“Discovering *Salmonella*”

Kejsi Dervishi

Rome, April 18th, 2024

Oleksandr Panasovskyi /Shutterstock image

General Features

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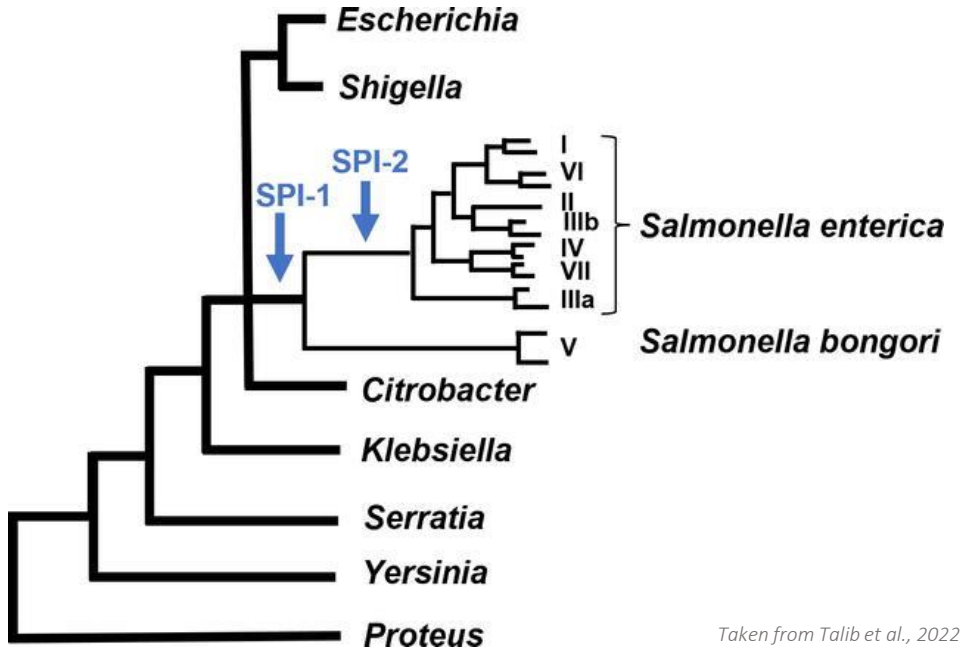
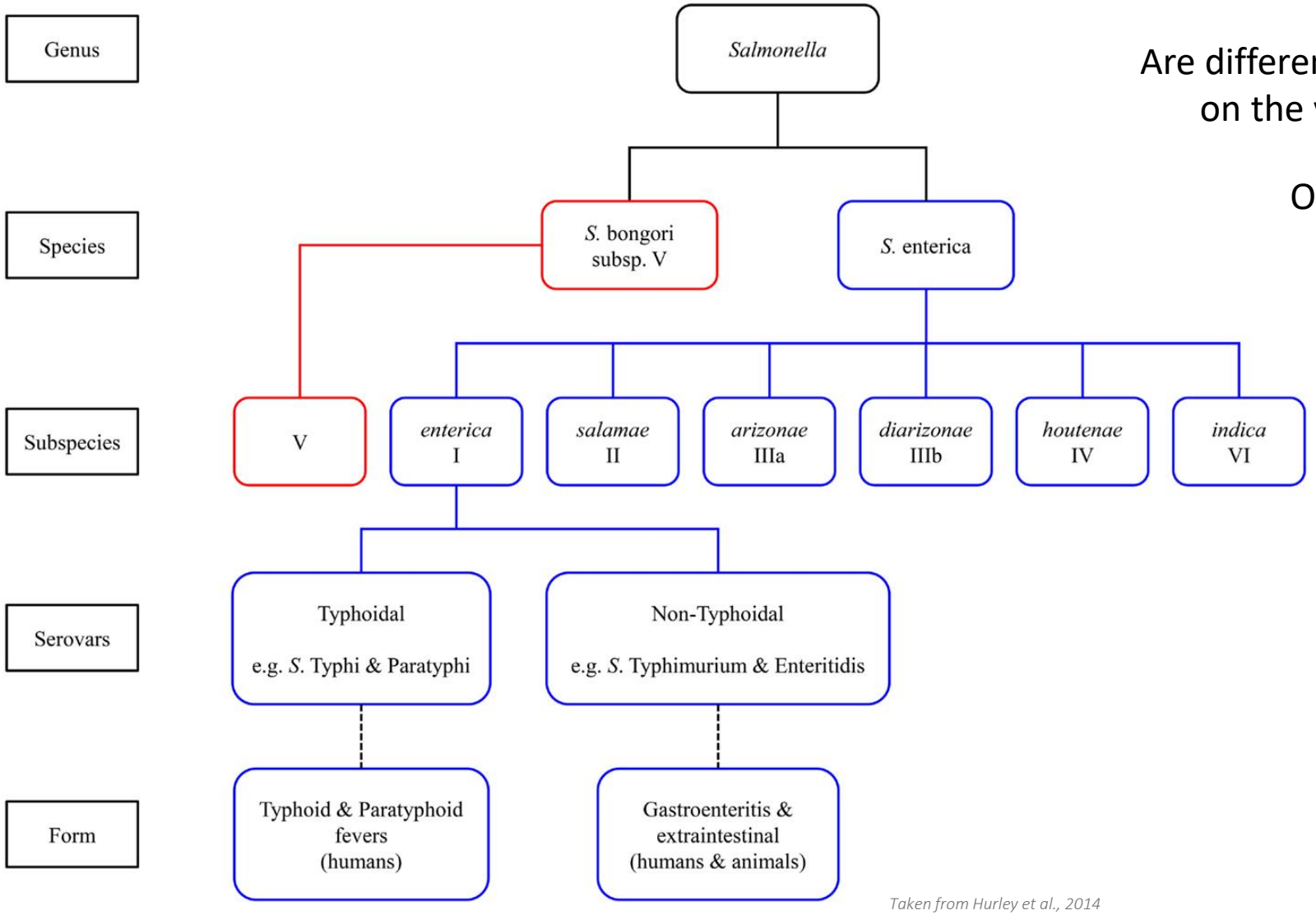


Taken from Talib et al., 2022

- Enterobacteriaceae
- Gram negative
- Rod – shaped bacilli (2μm x 0,5μm)
- Facultative anaerobe
- Motile → peritrichal flagella
- Numerous fimbriae

Classification

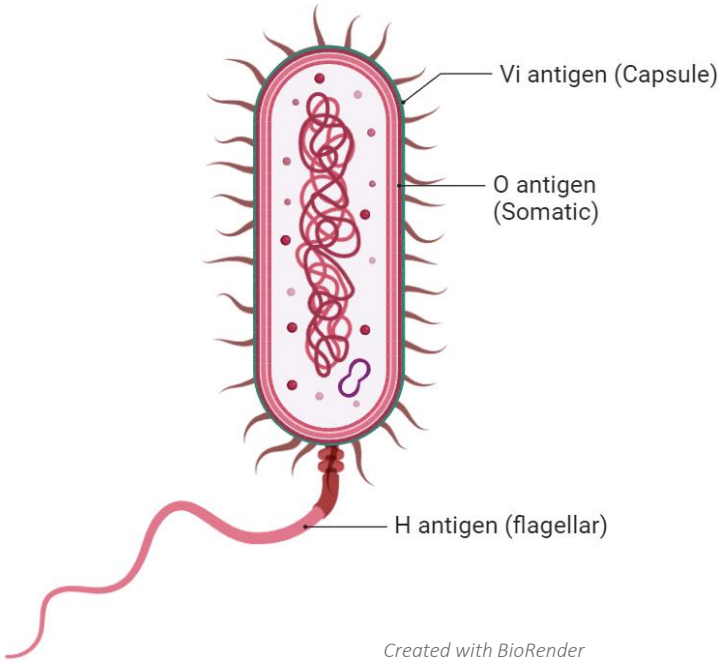


Taken from Hurley et al., 2014

Antigenic Types:

Are differentiated in serotypes/ serovars depending on the variation of the antigens on their surface

Over >2.500 serovars have been identified



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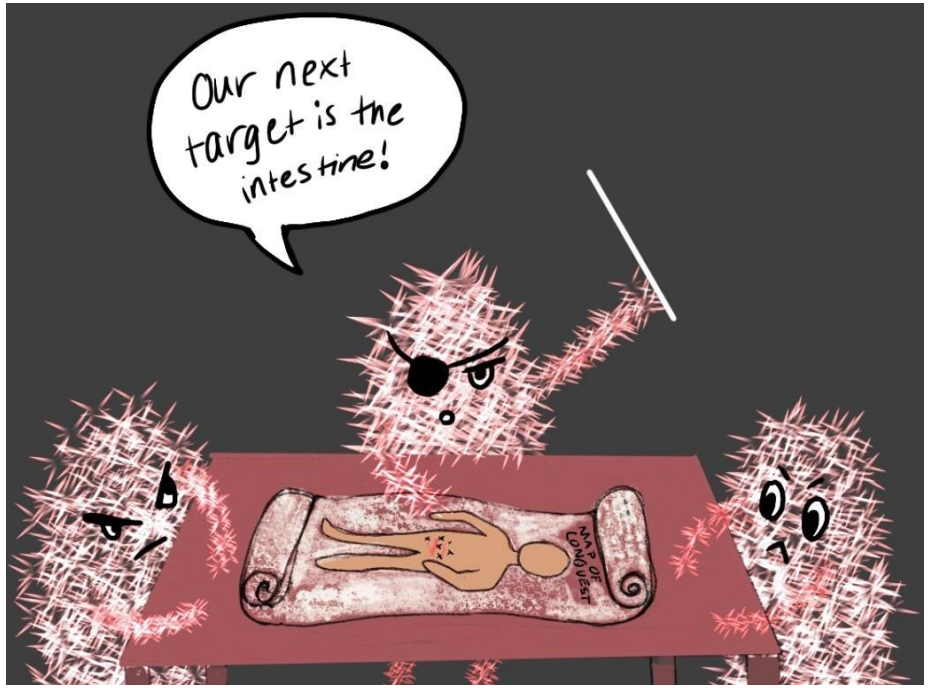
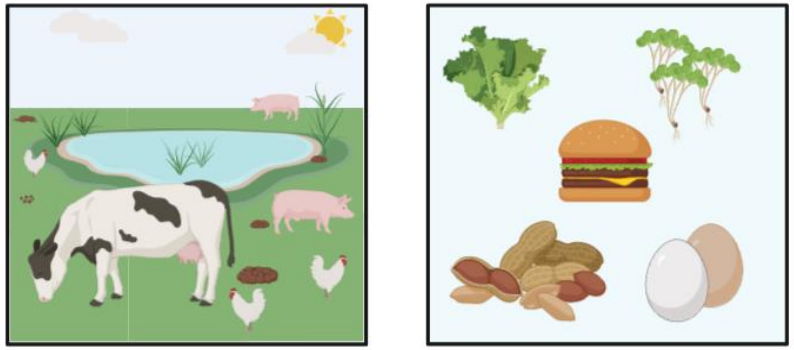
Salmonellosis

Food-borne infection caused by *Salmonella* bacteria



Valerie Pavilonis/ Yale News

- Transmission: oral – fecal route
- Sources: contaminated, improperly stored or handled food; contaminated water; household pets; environmental factors



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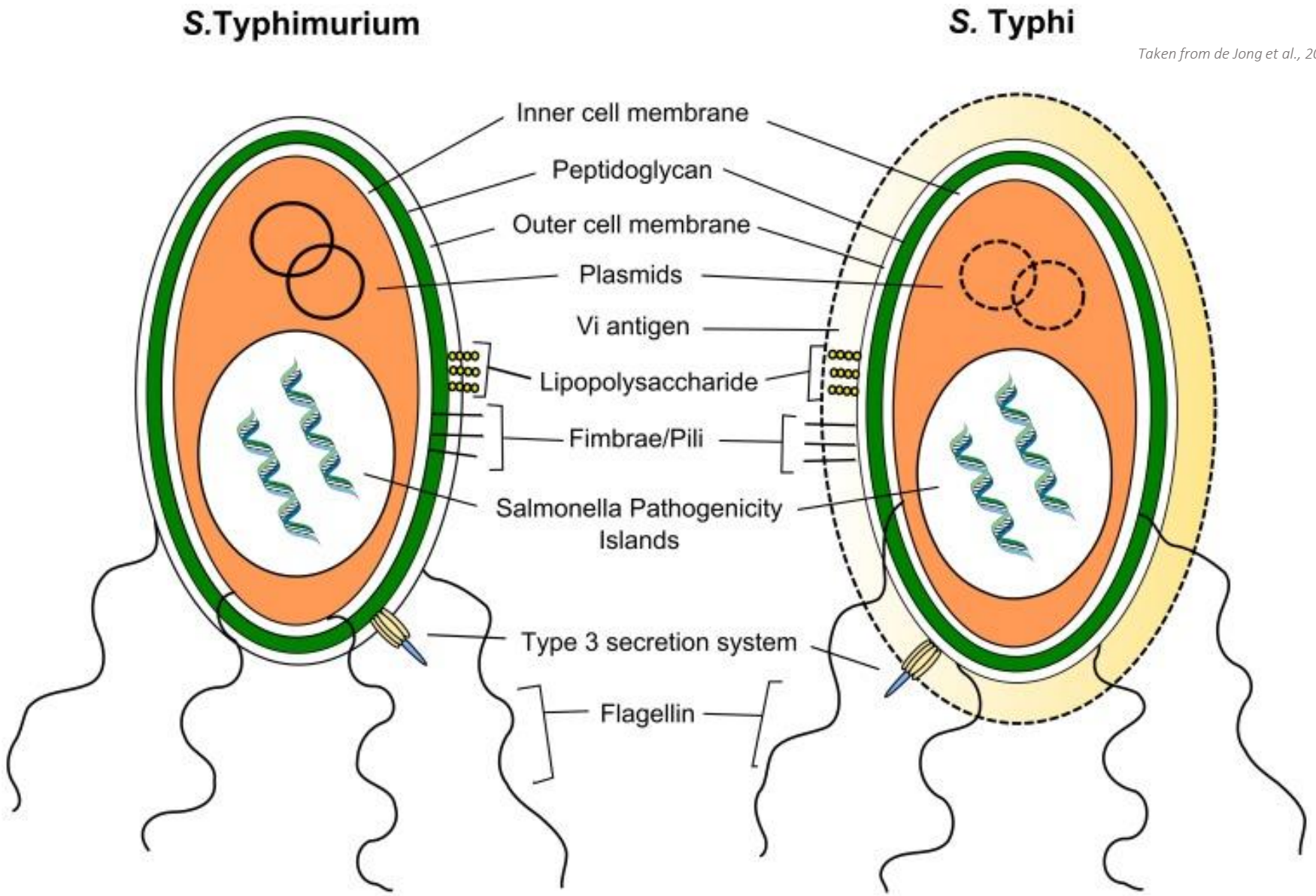
Infective dose: 10^6 bacteria (healthy)

Infection rate children <5 years is higher than all other diagnosed people

Elderly and immunocompromised individuals are more likely to present severe forms of the disease

Sharing about 90% genes, the residual 10% that differ include virulence factors (determines pathogenic potential)



Gastroenteritis

(NTS salmonellosis)

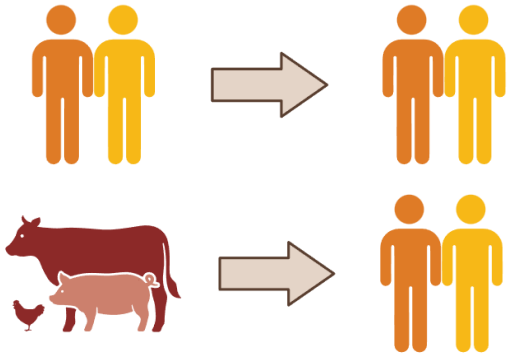
Incubation time: 6h to 2 days
Symptoms: < 10 days

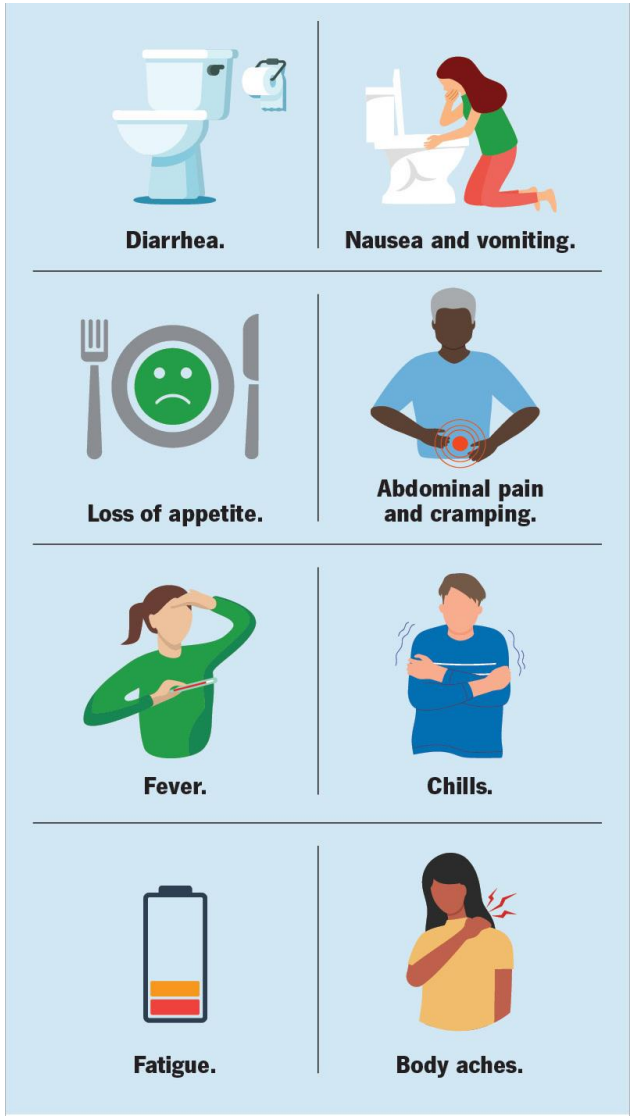
Tipically uncomplicated condition caused by non-typhoidal serotypes:
***S.* Typhimurium, *S.* Enteritidis**

→ self-limiting, resolve without antibiotics

→ serious complications may occur in immunocompromised patients, young children and ederly (appendicitis, pancreatitis..)

Worldwide disease → most common form of Salmonellosis





Cleveland Clinic

Typhoid Fever

(Enteric fevers)

Incubation time: 1 to 3 weeks
Symptoms: variable, even months

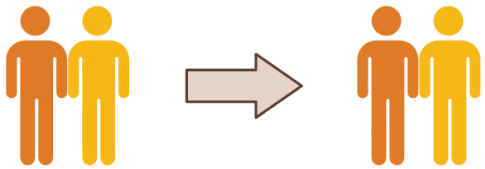
Caused by typhoidal serotypes: ***S.* Typhi and *S.* Paratyphi**

→ Enlarged liver and spleen

→ High mortality rate, especially if untreated (20%)

→ Chronic asymptomatic human carriers can spread the disease (Mary Mallon)

Serious health threat in developing countries, especially for children (Africa, Latin America, Asia) → inappropriate sewage disposal and poor sanitation



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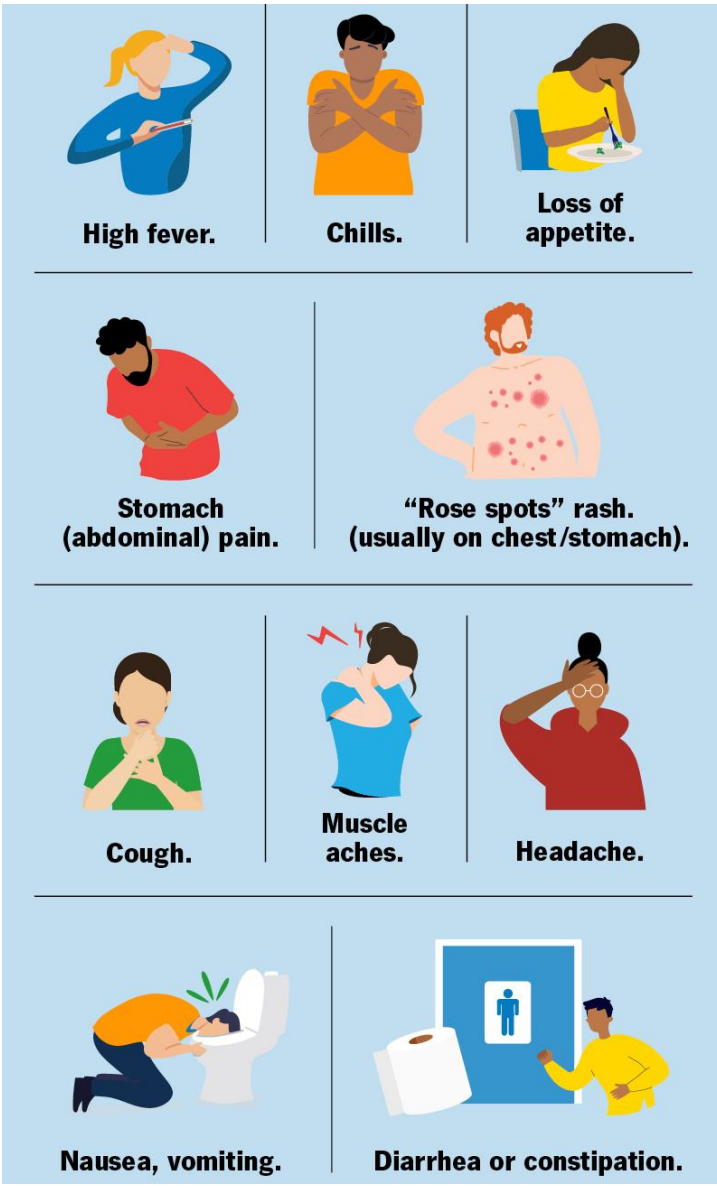


Cleveland Clinic

Other Clinical Features:

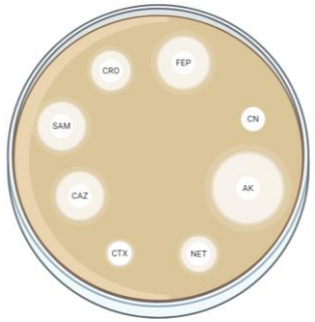
Treatments and Antibiotics

Non-typhoidal salmonellosis:

- No medical treatment required
- Fluid and electrolyte replacement: sodium, potassium and chloride ions
- Antibiotic treatment important in neonates

Typhoid fever:

- Bacteremia → dissemination to multiple organs (gallbladder = bacterial reservoir)
- Travel-associated disease
- Antibiotic treatment required: Ceftraxione, Ciproflaxin



Multiple drug resistance transmitted genetically by plasmids among bacteria → susceptibility testing for proper antibiotic treatment

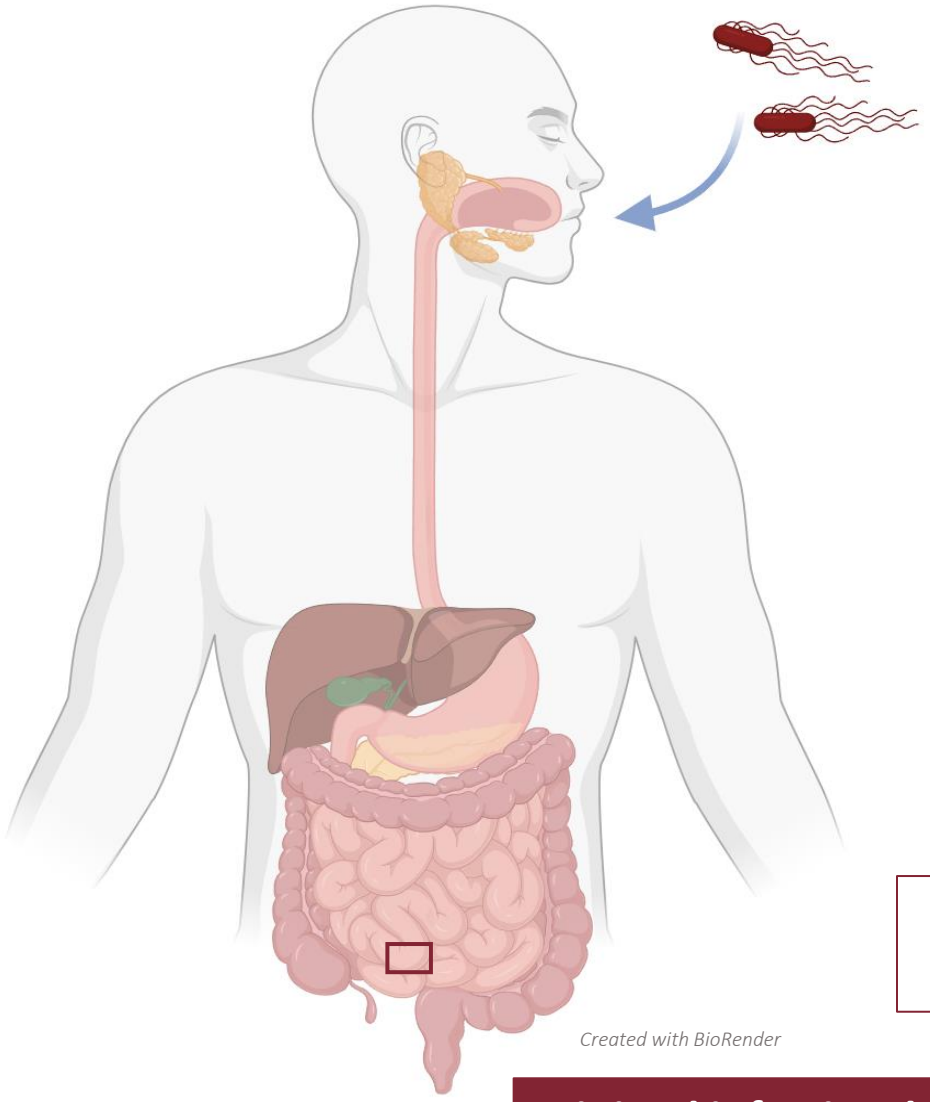
Vaccines to prevent typhoid fever:

- Capsular polysaccharide vaccine (Vi antigen) → intramuscularly
- Live, attenuated (weakened) vaccine → administered orally

Typhim Vi

Ty21a

Pathogenetic mechanisms



1. Microorganism ingested with contaminated food and/or water
2. Crosses the gastric barrier
3. Passes through the intestine to the distal part of the ileum (final part of the small intestine) and colonises it

Transit time: 90 minutes

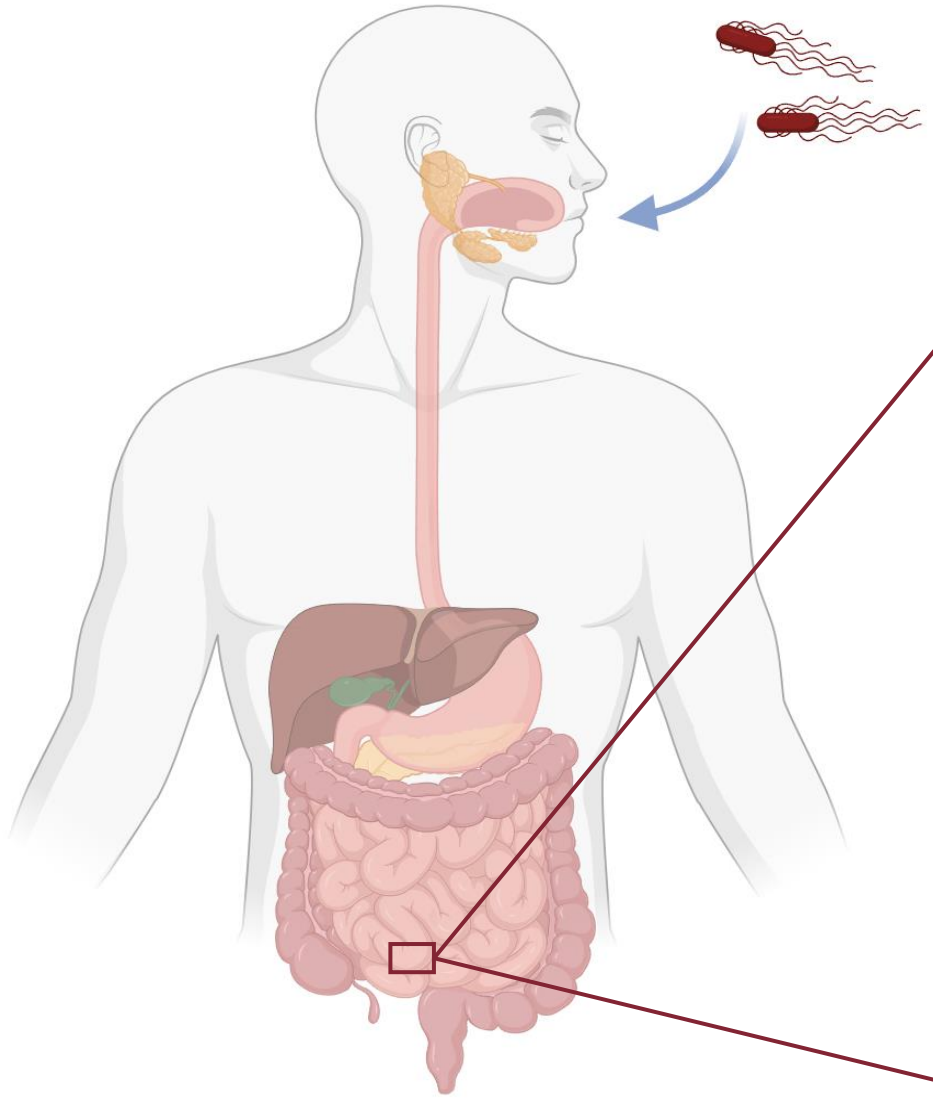
Several adaptive strategies to neutralise aggressive action of the acidic pH of the stomach and bile salts in the small intestine:

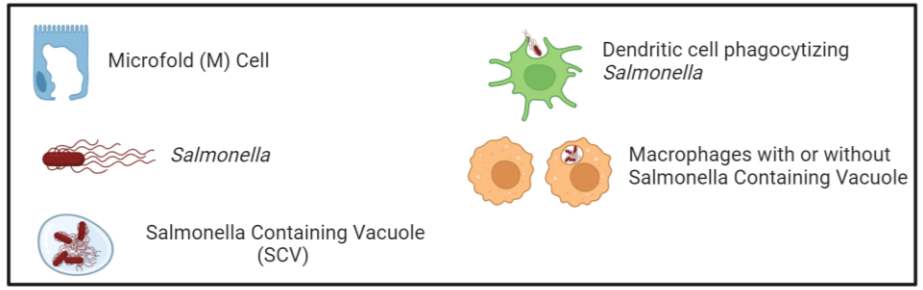
- LPS with protective function
- Modifications in membrane composition
- ATR (acid tolerance response) to preserve against acid shock

ATR: expression of enzymes to increase intracellular pH and synthesis of ASPs (acid shock proteins) to protect and repair DNA and proteins

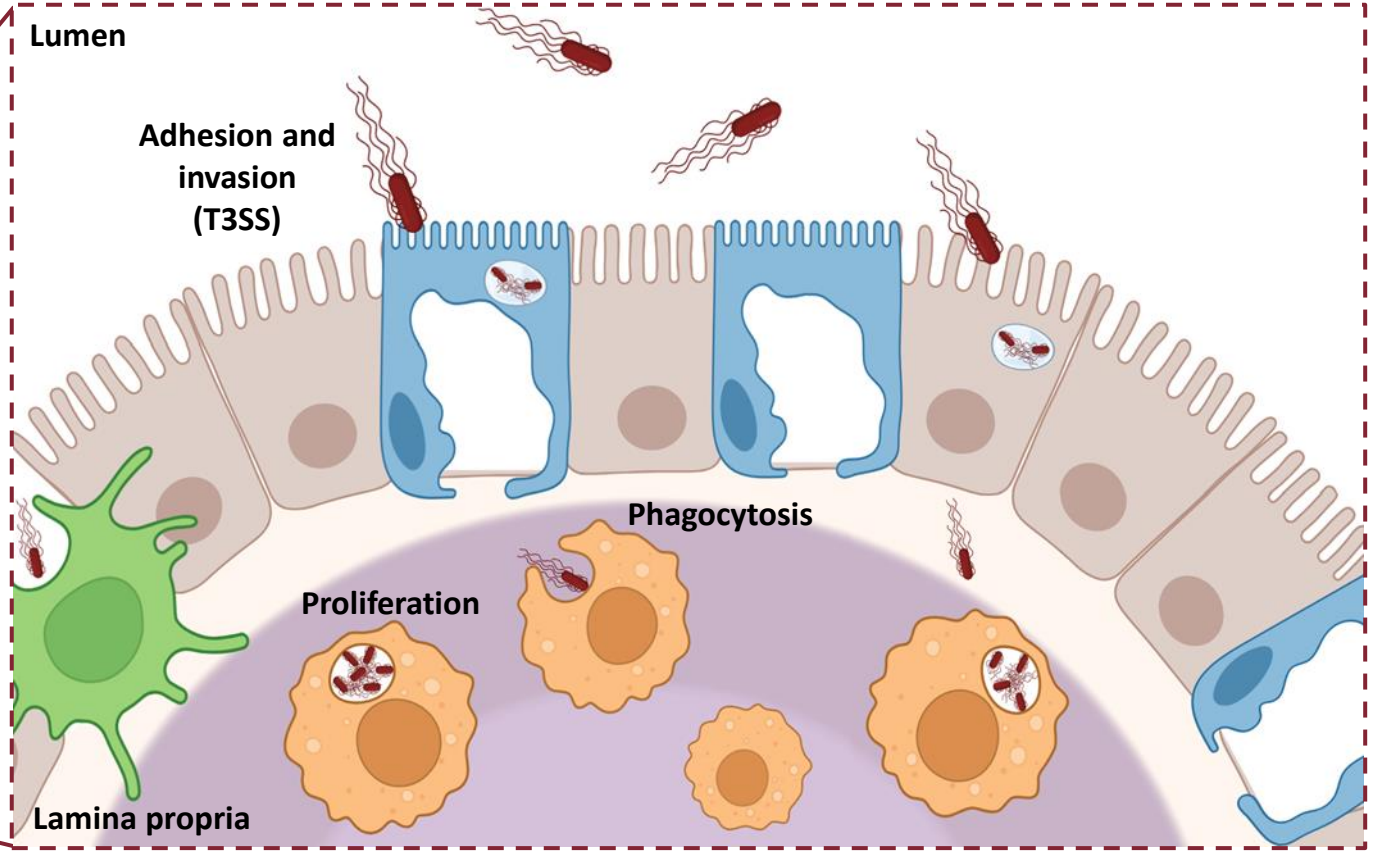
Minimal infective dose varies with serotype and physiological host conditions

Pathogenetic mechanisms





Acute inflammatory response



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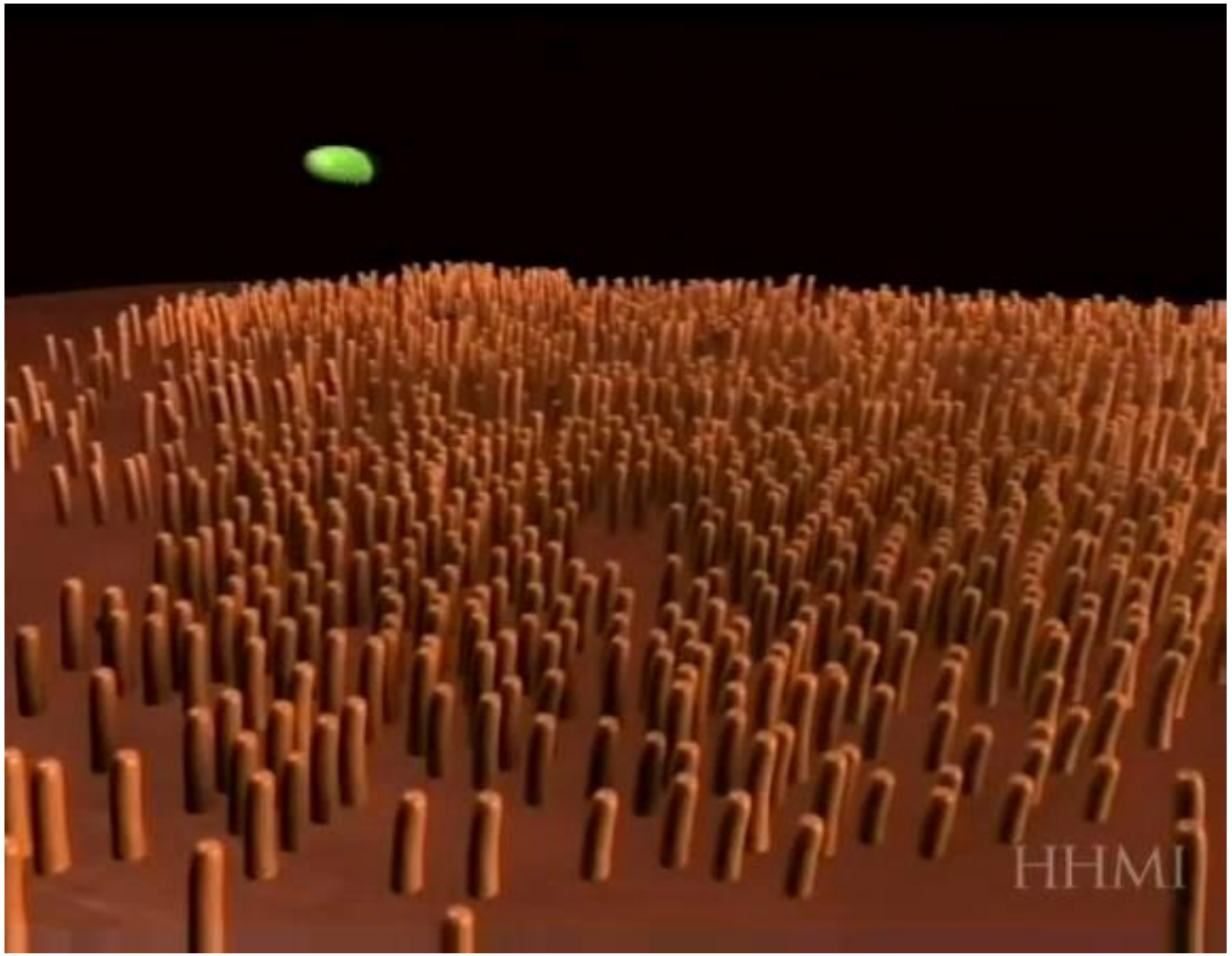
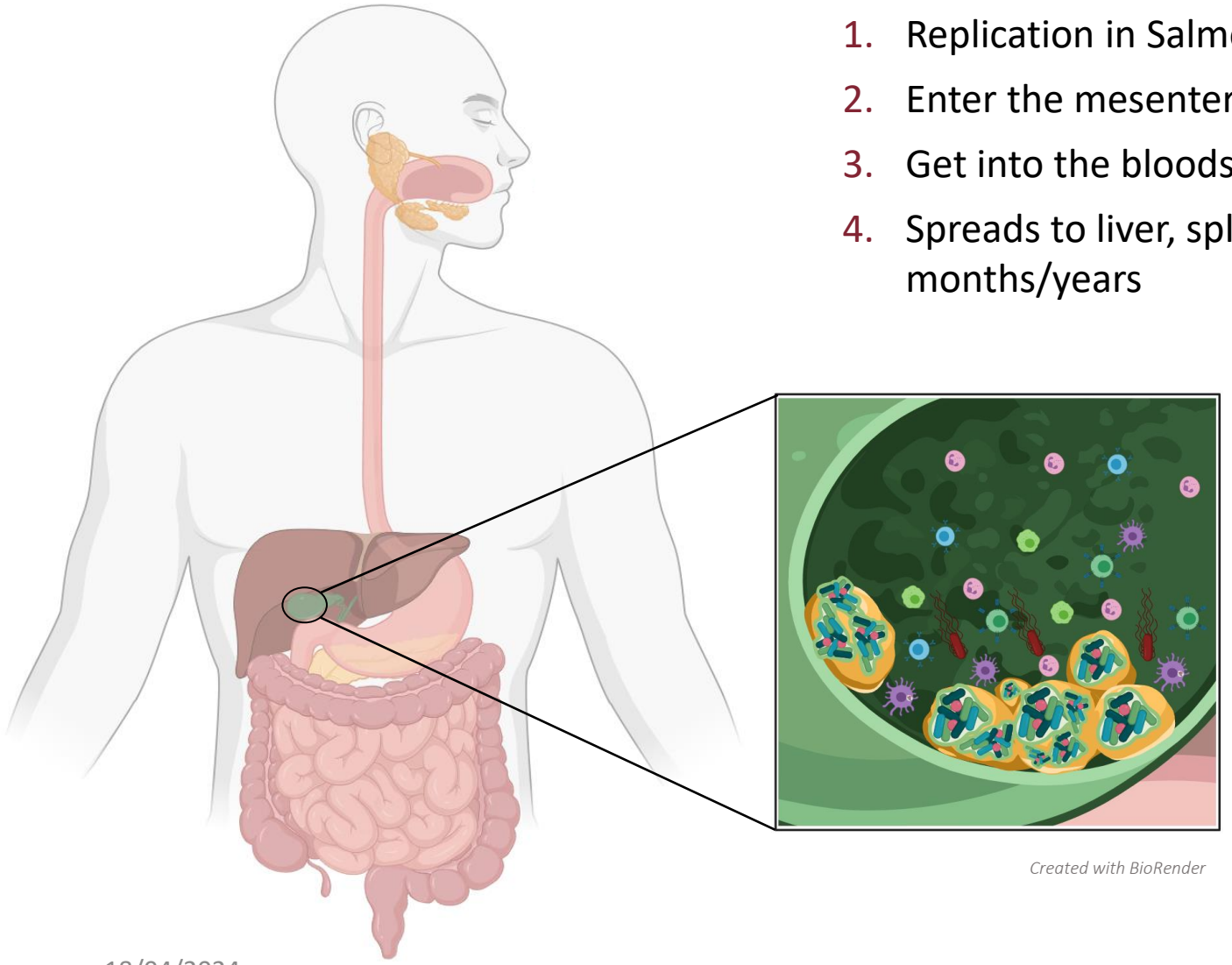
Lumen of Small Intestine → Peyer's Patch



https://youtu.be/q5-sxUbEu5M?feature=shared

Pathogenetic mechanisms



1. Replication in Salmonella-Containing Vacuole (SCV)
2. Enter the mesenteric lymph nodes and then the thoracic duct
3. Get into the bloodstream
4. Spreads to liver, spleen and gallbladder (bacteremia) → persists for months/years

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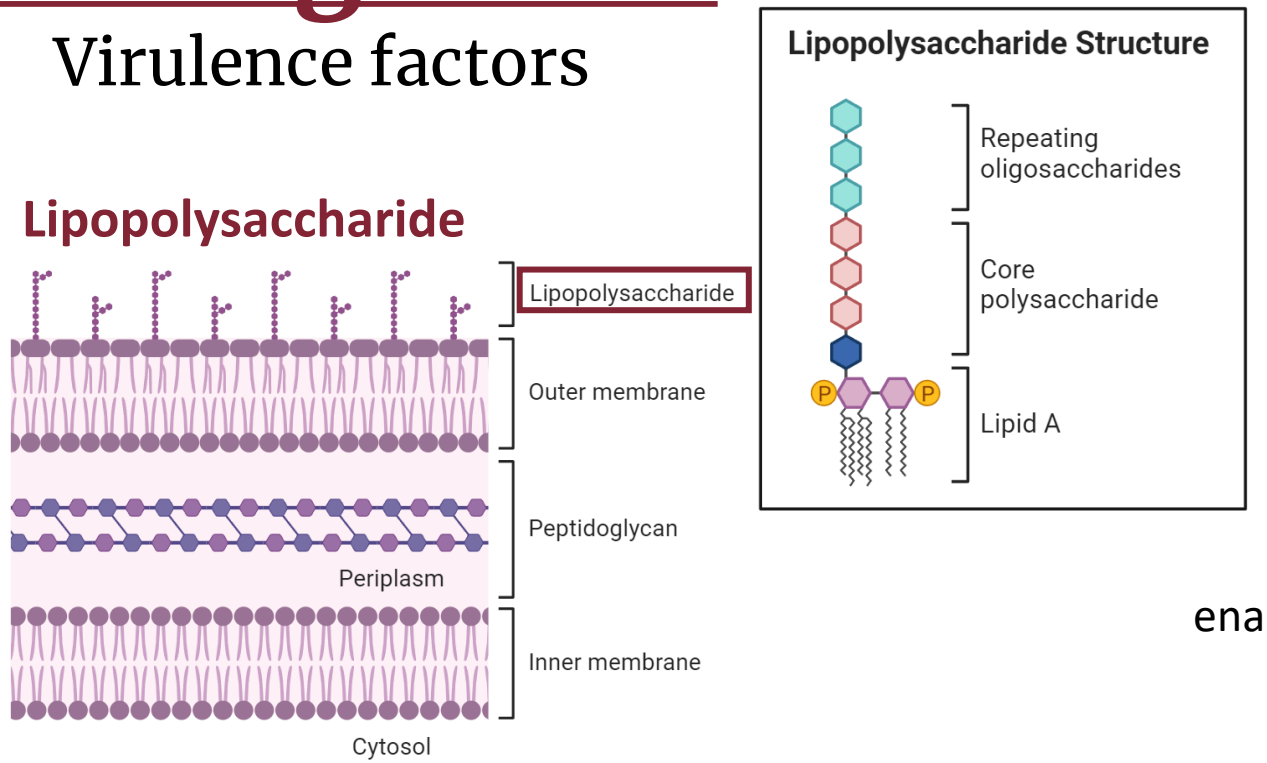
Gallbladder:

- acts as a reservoir in chronic cases of *S*. Typhi (chronic carriers)
- **biofilm** formation on gallstones
- protects bacteria from the host immune system and environmental stress

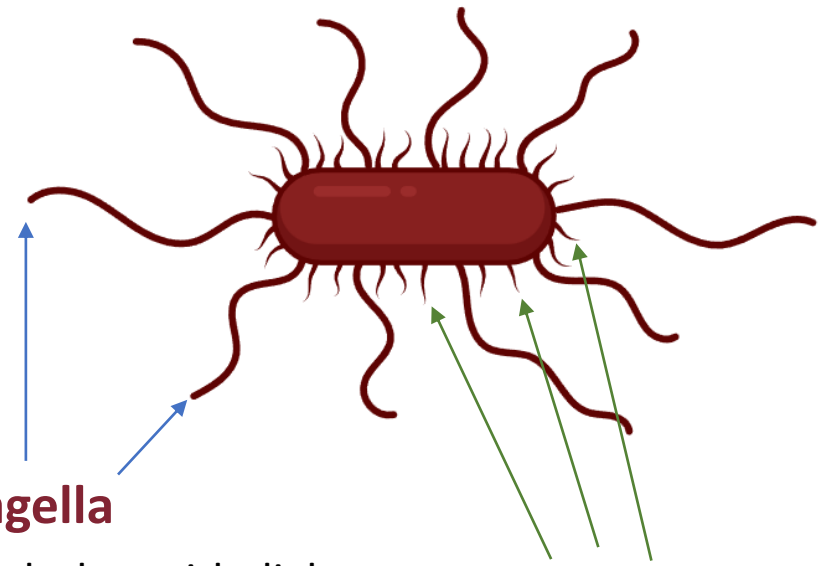
Pathogenesis

Virulence factors

Lipopolysaccharide



provides an effective protective barrier and incite inflammation in tissues



Flagella

enable *S.* to reach the epithelial barrier after ingestion

Fimbriae

important for initial adhesion, colonisation (peristaltic movement), biofilm formation

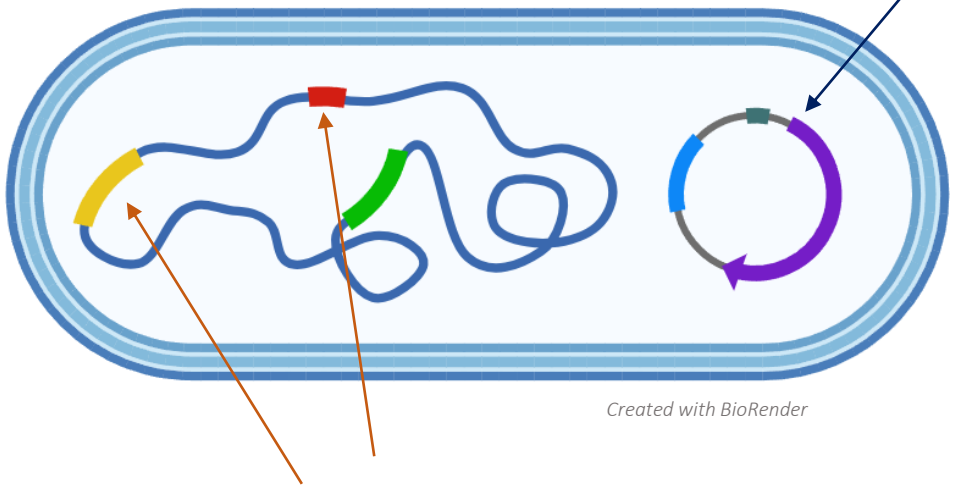
Stn

Salmonella enterotoxin

- all Salmonella spp.
- contributes to the integrity of the OM (OmpA localisation)
- key factor in acute gastroenteritis and diarrhea (Na+ and Cl- ions in the lumen)

Pathogenesis

Virulence factors



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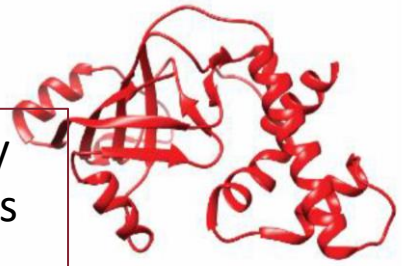
Plasmids

Genes associated with virulence and antimicrobial resistance

- In *S*. Typhimurium LT2: **pSLT** → *spv* genes encoding SpvB toxin
- In *S*. Typhi: **pR(ST98)** → genes involved in drug resistance and induction of apoptosis in macrophages

SpvB (**ADP-ribosylating toxin**) → secreted by T3SS SPI-2 into the cytoplasm where it causes host cytotoxicity = actin depolymerisation



SpvB

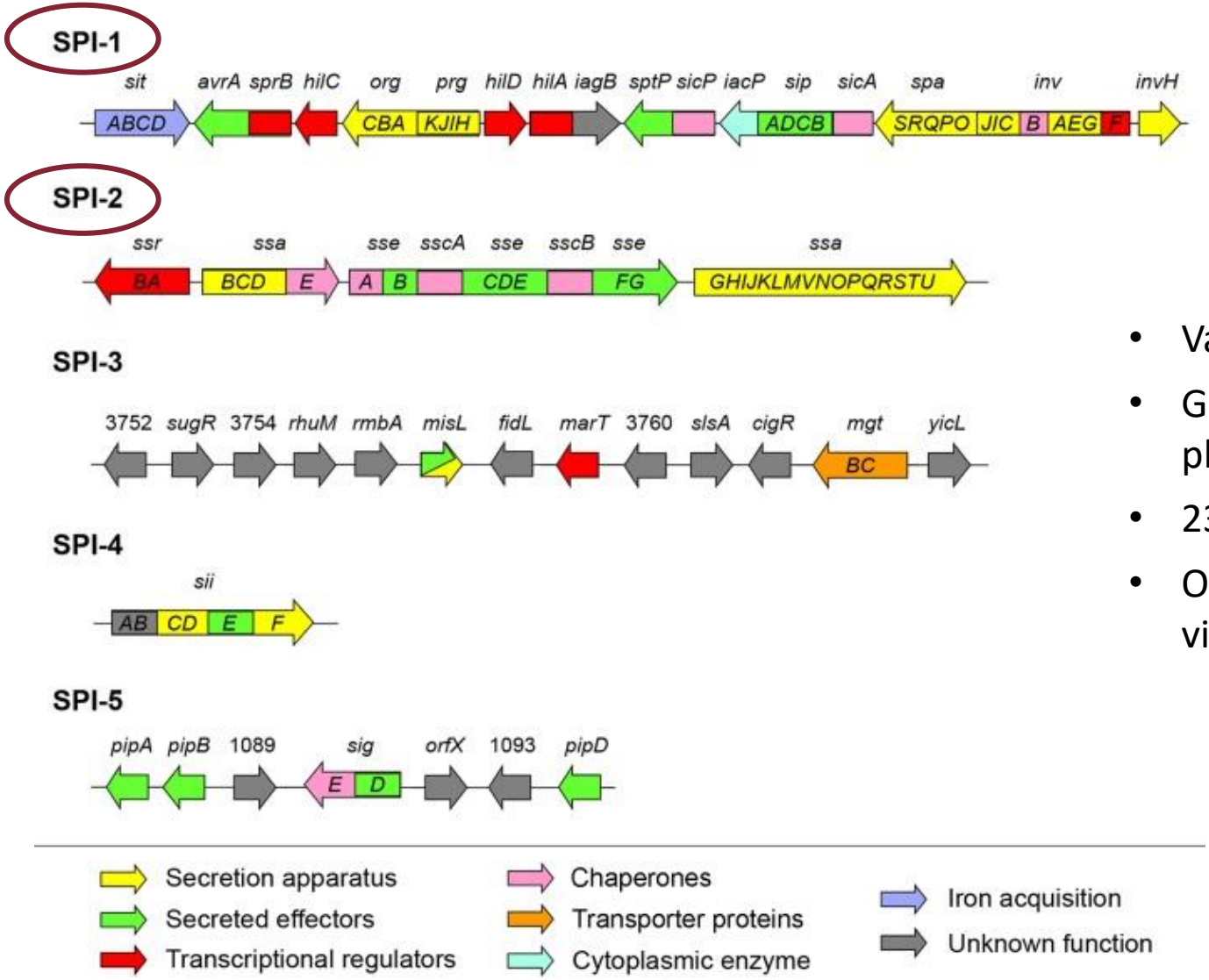
Taken Cheng and Wiedmann, 2019

Salmonella Pathogenicity Islands (SPI)

Genomic islands coding for virulence factors or adhesion and invasion proteins infected host

- Acquired by **horizontal gene transfer** (HGT) → flanked by repeated sequences (IS elements), different G+C content (37-47%)
- Gene expression coordinated by environmental stimuli (T, pH, osmotic pressure)

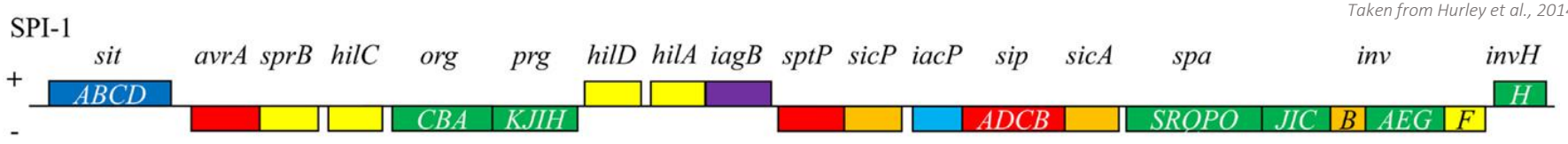
Salmonella Pathogenicity Islands (SPIs)



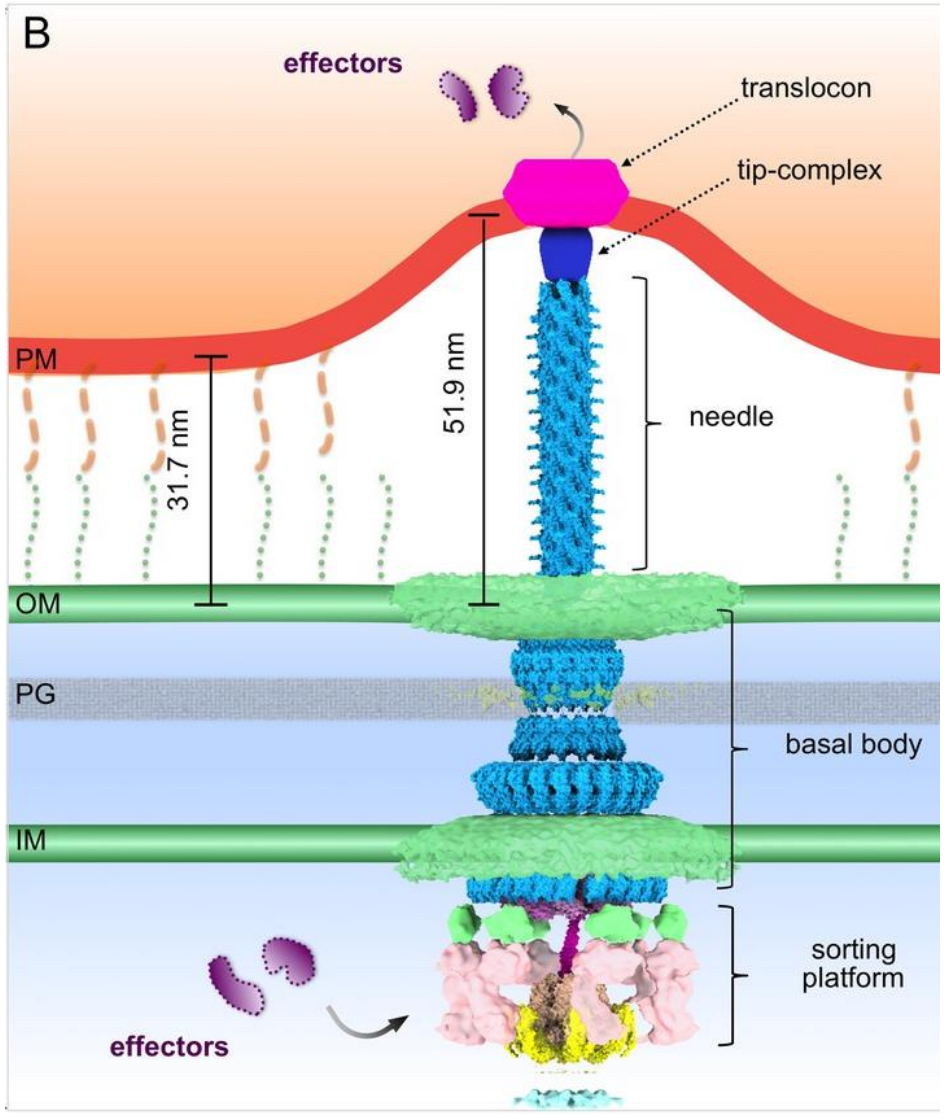
- Variable dimensions (10-40 kb)
- Generally located on bacterial chromosomes (or plasmids)
- 23 SPIs identified (to date)
- Only 5 present in all serotypes and relevant for virulence of the bacterium

Taken from Fàbrega and Vila, 2013

SPI-1



Taken from Hurley et al., 2014

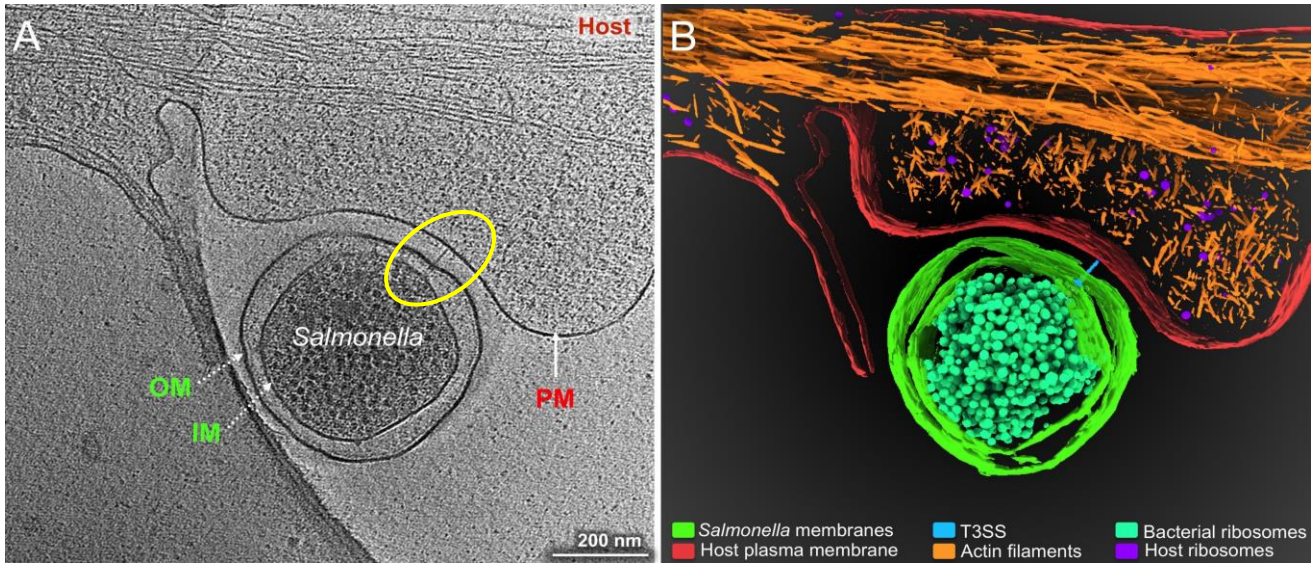


Taken from Park et al., 2018

T3SS-1 (Type III Secretion System):

- Sophisticated nanoinjection multi-protein system (20-30 proteins)
- 3 structures (needle complex, export apparatus, sorting platform)
- Contact-dependent release of effector proteins into the host cell cytoplasm
- SPI-1 translocated effectors drive the cell invasion process

Iron transport protein
T3SS secreted effector protein
Transcriptional regulator
Secretion apparatus
Cell invasion protein
Chaperone
Probable acyl carrier protein

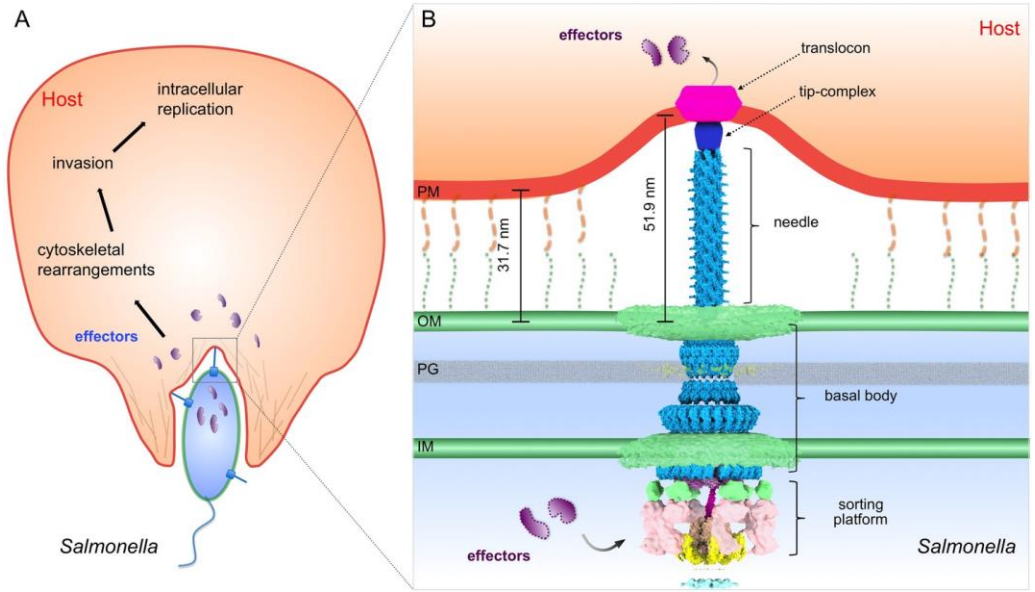


Taken from Park et al., 2018

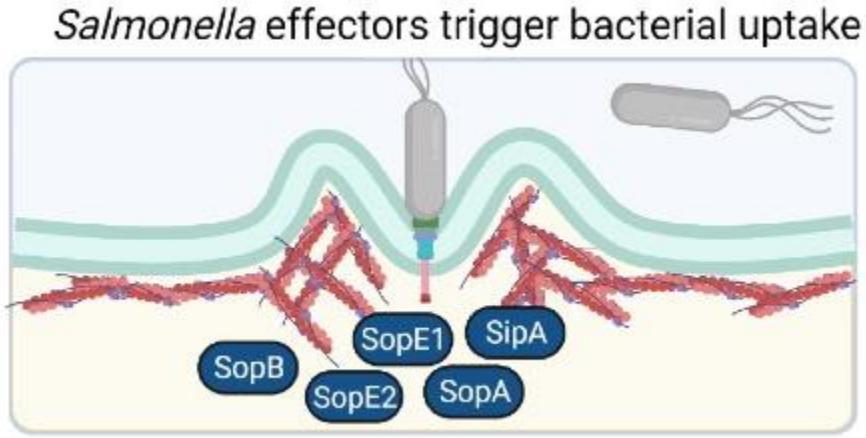
Cell invasion

Mechanism of bacterial infectivity: **TRIGGER**

(reorganisation of the actin cytoskeleton → ruffling of the membrane and bacterium enclosed within)



Taken from Park et al., 2018



Taken from Davidson et al., 2023

1. **SipB and SipC** (trasclocases) → binds caspase-1 (pro-inflammatory cytokines IL-18 and IL-1β)
2. **SipD** (tip protein) → mediates the sensing phase

First effector to be released: **DsbA** → verifies correct assembly and function of T3SS

All three effectors are injected into the host membrane to form the translocon channel

Cell invasion

Mechanism of bacterial infectivity: **TRIGGER**

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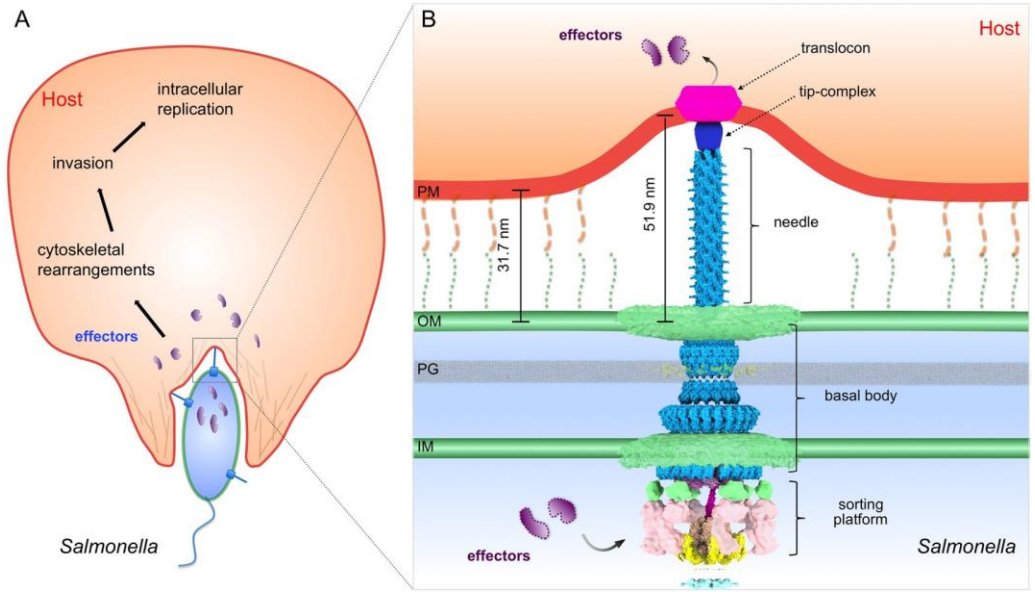

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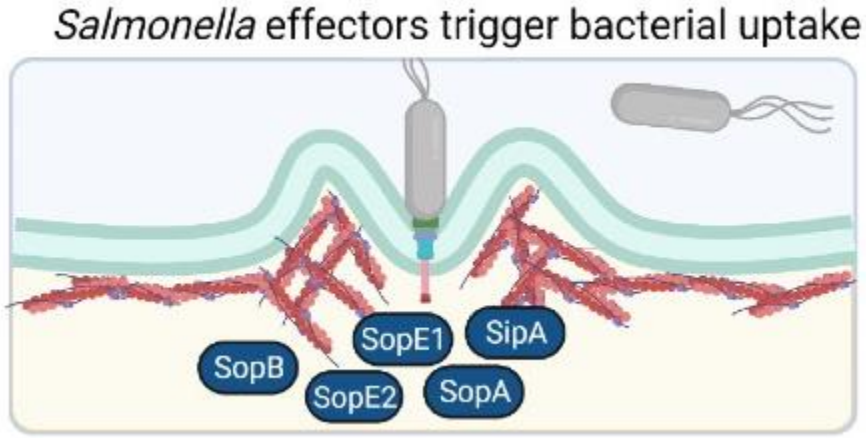
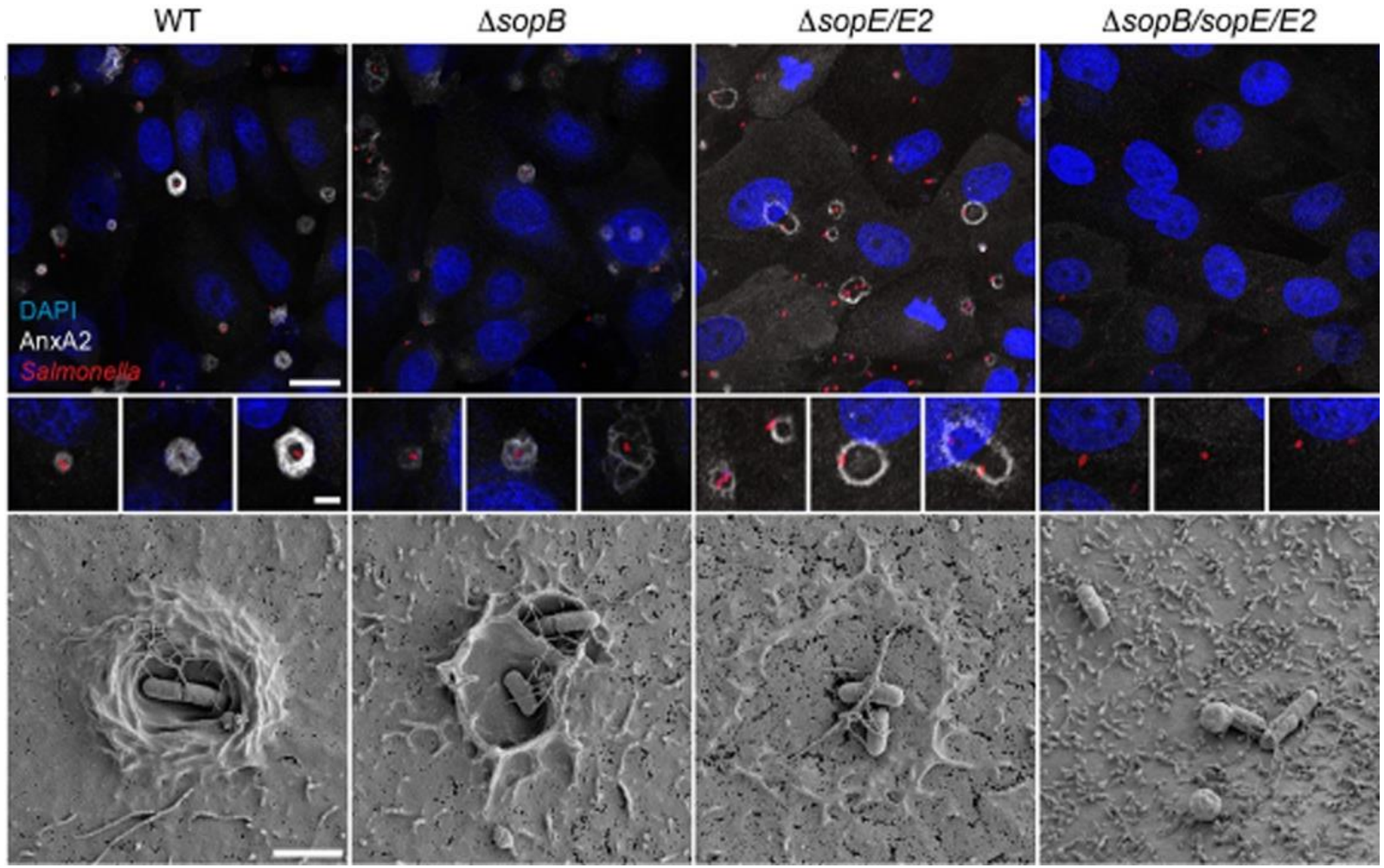


Taken from Davidson et al., 2023

1. **SipC** → translocon component promotes actin polymerisation (rapid growth)
2. **SipA** → recruits regulatory proteins to stabilise neosynthesised filaments, contributes to their localisation. Activates NF-kB and recruits neutrophiles.
3. **SopE1/SopE2** (SPI-5) → target Rho family GTPases (Rac-1 and Cdc42) that modulate the cytoskeleton (ramification) and, via NF-kB, induce pro-inflammatory cytokines (IL-8)
4. **SopB** → actin rearrangements

Cell invasion



Taken from Jolly et al., 2014

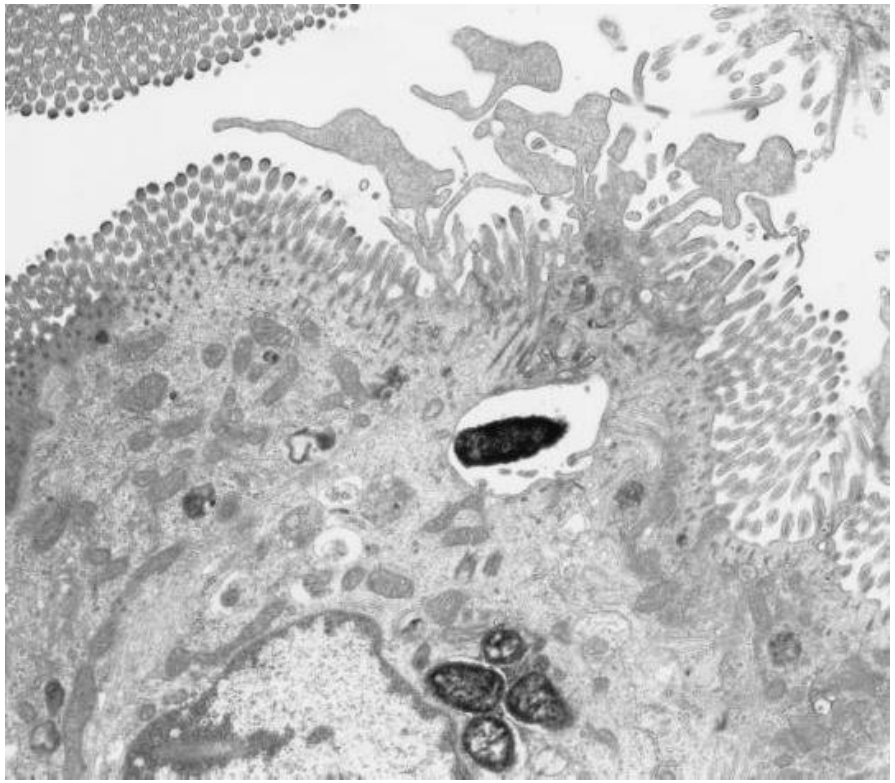
Single mutants induce ripples with lower efficiency than WT (smaller and less distinct)

ΔsopB/sopE/E2 triple mutant does not create ripples (no invasion)

SopE/E2 → Rho GTPase Rac-1 → **AnxA2** (enrichment at the invasion site)

with **AHNAK** → reorganisation of the actin cytoskeleton through activation of several small GTPases → contributing to invasion

Cell invasion

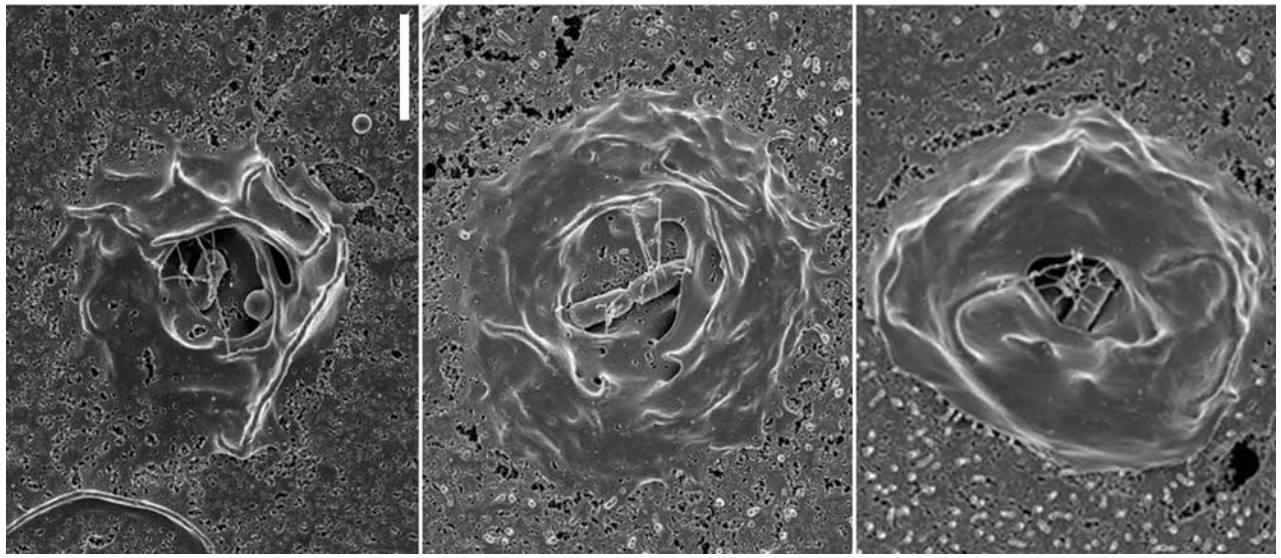


Taken from Darwin and Miller, 1999

Salmonella coordinates the expression of invasion genes and regulates them according to a time hierarchy → **progressively expressed effectors**

Cytoskeleton of the cell returns to its natural conformation and microvilli are completely reassembled:

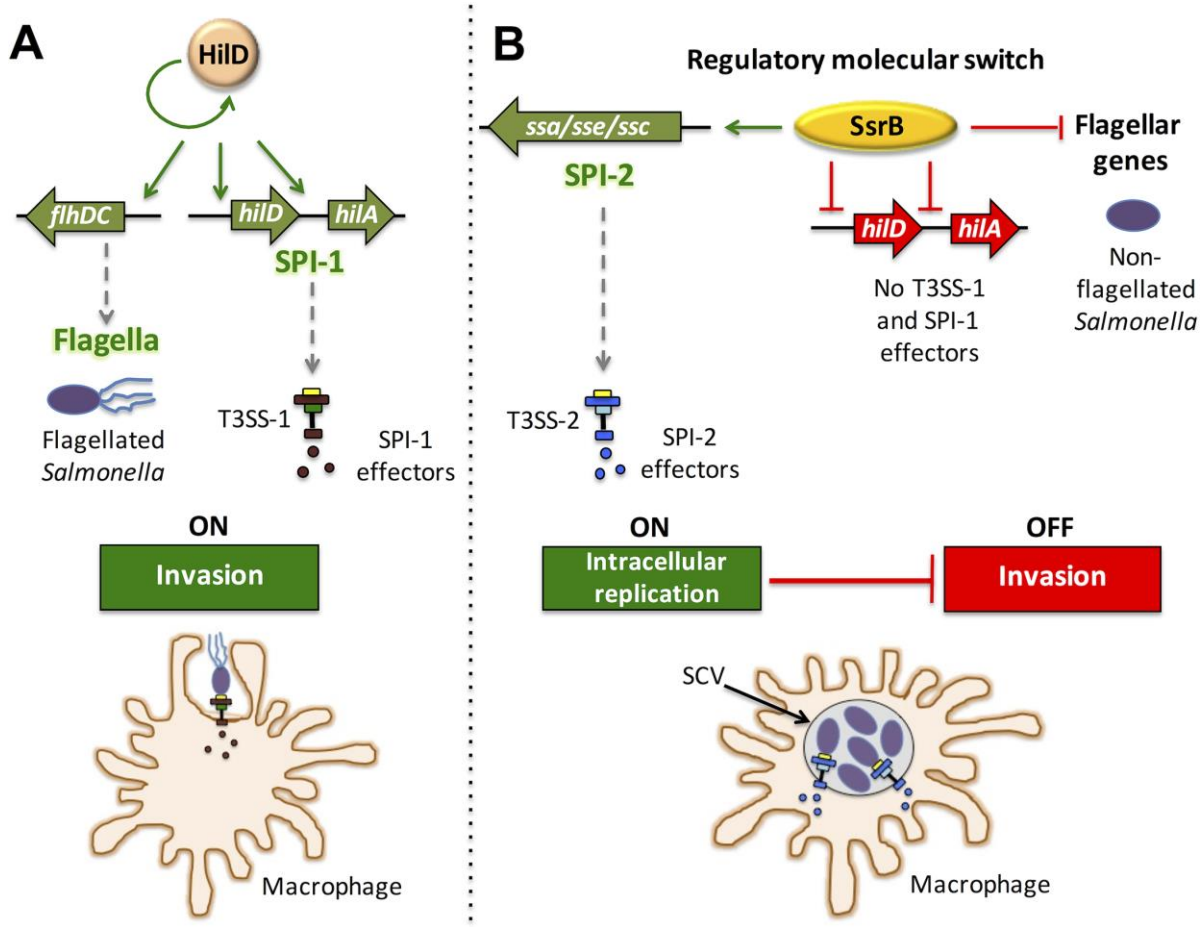
- **SptP** effector that inactivates Rac-1 and Cdc42
- **villin** which remodels the actin cytoskeleton of the brush border → constitution of microvilli and thus epithelial restitution after damage



Taken from Jolly et al., 2014

Transition to the intracellular lifestyle

Taken from Pérez-Morales, Banda et al., 2017



Invasion phase → **HilD** directly or indirectly activates the expression of

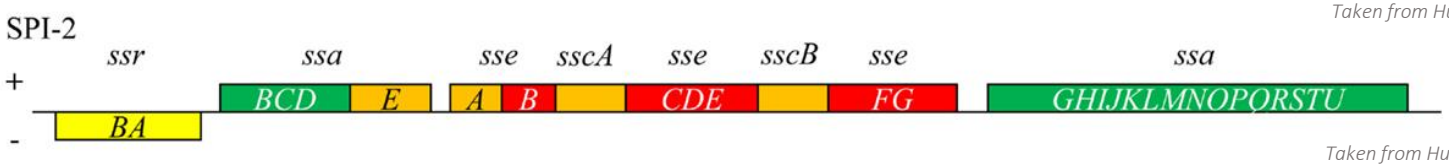
- SPI-1 genes
- many other genes located outside SPI-1 (T3SS)
- flhDC flagellar regulatory operon required for host cell invasion

Intracellular phase → After invasion, Salmonella in SCVs and here **SsrB** induces

- expression of
  - SPI-2 genes
  - other genes located outside SPI-2, which are necessary for survival and replication
- repression of
  - *hilD* and *hilA* regulatory genes
  - SPI-1 genes
  - flagellum-based motility genes

SsrB molecular regulatory switch that helps *Salmonella* transition to an intracellular lifestyle

SPI-2



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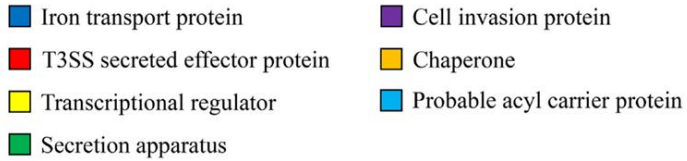
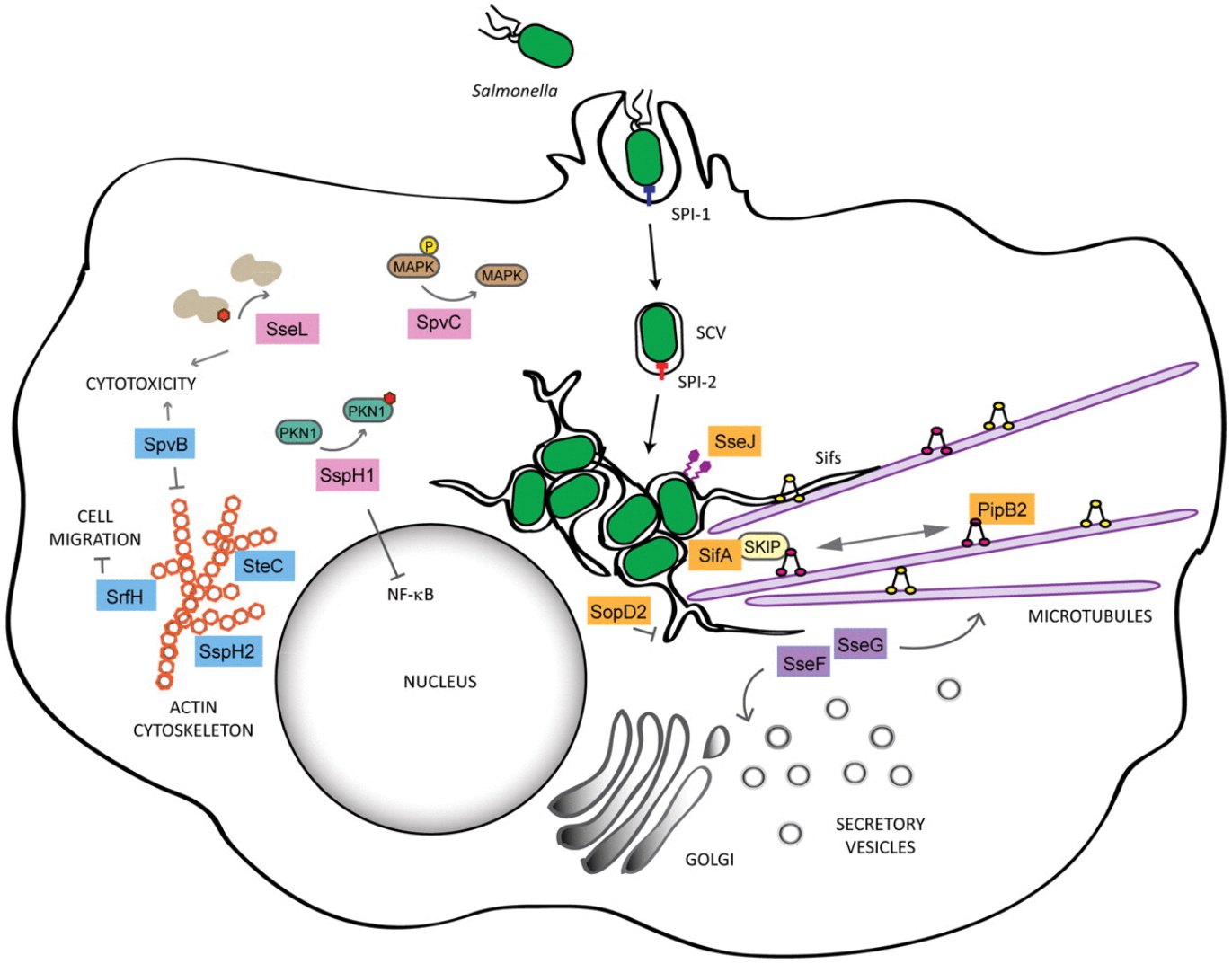




Taken from Figueira and Holden, 2012

Includes more than 40 genes organised in 4 operons:

- ***ssr*** → type III secretion apparatus (T3SS-2)
- ***ssa*** → secretion system regulators
- ***ssc*** → molecular chaperones
- ***sse*** → effector proteins

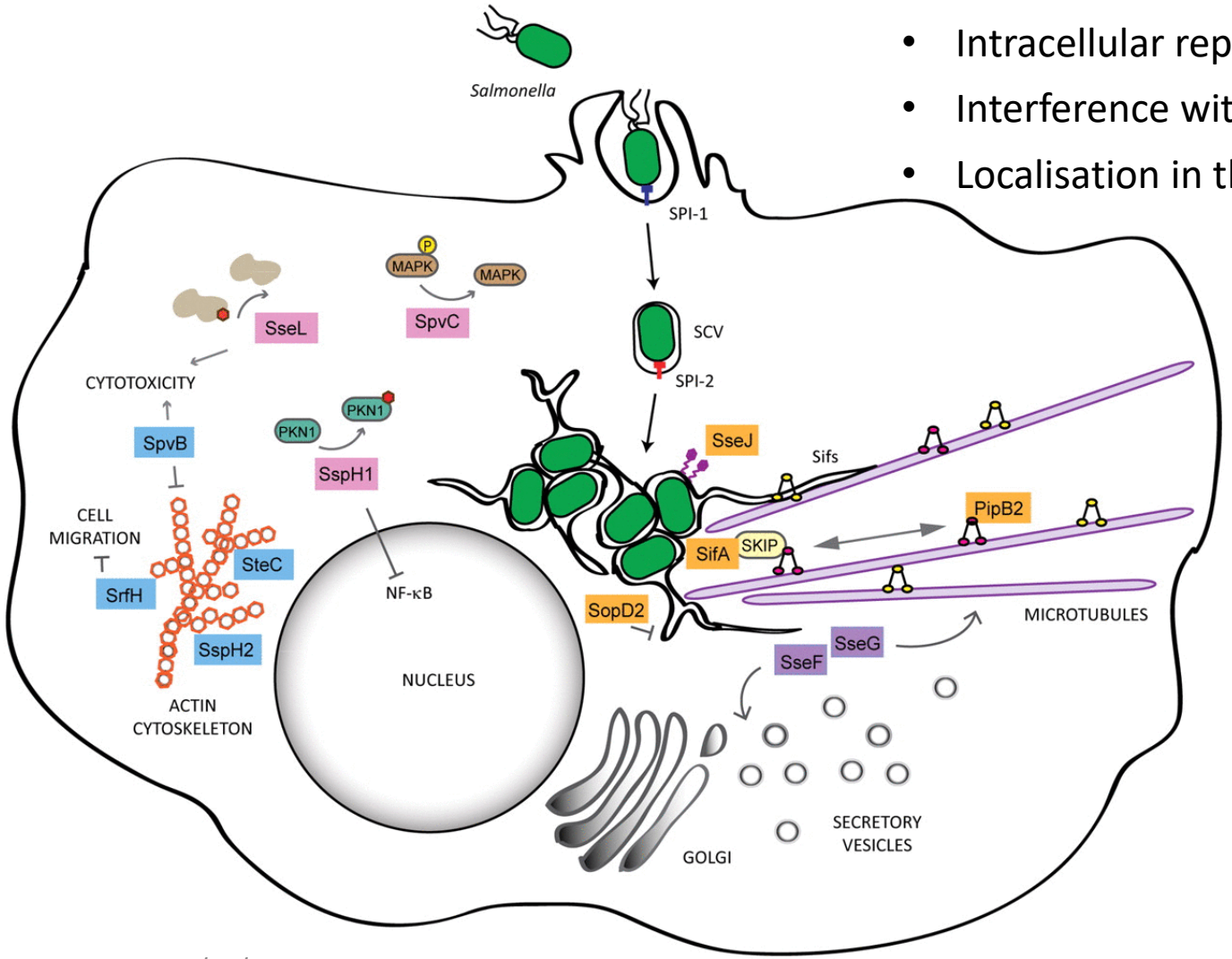
Main functions:

1. Survive and replicate within phagocytes and cells (4 hours after invasion)
2. Evade host phagosome oxidation mechanisms
3. Persist in target organs such as spleen and liver (systemic virulence in typhoid fever)

SPI-2

T3SS-2 → translocation of effector proteins (around 30) into the host cytoplasm leading to a series of bacterial adaptations

- Vacuolar remodelling
- Intracellular survival (maintenance of SCV integrity)
- Intracellular replication
- Interference with immune signalling
- Localisation in the peri-Golgi region



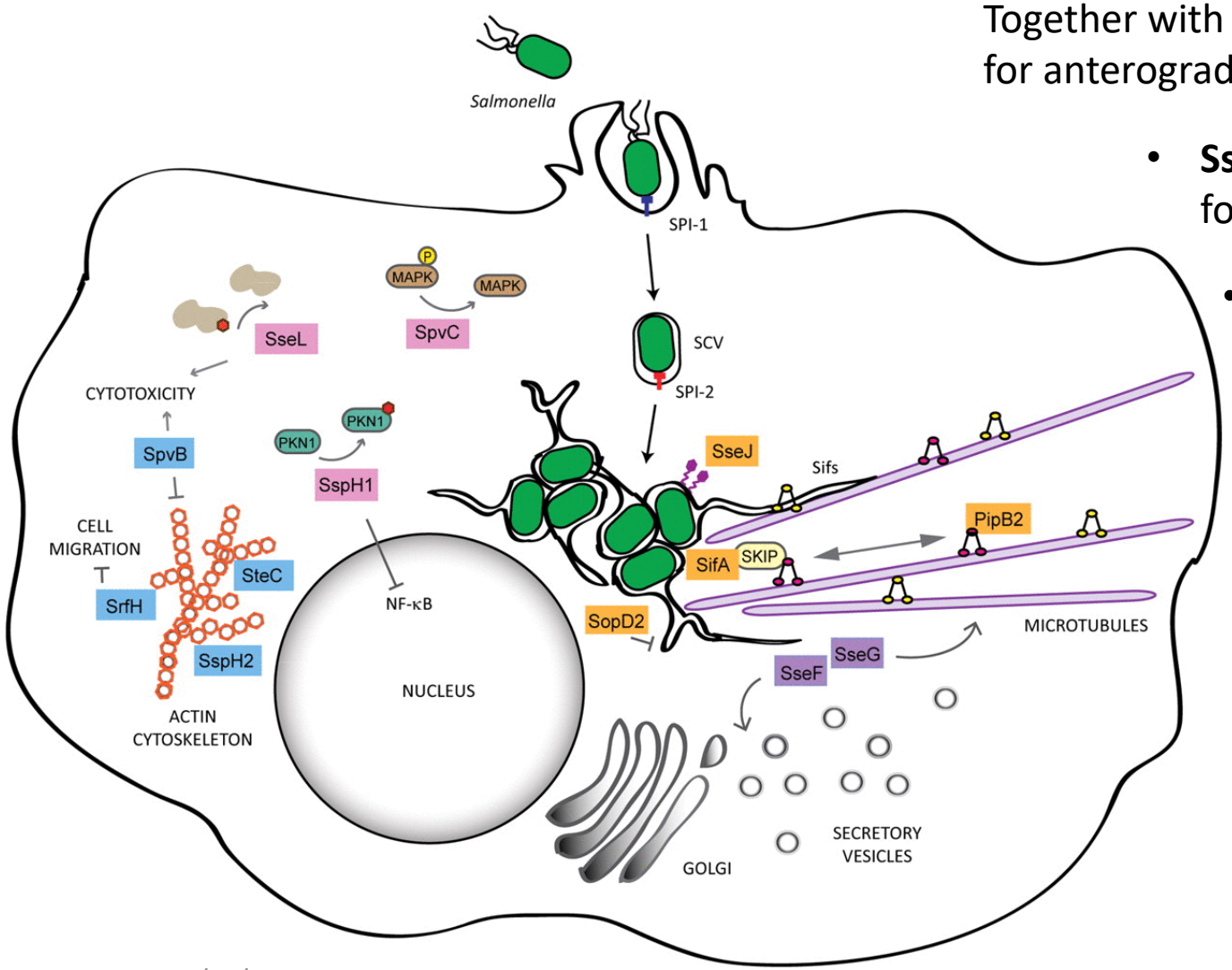
Taken from Figueira and Holden, 2012

Salmonella adapts to the intracellular environment and SPI-2 genes are differentially expressed.

Expression regulated by **two-component systems**:

- OmpR-EnvZ → regulates the expression of SsrA
- SsrA-SsrB → encoded by SPI-2
- PhoP-PhoQ → activated by the low pH intraphagosomal environment (PhoP regulates > 20 genes including SsrB)

T3SS-2 effectors



- **SifA** → main effector, Salmonella-induced filament formation (SIF). Together with **PipB2** interacts with **SKIP** protein (binds kinesin-1) for anterograde transport on microtubules (SCV localisation)
  - **SseF** → SCV localisation, microtubule clustering and SIF formation
    - **SpiC** → prevents fusion with phagolysosome
    - **SpvC** → anti-inflammatory effect (MAPK)

SifA is regulated by **SsrA** → for vacuolar membrane maintenance

Taken from Figueira and Holden, 2012

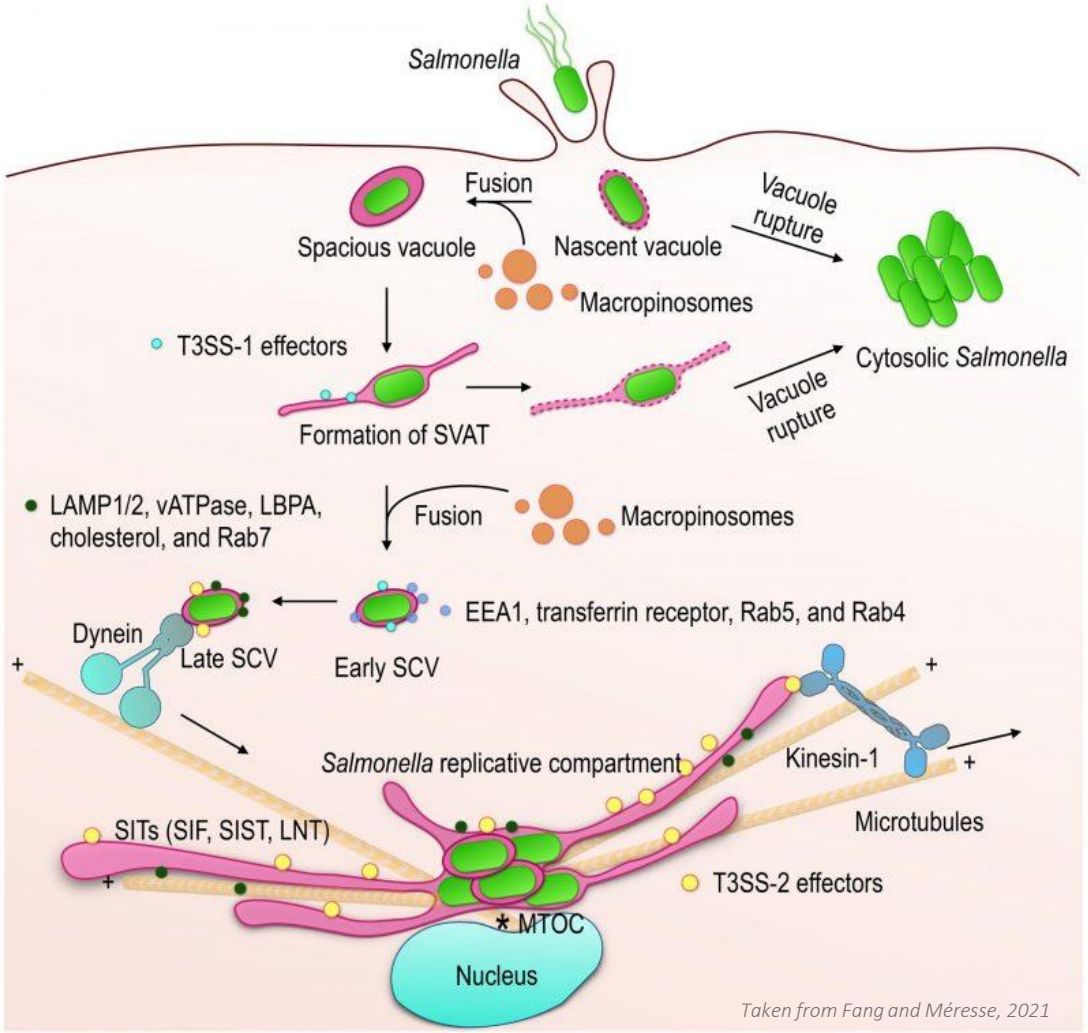
Maturation of the SCV

Evading antimicrobial activities arsenal by staying within the SCV

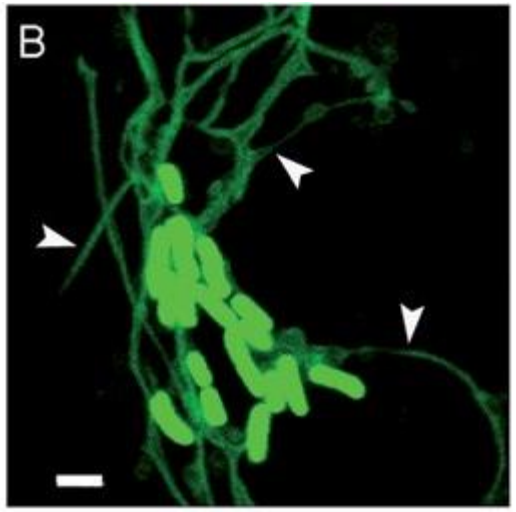
SCV undergo a maturation process (latency 2-3 hours) before cell replication takes place:

- **volume growth** by fusion with endocytic vesicles
- membrane remodelling → **lysosomal membrane glycoproteins** (LAMP1 and Rab7)
- lumen **acidification** (vacuolar-type V-ATPases)
- no fusion with lysosome (no acid hydrolases, no mannose 6-P receptors) → remains in **late endosome state**

Movement from the cell periphery to the perinuclear region → pH decrease→ inducing expression of T3SS-2 and its effectors



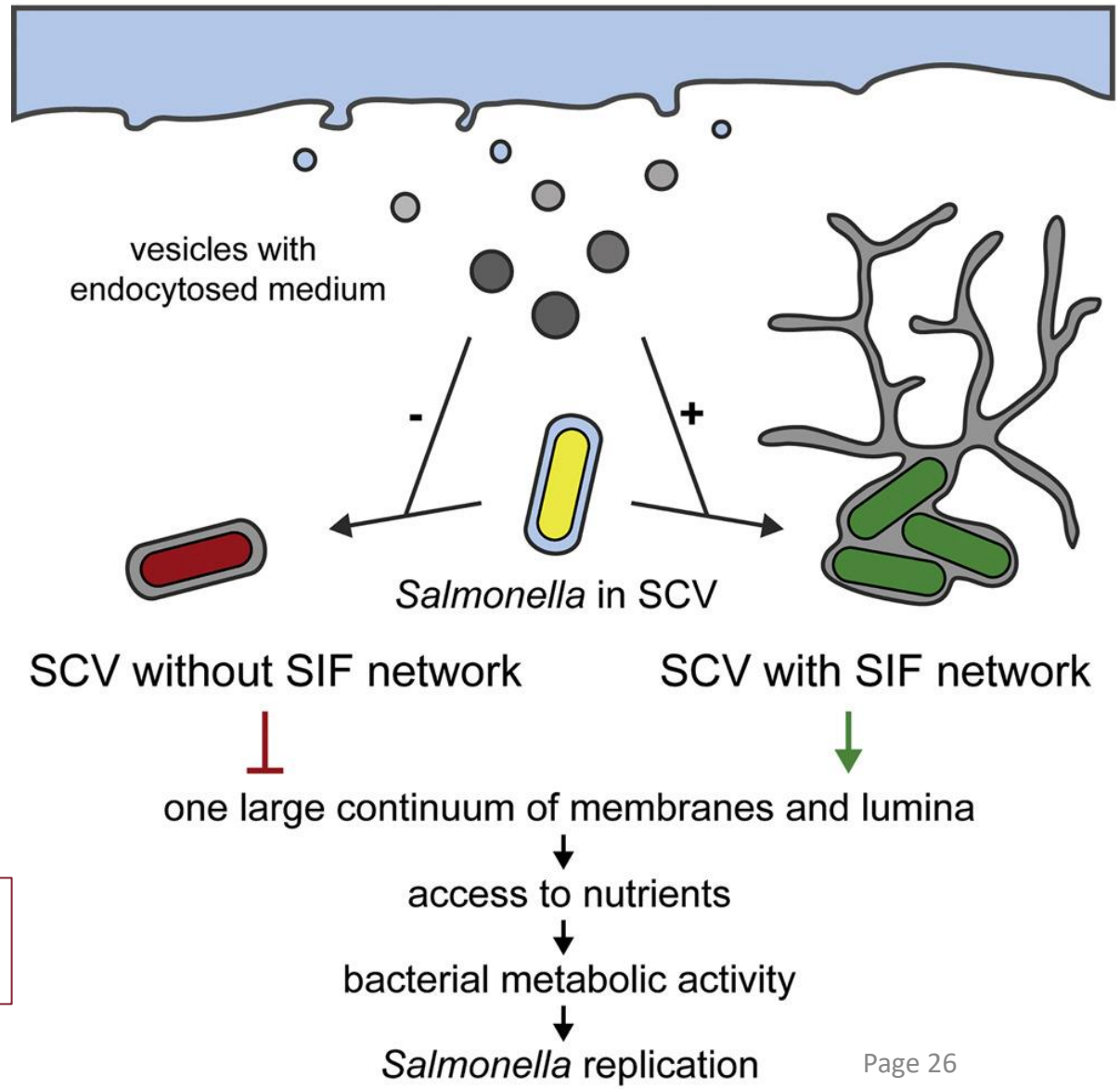
Salmonella-induced filaments (SIF)



Taken from Zhang and Hensel, 2013

Crucial for intracellular proliferation and survival

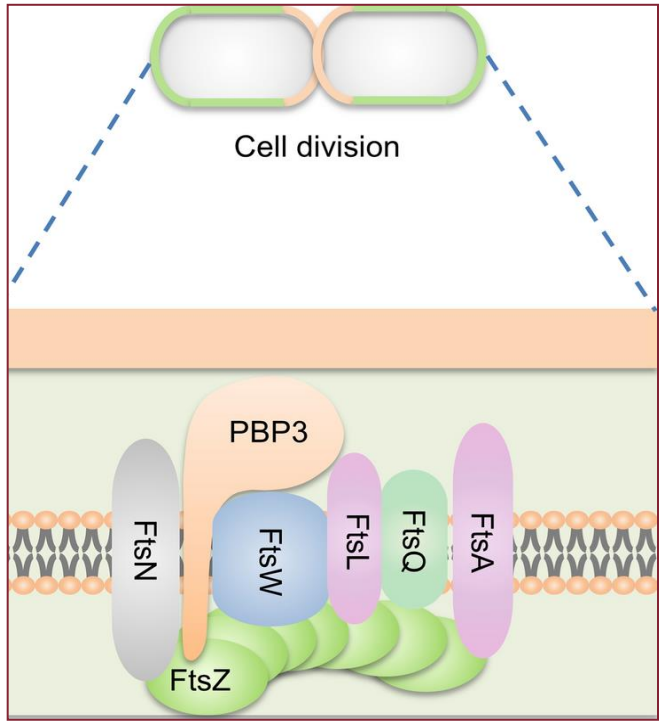
Taken from Liss et al., 2017



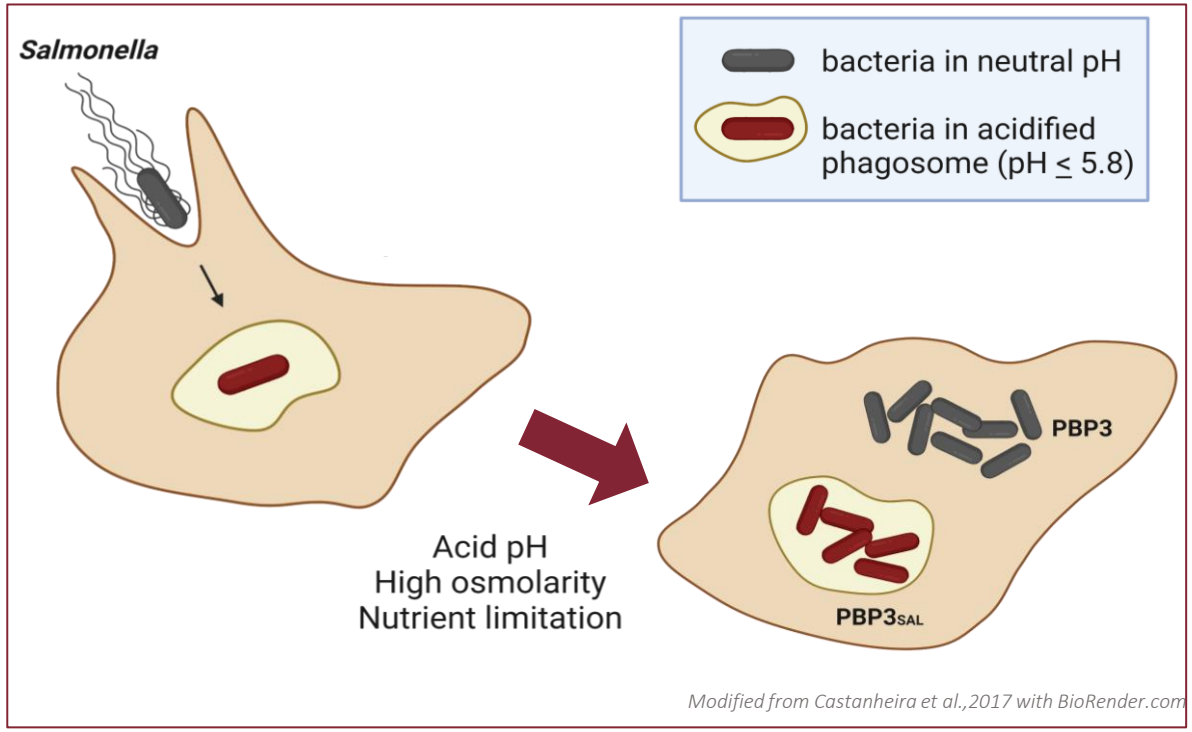
- Same composition as SCV (LAMP1 and Rab7)
- Support intracellular lifestyle by avoiding nutritional restriction → continuum with SIF (membranes and lumen) → endocytosed material
- SCV localisation

SIF formation coincides with initiation of *Salmonella* cell replication

Cell proliferation



Huo, Zhao, Zhang et al., 2020



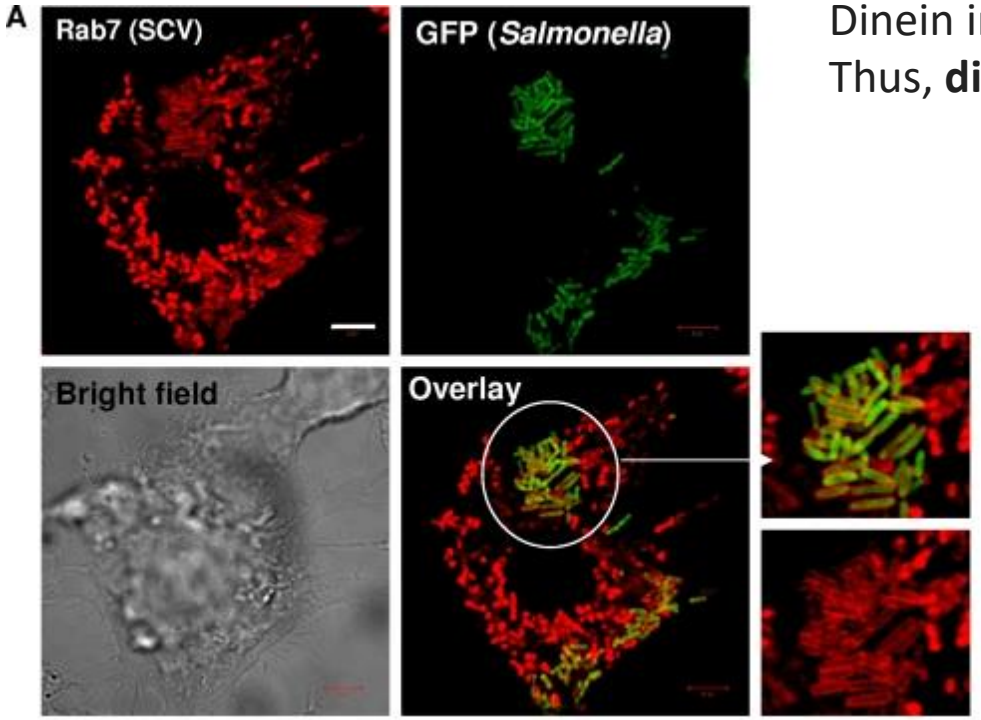
Salmonella encodes for a homologous to PBP3 → **PBP3sal** (63%) that promotes cell division independently from PBP3

- Allows *Salmonella* to grow in acidified media
- Contributes to the adaption of the bacterium to the intracellular lifestyle
- Low affinity for some β-lactam antibiotics wich binds PBP3 with high affinity

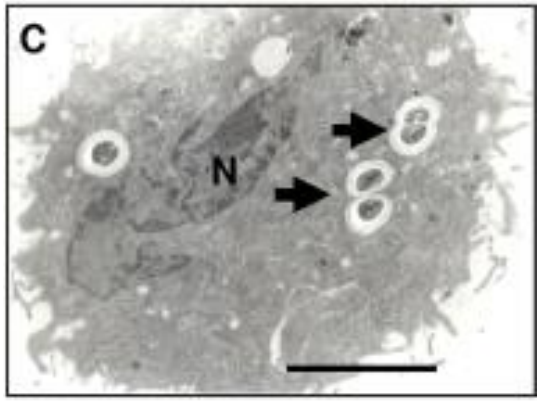
SCVs contain a single bacterium per vacuole

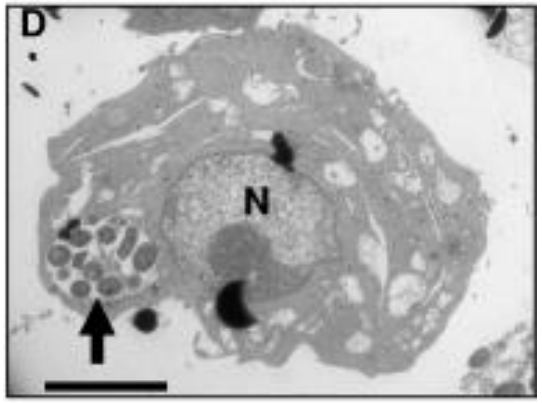
Image shows that many SCVs (96%) have a single bacterium (3 hours)

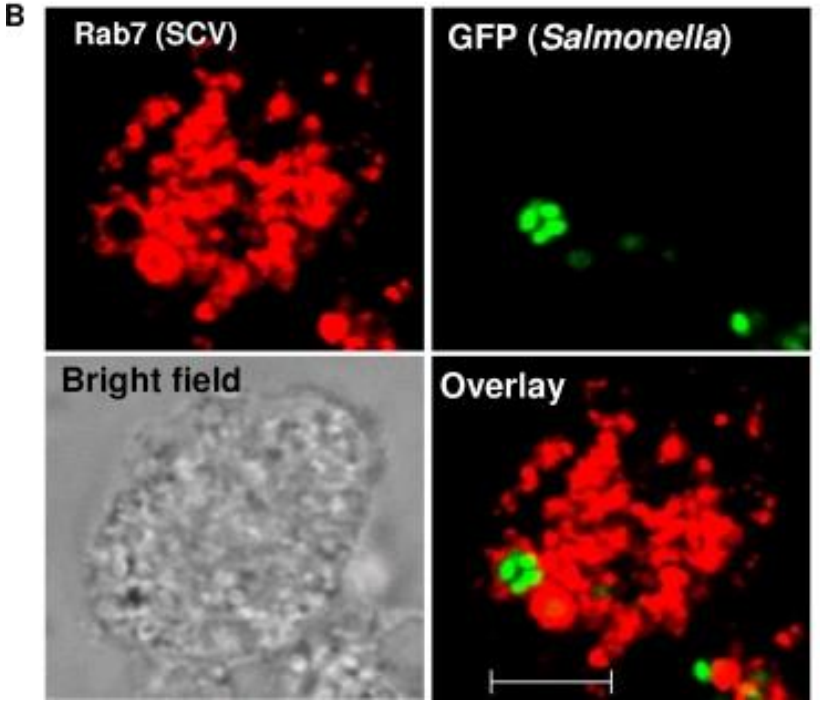
Taken from Eswarappa et al., 2010



Dinein inhibitor → increased number of bacteria per SCV.
Thus, **dinein** is involved in SCV division (concomitant with bacterial cell division)







Advantages for *Salmonella*

- No competition for nutrients
- One lysosome targets one SCV (n. lysosomes insufficient if n. SCV grows)

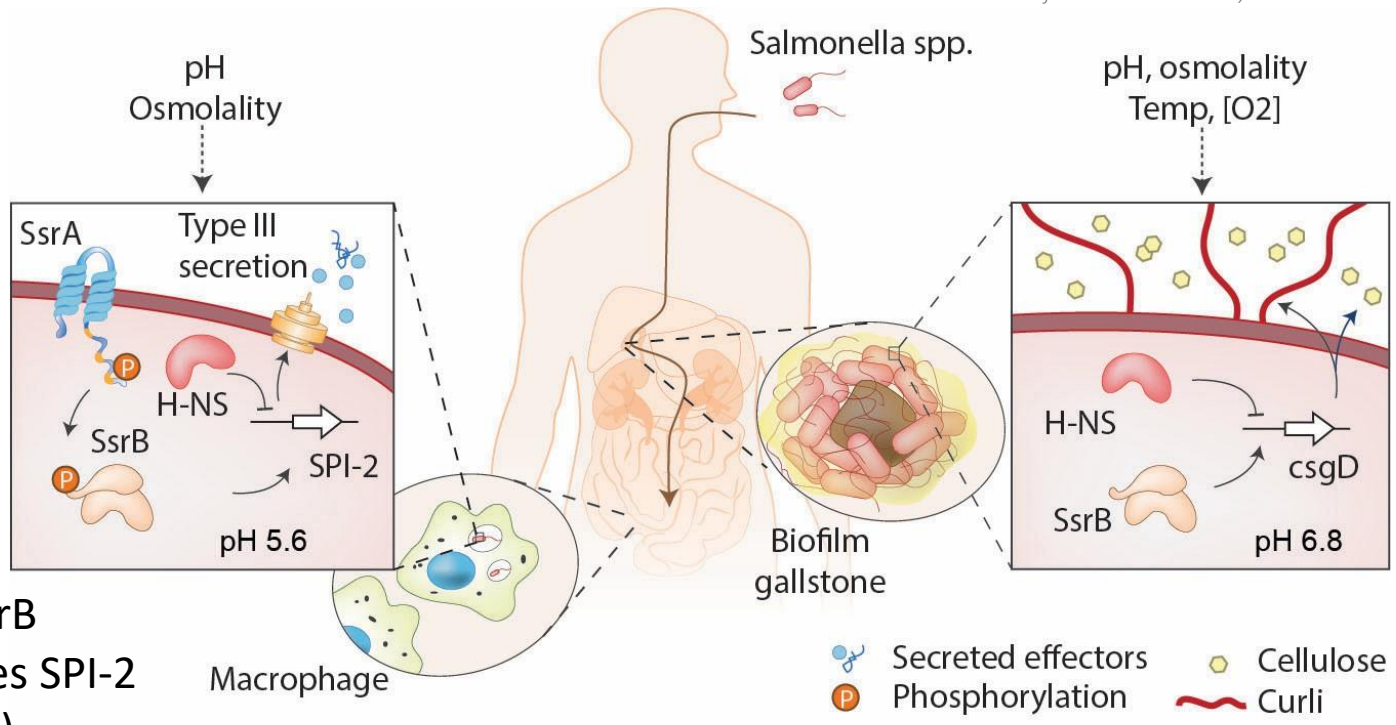
SCVs are targets of lysosomes → addressing lysosomal degradation in an intelligent way

Non-canonical function of SsrB: new lifestyle

EnZ/OmpR → OmpR regulates the promoter of SsrA

PhoP/Q → PhoP regulates the SsrB promoter

Taken from Tze Fui Liew et al., 2019



Acid pH (5.6) activates SPI-2 expression:
increase TCS expression SsrA/B
SsrA (HK) membrane phosphorylates and activates SsrB (cytoplasmic RR) → binds to DNA regions and activates SPI-2 gene transcription (by removing H-NS binding protein)

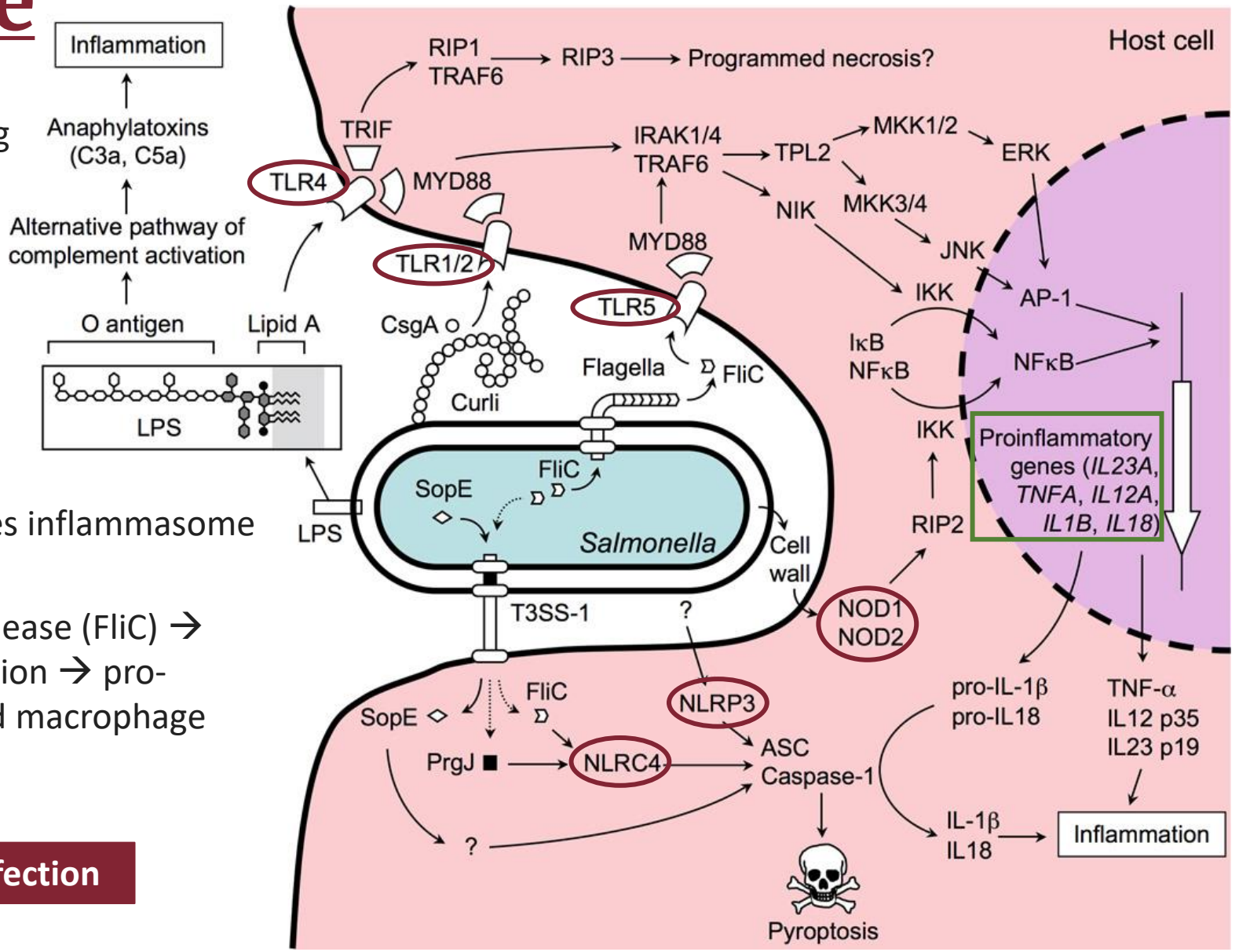
At neutral pH (6.8) SsrB also plays a non-canonical role: biofilm formation on gallstones in the gallbladder and establishment of carrier state
SsrA kinase is almost absent and SsrB is not phosphorylated → de-represses H-NS in the *csg*D promoter (main regulator of biofilms)
→ activates cellulose operon = structural role in biofilm (scaffold that protects and supports growth)

Immune response

Host-Salmonella complex dialogue culminating in **induction of host immune response**

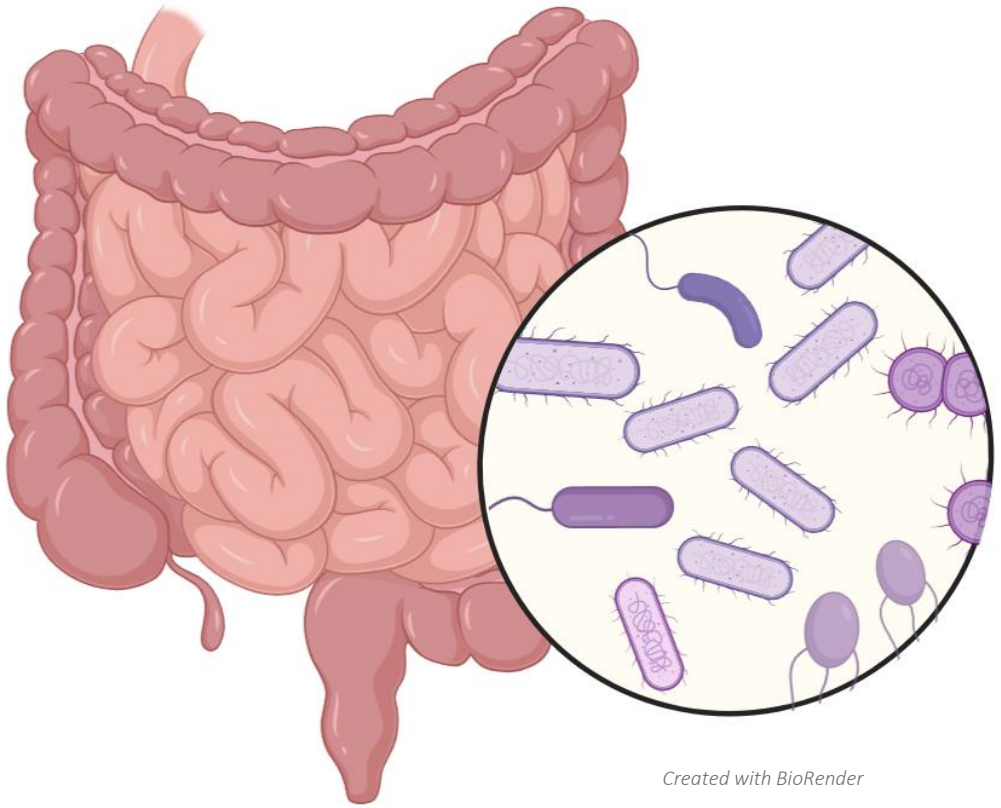
- Lipopolysaccharide (**lipid A**) → TLR4
- **FliC** of the flagellum → TLR5
- Cell wall (**PG**) → NOD1/NOD2
- **Curls** (biofilm fimbriae) → TLR1/2
- T3SS-1-dependent cytosolic process causes inflammasome activation (NLRC4 and NLRP3)
- Enterocyte-bacterium contact, flagellin release (FliC) → inflammatory response with NF-kB activation → pro-inflammatory cytokines for neutrophil and macrophage recall

Inflammation supports *Salmonella* infection



Taken from Thiennimitr et al., 2012

Salmonella – gut microbiota interactions



Created with BioRender

Gut microbiota:

- community of microorganisms that dwell in a mutualistic relationship with hosts in gastrointestinal tract
- contains about 10^{14} microbial cells (Bacteroides, Firmicutes and Actinobacteria)
- contributes to protection against pathogens (competition for nutrients; activation and support of immune response)

Salmonella – gut microbiota interactions

- Prolonged treatment with antibiotics → negative effects on the microbiota
- *S.* exploits **ETHANOLAMMINE** to gain significant growth advantage
- *S.* synthesises **SALMOCHELIN** to acquire iron in the inflamed gut
- *S.* exploits **TETRATHIONATE** for anaerobic respiration

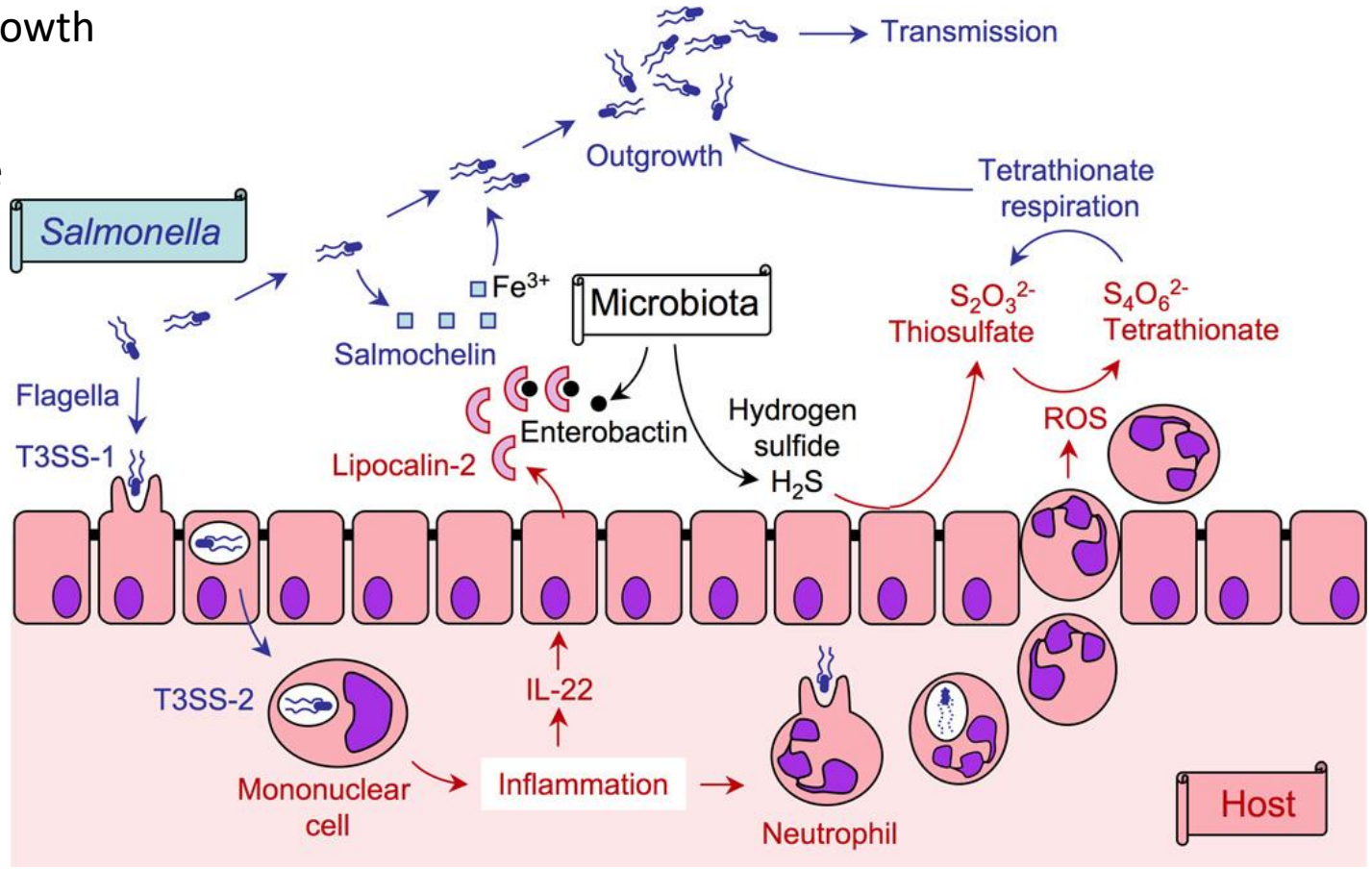
Microbiota depletion

↓

Growth advantage for *Salmonella*

↓

Established carrier state



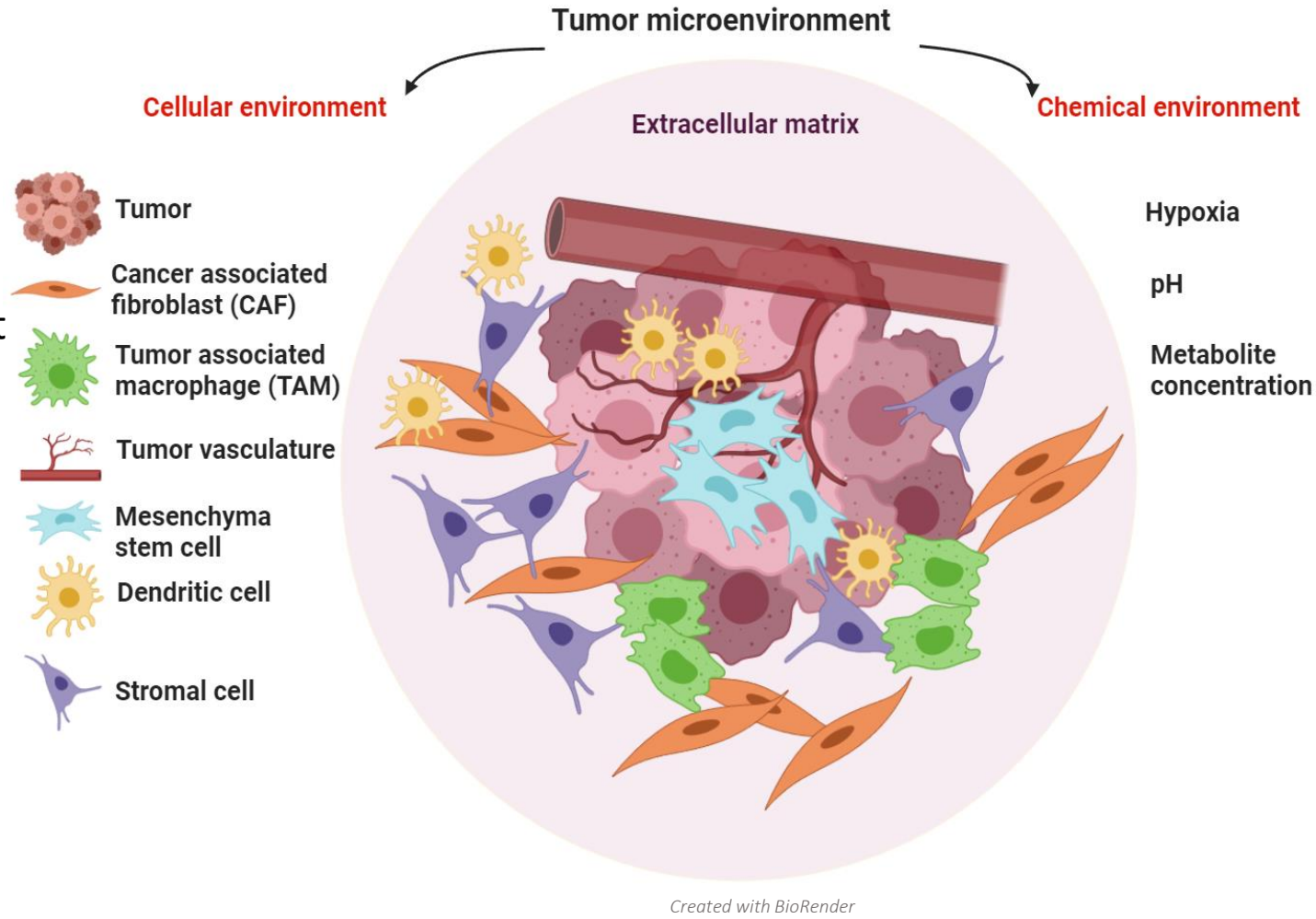
Taken from Thiennimitr et al., 2012

Bacteria-mediated cancer therapy (BMCT)

In the 20th century orthopaedic surgeon (Coley) injected heat-inactivated *Streptococcus pyogenes* into bone sarcomas → tumour regression

Triggering anti-tumour immune responses and destroying the **tumour microenvironment**

- Down-regulation of tumour antigens
- Formation of an immunosuppressive environment
  - Kill NK cells
  - Inhibition of DC activity (IL-10 and TGF-α)
  - Recruit T reg → suppress immune response
  - M2 macrophages (TAM) → immunosuppression
  - VEGF production→ promotes angiogenesis
- Surrounding matrix of fibrillar collagen, elastin, fibronectin



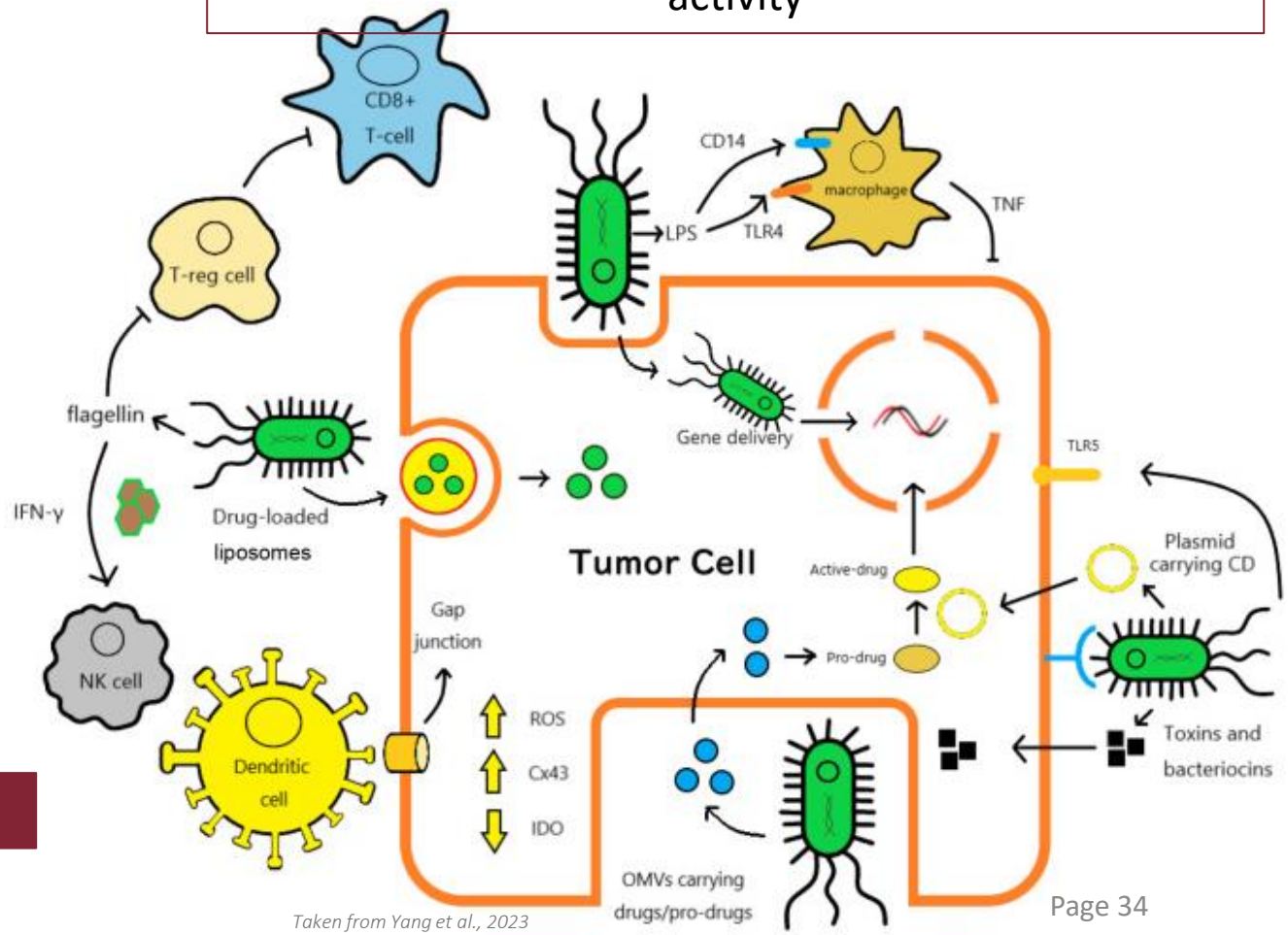
Bacteria-mediated cancer therapy (BMCT)

***S.* Typhimurium = nanomachines**

- Colonises tumour tissues (1000 times more)
- Prefers hypoxic, poorly vascularised and acidic environment
- Induces anti-tumour immune responses

The genome of *S.* enterica can be **genetically engineered** like a programmable robot to ensure safety (attenuated) and increase its therapeutic activity

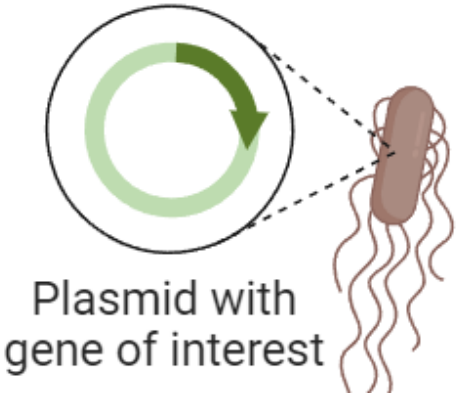
1. Gene transport system (**Bactofection** → plasmids encoding tumour genes under eukaryotic promoter)
2. Drug transport system to directly target the tumour (reduces toxicity dosages)
3. Induces **apoptosis** in tumour cells via toxin release
4. Induces anti-tumour responses by expression of **pro-inflammatory chemokines and cytokines** → increase in immune cell numbers (CD4+ helper T cells, CD8+ cytotoxic T cells, NK cells, macrophages)



Taken from Yang et al., 2023

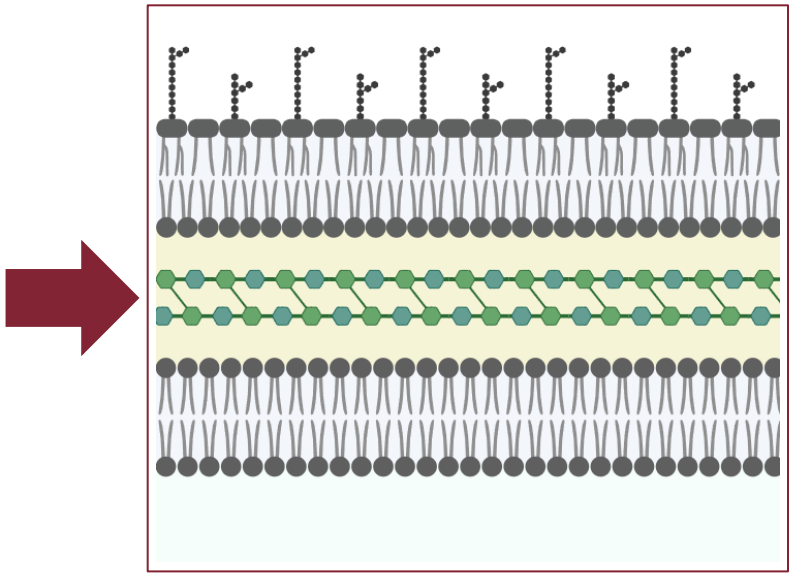
Therapeutic option with great potential

“Chassis” for exogenous proteins study



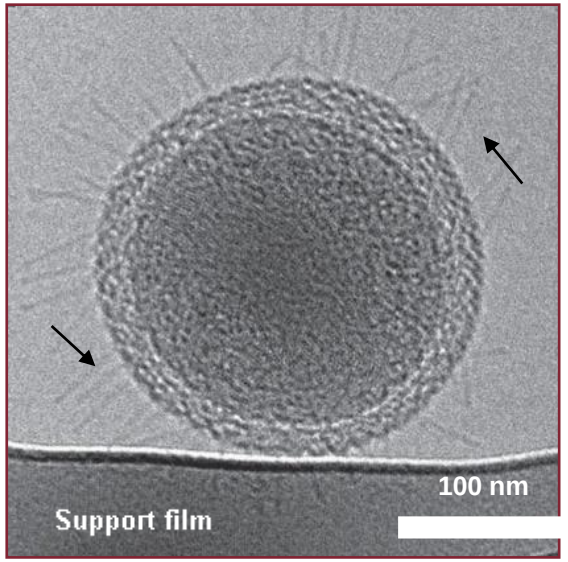
Focused on the **peptidoglycan** of uncultured bacteria of the Candidate Phyla Radiation (CPR):

- understudied bacteria
- monophyletic radiation making up 15% of the bacterial domain
- tiny cells (expected size from 100 to 300 nm)
- streamlined genomes (~1.0-1.2 Mb) → influence metabolic activity
- predicted a (epi)-symbiotic lifestyle (type IV pili)
- unusual ribosomal composition



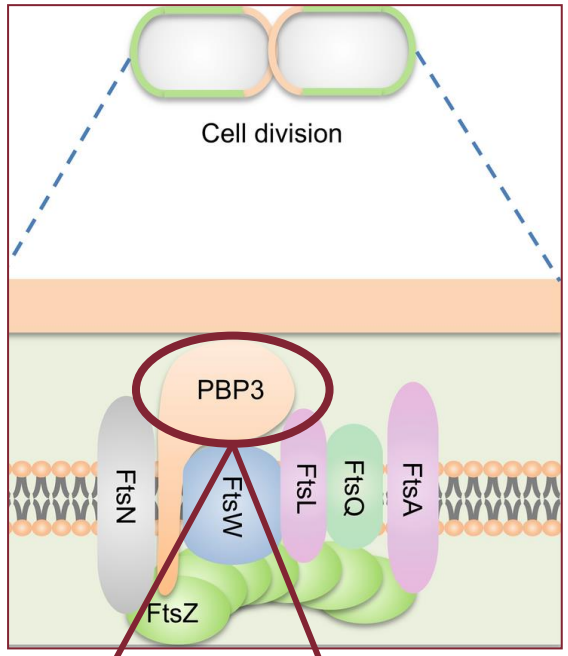
Created with BioRender

***Salmonella enterica serovar* Typhimurium**
(*S.* Typhimurium) cells as “**chassis**” to express enzymes of non-cultured microbes



Taken from Luef, Frischkorn, Wrighton et al., 2015

Study of enzymes related to PG metabolism of non-cultured microbes



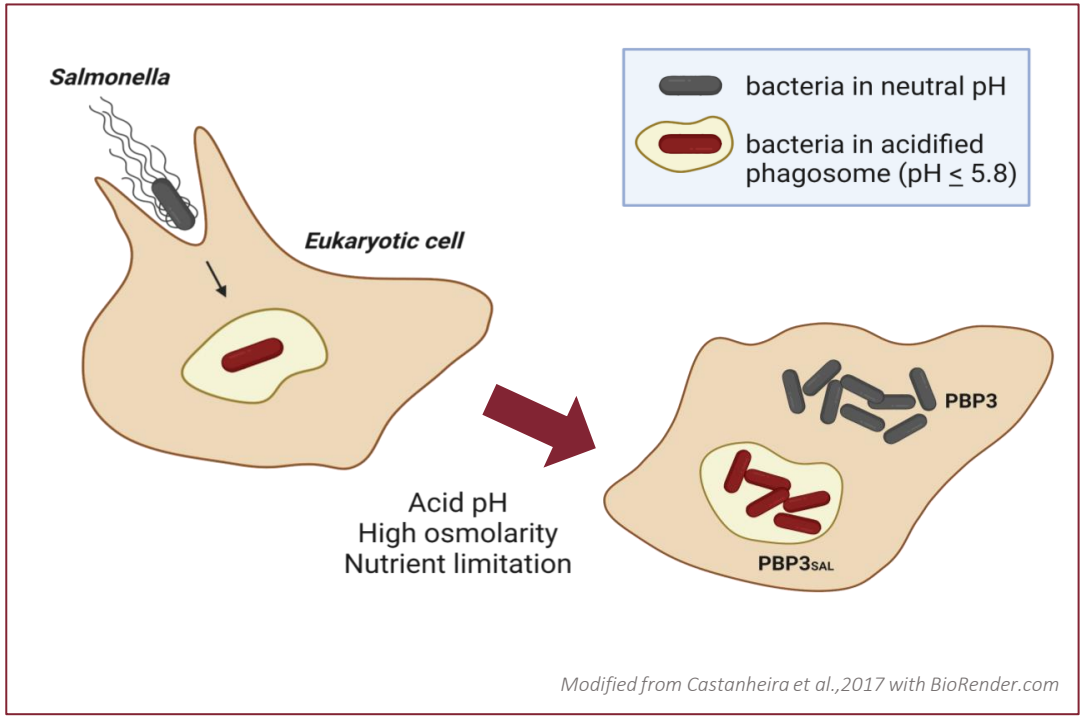
Hu, Zhao, Zhang et al., 2020

Evo-4 Evo-5

Both two candidate enzymes retained intact the **essential motifs** for catalytic activity (SXXK, SXN/D and KTG) present in the **transpeptidase** (TP) **domain**.

Expression in different genetic backgrounds and at different pH

observation of any **impact on cell shape** of *Salmonella*



Modified from Castanheira et al.,2017 with BioRender.com

Thank you for your attention!

Picture from Robert Koch-Institut