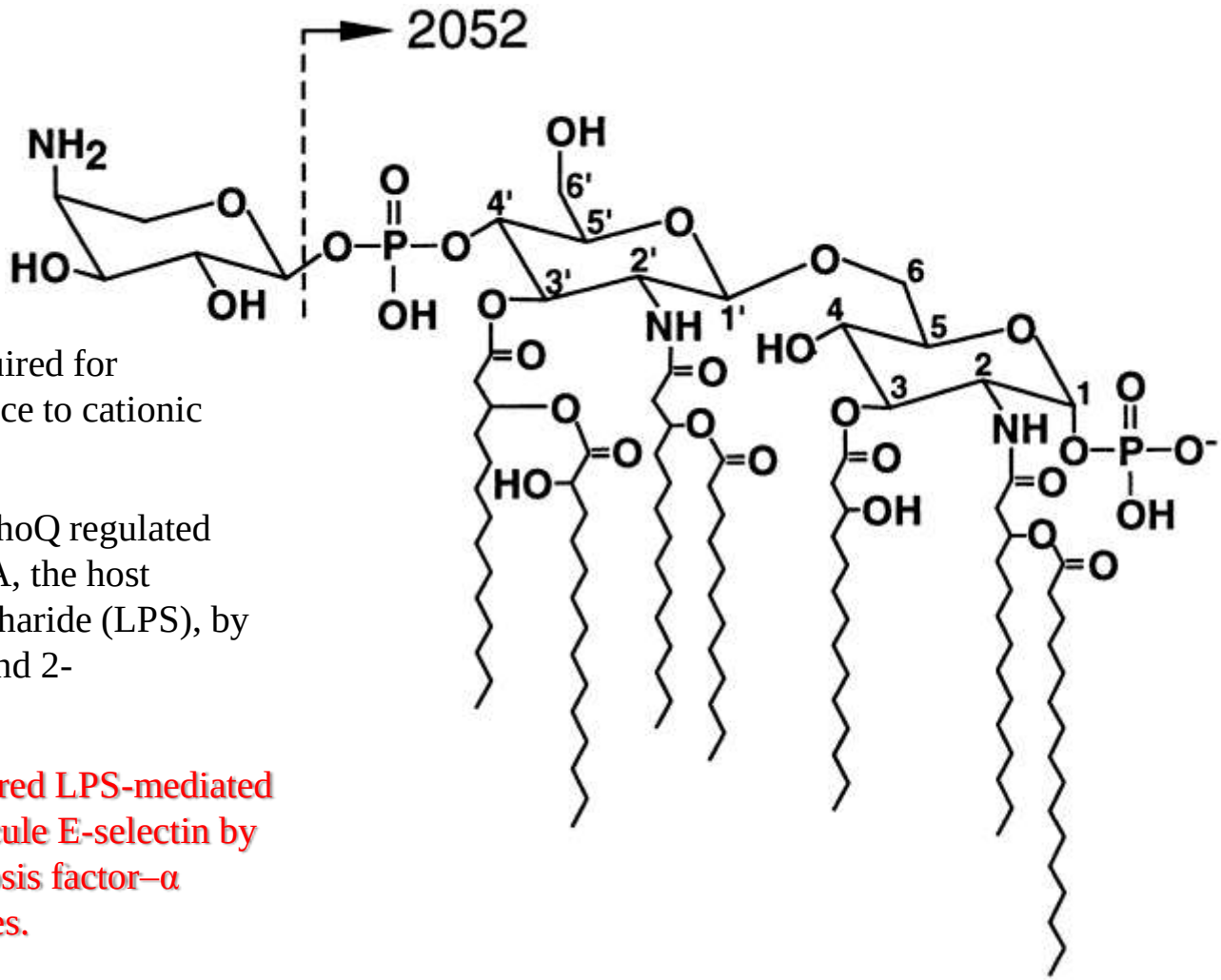
Model popisující regulační cíle PhoP-PhoQ systému a interakci s PmrA-PmrB systémem

Antibacterial peptide resistance



- addition of aminoarabinose
- acylaction of lipid A
- phosphate modification

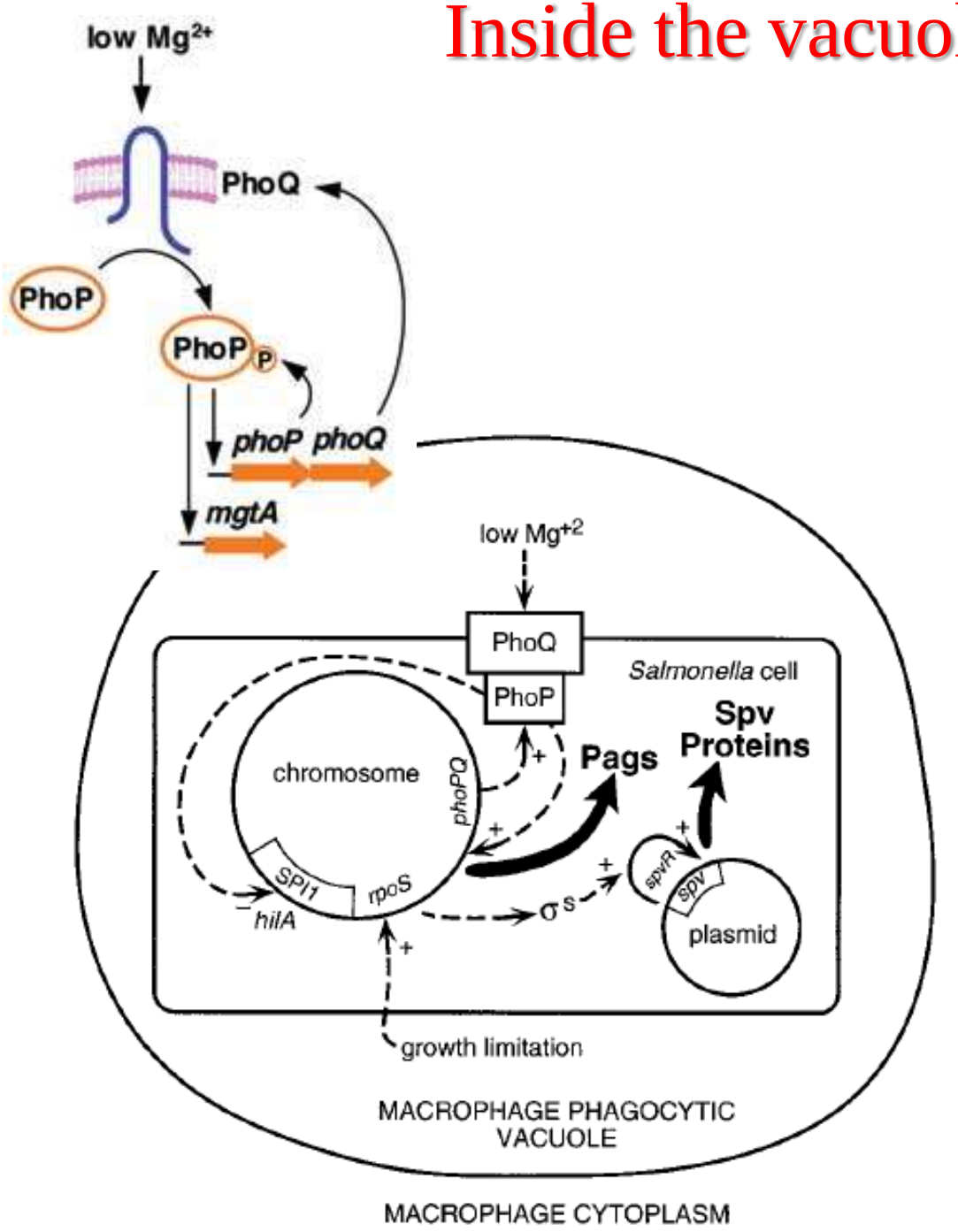
Salmonella amino-arabinosylates lipidA in the vacuole to resist cationic peptides attack



- PhoP-PhoQ regulates genes required for intracellular survival and resistance to cationic peptides.
- *Salmonella typhimurium* PhoP-PhoQ regulated structural modifications of lipid A, the host signaling portion of lipopolysaccharide (LPS), by the addition of aminoarabinose and 2-hydroxymyristate.
- Structurally modified lipid A altered LPS-mediated expression of the adhesion molecule E-selectin by endothelial cells and tumor necrosis factor–α expression by adherent monocytes.
- Altered responses to environmentally induced lipid A structural modifications may represent a mechanism for bacteria to gain advantage within host tissues.

L Guo et al. Science 1997;276:250-253

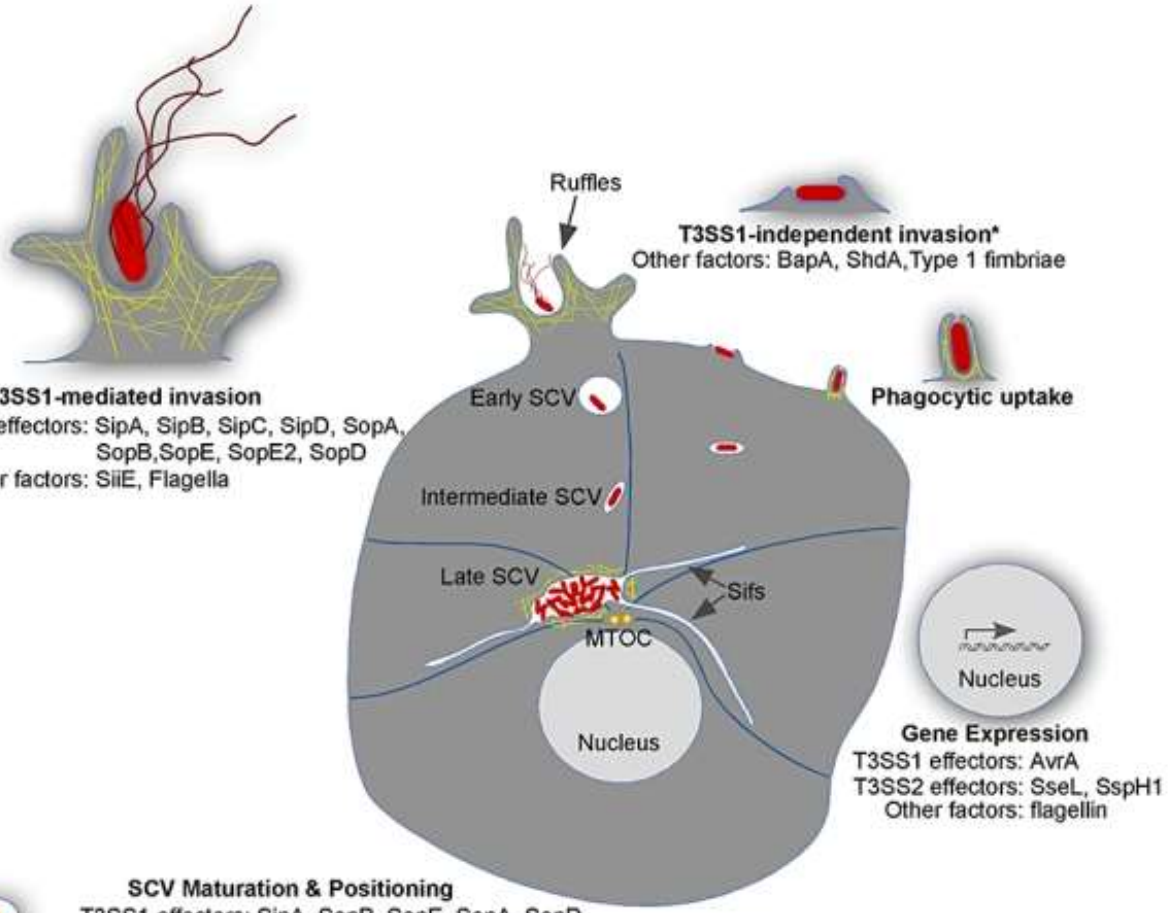
Inside the vacuole…



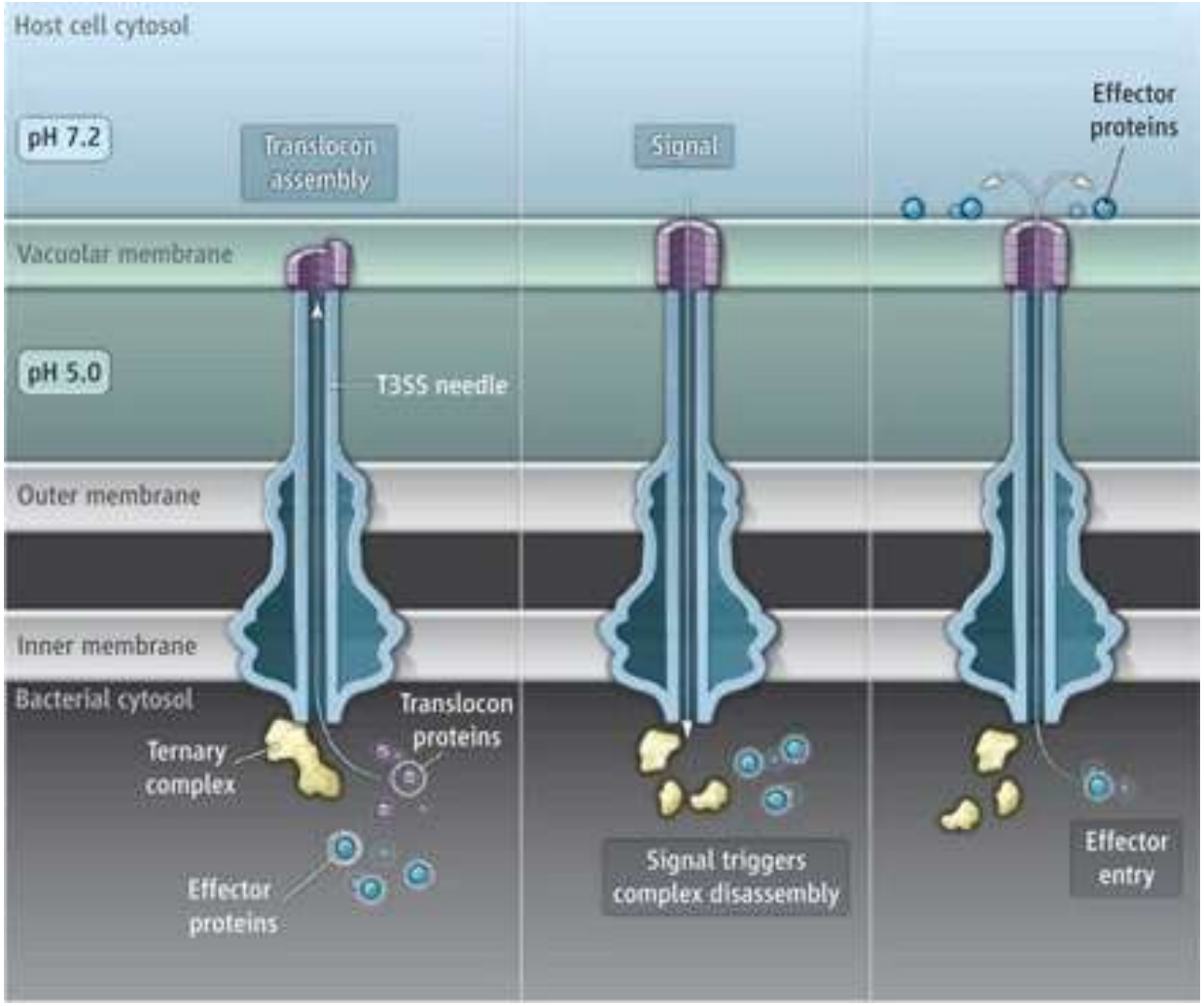
Model for the regulation of *Salmonella virulence* genes involved in the extraintestinal infection of tissue macrophages.

After phagocytosis, S*almonella* cells remain within the phagocytic vacuole. This environment limits the growth of the organism, leading to increased levels of the alternative sigma factor ss, the product of the chromosomal *rpoS gene.* ss increases synthesis of the transcriptional activator SpvR which acts together with ss to induce the *spv operon* on the virulence plasmid. The Spv proteins appear to enhance proliferation of the *Salmonella* in the intracellular environment. During maturation of the phagocytic vacuole, low Mg^{2+} levels activate the two-component PhoPQ regulatory system, leading to synthesis of several proteins designated Pags, the products of PhoP-activated genes. The Pags appear to be involved in survival of the bacteria inside macrophages. PhoP also negatively regulates *hilA*
production of invasion gene products.

The intracellular Salmonella life style



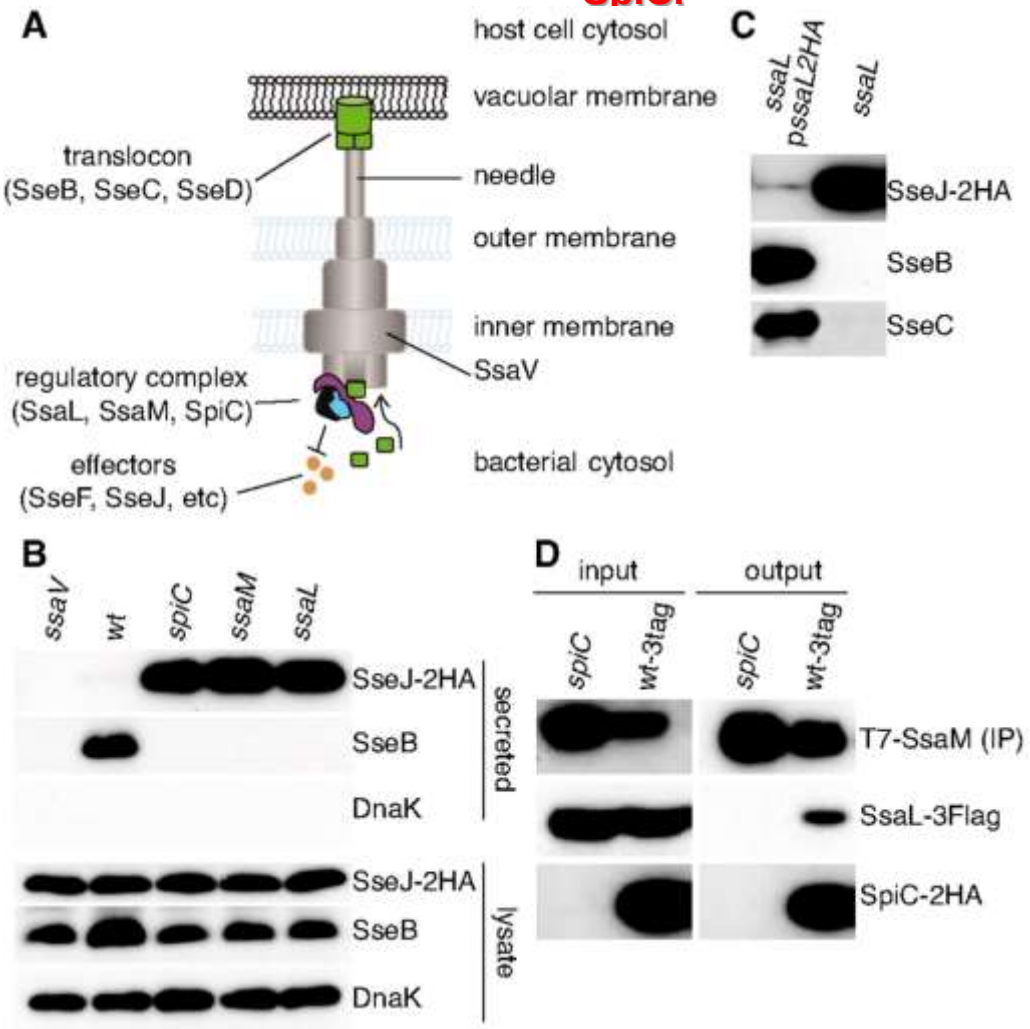
Salmonella's Safety Catch



(**Left**) After *S. enterica* has entered a host cell, the acidity of the vacuole within which the bacterium resides induces assembly of the T3SS needle complex. A regulatory complex (yellow) formed from SsaL, SsaM, and SpiC at the entry portal of the needle prevents secretion of effectors (blue), but allows translocon proteins (purple) to enter and proceed to the tip of the needle, where they form a pore in the vacuolar membrane. (**Middle**) The pore enables the elevated pH of the host cell cytosol to be sensed, causing the disassembly of the SsaL/SsaM/SpiC (ternary) complex. (**Right**) Effector proteins can now pass through the needle complex and enter the cytosol of the host cell.

Science 21 May 2010: Vol. 328 no. 5981 pp. 1040-1043

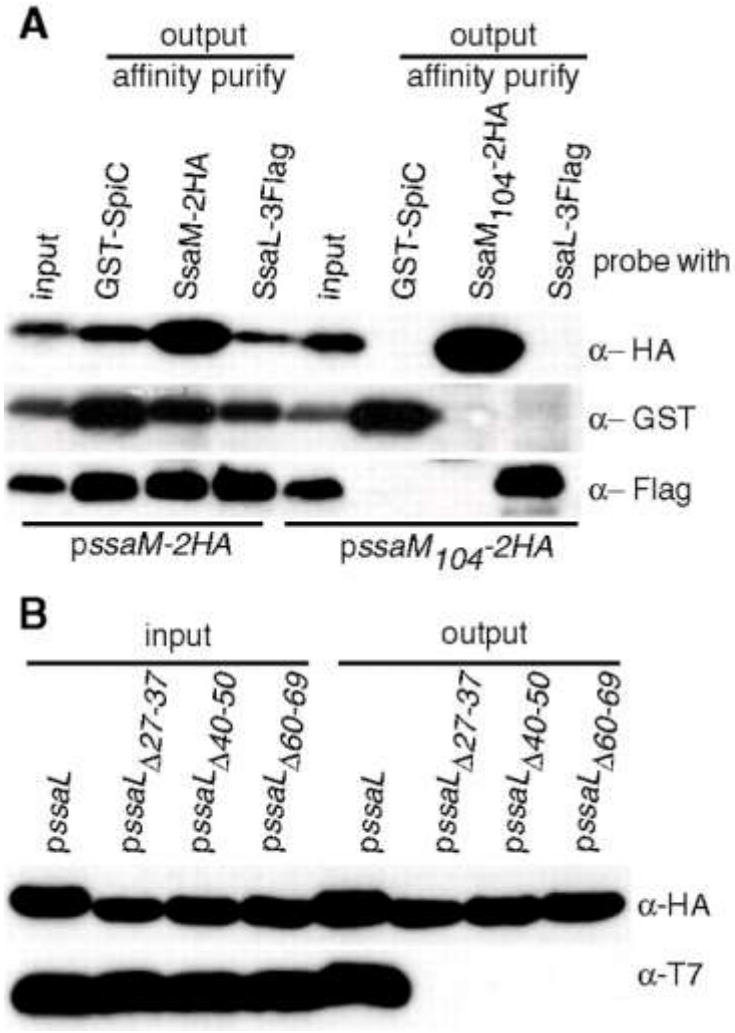
SsaL is required for translocon protein secretion, suppresses effector secretion, and interacts with SsaM and SpiC.



SsaL is required for translocon protein secretion, suppresses effector secretion, and interacts with SsaM and SpiC. (**A**) Model of the SPI-2 T3SS spanning the inner and outer membranes of the bacterial cell and connected to a translocon pore formed in the vacuolar membrane. Translocon proteins must be secreted before effectors can be translocated. SsaV is thought to be located in the inner membrane and is essential for the function of the secretion system. (**B**) Wild-type (wt), *ssaV*, *spiC*, *ssaM*, or *ssaL* deletion mutant strains expressing 2HA-tagged SseJ from chromosome were grown in minimal medium pH 5.0, and secreted and bacterial-associated (lysate) proteins were examined by means of immunoblotting to detect the HA epitope, SseB, and DnaK. (**C**) A plasmid expressing SsaL-2HA was introduced into the *ssaL* deletion mutant, and secreted levels of SseJ-2HA, SseB, and SseC were compared with the *ssaL* mutant by means of immunoblotting. (**D**) Interaction between SpiC-2HA, T7-SsaM, and SsaL-3Flag. In the wt-3tag strain, *spiC*, *ssaM*, and *ssaL* are replaced with versions expressing epitope-tagged proteins. This and an isogenic strain lacking SpiC (*spiC*) were grown in minimal medium pH 5.0, and whole-cell lysates were immunoprecipitated with an antibody to T7. The presence of the three proteins was detected in input samples (input) and after immunoprecipitation (output) by means of immunoblotting.

Published by AAAS X Yu et al. Science 2010;328:1040-1043

SsaL and SsaM variants block ternary complex formation.



Phenotypes of SsaL and SsaM variants that block ternary complex formation. (**A**) The SsaL/SpiC/SsaM complex requires the C-terminal 18 amino acids of SsaM. A strain expressing SsaL-3Flag, glutathione *S*-transferase (GST) –SpiC, and either SsaM-2HA or a nonfunctional version lacking its C-terminal 18 amino acids ($SsaM_{104}$-2HA) (4) were grown in minimal medium pH 5.0, and whole lysates were used for GST pull-down (GST-SpiC) or immunoprecipitation (SsaL-3Flag, SsaM-2HA, and $SsaM_{104}$-2HA). (**B**) Plasmids encoding T7-SsaM and 2HA-tagged SsaL or mutant variants were introduced into an *ssaL* deletion mutant. Whole bacterial lysates were immunoprecipitated with antibody to HA. SsaL-2HA and T7-SsaM were detected in input samples (input) and after immunoprecipitation (output) by means of immunoblotting. (**C**) The *ssaL* deletion strain expressing SseB and SseJ-2HA, and SsaL or mutant variants from a plasmid, were grown in minimal medium pH 5.0 for 5 hours. Secreted fractions were analyzed by means of immunoblotting for SseB and SseJ-2HA. (**D**) The wild-type strain, an *ssaM* mutant, and the mutant with or without a plasmid expressing SsaM-2HA or $SsaM_{104}$-2HA were grown at pH 5.0 and analyzed as in (C).

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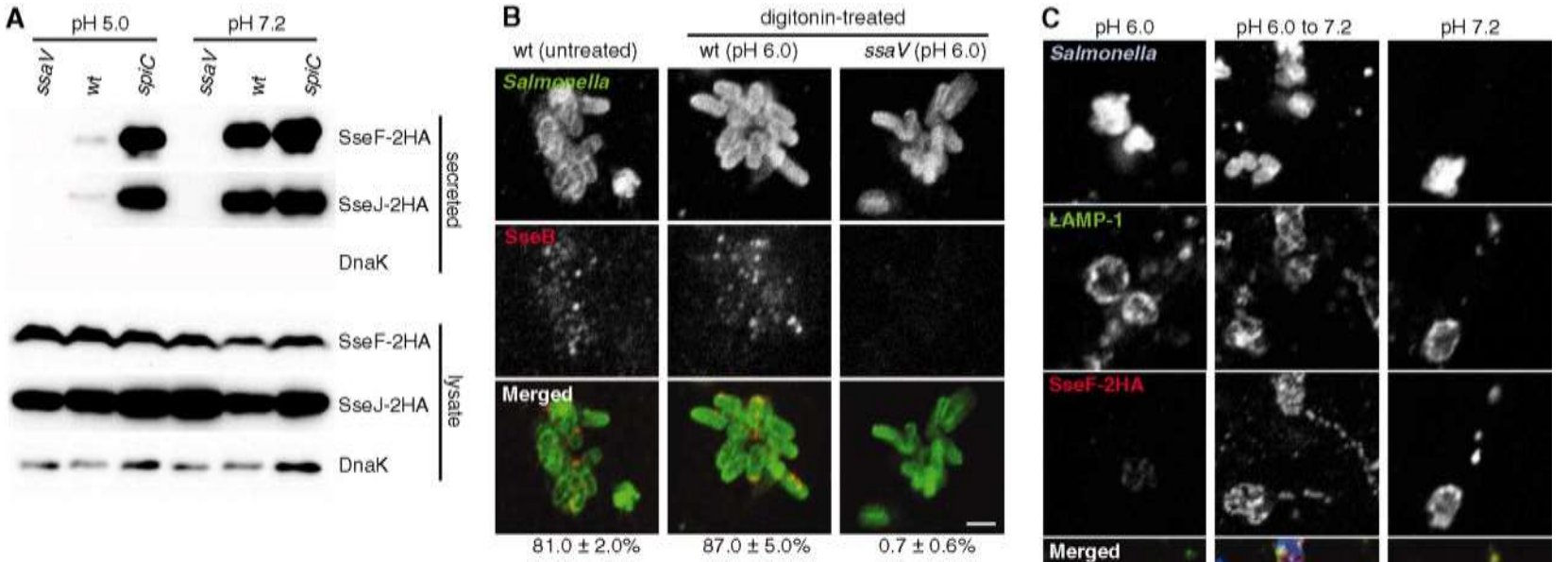
Effect of pH on effector secretion and translocation.



Effect of pH on effector secretion and translocation. (**A**) Bacterial strains were grown in minimal medium pH 5.0 for 4 hours, then exposed to pH 5.0 or 7.2 for 90 min. Secreted and bacteria-associated (lysate) 2HA-tagged effectors and DnaK were examined by means of immunoblotting. (**B**) HeLa cells were infected with wild-type or *ssaV* mutant *Salmonella* for 3.5 hours in order to allow expression of the SPI-2 T3SS, then some samples were permeabilized with digitonin and exposed to pHe 6.0. Cells were fixed 2.5 hours later and immunolabeled in order to detect *Salmonella* and secreted SseB. (**C**) HeLa cells were infected for 3.5 hours with wild-type *Salmonella* expressing SseF-2HA, then permeabilized with digitonin and exposed to pH 6.0 or 7.2 for a further 2.5 hours. In one sample, pH was changed from 6.0 to 7.2, 1 hour before fixation. Fixed cells were immunolabeled to detect *Salmonella*, LAMP-1, and SseF-2HA. In (B) and (C), values below the images represent the percentage of cells in which secreted SseB or translocated SseF-2HA was detected, ±SE of three experiments (n > 100 cells per experiment). Scale bars, 2 μm.

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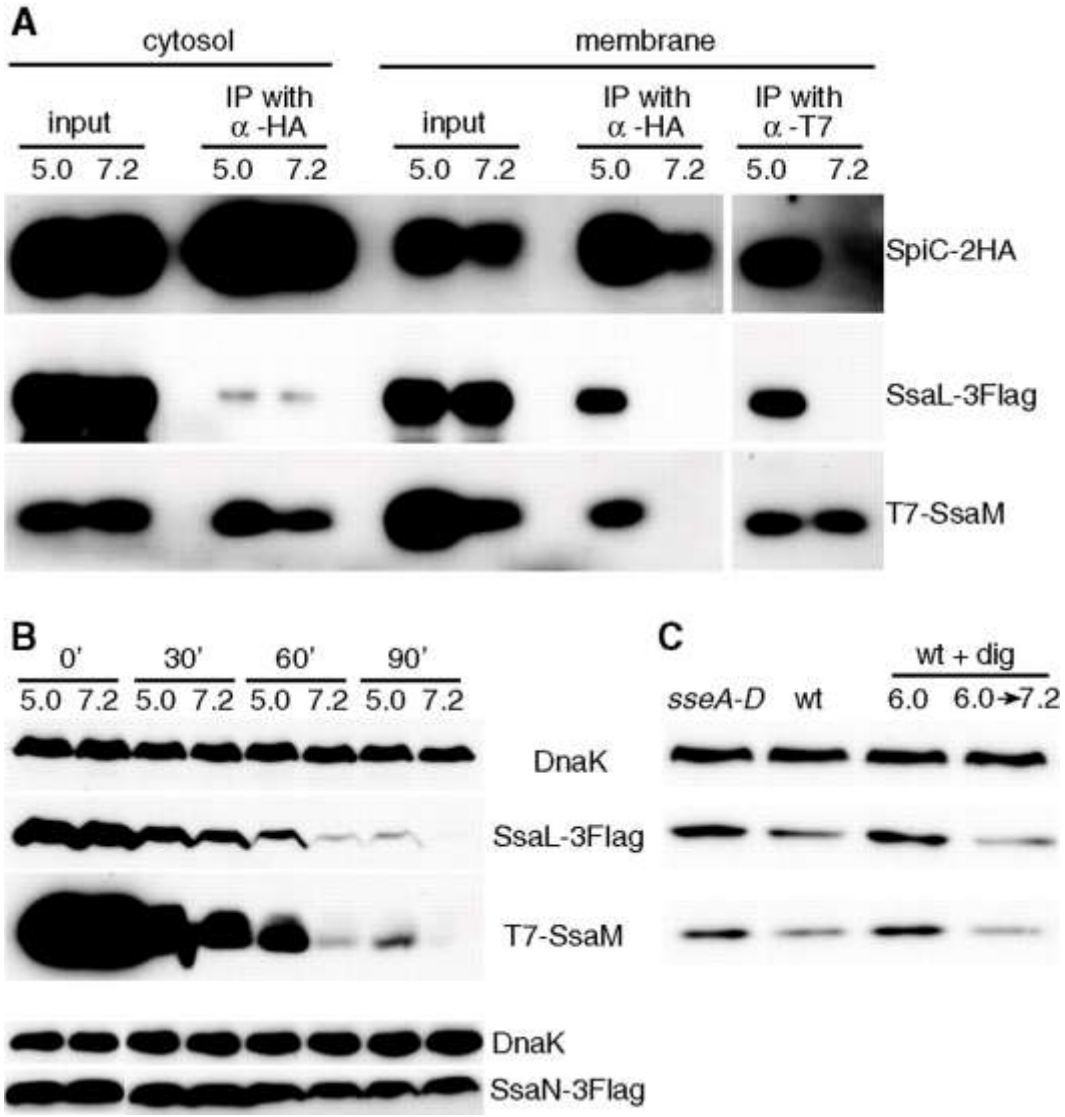
Effect of pH on the SsaL/SsaM/SpiC complex.



Effect of pH on the SsaL/SsaM/SpiC complex. (**A**) The wt-3tag strain was grown in minimal medium at pH 5.0 for 4 hours, then exposed to pH 5.0 or 7.2 for 1 hour, then membrane-associated and cytosolic SpiC-2HA and membrane-associated T7-SsaM were immunoprecipitated (IP). Proteins were detected in input samples (input) and after immunoprecipitation (output) by means of immunoblotting. (**B**) The wt-3tag strain and a strain in which the chromosomal copy of *ssaN* is replaced with a functional version carrying a 3Flag epitope (bottom) were subjected to pH shift in the presence of tetracycline. Samples were removed at various times, and lysates were analyzed by means of immunoblot. (**C**) HeLa cells were infected with the wt-3tag strain (wt) or an isogenic translocon mutant (*sseA-D*). At 3.5 hours after invasion, some wt-infected cells were treated with digitonin (wt + dig), and exposed to pHe 6.0 for 2.5 hours (6.0) or pHe 6.0 for 1.5 hours, then changed to pHe 7.2 for another 1 hour (6.0 → 7.2). Cells were lysed at 6 hours after invasion and analyzed by means of immunoblotting.

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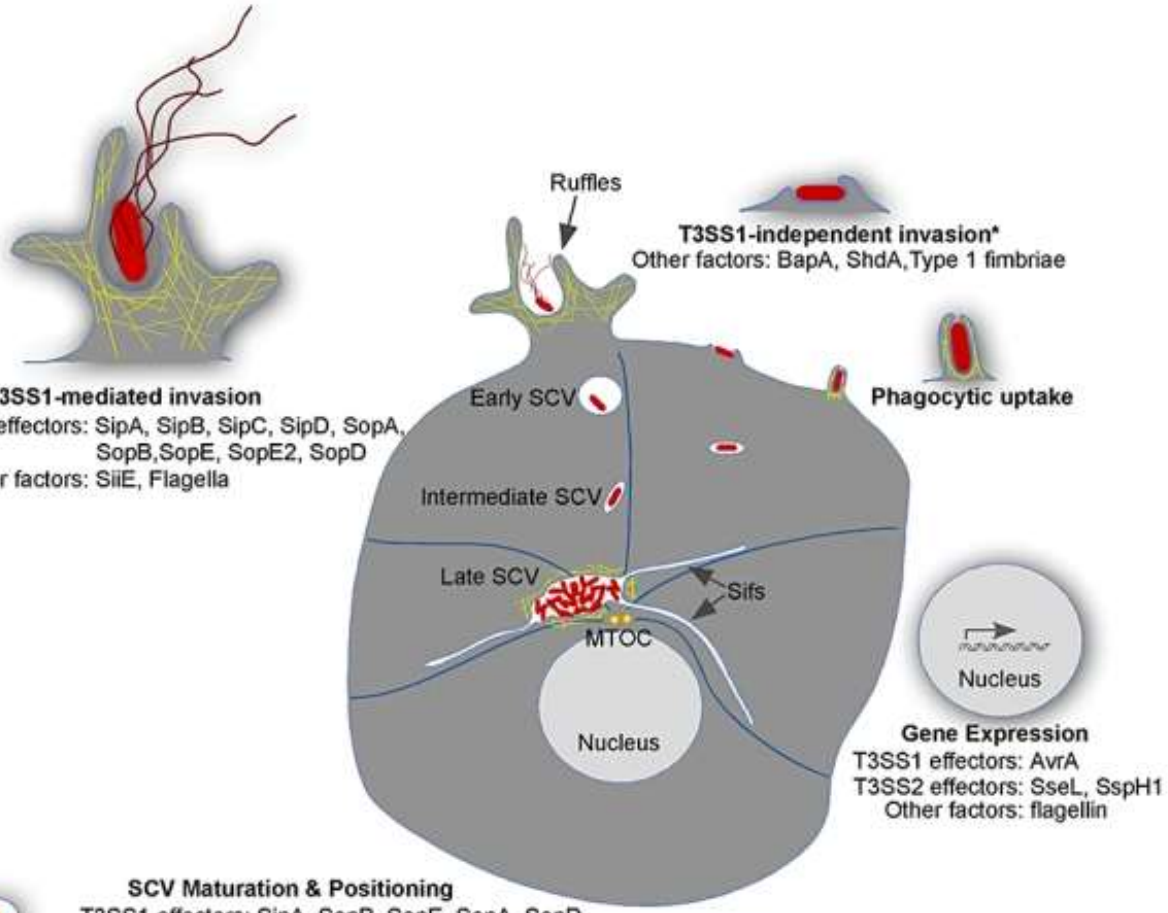
The intracellular Salmonella life style



Intracellular life

- Survival in SCV (*Salmonella*-containing vacuole),
- part of bacteria replicates also in cytosol and can egress from cells…

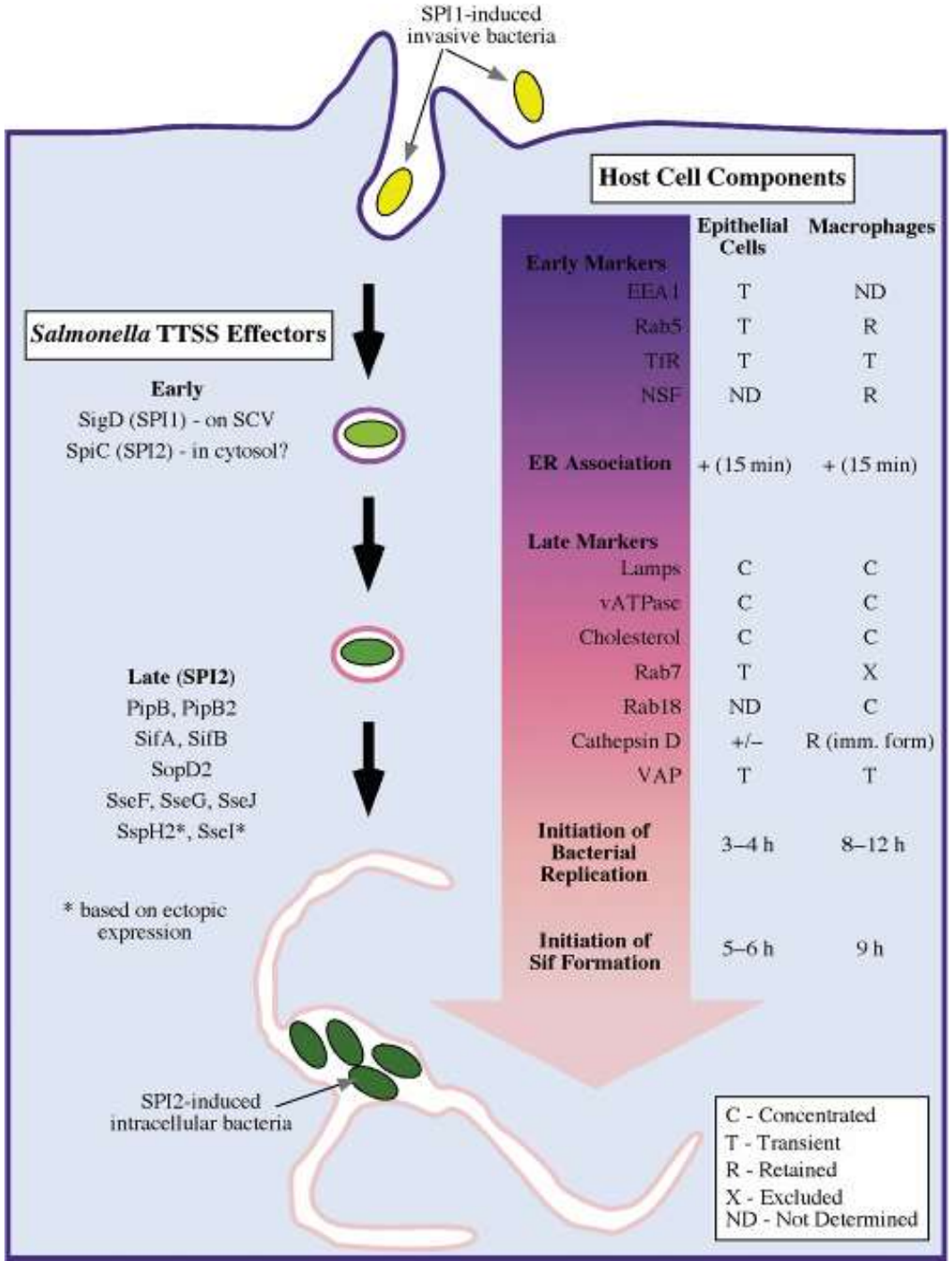
- **SCV biogenesis:**
  - Directed by the second T3SS from SPI-2 gene effectors SifA, SseF and SseG induce formation of tubular protrusions
  - Vacuole fuses with early endosomes and recruits EEA1, Rab5 and transferrin receptors (iron source…)
  - Early endosomal proteins recruited by vesicle fusion: Lamp1, Lamp2, vacuolar ATPase, mannose-6-P receptor (sorting) excluded from SCV a and lysosomal enzymes dependent for sorting on manose-6-P receptor are not recruited – fusion with late endosome restricted
  - SCV enrichment in cholesterol

Traffic. 2003 Sep;4(9):587-99
Taking possession: biogenesis of the Salmonella-containing vacuole

Intracellular life

SCV biogenesis:

- in 2-3 hours *Salmonella* modifies the vacuole so that it can survive
- formation of Sif (Salmonella induced filaments) = SCV membrane tubule protrusions initiated by T3SS effector SifA
- Fusion with lysosome inhibited – survival - due to T3SS effector SpiC action from SPI-2 (SpiC binds and inhibits Hook3 protein action required for endosome-lysosome fusion)
- *Salmonella* can egeress into cytosol and multiply in enterocytes, but does not proliferate in macrophage cytosol?



Traffic. 2003 Sep;4(9):587-99
Taking possession: biogenesis of the Salmonella-containing vacuole
Mol Microbiol. 2003 Sep;49(6):1565-76
The Salmonella SpiC protein targets the mammalian Hook3 protein function to alter cellular trafficking
Mol Microbiol. 2002 May; 44(3):645-61
Complementary activities of SseJ and SifA regulate dynamics of the Salmonella typhimurium vacuolar membrane

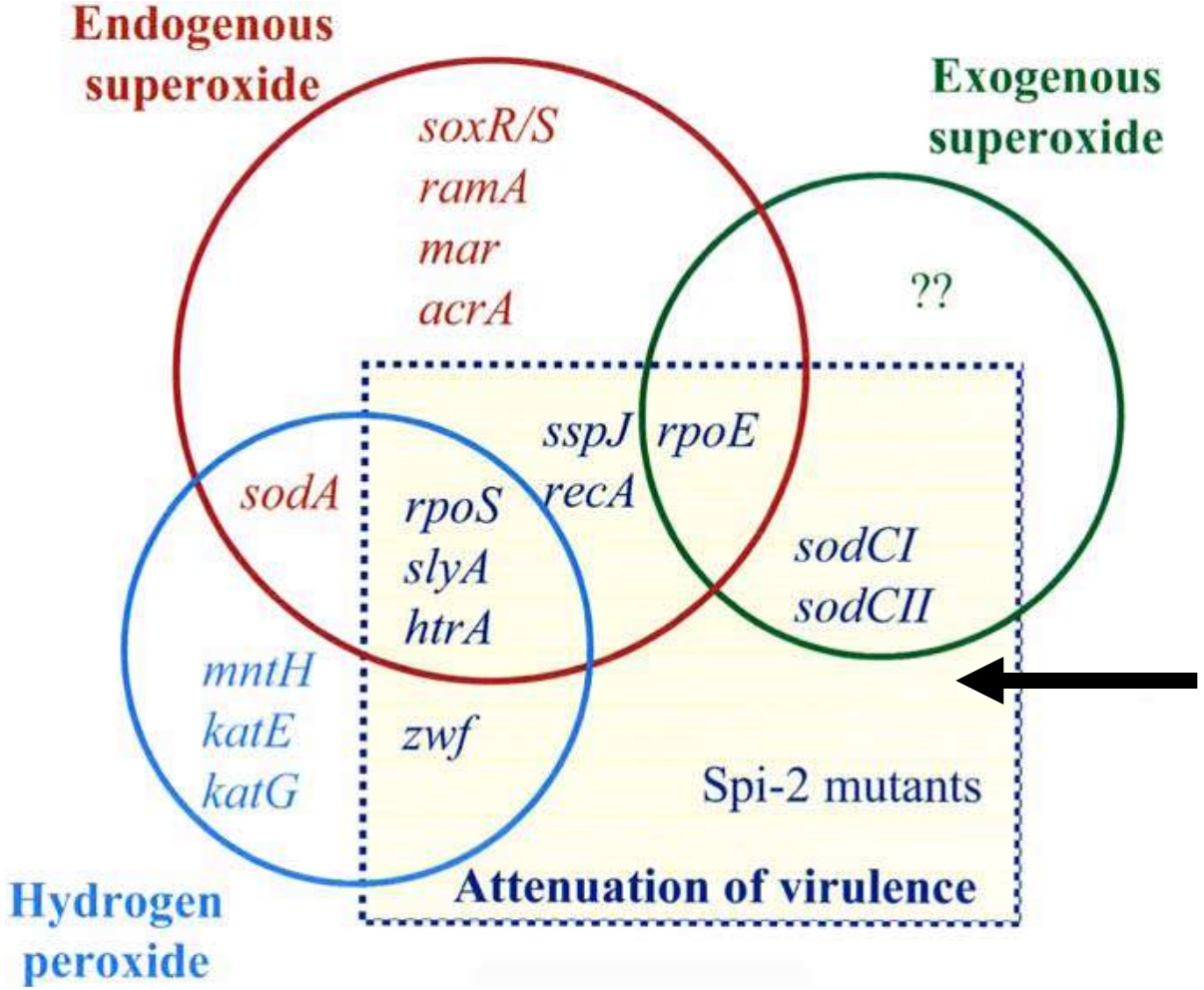
Table 2: *Salmonella* TTSS effector proteins, which affect SCV biogenesis or are localized to SCV/Sifs

Effector (location on chromosome)	SPI1 or SPI2 substrate	Intracellular localization	Function	Virulence of mutant in animal model of infection	Refs
PipB (SPI5, 24 cs)	SPI2	SCV, Sifs (DRMs)		*Mur* – not attenuated *Bov* – decreased intestinal secretory and inflammatory response	(99,109)
PipB2 (60 cs)	SPI2	SCV, Sifs Peripheral vesicles (DRMs)		*Mur* – slight attenuation	(15)
SifA (27 cs)	SPI2	SCV, Sifs	SCV membrane integrity Sif formation	*Mur* – attenuated	(13,24,42,73, 104–106, 110)
SifB (35 cs)	SPI2	SCV, Sifs (LAMP + ve vesicles in RAW cells)		*Mur* – not attenuated	(44,111)
SopB/SigD (SPI5, 24 cs)	SPI1	SCV	Inositol polyphosphate phosphatase, Akt/PKB Activation	*Mur* – not attenuated *Bov* – decreased intestinal secretory and inflammatory response	(75,76,82, 109,112)
SopD2 (22 cs)	SPI2	SCV, endosomes		*Mur* – slight attenuation	(113)
SpiC/SsaB (SPI2, 30 cs)	SPI2	Cytosol	Inhibition of SCV fusion with lysosomes/endosomes Sif formation Interacts with NIPSNAP homologue TassC Required for translocation of SPI2 effectors	*Mur* – attenuated	(100–103)
SpvB (pSLT virulence plasmid)		Cytosol	ADP-ribosylation of actin Inhibits VAP formation	*Mur* – attenuated	(68,70–72, 114,115)
SseF (SPI2, 31 cs)	SPI2	SCV, Sifs	Aggregation of endosomes Sif formation	*Mur* – slight attenuation	(6,116,117)
SseG (SPI2, 31 cs)	SPI2	SCV, Sifs	Aggregation of endosomes Sif formation	*Mur* – slight attenuation	(6,116,117)
SseI/SrfH (Gifsy-2 prophage, 23 cs)	SPI2	VAP Actin remodeling?	Interacts with filamin	*Mur* – not attenuated	(44,86)
SseJ (35 cs)	SPI2	SCV, Sifs (LAMP +ve vesicles in RAW cells)	Aggregation of endosomes	*Mur* – slight attenuation	(44,111,117)
SspH 2 (48 cs)	SPI2	VAP	Interacts with filamin and profilin Actin remodeling?	*Mur* – not attenuated *Bov* – reduced severity of intestinal lesions	(86,118)

Bov: bovine. DRMs: detergent-resistant microdomains. *Mur*: murine. SCV: *Salmonella*-containing vacuole. Sifs: *Salmonella*-induced filaments. VAP: vacuole-associated actin polymerization.

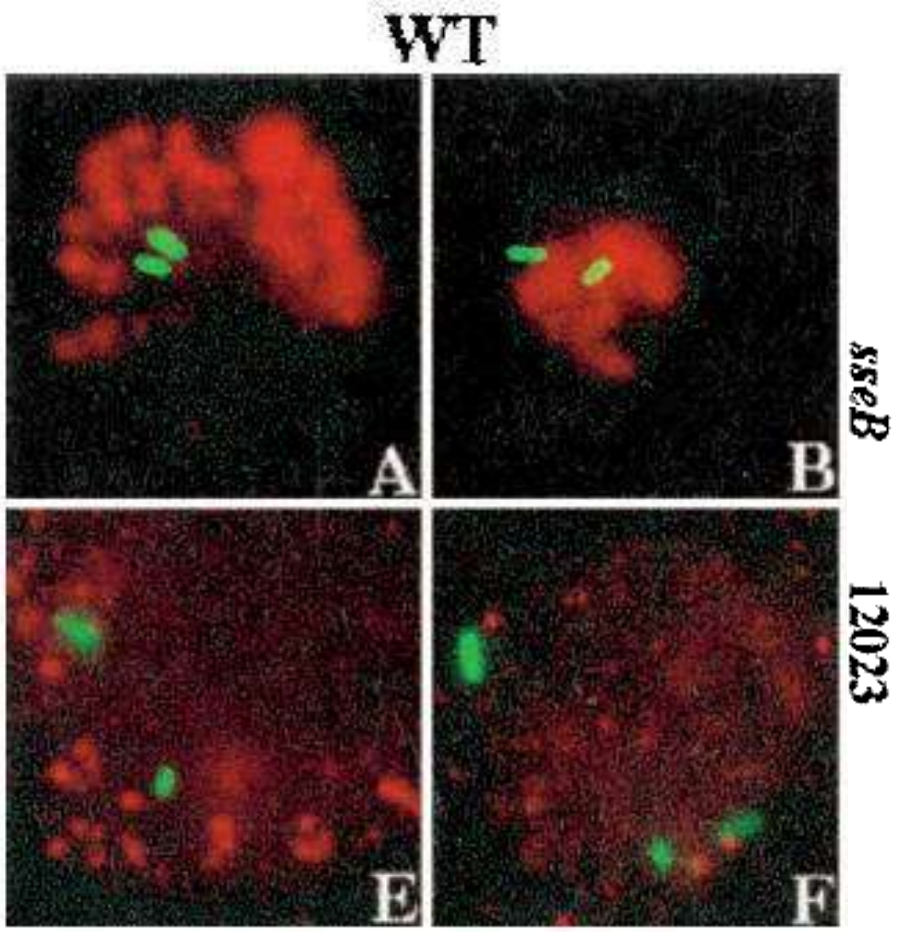
Intracellular life

- Resistance to ROS a RNS radical important – genetic screen for resistance:



Intracellular life

- Some T3SS effectors block recruitement of NADPH oxidase into SCV to promote survival...



localization of p22*phox* subunit of NADPH oxidase in SCV containing virulent 12023 strain and *sseB* mutant

Intracellular life

Infected phagocytes are eventually killed:

- **2 ways:**
  - Necrotic death due to cytotoxix action of T3SS effector SipB that induces caspase 1 and autogphagy
  - 5 h later some cells die from apoptosis due to OmpR-regulated expression of SPI-2 genes

Pathogenicity islands

Table I. Selected *S. typhimurium* virulence factors associated with pathogenicity islands.

Location	*Gene*	*Type*	*Biochemical activity*	*Effect on host cell*	*Mutant phenotype*	*Reference*
SPI (b3c5)	*invA*	Structural (inner membrane)	Translocation	Delivery of type III secreted effectors	LD_{50} attenuated after oral infection but not intraperitoneal. Non-invasive in vitro	[15]
	orgA	?	Translocation?	Delivery of type III secreted effectors	Same as *invA*. Regulation of *orgA* by low oxygen levels	[16]
	sptP	Translocated effector	Tyrosine phosphatase	Actin rearrangements?	Invasion unaffected but attenuated in colonization of spleen in mice	[25, 26]
	sipA	Translocated effector	Binds actin, activates plastin	Actin rearrangements and bundling	Invasion slightly attenuated in vitro, loss of actin polymerization in host cell at site of entry	[29, 30]
	sipB	Translocated effector	Translocation, caspase 1 activation	Apoptosis of macrophages		[31]
	sipC	Translocated effector?	Translocation, other?	Delivery of type III secreted effectors, other?		[33]
61 cs	*sopE**	Translocated effector	Activates Cdc42 and Rac GTPases	Actin rearrangements, cytokine production	Invasion slightly attenuated in vitro. Virulence unaffected in vivo	[37, 39]
SPI2 (31 cs)	*ssaJ*	YscJ/MxiJ/PrgK family of lipoproteins (structural?)	Translocation?	Delivery of type III secreted effectors?	Virulence attenuated in mice and bacteria unable to spread to mesenteric lymph nodes. Mutants unable to replicate in the spleen.	[44, 47]
	sseABC	Translocated effectors?	?	?	Virulence attenuated in mice. Mutants unable to replicate in macrophages in vitro.	[46]
	spiC	Translocated effector	Inhibits endosome-endosome fusion in vitro	Inhibits fusion of SCV with lysosomes and endosomes. Interferes with normal trafficking of the transferrin receptor	Attenuated virulence in mice and survival in macrophages in vitro	[51]
SPI3 (82 cs)	*mgtCB*	Cation transporters?	Mg^{2+} uptake, others?	?	Attenuated virulence in mice and survival in macrophages in vitro	[57, 58]
SPI4 (92 cs)	Various	Type I Secretion? Others?	Toxin delivery?	Apoptosis?	Intramacrophage survival	[61, 66]
SPI5 (25 cs)	*sopB/sigD**	Translocated effector	Inositol phosphate phosphatase	Chloride secretion	Attenuated enteropathogenesis in ileal loop model	[70, 71]
	pipB				Attenuated virulence in mice	[53]

* Note: Translocated via the SPI1 type III secretion system into the host cell cytosol.

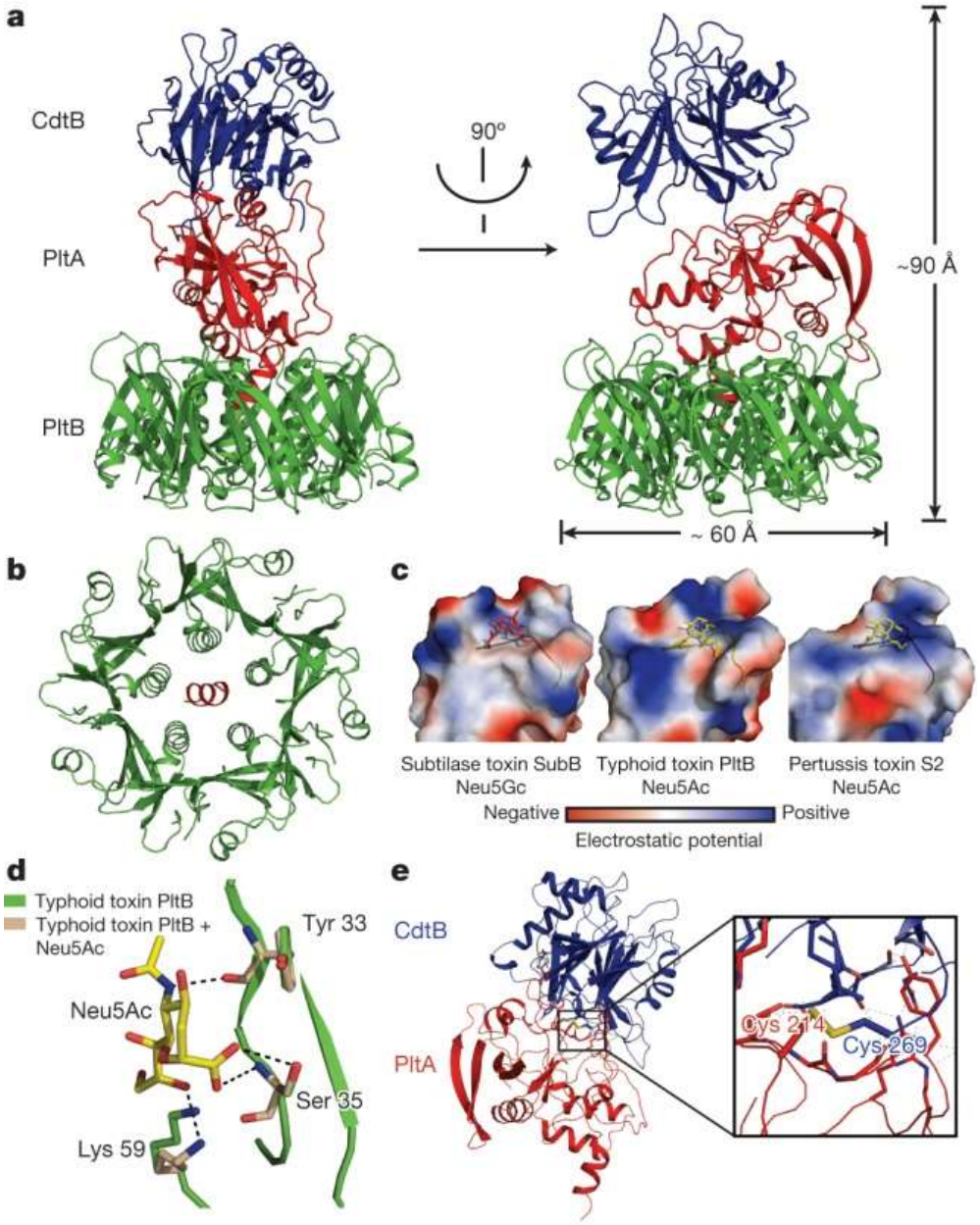
S. typhi

- Strains are monomorphic, 97% of identical genes
  - Variations in Vi antigen conferring serum resistance, high virulence
- Typhoid fever – life-threathenic systemic disease
- Assymptomatic carriers walking and shedding around…
- Symptoms in 10-14 days (high fever, halucinating, spasms, no diarrhoea)
- Spleen and liver infected and inflammed
- In asymptomatic carriers a a colonization of gallbladder and years to life-long shedding in bile can occur …
- ampicilin, amoxicillin, co-trimoxazol for sensitive strains

Salmonella typhi – the human killer

- Unlike other *Salmonella* serovars, which typically cause self-limiting gastroenteritis, *S. Typhi* causes a systemic, life-threatening systemic infection known as typhoid fever
- *Salmonella enterica* serovar Typhi (*S.* Typhi) causes more than 200,000 annual deaths
- One of the few *S.* Typhi-specific factors that have been shown to directly affect its interaction with host cells is an AB-type toxin dubbed typhoid toxin that binds carbohydrate moieties on specific surface glycoproteins of many cell types
- systemic administration of typhoid toxin, a unique virulence factor of *S.* Typhi, reproduces many of the acute symptoms of typhoid fever in an animal model.
- Unlike typical AB toxins, typhoid toxin is composed of two A subunits, PltA and CdtB, which are homologues of the A subunits of the pertussis and cytolethal distending toxins, respectively.
- Its single B subunit, PltB, is a homologue of one of the components of the heteropentameric B subunit of pertussis toxin.
- Cellular targets of the ADP-ribosyl transferase activity of PltA have not yet been identified
- CdtB is a DNase that inflicts DNA damage and induces cell-cycle arrest
- S. Typhi produces typhoid toxin only within mammalian cells, and the toxin is then ferried to the extracellular environment by a unique transport mechanism that involves vesicle carrier intermediates

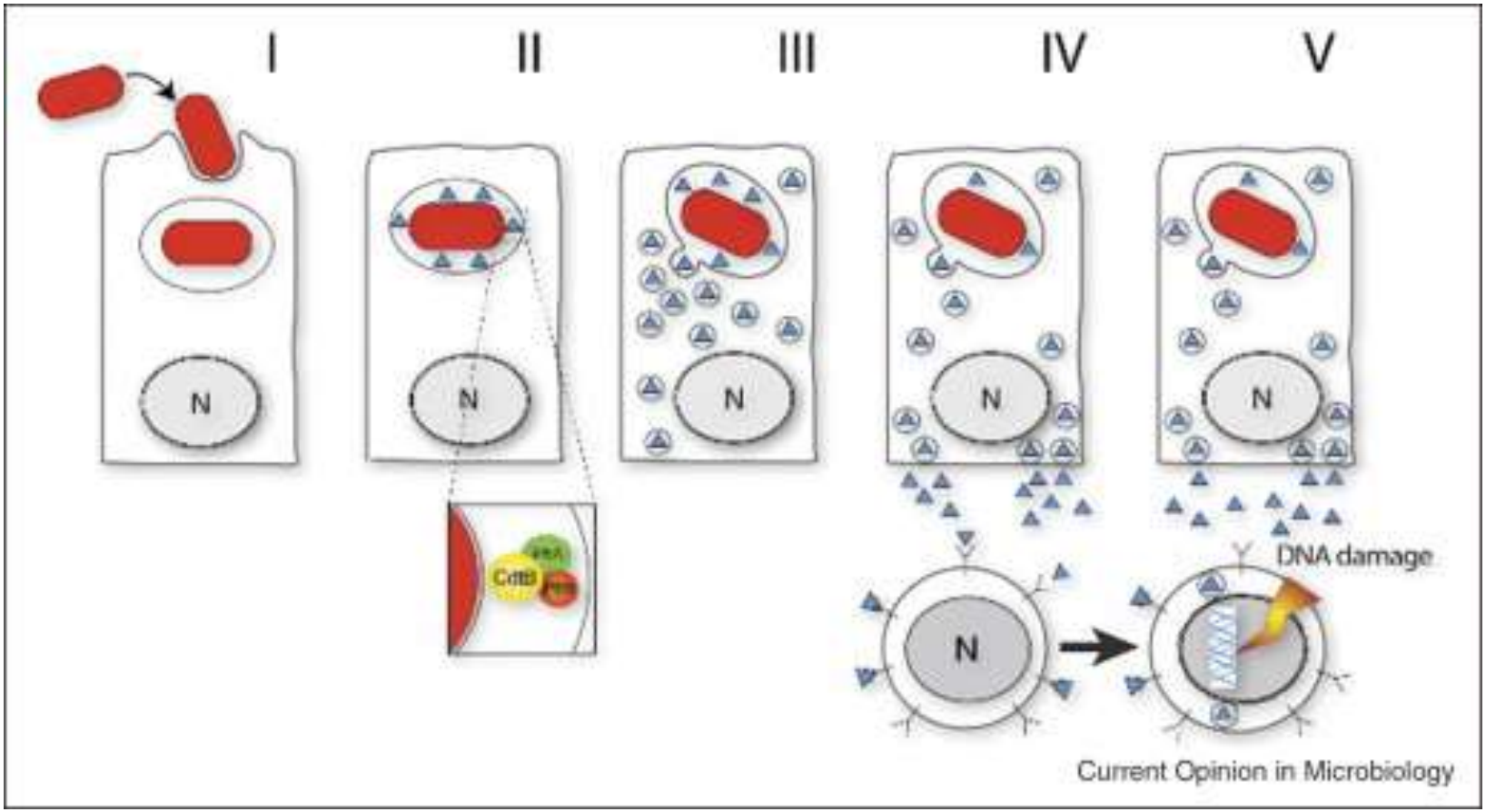
The crystal structure of typhoid toxin depicts a unique architecture.



a, Two views of the overall structure of the typhoid holotoxin complex, CdtB, PltA and PltB are shown in blue, red and green, respectively.
b, Bottom view of the channel formed by the PltB pentamer (in green), depicting the PltA C-terminal α-helix (in red) within it.
c, Surface charge distribution of the predicted sugar-binding pockets of different B subunit homologues of the indicated AB_5 toxins (SubB for subtilase and S2 for pertussis toxins). A highly conserved serine residue critical for sugar binding is indicated within the sugar-binding pocket. The sugars N-glycolylneuraminic acid (Neu5Gc; within SubB) and N-acetylneuraminic acid (within typhoid and pertussis toxins) are shown.
d, Molecular modelling of N-acetylneuraminic acid within the typhoid toxin binding pocket. Critical residues engaged in this interaction are shown.
e, Atomic interface between CdtB and PltA. The inset shows a detailed view of a critical disulphide bond between PltA Cys 214 and CdtB Cys 269.

J Song *et al.* 499, 350–354 (18 July 2013) doi:10.1038/nature12377

Salmonella typhi – the human killer making the typhoid toxin

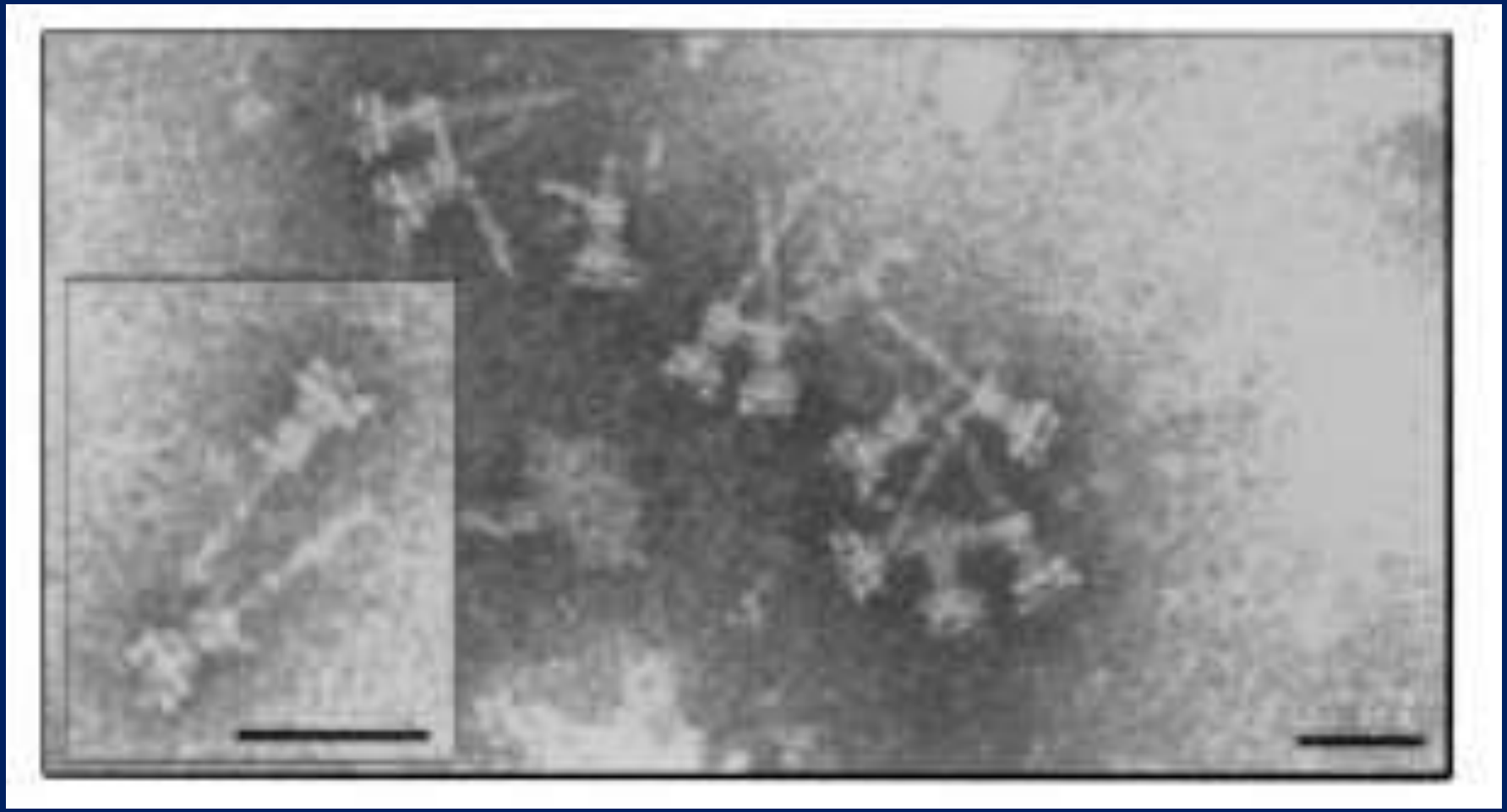


Salmonella Pathogenicity Island 1

- SPI-1 hraje roli hlavně v počáteční fázi infekce = invazi do buněk sliznice, a dále k indukci smrti infikovaných makrofágů
- Zahrnuje nejméně 30 genů:
  - 17 genů kóduje stukturní komponenty TTSS *inv/Spa*, např. *prgHIJK* operon
  - dále geny pro injektované proteiny pomocí TTSS *inv/Spa*
    - Efektorové molekuly ze SPI-1 např. : SipA, AvrA a SptP
    - Efektorové molekuly ležící mimo SPI-1 např.: SopA, SopB, SopD, SopE1, SopE2, SspH1 a SlrP
  - geny pro regulaci genů virulence:
    - *hilA*, *invF* (transkripční aktivátor genů pro proteiny secernované TTSS)
    - *sirA* a *phoP/phoQ* dvoukomponentový regulační systém
  - geny pro chaperony, např. *sicA*

Proc Natl Acad Sci U S A. 2000 Sep 26;97(20):11008-13.
Contribution of Salmonella typhimurium type III secretion components to needle complex formation

Salmonella Pathogenicity Island 1



Proc Natl Acad Sci U S A. 2000 Sep 26;97(20):11008-13.
Contribution of Salmonella typhimurium type III secretion components to needle complex formation

Salmonella Pathogenicity Island 1

Table I. SPI-1 effector proteins.

Protein	Biochemical activity	Known in vivo function	Reference
AvrA	unknown	unknown	[16]
SipA	stabilization of F-actin, reduction of the critical concentration for actin polymerization	actin polymerization and reorganization	[48, 49]
SipB	binding of caspase-I	translocation of SPI-1 effectors and induction of apoptosis	[58]
SipC	actin nucleation and bundling	translocation of SPI-1 effectors and actin polymerization	[50]
SipD	unknown	translocation of SPI-1 effectors	[13]
SlrP	unknown	putative host adaptation factor	[14]
SopA	unknown	induction of enteritis	[26]
SopB	inositol phosphatase	chloride secretion and actin polymerization	[38, 39]
SopD	unknown	induction of enteritis	[23]
SopE	small G protein GEF	actin polymerization	[18]
SopE2	small G protein GEF	actin polymerization	[19]
SspH1	unknown	lethal infection in calves	[15]
SptP	small G protein GAP and tyrosine phosphatase	recovery of actin cytoskeleton rearrangements	[42, 59]

Microbes and Infection, 3, 2001, 1293–1298
Salmonella entry into host cells: the work in concert of type III secreted effector proteins

Salmonella Pathogenicity Island 2

- SPI-2 hraje roli hlavně v přežití salmonel v makrofázích a ve vyvolání systémové infekce
- Zahrnuje nejméně 40 genů:
  - 13 genů kóduje komponenty TTSS *Spi/Ssa*, který traslokuje efektory přes fagosomální membránu
  - efektorové proteiny:
    - SpiC z SPI-2, který zajišťuje inhibici fúze s lysozomy
    - SifA mimo SPI-2, který indukuje tvorbu sifů
    - SseF a Sseg z SPI-2, které se spolupodílí na tvorbě sifů
  - geny pro regulaci genů virulence:
    - ssrA-ssrB dvoukomponentový regulační systém (je pod kontrolou jiného dvoukomponentového regulačního systému OmpR-EnvZ), který např. reguluje expresi *sifA*
  - spousta genů z SPI-2 je podobných genům virulence jiných bakterií

Salmonella Pathogenicity Island 3

- Zahrnuje nejméně 10 genů:
  - *mgtCB* operon kóduje macrophage survival protein MgtC, což je Mg^{2+} transportér, který bakterii zabezpečuje přísun Mg^{2+} ve fagosomu, který je na něj chudý
  - Proteiny sekvenčně podobné známým proteinům jiných patogenů

Salmonella Pathogenicity Island 4

Zahrnuje nejméně 18 genů:

- Pravděpodobně kóduje sekreční systém typu 1

Salmonella Pathogenicity Island 5

Zahrnuje nejméně 6 genů:

- Účastní se vzniku enterické formy salmonelózy
- Sop (Salmonella outer proteins) a Pip proteiny (Pathogenicity island encoded protein)
  - Sop jsou secernovány TTSS SPI-1

Plasmid virulence pSLT

- Bez tohoto plasmidu je S. typhimurium avirulentní
- Rck protein
  - Zvyšuje sérovou resistenci
- skupinu genů *spv* (*spvABCD* a *spvR*)
  - *spvR* reguluje transkripci *spv* genů, *spvABCD* většinou neznámé funkce, celkově zabezpečují přežití v makrofágu
    - u *spvB* byla identifikována ADP rybosylační aktivita, následkem je rozvrat v aktinu a je s ním spojena těžká systémová infekce na zvířecích modelech

Microb Pathog. 2003 Jul;35(1):43-8

Intracellular expression of the Salmonella plasmid virulence protein, SpvB, causes apoptotic cell death in eukaryotic cells

Fágové geny virulence

- Gifsy-1 a Gifsy-2
  - Gifsy-2 nese např. gen *SodCI* pro Cu/Zn superoxid dismutázu a další zatím neznámý silný faktor virulence
  - Salmonella typhimurium bez Gifsy-2 je signifikantně oslabena ve virulenci u myší

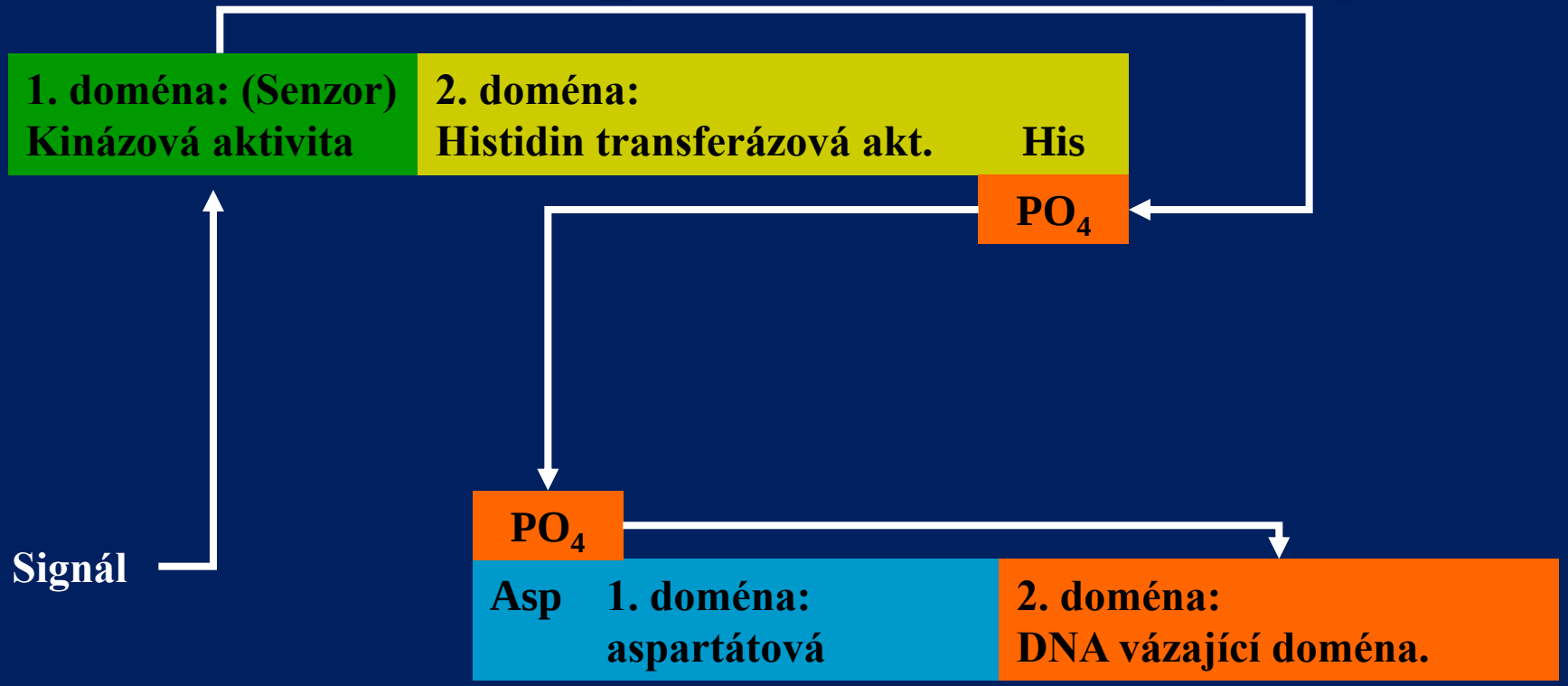
Bacteriol. 2002 Oct;184(19):5234-9

Identification of GtgE, a novel virulence factor encoded on the Gifsy-2 bacteriophage of Salmonella enterica serovar Typhimurium

Regulace genů virulence

- Dvoukomponentové regulační systémy
  - jsou rozhodující pro regulaci exprese virulentních genů
  - složky: senzor signálu a regulátor odpovědi
- Quorum sensing systémy
  - rozpoznání denzity populace bakterií
- Transkripční faktory

Dvoukomponentové reg. sys.



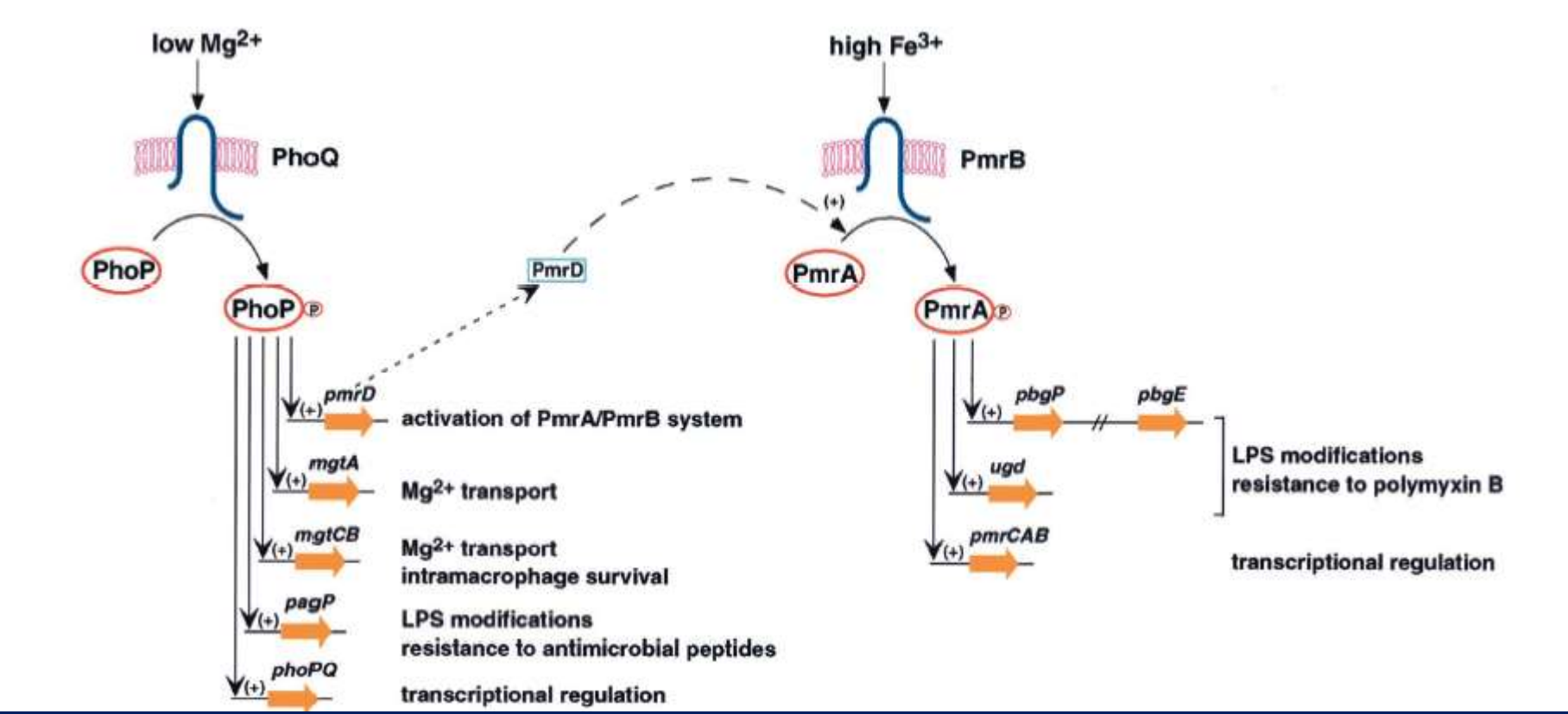
Dle textu: Macela et al.
Intracelulární parasitismus bakterií

PhoP-PhoQ systém

- SPI-1
- sensor vnitřní membrány PhoQ reaguje na nízké hladiny extracelulárního Mg2+ a Ca2+ aj.
  - pravděpodobně indukovaný vstupem salmonel do makrofágových fagosomů
- je aktivován též přítomností subletální koncentrací antibakteriálních peptidů
- mutant defektní v genech *phoP* nebo *phoQ* je vysoce oslabený ve virulenci:
  - je defektní v přežití uvnitř makrofágů
  - mají zvýšenou citlivost k antibakteriálním peptidům, solím žlučových kyselin a nízkému pH
- systém kontroluje expresi přibližně 40 genů: skupina pag (PhoP activated genes) je důležitá pro intracelulární přežití, skupina prg (PhoP repressed genes) je důležitá pro invazi do epitelií
  - Mimo jiné jiný dvoukomponentový systém PmrA-PmrB

Molecular Microbiology (2003) 50 (1), 219–230
Regulation of Salmonella typhimurium virulence gene expression by cationic antimicrobial peptides
JOURNAL OF BACTERIOLOGY Mar. 2001, p. 1835–1842
The Pleiotropic Two-Component Regulatory System PhoP-PhoQ

PhoP-PhoQ systém



Model popisující regulační cíle PhoP-PhoQ systému a interakci s PmrA-PmrB systémem

JOURNAL OF BACTERIOLOGY Mar. 2001, p. 1835–1842
The Pleiotropic Two-Component Regulatory System PhoP-PhoQ

PhoP-PhoQ systém

Gene	Function and /or properties of gene product	Present in *E. coli* K-12?
hilA	Transcriptional regulator of invasion genes	No
mgtA	P-type ATPase Mg^{2+} transporter	Yes
mgtB	P-type ATPase Mg^{2+} transporter	No
mgtC	Mg^{2+} acquisition; intramacrophage survival	No
pagC	Outer membrane protein with sequence similarity to *Enterobacter* OmpX, *Yersinia* Ail, and phage lambda Lom	No
pagP	Outer membrane enzyme mediating transfer of palmitate to lipid A; resistance to peptide C18G	Yes
pbgPE operon	Synthesis and/or incorporation of 4-aminoarabinose into lipid A; resistance to polymyxin	Yes
pcgL	Periplasmic D-Ala-D-Ala dipeptidase	No[a]
pgtE	Outer membrane protease; resistance to peptide C18G	No[a]
phoN	Periplasmic nonspecific acid phosphatase	No
phoPQ	Mg^{2+} -responding two-component system	Yes
pmrAB	Fe^{3+} -responding two-component system	Yes
pmrD	Mediator of transcriptional activation of *pmrA*-regulated genes during growth in low Mg^{2+}	Yes
prgHIJK	Components of Inv-Spa type III secretion system	No
spvB	Mono(ADP-ribosyl)transferase encoded in *Salmonella* virulence plasmid	No
ugd	UDP-D-glucose dehydrogenase	Yes
ugtL	Putative membrane protein	No

Geny regulované Phop-PhoQ systémem

JOURNAL OF BACTERIOLOGY Mar. 2001, p. 1835–1842
The Pleiotropic Two-Component Regulatory System PhoP-PhoQ

PmrA-PmrB systém

- Reaguje na koncentraci Fe a přes PmrD na koncentraci Mg
- Reguluje geny pro modifikace na LPS
- In-silico analýza odhalila další potenciální cíle pro vazbu PmrA-PmrB
  - Např.: *yibD*, *aroQ*, *mig-13* a *sseJ*

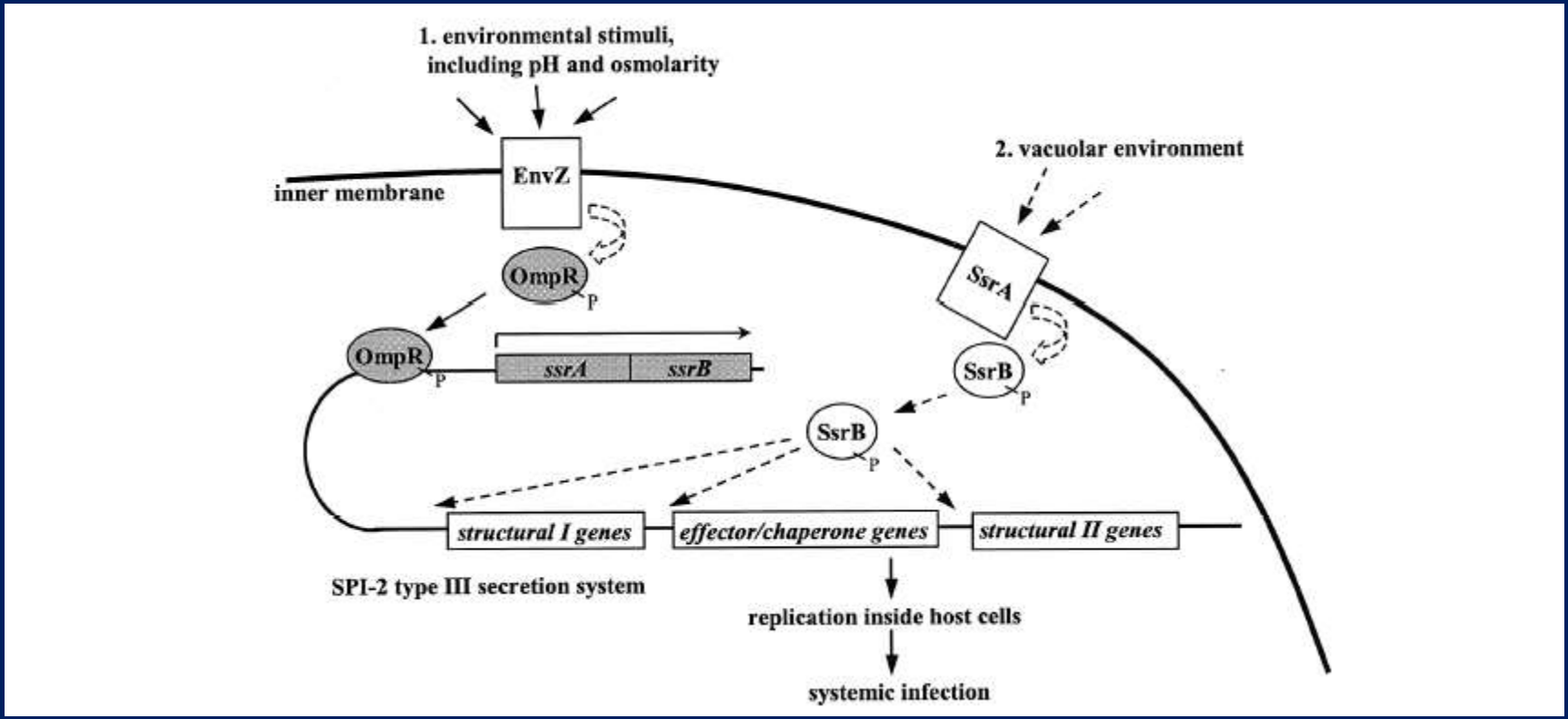
Genome Biol. 2004;5(2):R9. Epub 2004 Jan 29
In silico identification and experimental validation of PmrAB targets in Salmonella typhimurium by regulatory motif detection

OmpR-EnvZ systém

- reaguje na nízké pH
- mmj. je regulátorem jiného dvoukomponentového systému SsrA-SsrB (z SPI-2), přímou vazbou OmpR složky na ssrAB promotor
  - Takto nepřímo reguluje komponenty TTSS SPI-2 a efektory

Microbiology. 2003 Sep;149(Pt 9):2385-96
The roles of SsrA-SsrB and OmpR-EnvZ in the regulation of genes encoding the Salmonella typhimurium SPI-2 type III secretion system

OmpR-EnvZ systém



Model SPI-2 regulace uvnitř makrofágů: OmpR-EnvZ odpovídá na intracelulární prostředí – OmpR se váže na *ssrA* promotor a aktivuje expresi *ssrAB* genů. Později SsrA detekuje změněné signály ve vakuole a SsrB aktivuje TTSS na SPI-2

J Bacteriol. 2000 Feb;182(3):771-81.
OmpR regulates the two-component system SsrA-ssrB in Salmonella pathogenicity island 2

SsrA-SsrB systém

- je kódovaný na SPI-2
- kontroluje:
  - expresy komponent TTSS z SPI-2
  - expresy translokovaných efektorů, ty nemusí být jen SPI-2 např. *SiFA*
  - indukuje expresy genů SPI-2 v prostředí s nízkou osmolaritou, kyselým pH a s absencí Ca2+
- systém je regulován OmpR-EnvZ systémem,

RcsB-RcsC systém

- reaguje na osmolaritu
- ovlivňuje expresi Sip proteinů (SPI-1), flagelinu ale i Vi antigenu u *S. typhi*
- s rostoucí osmolaritou vzrůstá i exprese sipB, ale značně klesá syntéza Vi antigenu
- toto odráží odpověď bakterie ke změnám prostředí, ke kterým dochází v průběhu patogeneze
  - nízká osmolarita = mimo hostitele Sip není exprimován, ale Vi antigen se vyskytuje hojně, působí jako ochrana proti škodlivým vlivům prostředí
  - vysoká osmolarita = v tenkém střevu začíná vzrůstat produkce flagelinu zabezpečující pohyb bakterie a produkce Sip proteinu
  - střední osmolarita = v krevním řečišti je snížena exprese flagelinu a Sip, ale vzrůstá syntéza Vi antigenu

Mol Microbiol. 1998 Aug;29(3):835-50.
The RcsB-RcsC regulatory system of Salmonella typhi differentially modulates the expression of invasion proteins, flagellin and Vi antigen in response to osmolarity

4. Imunitní odpověď hostitele

- první obranná linie = lokálním lymfatickým systém
  - již v buňkách epitelu dochází k interakci flagelinu s TLR5 s následnou aktivací Nf κB
  - produkce cytokinů a chemokinů vyvolá vznik zánětu
  - likvidace profesionálními fagocyty (platí pouze u salmonel s neschopností přežít v SCV v profesionálních fagocytech = přežití fagocytózy je pro patogenezu klíčové)
  - význam dendritických buněk: presentace antigenů
  - Mechanismy specifické imunity = zesílení odpovědi vůči nitrobuněčným parazitům:
    - MHC II reaguje s CD4+ buňkami s TCR α/ß+ generující Th1 odpověď (podpora činnosti monocytů, makrofágů a cytotoxockých lymfocytů NK a CD8+) a produkce IL-2 a IFN-γ
    - Cytotoxické CD8+ T buňky
    - B buňky produkcí protilátek namířených proti O antigenu a jádru LPS, Vi antigenu, fimbriím, atd