

# Intracellular lifestyles of pathogens

Dr. Janine Bossé

Molecular Infectious Disease Group  
Dept. of Paediatrics, Imperial College  
[j.bosse@ic.ac.uk](mailto:j.bosse@ic.ac.uk)

# Intracellular pathogens

- **Obligate intracellular pathogens**
  - Viruses and prions
  - Parasites: *Leishmania*
  - Bacteria: Rickettsias, Chlamydias, *Ehrlichia*, *Mycobacterium leprae*
- **Facultative intracellular pathogens**
  - Fungi: *Aspergillus*, *Cryptococcus*, *Histoplasma*, *Candida*
  - Parasites: *Toxoplasma*, *Plasmodium*, *Cryptosporidium*, *Eimeria*, *Trypanosoma*
  - Bacteria: *Bartonella*, *Brucella*, *Campylobacter*, *Citrobacter*, *Haemophilus*, *Legionella*, *Leptospira*, *Listeria*, *Mycobacterium*, *Neisseria*, *Nocardia*, *Shigella*, *Salmonella*, *Treponema*, *Yersinia*
  - NB intracellular state is often transient!

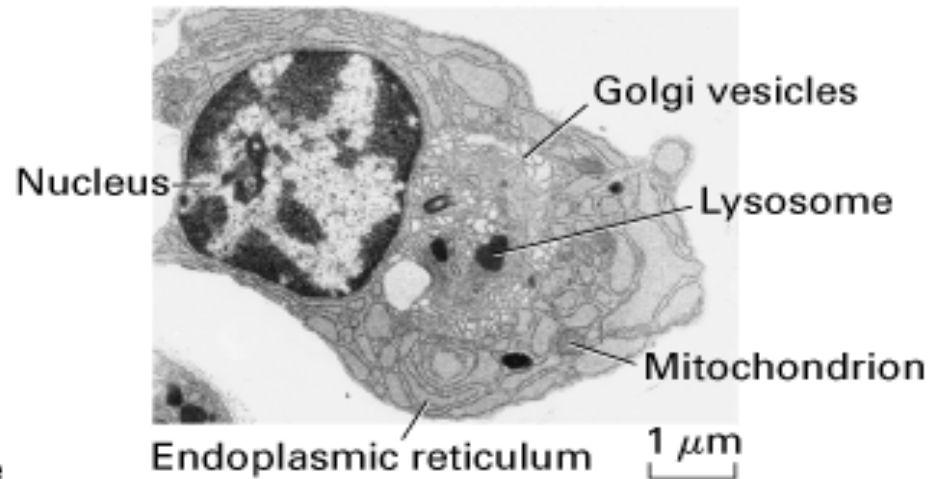
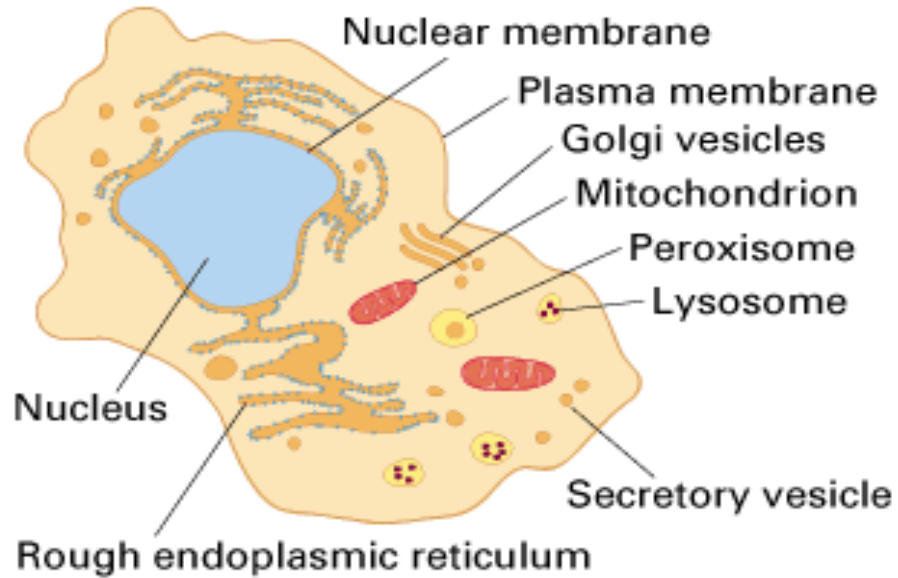
# Why live inside host cells?

- **Advantages**
  - privileged environment (no competition)
  - inaccessible to complement and antibodies
  - protected from antibiotics
  - ready access to nutrients
  - require host cell environment (obligate intracellular pathogens)
- **Disadvantages**
  - hostile environment if pathogen is unadapted

# Killing by professional phagocytes

- Oxygen dependent
  - Respiratory burst (NADPH oxidase)
  - $O_2^-$ ,  $H_2O_2$ ,  $OCl^-$
  
- Oxygen independent
  - Low pH (lysosome)
  - Proteolytic enzymes
  - Lysozyme
  - Lactoferrin
  - Cationic membrane damaging proteins

# Eukaryotic cell



# Membrane traffic

- **Organelles**
  - Distinct membrane-bound compartments
  - Specialised functions
  - Unique combination of lipids and proteins
- **Vesicles**
  - Transport proteins and lipids between donor and acceptor compartments
  - Specificity of transport dictated by membrane markers

# Endocytosis and membrane traffic

- Means of acquiring nutrients and/or transmitting signals
- Internalise receptors and their ligands, as well as particles and/or solutes in the extracellular environment
- Receptors are returned to the PM; constant turn-over (90%/h)
- Macromolecules for degradation are targeted to late endosomes and lysosomes-- metabolites released into cytoplasm
- Biosynthetic pathway: proteins for secretion are synthesised on ribosomes, passed through the ER and Golgi apparatus, and are packaged into specific vesicles by the trans-Golgi network
- Vesicles are targeted (via marker proteins) to endosomes or to plasma membrane for exocytosis

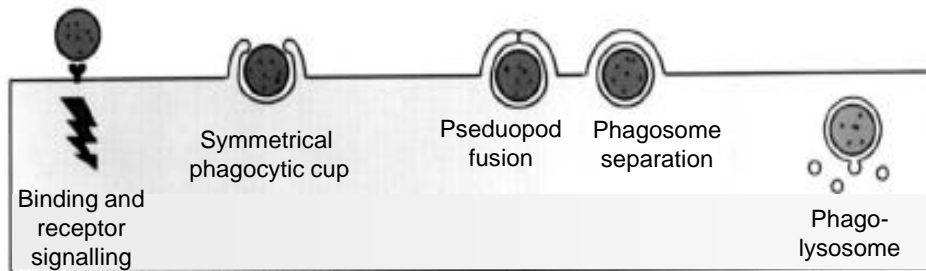
# Pathogen entry into host cells

- Professional phagocytes (m $\phi$  and PMNs)
  - FcR or CR mediated zipper phagocytosis (*Mycobacterium*)
  - Coiling phagocytosis (*Legionella*, spirochetes, *Leishmania*)
  - Triggered macropinocytosis (*Salmonella*)
- Non-professional phagocytes
  - Type of cell invaded dictated by ligand-receptor interaction
  - Receptor mediated zipper phagocytosis (*Yersinia*, *Listeria*)
  - Triggered macropinocytosis (*Salmonella*, *Shigella*)

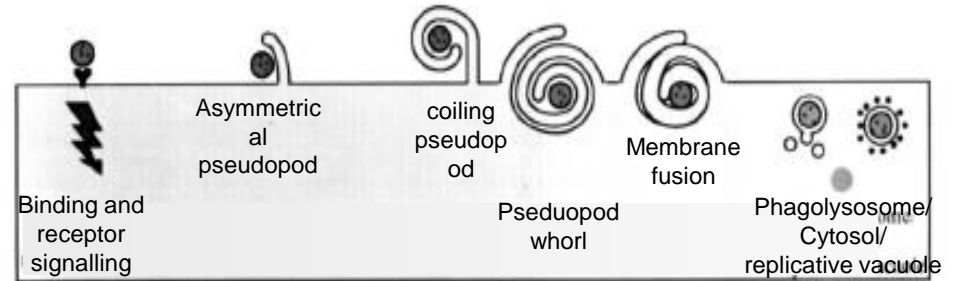


# Phagocytosis and Macropinocytosis

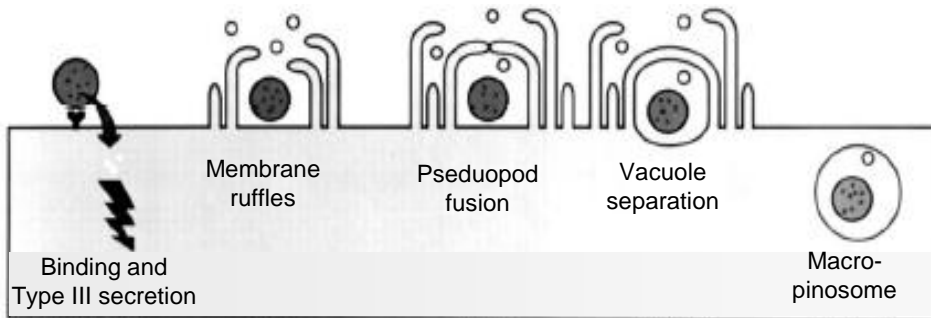
1. Attachment    2. Engulfment    3. Internalisation    4. Processing



Zipper phagocytosis

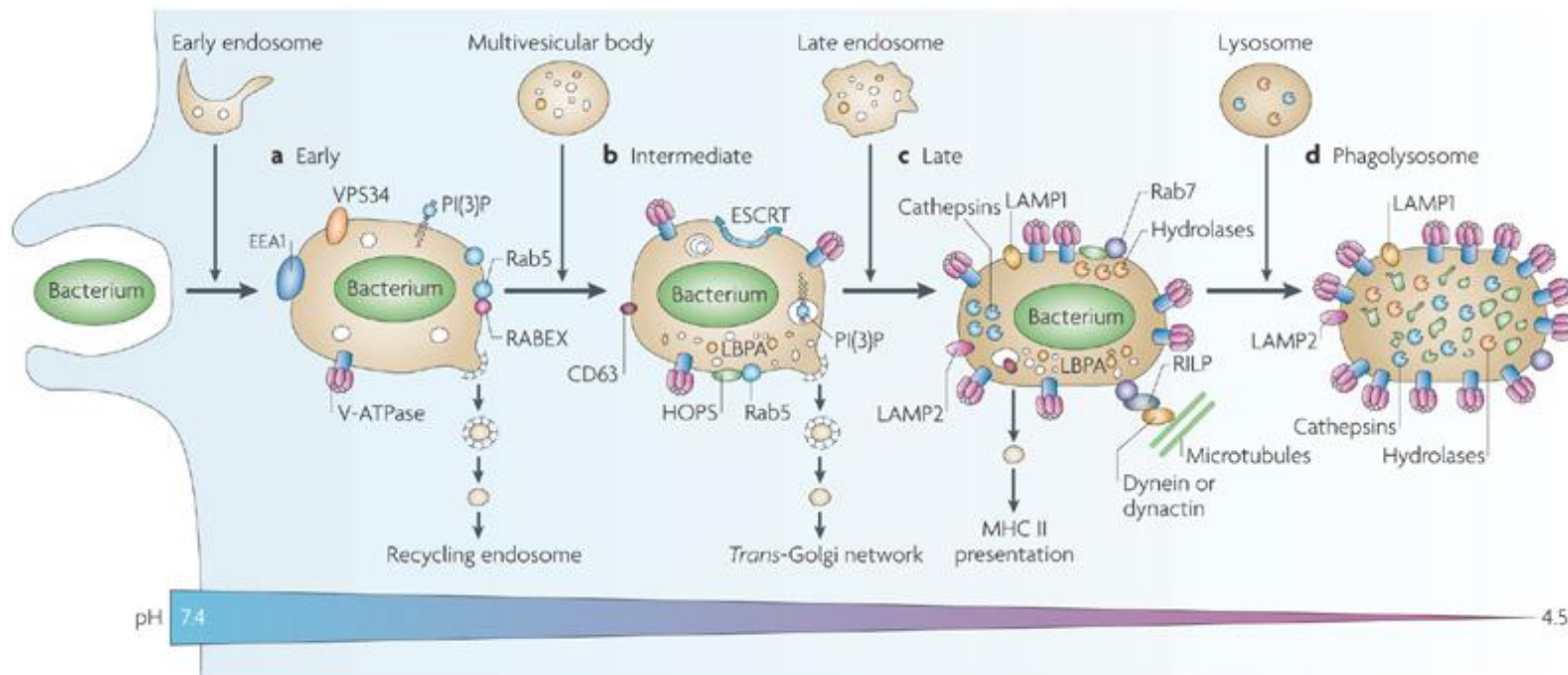


Coiling phagocytosis



Triggered macropinocytosis

# Phagocytosis and the endocytic pathway



## Early phagosome

recycling proteins back to PM; transient fusion to early endosomes; pH 6-6.5; LDL receptor, transferrin receptor, Rab4, Rab5, EEA1

## Late phagosome

transient fusion to late endosomes; pH 5.5-6; mannose 6 phosphate receptor, Rab7, LAMP1, vacuolar ATPase

## Phagolysosome

transient fusion to lysosomes; pH < 5.5; lysosomal enzymes, NADPH oxidase, nitric oxide synthase, cathepsin D; high density of LAMP1

# Resistance to intracellular killing

- Escape from phagosome
- Block phagosome lysosome fusion
- Modify vacuole or resist damage
  - HMW polysaccharides can scavenge free toxic oxygen radicals and protect peptidoglycan from lysosyme
  - SOD and catalase neutralise toxic oxygen radicals
  - Urease can neutralise acidity
  - Stress response proteins
  - Iron scavenging systems (Tbps, siderophores)

# Mechanisms of resistance

Bacterium	Type of Interference	Mechanism
<i>Rickettsia</i>	Escape phogosome	Phospholipase A
<i>Listeria</i>	Escape phogosome	LLO, phospholipase C
<i>Shigella</i>	Escape phogosome	IpaB
<i>Trypanosoma</i>	Escape phogosome	TcTox
<i>Legionella</i>	Block lysosome fusion	Dot/Icm
<i>Toxoplasma</i>	Block lysosome fusion	Entry via caveolae
<i>Chlamydia</i>	Block lysosome fusion	Chlamydial protein
<i>Mycobacterium</i>	Block lysosome fusion	Entry via caveolae
<i>Salmonella</i>	Resist killing	TTSS, stress proteins
<i>Brucella</i>	Resist killing	LPS
<i>Coxiella</i>	Resist killing	Low pH metabolism

# Intracellular niche

- **Intralysosomal**
  - Low pH environment (pH 4.7-5.2)
  - Access to nutrients but risk hydrolytic attack
  - Compartment still interacts with the endosomal network of cell
- **Intravacuolar**
  - Pathogen blocks normal phagosome maturation
  - Vacuole may exist outside normal membrane trafficking pathways
- **Cytosolic**
  - escape into cytoplasm: avoid hostile endosome
  - pathogen exhibits membrane disrupting activity

# Intracellular niche: Intralysosomal

- *Coxiella burnetii*
  - Organism grows optimally at pH < 5 !!
  - Resists degradation by enzymes
- *Mycobacterium leprae*
  - Waxy, hydrophobic cell wall and capsule components (mycolic acids) are not easily attacked by lysosomal enzymes
  - Stress response proteins-- resistance to oxidative stress

# Intracellular niche: Intravacuolar

Pathogen	Type of vacuole	Acidified	Features
<i>Salmonella</i>	Endosome	Yes	Some markers
<i>Leshmania</i>	Early endosome	Yes	
<i>Legionella</i>	ER	No	
<i>Brucella</i>	ER	Yes	No markers
<i>Chlamydia</i>	Inclusion	No	Chlamydial protein
<i>Toxoplasma</i>	Parasitophorous	No	No host proteins

# Intracellular niche: Cytosolic

- *Shigella flexneri*
  - IpaB
- *Listeria monocytogenes*
  - listeriolysin O (LLO); pore forming toxin
  - phospholipases
- *Rickettsia prowazekii*
  - two phospholipases



# Selected intracellular pathogens

- Cytosolic
  - *Listeria monocytogenes*
  - *Shigella dysenteriae*
- Intravacuolar
  - *Salmonella typhimurium*
  - *Legionella pneumophila*

# *Listeria monocytogenes*

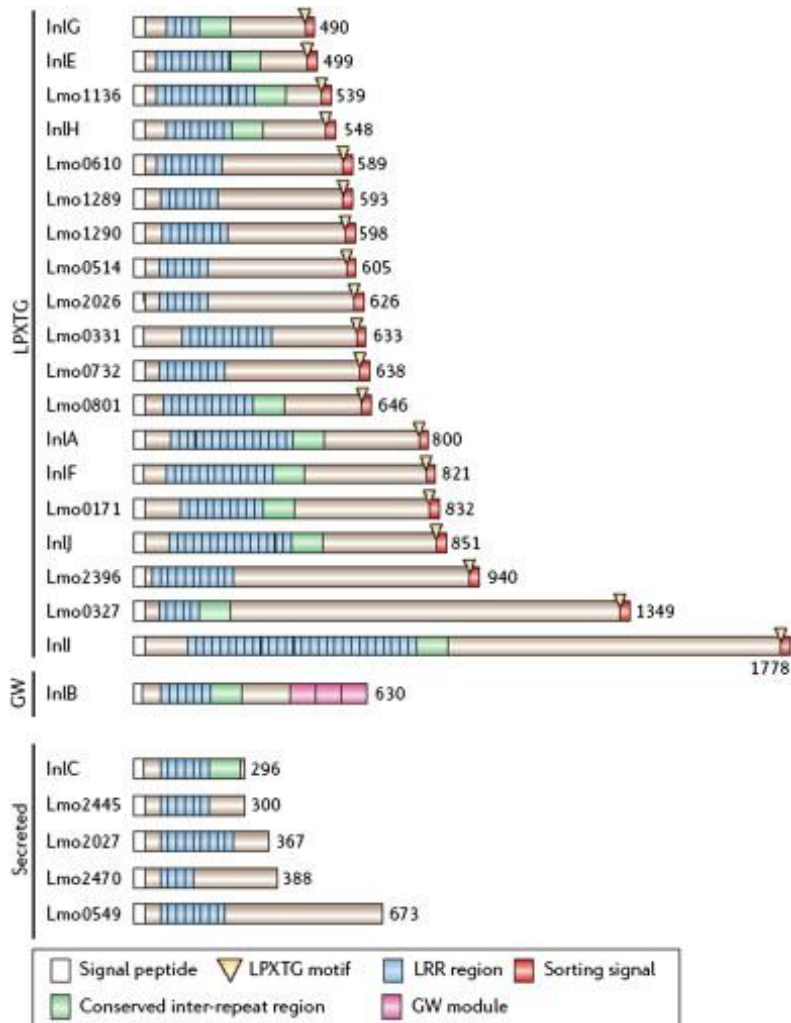
- *L. monocytogenes*
  - ubiquitous Gm<sup>+</sup> motile bacterium
- Disease
  - Listeriosis
  - Normally not highly virulent
    - Special risk to pregnant women (abortion)
    - Special risk to immunocompromised people (meningitis/encephalitis)
- Symptoms
  - Fever, muscle aches, nausea, diarrhoea
  - In CNS infection: headache, stiff neck, loss of balance, convulsions
- Transmission
  - Ingestion of contaminated food
    - Can survive at low temperature (in refrigerated food)

# *Listeria monocytogenes*

- Pathogenesis

- Crosses intestinal mucosa
  - May use M cells, or directly invade epithelial cells
  - Capable of invading wide range of cell types (including macrophages, PMN, endothelial cells, hepatocytes) via receptor-mediated (zipper) endocytosis
- Escape from phagosome, multiply in cytosol
  - LLO, Phospholipases
- Cell to cell spread
  - ActA mediated actin polymerisation
- Systemic spread
  - Carried by lymph or blood to spleen and liver
  - favored niche is hepatocytes

# Listeria internalins



- Family of proteins with LRR thought to be involved in specific protein-protein interactions

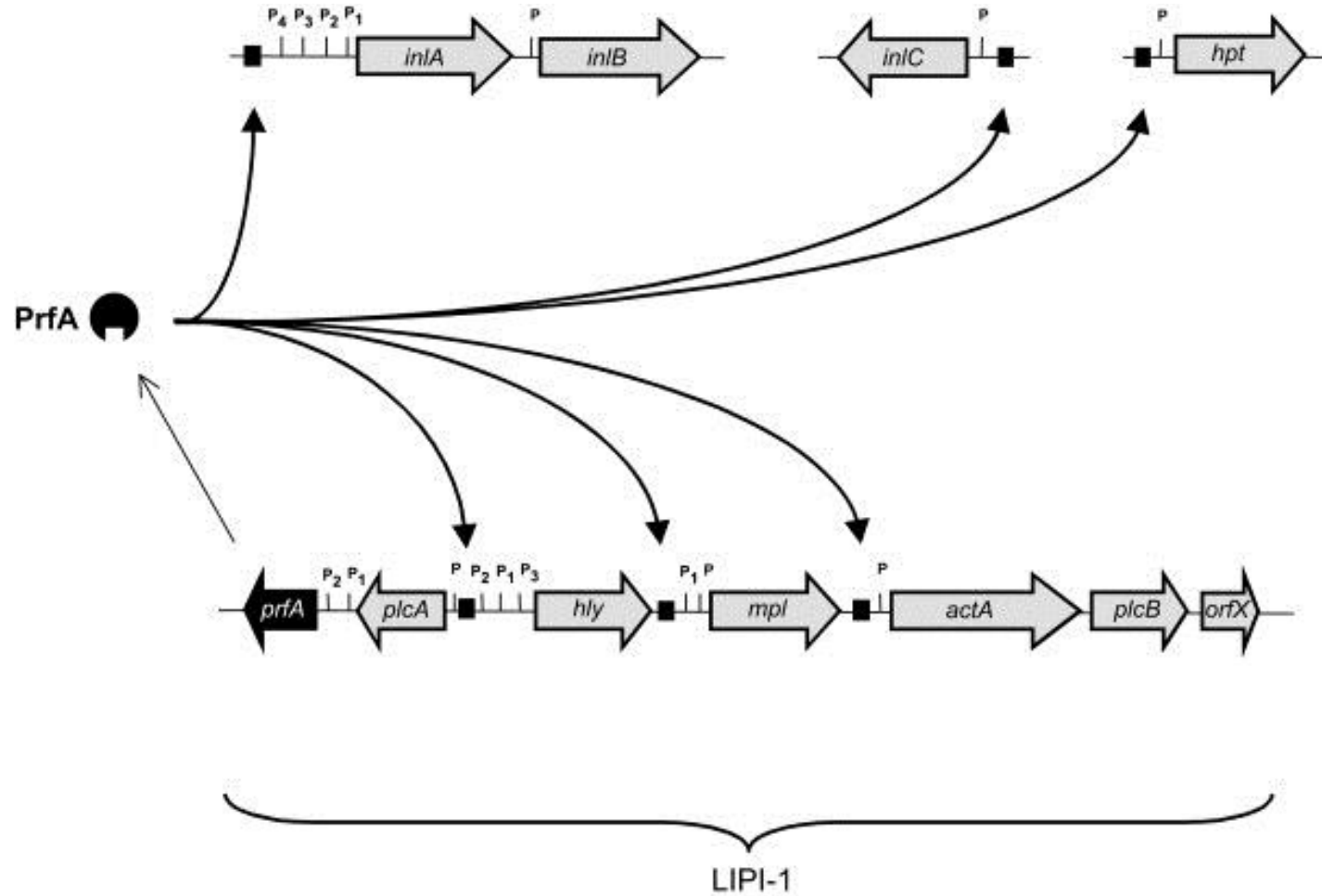
- **InA**

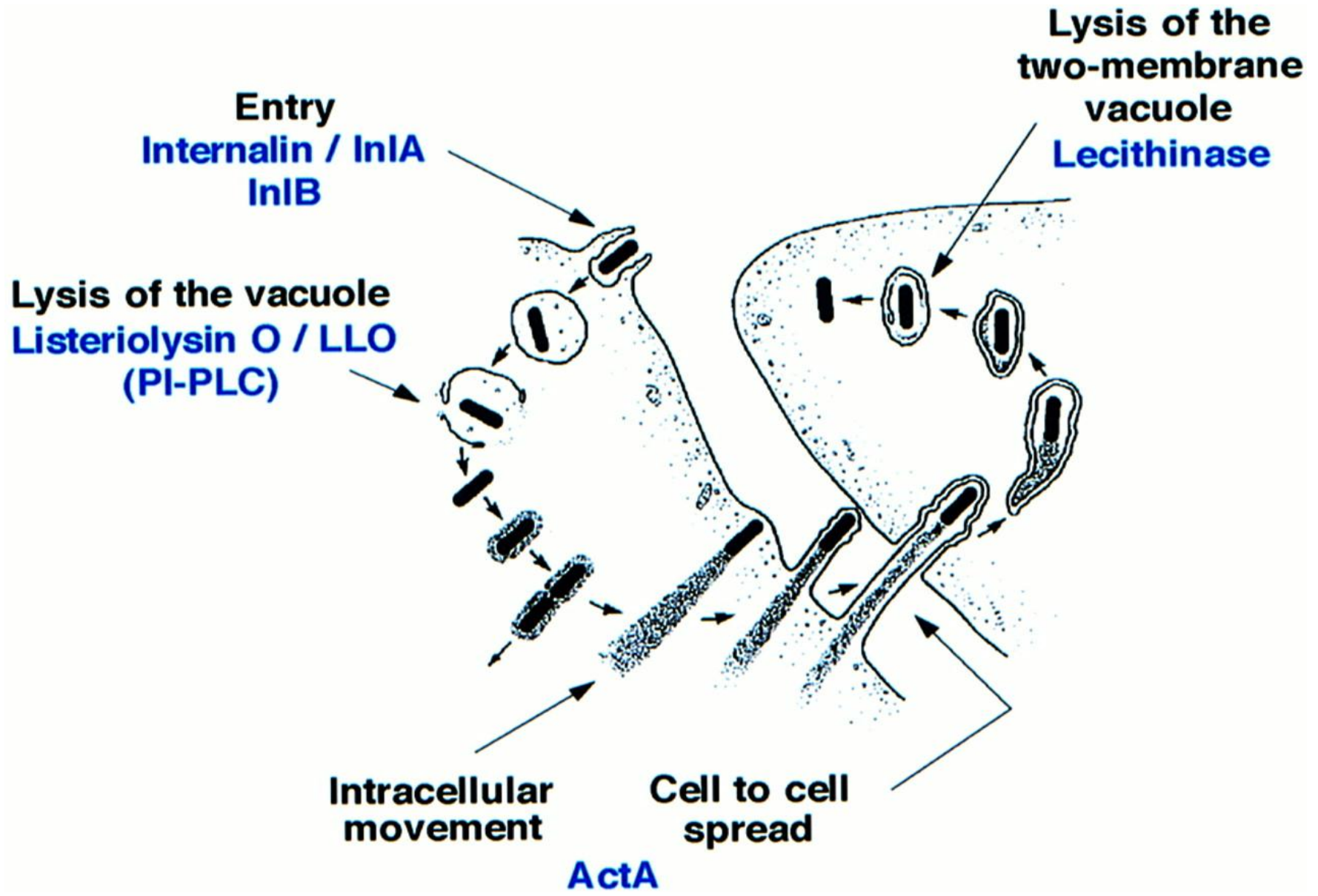
- Binds E-cadherin, a protein found on the surface of intestinal epithelial cells, hepatocytes, dendritic cells, brain microvascular endothelial cells, and epithelial cells of choroid plexus and placental chorionic villi
- Binding leads to actin cytoskeleton rearrangements via  $\alpha$ - and  $\beta$ -catenins

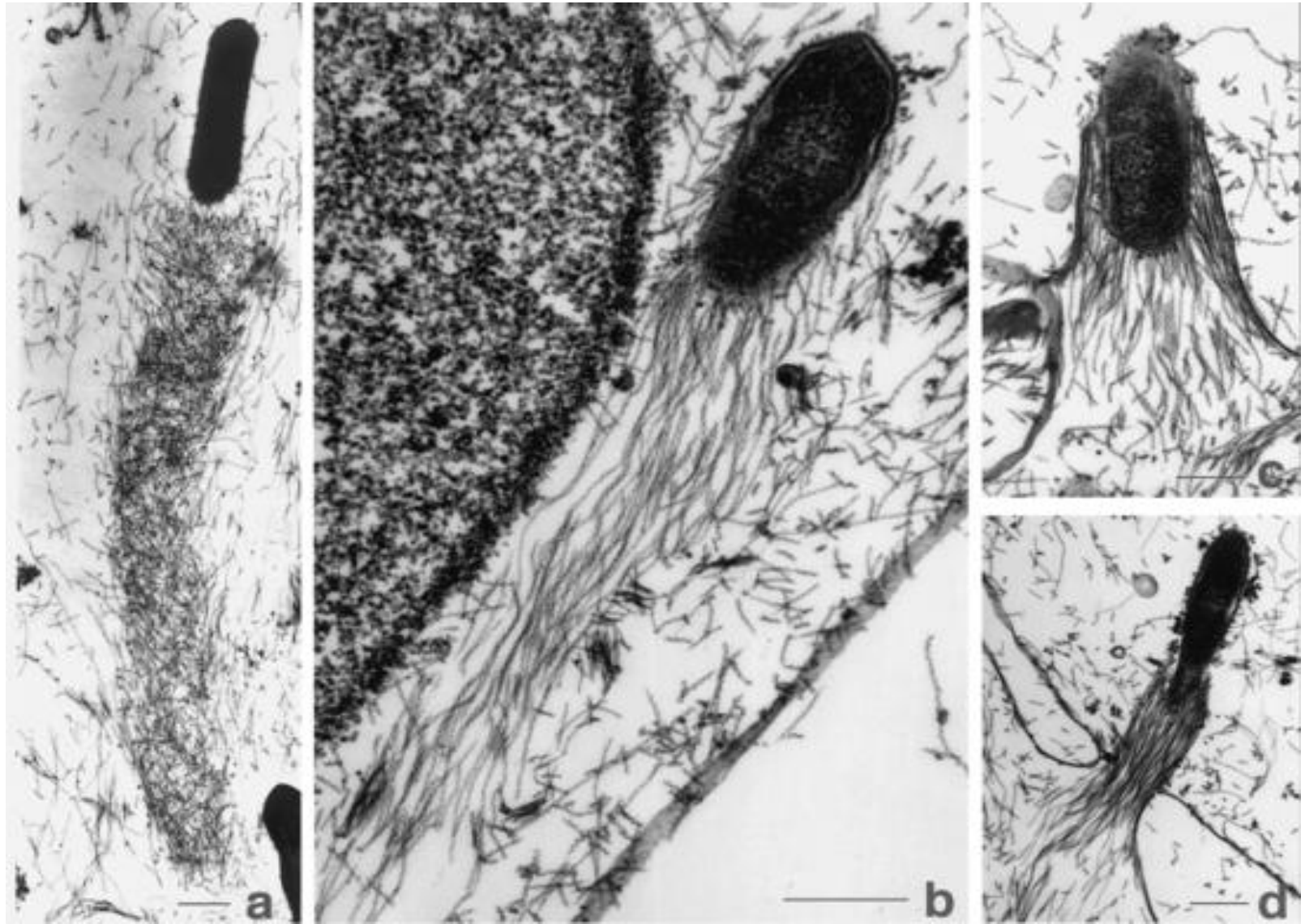
- **InB**

- Binds gC1q-R found on wide range of cells
- Binding induces membrane ruffling and tyrosine phosphorylation of several host proteins

# Co-ordinate gene regulation

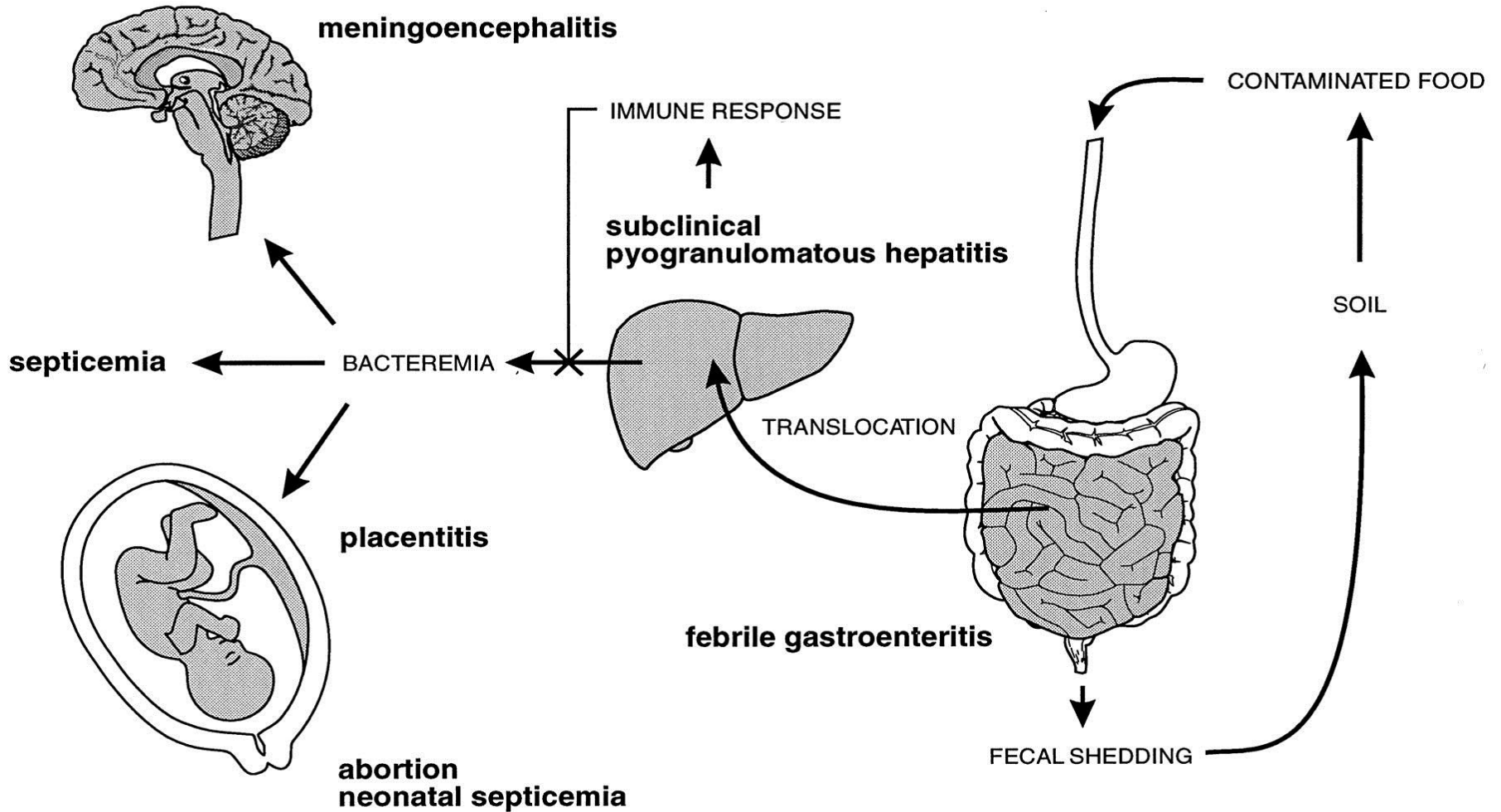








# Pathogenesis of *Listeria* infection





# *Shigella dysenteriae*

- *S. dysenteriae*
  - Gm<sup>-</sup> non-motile enteric bacterium
- Disease
  - shigellosis or bacillary dysentery in humans
- Symptoms
  - fever, intestinal cramps and bloody diarrhoea with mucopurulent discharge
- Transmission
  - Fecal/oral, spread through contaminated food/water

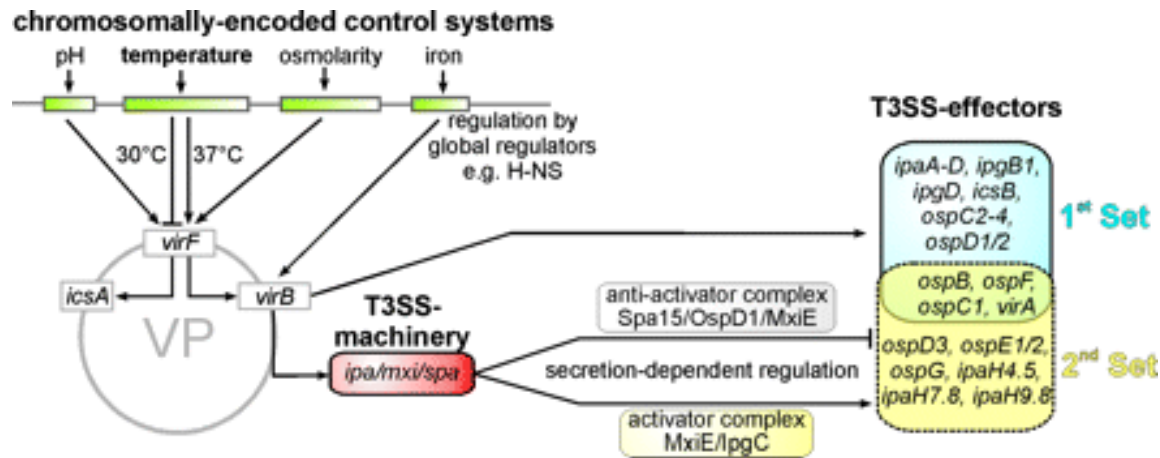
# *Shigella dysenteriae*

- Pathogenesis
  - Cross intestinal epithelium via M cells
  - Taken up by macrophages, cause apoptosis via IpaB
  - Invade epithelial cells via basolateral surface using Type III secretion system
  - Cells released into cytoplasm via IpaB
  - Cell to cell spread via IcsA mediated actin polymerisation

# *Shigella* Type III secretion system

- Pathogenicity island
  - Mxi-Spa proteins
  - Ipa proteins
- Mxi-Spa proteins
  - Secretion apparatus
- Ipa proteins
  - IpaB and IpaC form extracellular complex and insert into host cell membrane to form a pore
  - IpaC intracellular domain induces actin polymerisation
    - Uptake of bacteria by trigger macropinocytosis
  - IpaA injected into cell induces actin depolymerisation
    - Formation of pseudoadherence plaque

# Co-ordinate gene regulation



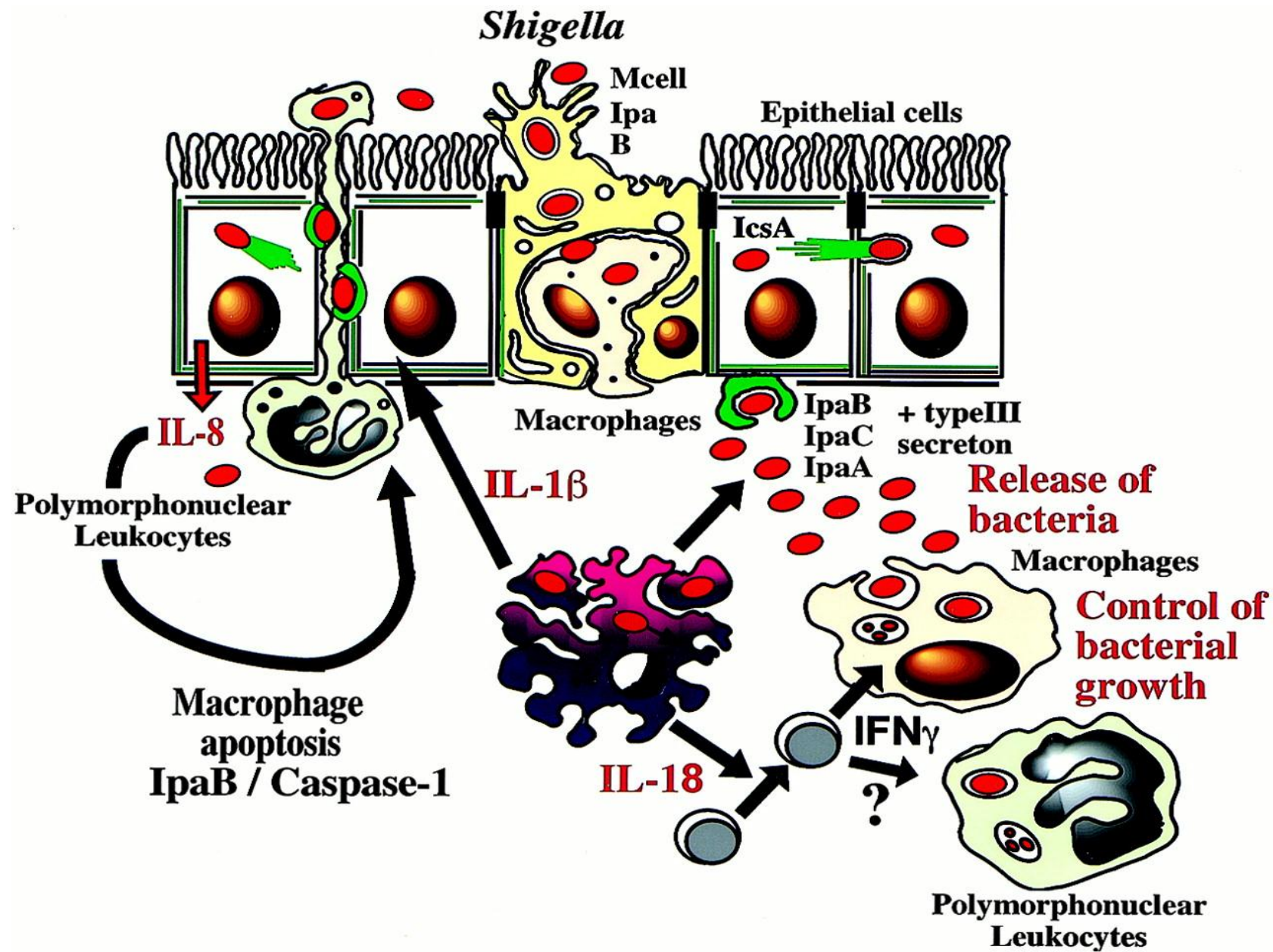
## Effectors, translocators, chaperones



## Assembly & function of the T3SS needle complex



T3SS-translocators/-substrates
  Chaperones
  T3SS machinery
  Regulators



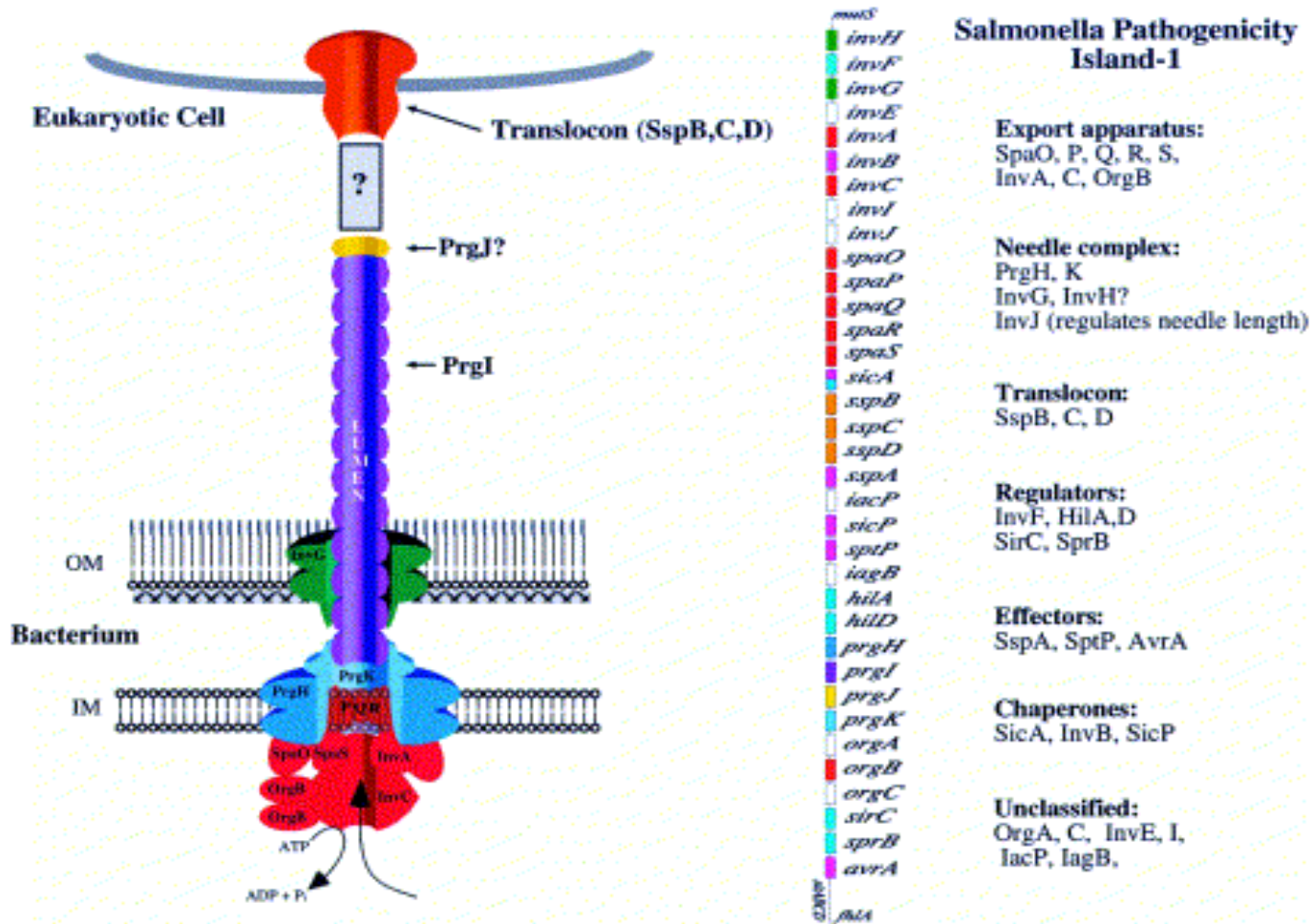
# *Salmonella typhimurium*

- *S. enterica* serovar *typhimurium*
  - Gm<sup>-</sup> motile enteric bacteria
- Disease
  - Gastroenteritis
- Symptoms
  - Fever, intestinal cramps, nausea and vomiting, diarrhoea often includes mucous and is occasionally bloody
- Transmission
  - Fecal/oral
  - spread both through food and by person-to-person contact

# *Salmonella typhimurium*

- Pathogenesis
  - Invade epithelial cells and macrophages (SPI-1 TTSS)
  - Remain inside spacious phagosome (SPI-2 TTSS)
  - Acidification of phagosome required for induction stress response factors
  - In macrophages, can cause apoptosis via SipB
    - Activated macrophages more prone to apoptosis

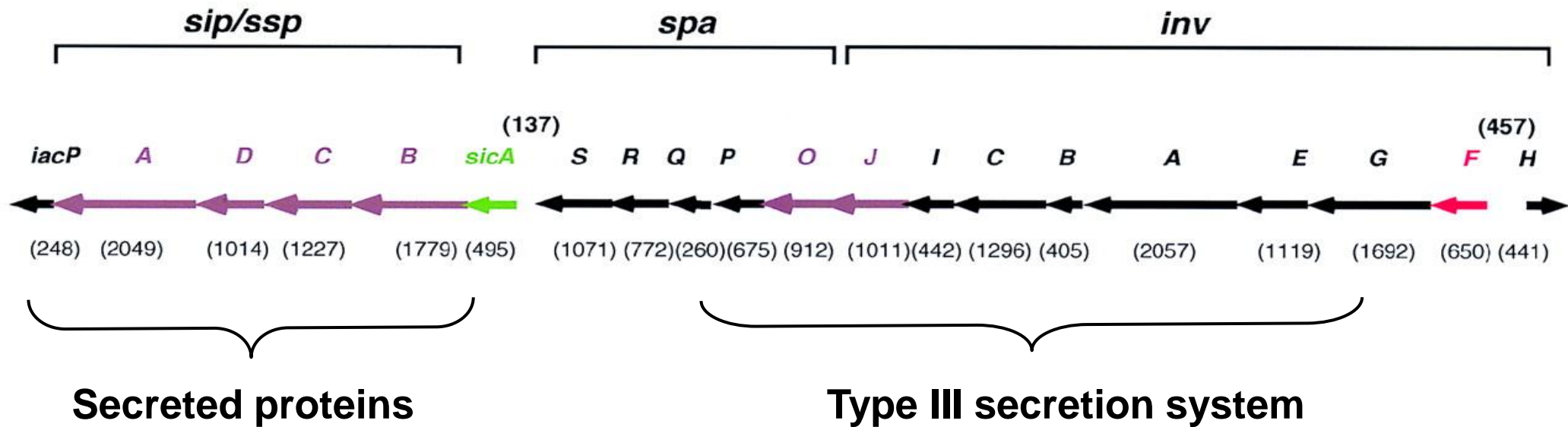
# SPI-1 encoded Type III secretion system



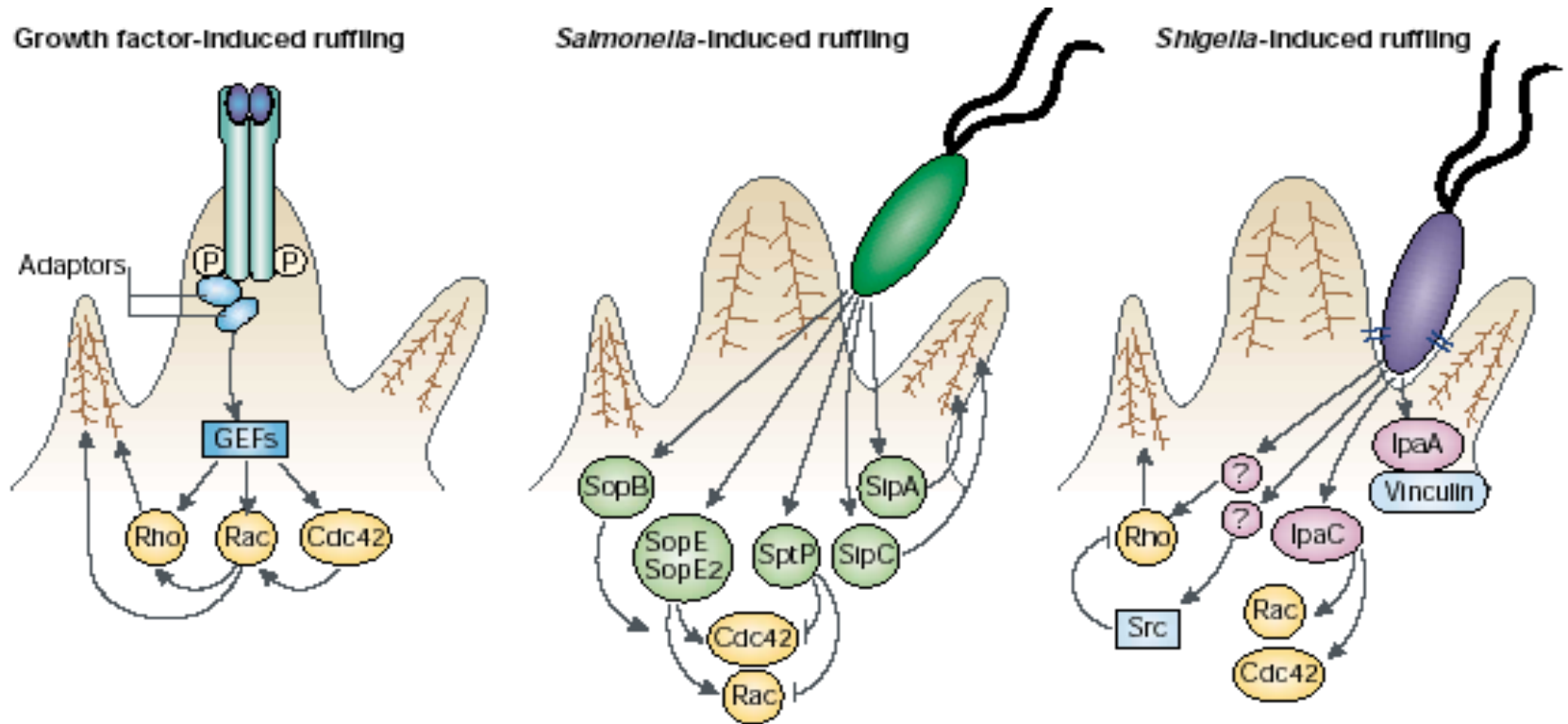


# Co-ordinate gene regulation: invasion

Membrane ruffling - requires SPI1

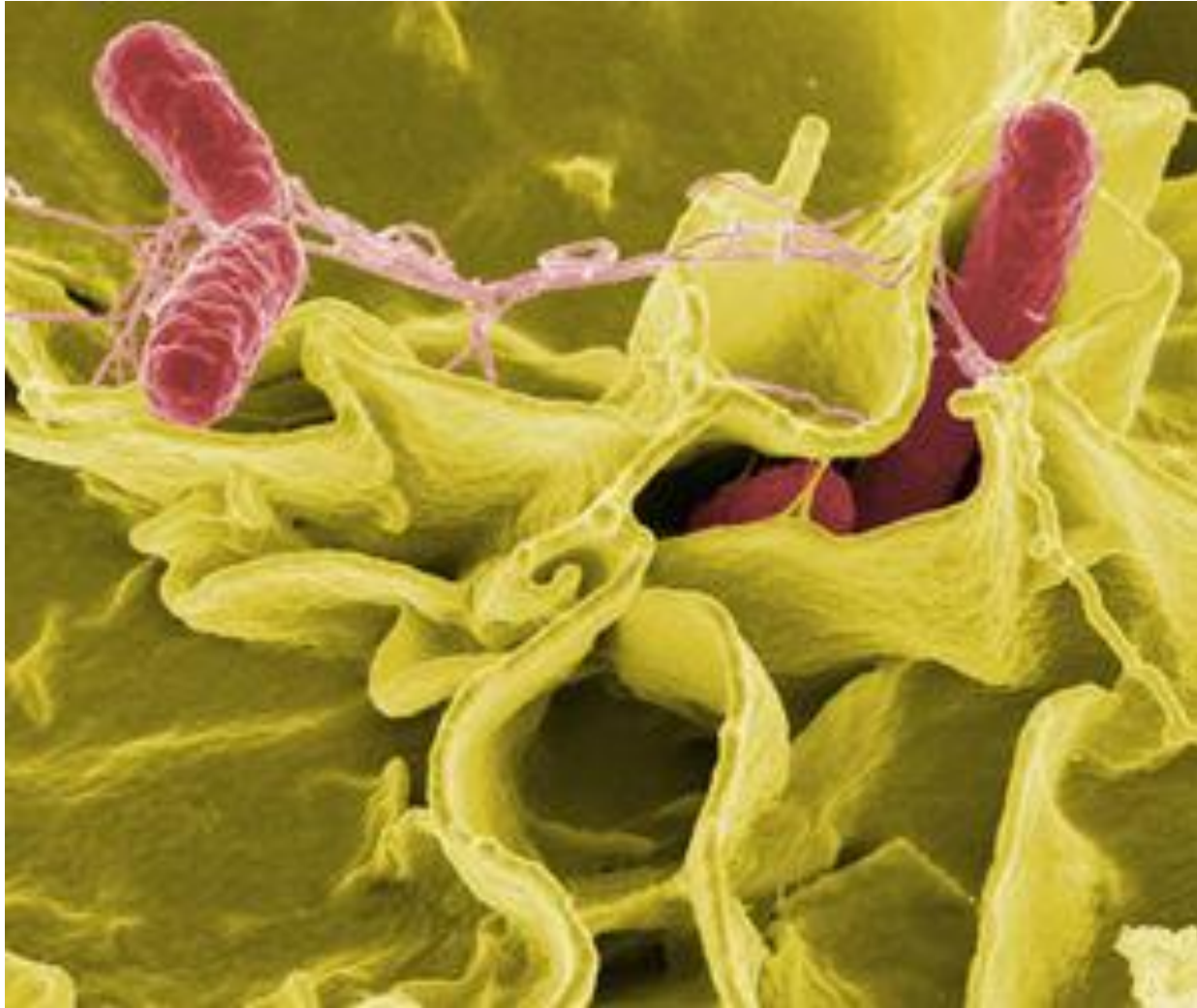


# Invasion by *Salmonella* and *Shigella*

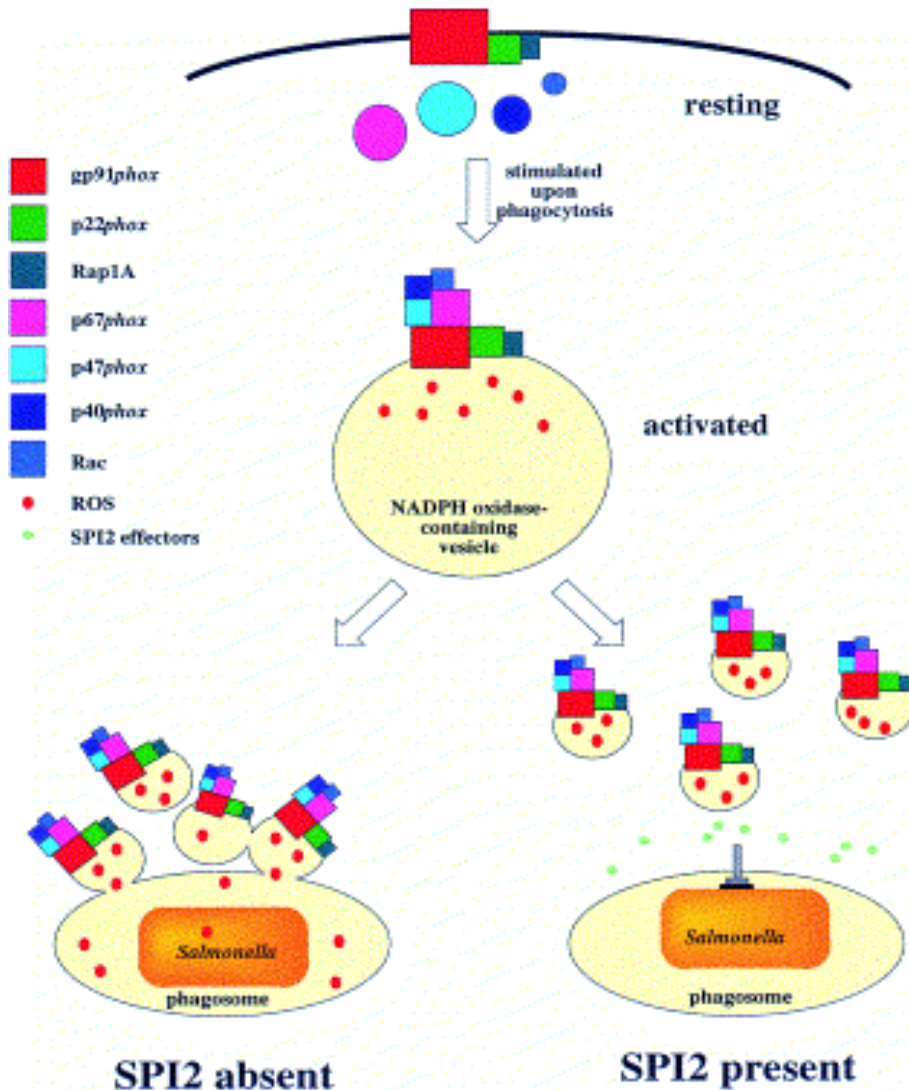


**SipA, SipC and SipB** homologous to **Ipa A, IpaB and IpaC**  
presumed to have same function and mechanism of action

# Membrane ruffling



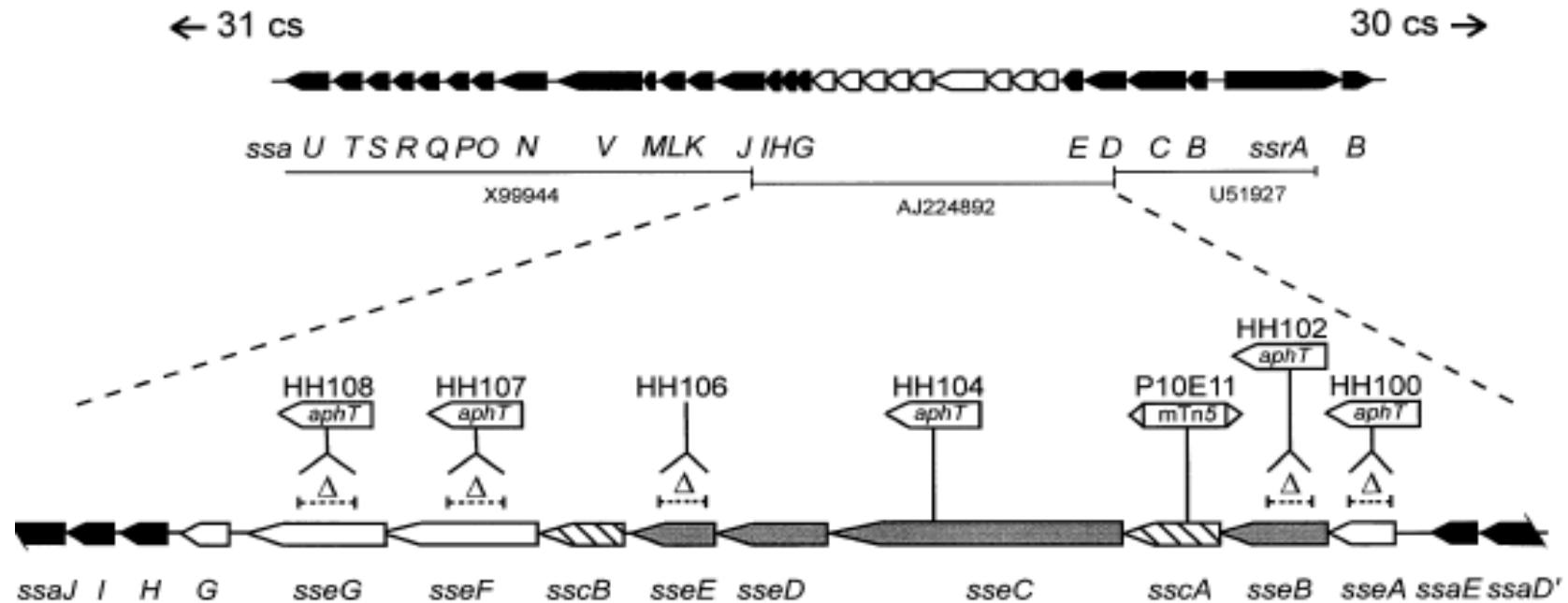
# Survival of *Salmonella* in SCVs



- SPI-2 proteins prevent fusion of NADPH oxidase-containing endosome to SCV
- Production of stress response proteins, enzymes, and scavengers that detoxify reactive oxygen species (ROS), also contribute to survival in phagosomes

# Co-ordinate gene regulation: survival

Requires SPI-2 encoded type III secretion system







# *Legionella pneumophila*

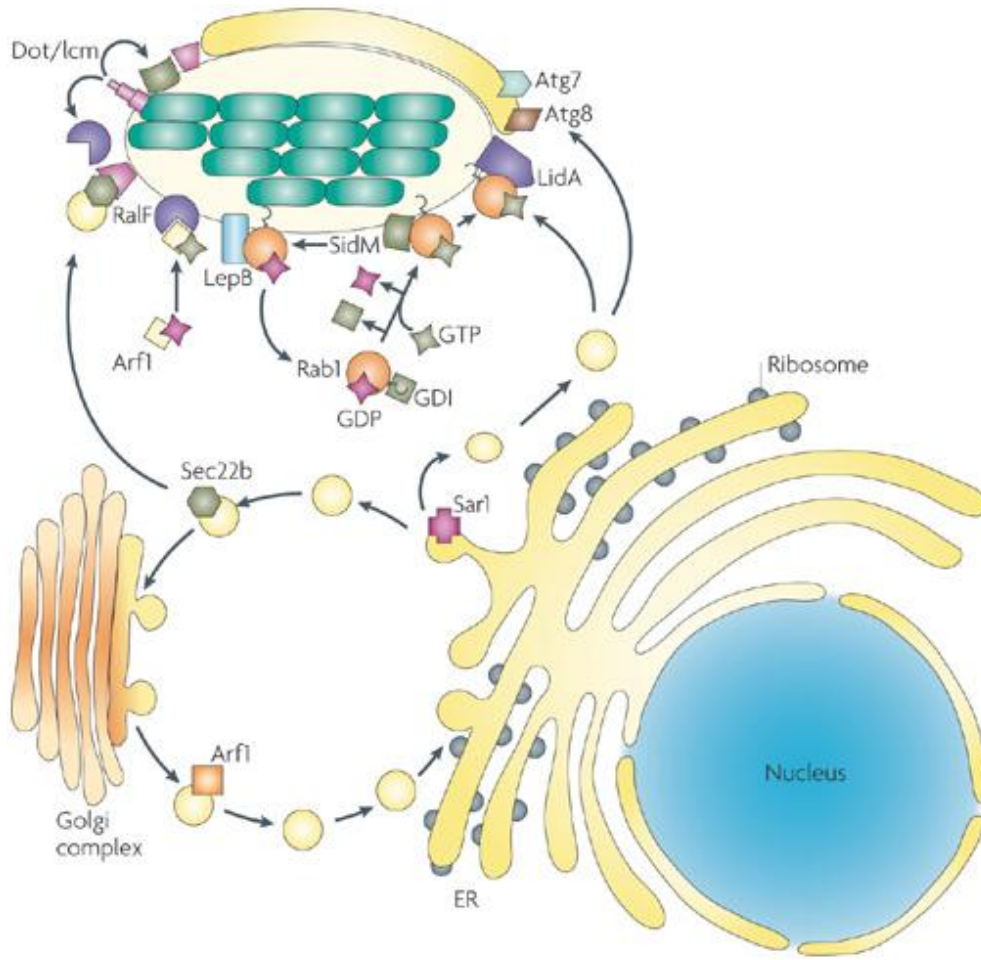
- *L. pneumophila*
  - Gm<sup>-</sup> motile bacterium
- Disease
  - Legionnaire's disease, Pontiac fever
  - More common in immunocompromised people or those with emphysema or chronic lung disease
- Symptoms
  - Acute pneumonia, watery diarrhoea, kidney and liver abnormalities
  - Pontiac fever is milder flu-like version with cough and chest pain
- Transmission
  - Inhalation of aerosols from environmental sources
    - NO PERSON TO PERSON SPREAD

# *Legionella pneumophila*

- Pathogenesis
  - Inhalation of bacteria leads to ingestion by alveolar macrophages (via coiling phagocytosis)
  - Phagosomes containing stationary phase bacteria fail to acidify or fuse with lysosomes (Dot/Icm)
  - Phagosomes transiently associate with mitochondria, then RER
    - Bacteria convert to replicative form
    - Rapid replication leads to lysis of vacuole and host cell death

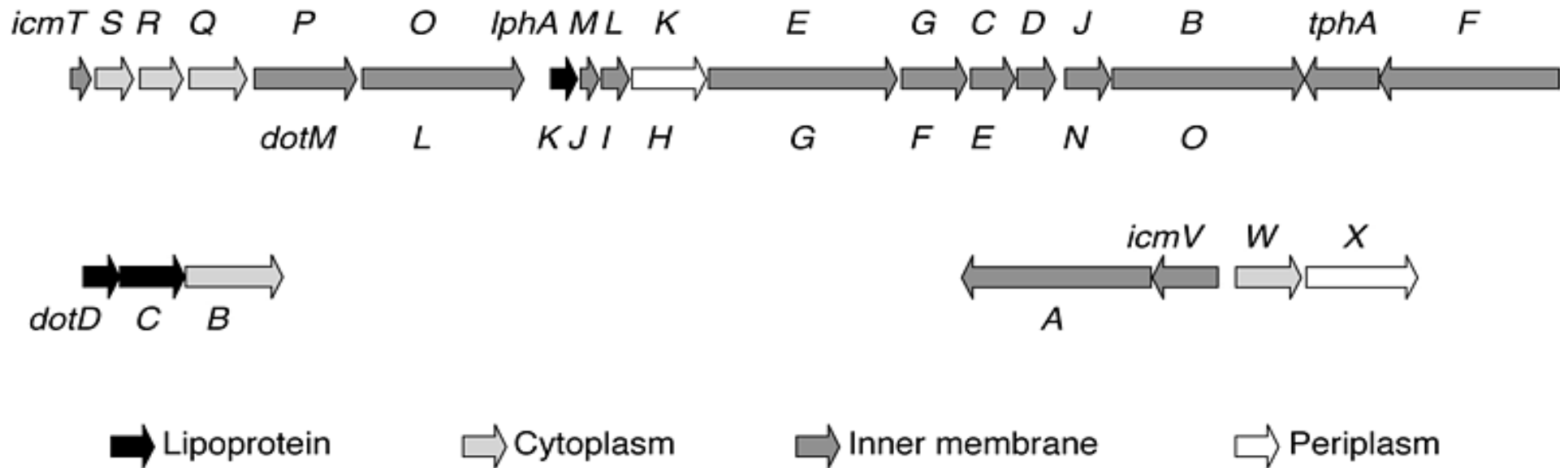


# Type IV secretion system of *Legionella*

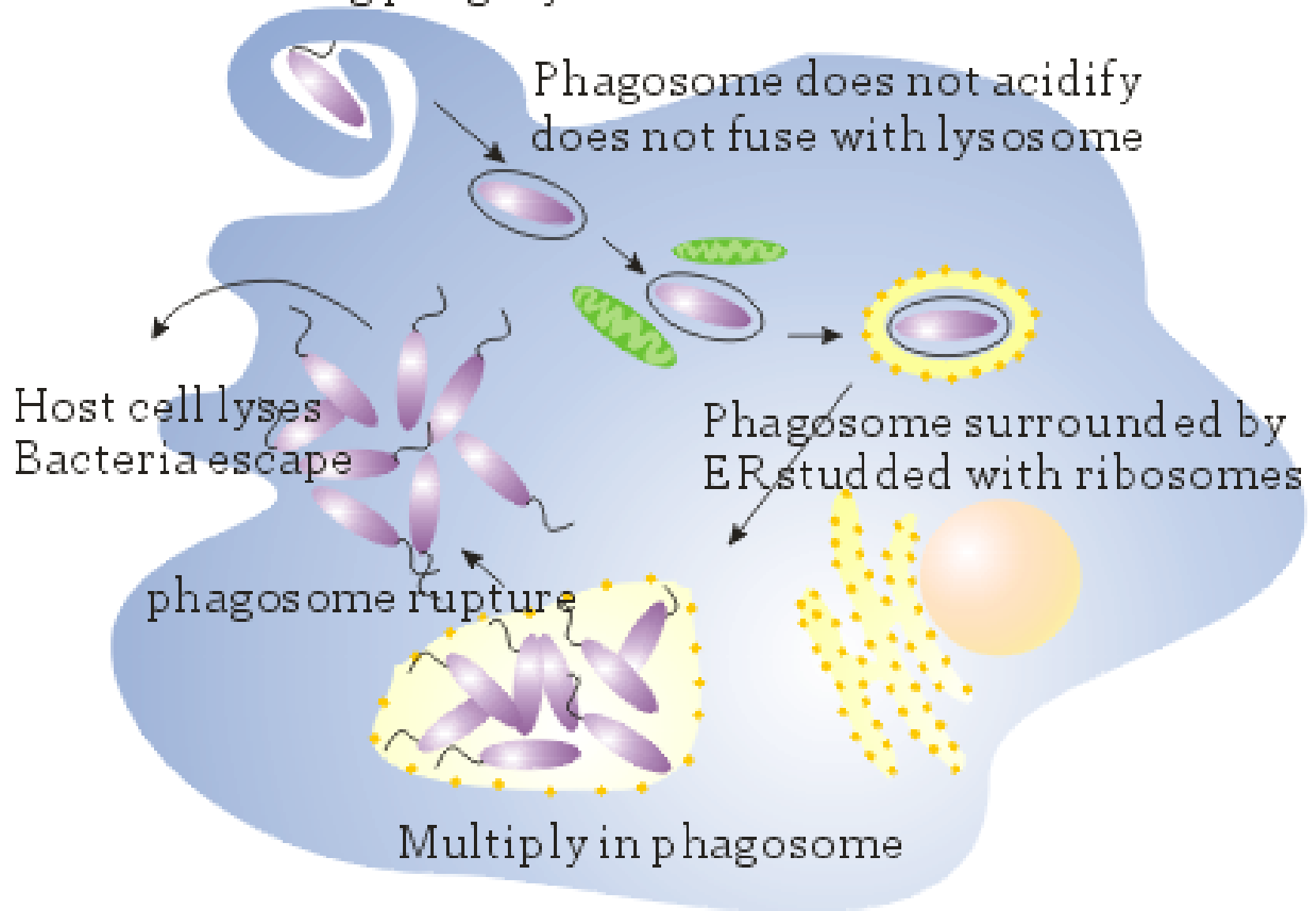


- Some Dot/Icm proteins form a channel through vacuole membrane
- Other Dot/Icm proteins are effectors that interact with host cell vesicle trafficking proteins
- Allows association with mitochondria and RER, avoiding normal targeting to lysosome
- *Legionella* replicate rapidly in the vacuole

# Co-ordinate gene regulation



# Coiling phagocytosis



# Summary

- Intracellular pathogens represent a diverse group of organisms
- organisms are specifically adapted to intracellular niche
  - involves subversion of normal host cell functions
  - different bacteria can use different mechanisms
  - requires co-ordinated expression of numerous genes
- occupation of an intracellular niche is often a transient step
- pathogen must escape and spread to new host cells and new hosts

