Intracellular lifestyles of pathogens

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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)

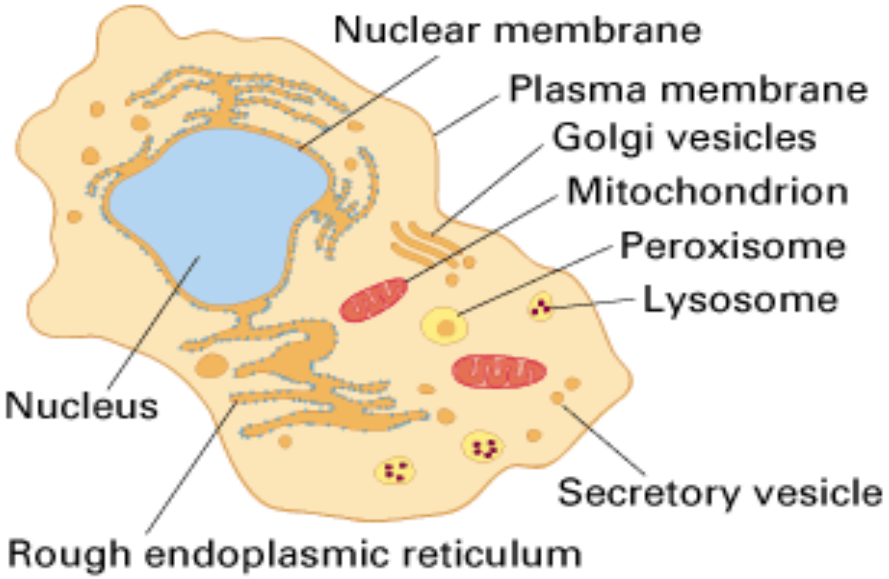
- Disadvantages
  - hostile environment if pathogen is unadapted

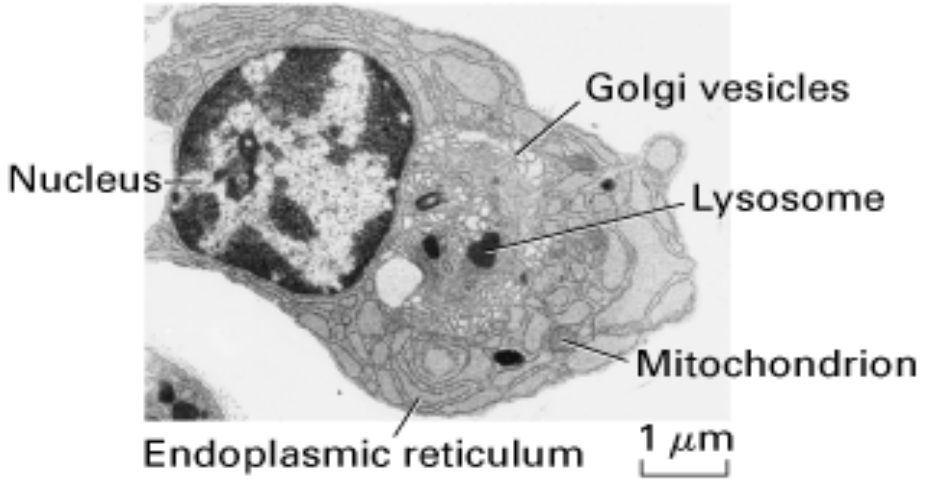
Killing by professional phagocytes

- 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

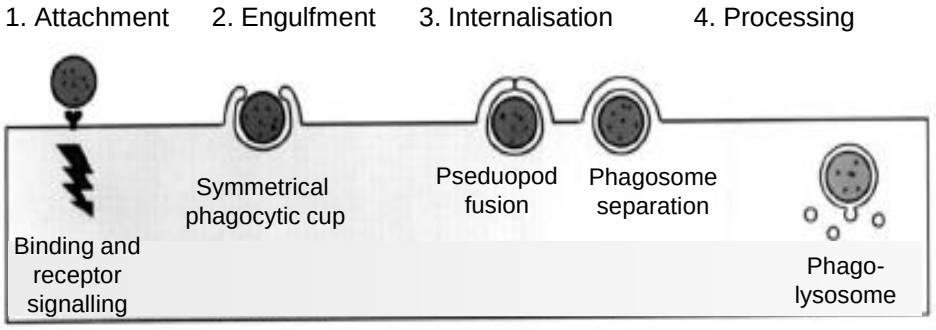
- Means of acquiring nutrients and/or transmitting signals
- Internalise receptors and their ligands, as well as particles and/or solutes in the extracellular environment
- Receptors are returned to the PM; constant turn-over (90%/h)
- Macromolecules for degradation are targeted to late endosomes and 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

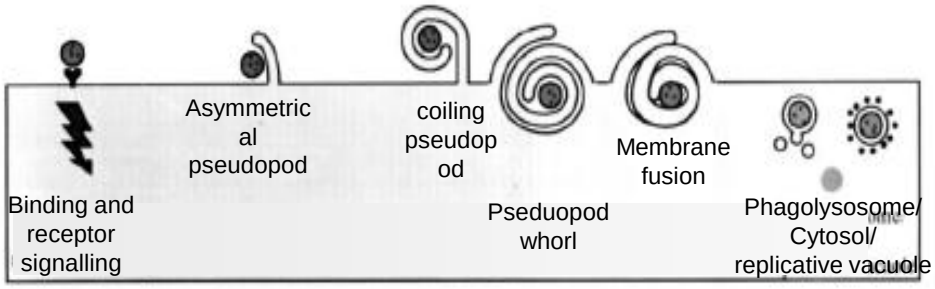
- Professional phagocytes (mϕ and PMNs)
  - FcR or CR mediated zipper phagocytosis (*Mycobacterium*)
  - Coiling phagocytosis (*Legionella*, spirochetes, *Leishmania*)
  - Triggered macropinocytosis (*Salmonella*)

- Non-professional phagocytes
  - Type of cell invaded dictated by ligand-receptor interaction
  - Receptor mediated zipper phagocytosis (*Yersinia, Listeria*)
  - Triggered macropinocytosis (*Salmonella, Shigella*)

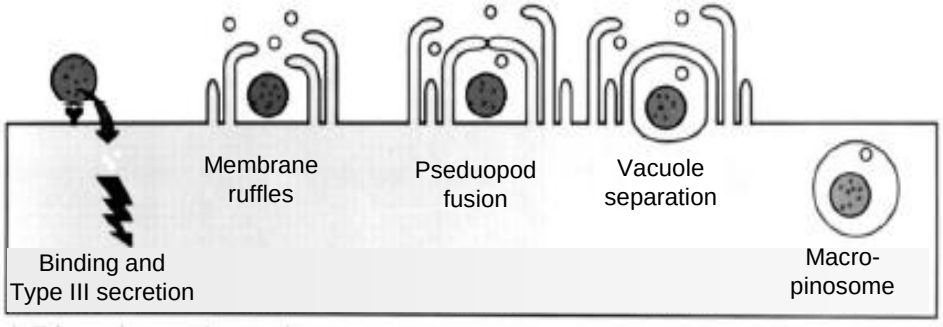
Phagocytosis and Macropinocytosis



Zipper phagocytosis



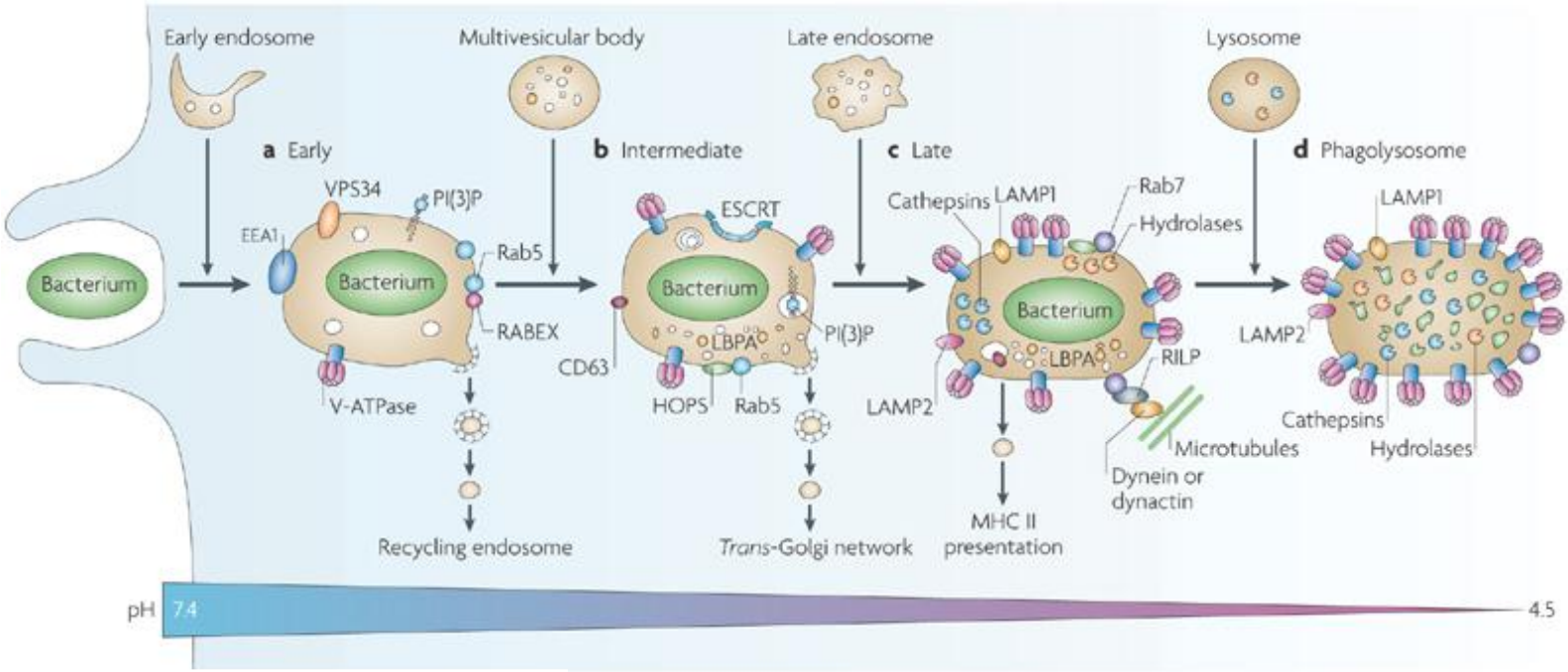
Coiling phagocytosis



Triggered macropinocytosis

Rittig *et al*., 1999

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

Flannagan *et al.* 2009

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
Ricketsia	Escape phogosome	Phospholipase A
Listeria	Escape phogosome	LLO, phospholipase C
Shigella	Escape phogosome	IpaB
Trypanosoma	Escape phogosome	TcTox
Legionella	Block lysosome fusion	Dot/Icm
Toxoplasma	Block lysosome fusion	Entry via caveolae
Chlamydia	Block lysosome fusion	Chlamydial protein
Mycobacterium	Block lysosome fusion	Entry via caveolae
Salmonella	Resist killing	TTSS, stress proteins
Brucella	Resist killing	LPS
Coxiella	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
Salmonella	Endosome	Yes	Some markers
Leshmania	Early endosome	Yes	
Legionella	ER	No	
Brucella	ER	Yes	No markers
Chlamydia	Inclusion	No	Chlamydial protein
Toxoplasma	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*

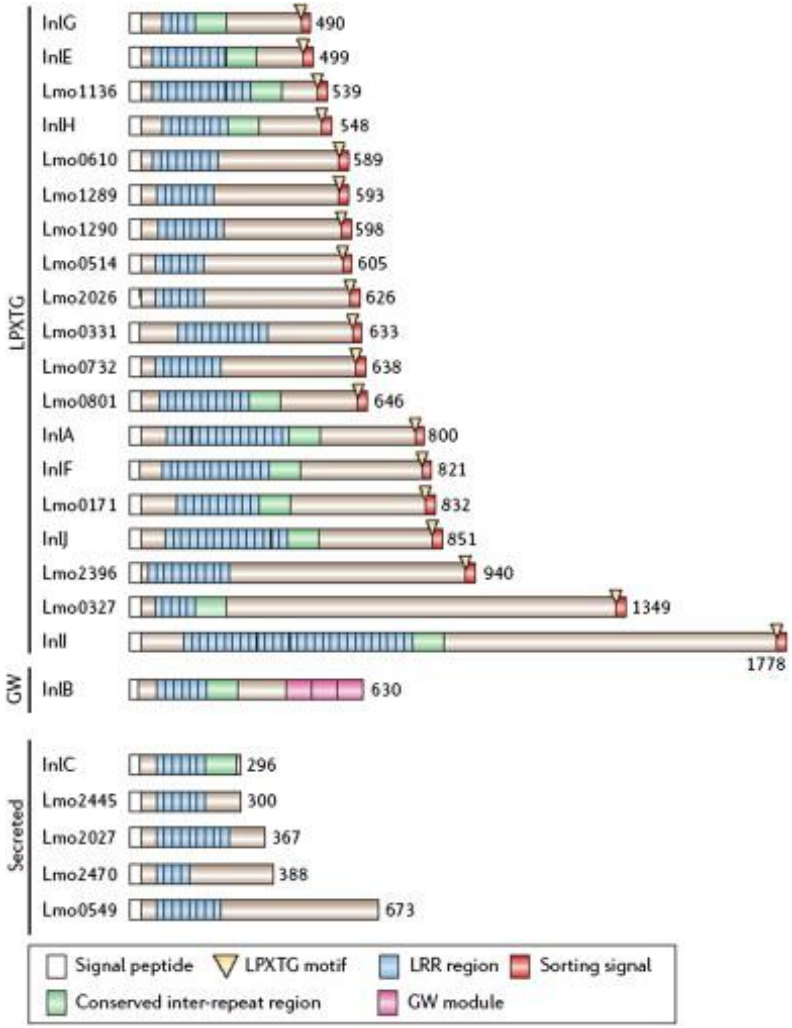
Listeria monocytogenes

- *L. monocytogenes*
  - ubiquitous Gm^+ 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
  - Injestion 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

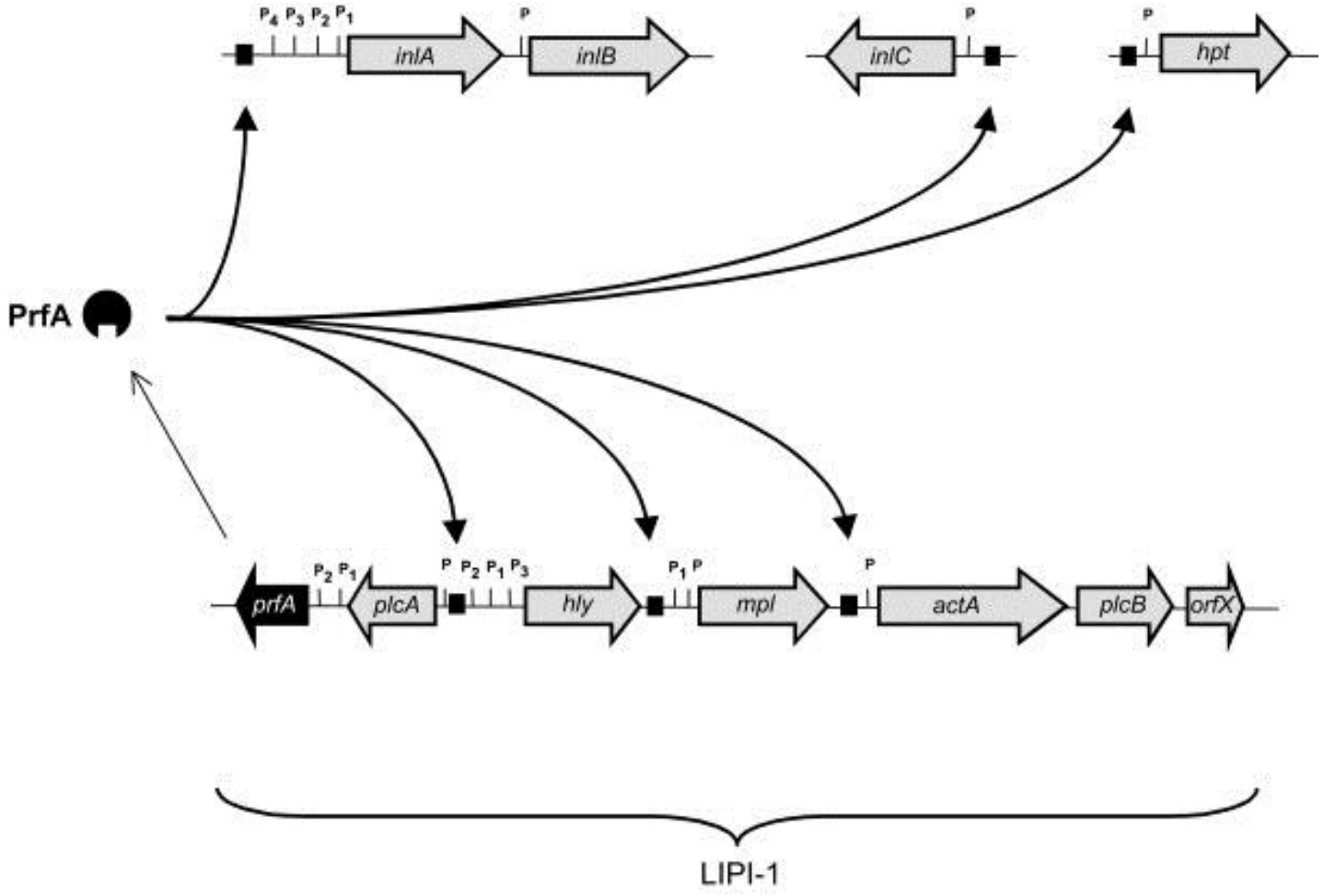
Listeria internalins



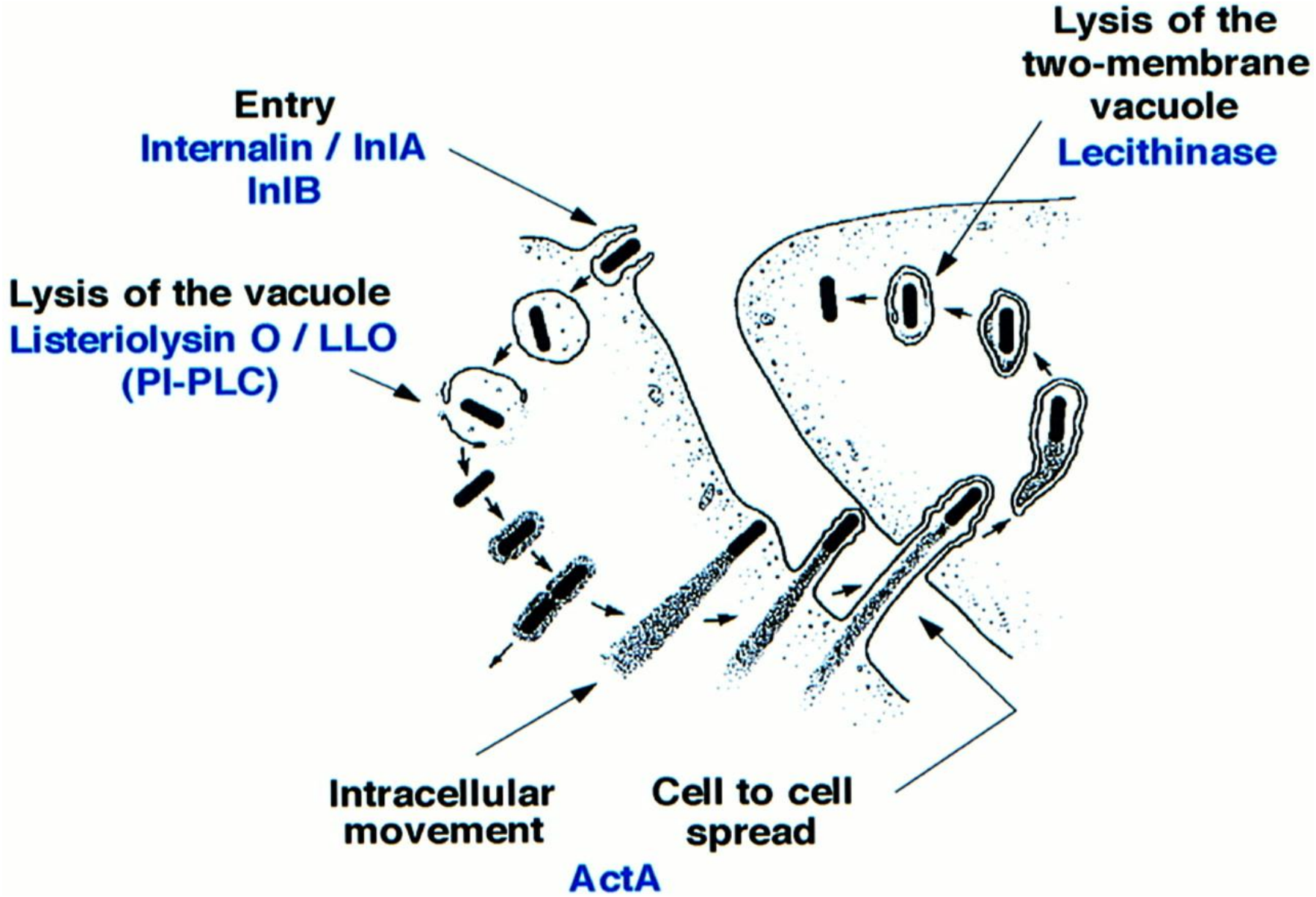
- Family of proteins with LRR thought to be involved in specific protein-protein interactions
- InlA
  - Binds E-cadherin, a protein found on the surface of intestinal epithelial cells, hepatocytes, dendritic cells, brain microvascular endothelial cells, and epithelial cells of choroid plexus and placental chorionic villi
  - Binding leads to actin cytoskeleton rearrangements via α- and β-catenins
- InlB
  - Binds gC1q-R found on wide range of cells
  - Binding induces membrane ruffling and tyrosine phosphorylation of several host proteins

Seveau *et al*., 2007

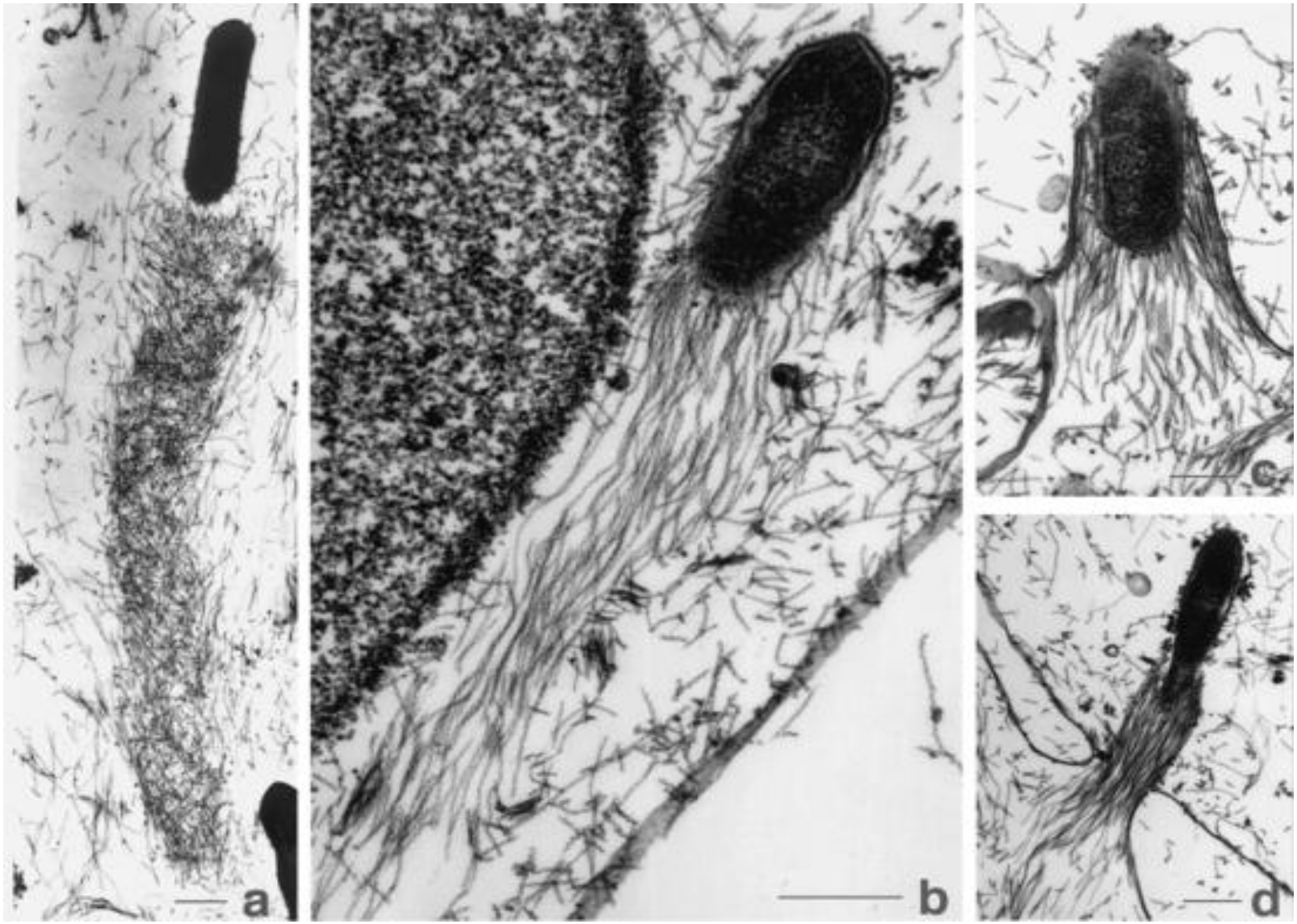
Co-ordinate gene regulation



Scortti *et al.*, 2007

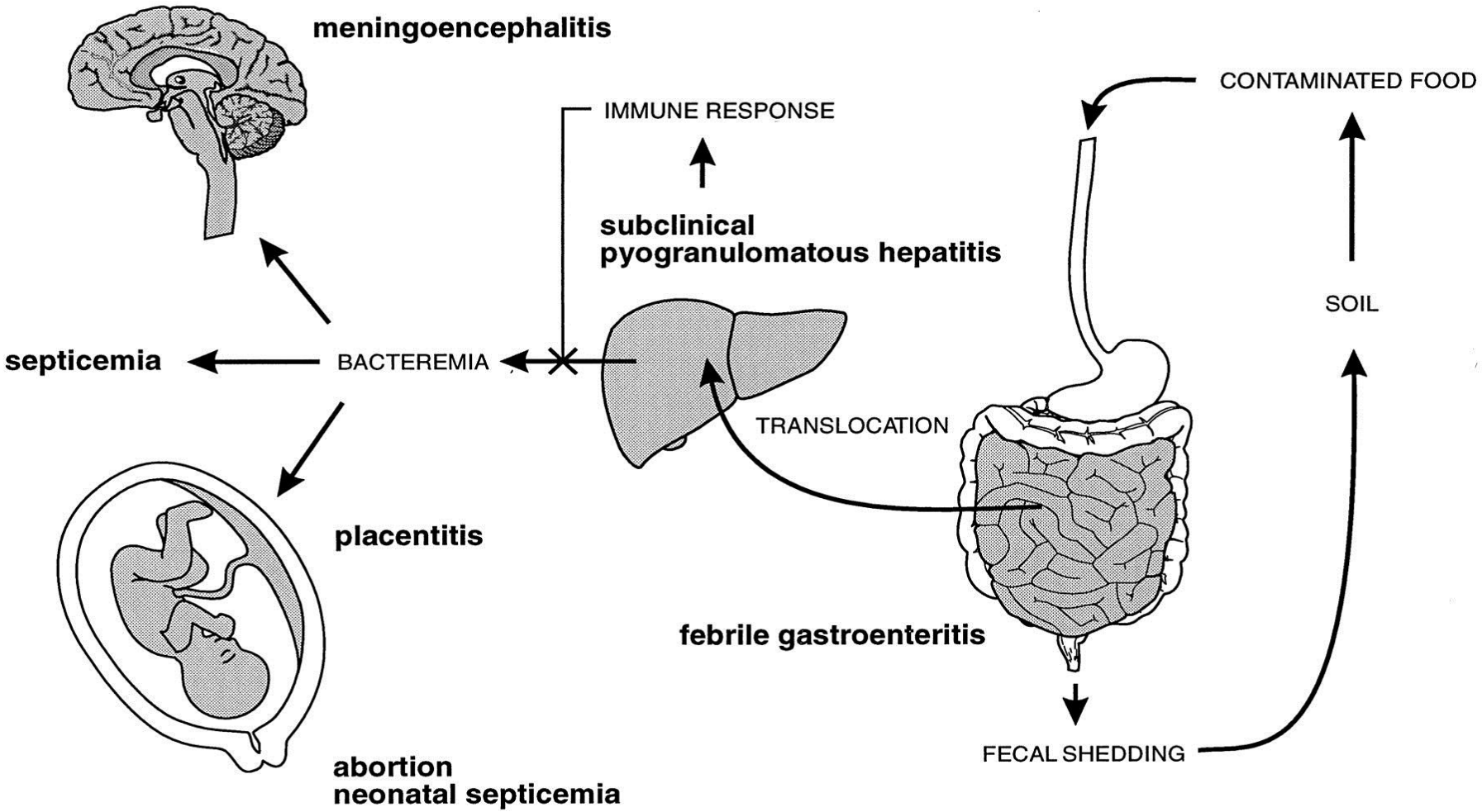


Tilney & Portnoy 1989



Cossart, 2000

Pathogenesis of *Listeria* infection



Vazquez-Boland et al., 2001

Shigella dysenteriae

- *S. dysenteriae*
  - Gm^- 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

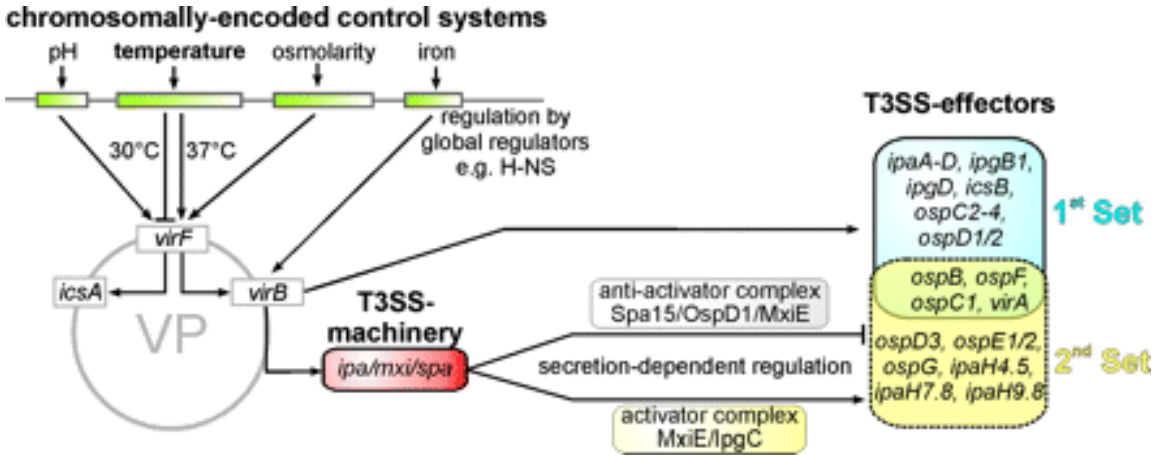
Shigella dysenteriae

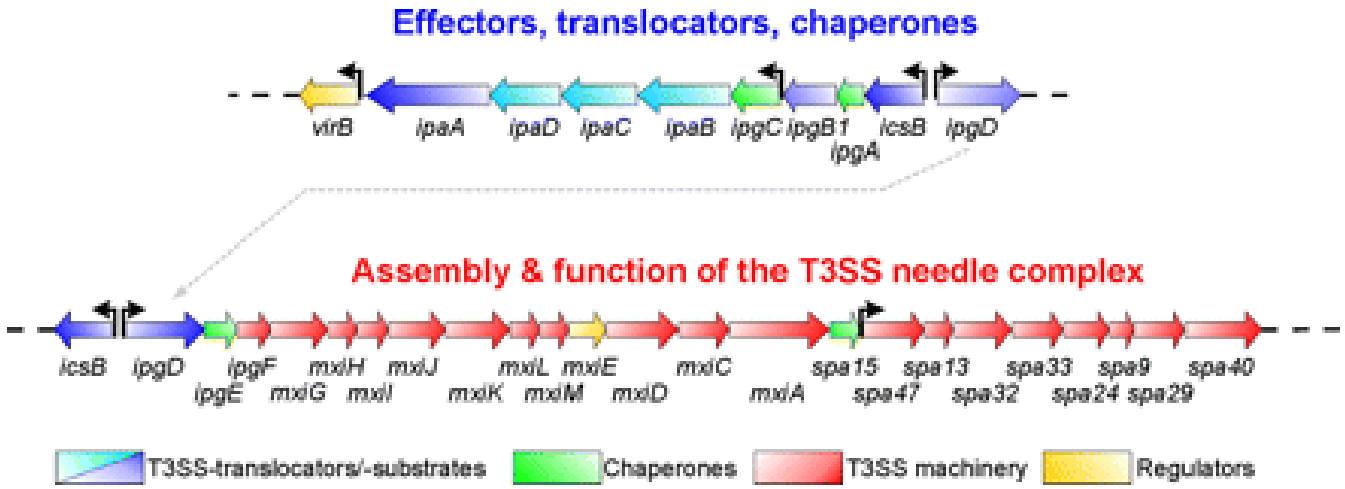
- Pathogenesis
  - Cross intestinal epithelium via M cells
  - Taken up by macrophages, cause apoptosis via IpaB
  - Invade epithelial cells via basolateral surface using Type III secretion system
  - Cells released into cytoplasm via IpaB
  - Cell to cell spread via IcsA mediated actin polymerisation

Shigella Type III secretion system

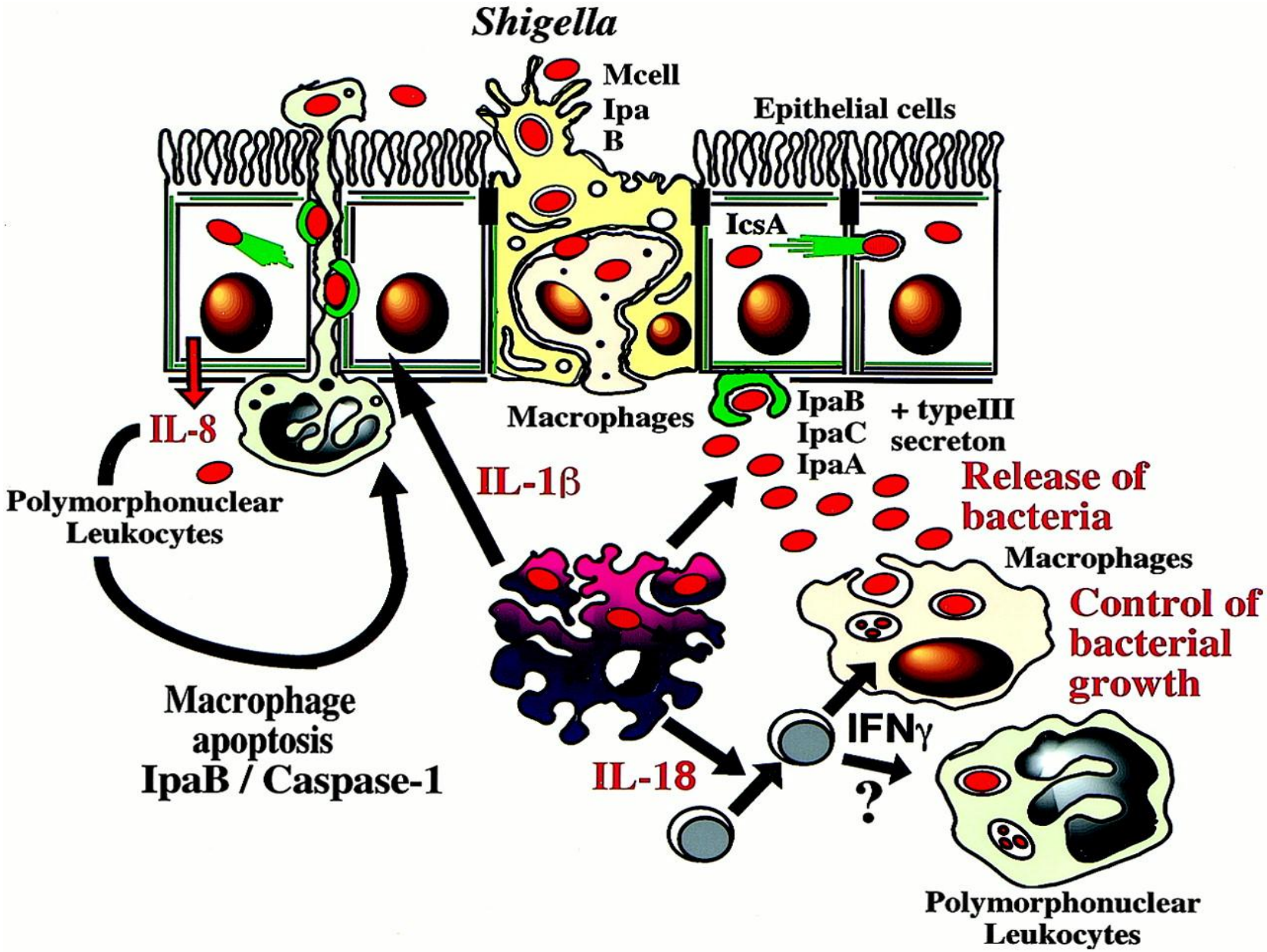
- Pathogenicity island
  - Mxi-Spa proteins
  - Ipa proteins
- Mxi-Spa proteins
  - Secretion apparatus
- Ipa proteins
  - IpaB and IpaC form extracellular complex and insert into host cell membrane to form a pore
  - IpaC intracellular domain induces actin polymerisation
    - Uptake of bacteria by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

Co-ordinate gene regulation





Schroeder and Hilbi, 2008


Shigella
Mcell
Ipa
B
Epithelial cells
IcsA
IL-8
Polymorphonuclear
Leukocytes
Macrophages
IpaB
IpaC
IpaA
+ typeIII
secreton
Release of
bacteria
IL-1β
Macrophages
Control of
bacterial
growth
Macrophage
apoptosis
IpaB / Caspase-1
IL-18
IFNγ
?
Polymorphonuclear
Leukocytes

Sansonetti

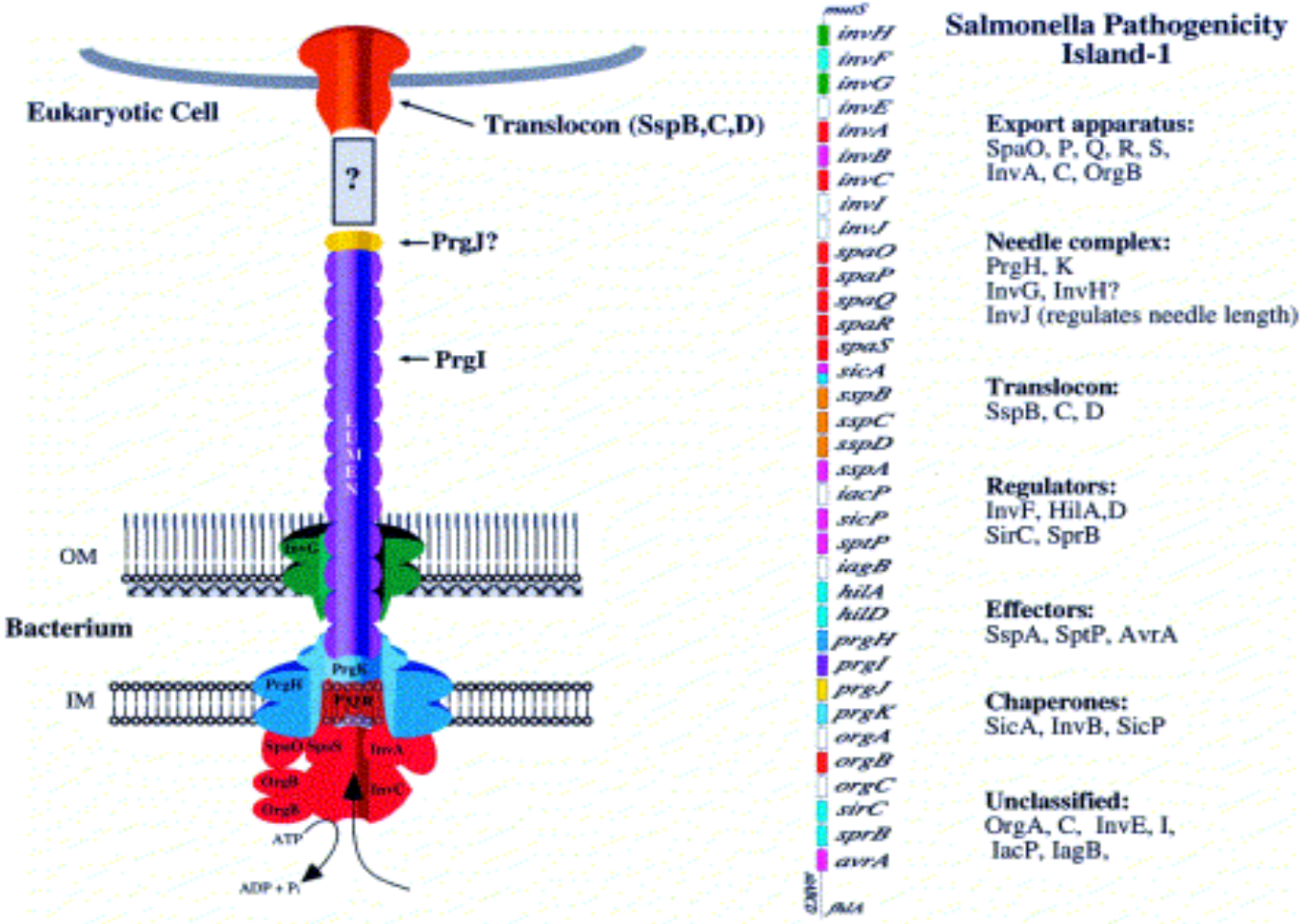
Salmonella typhimurium

- *S. enterica* serovar *typhimurium*
  - Gm^- 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

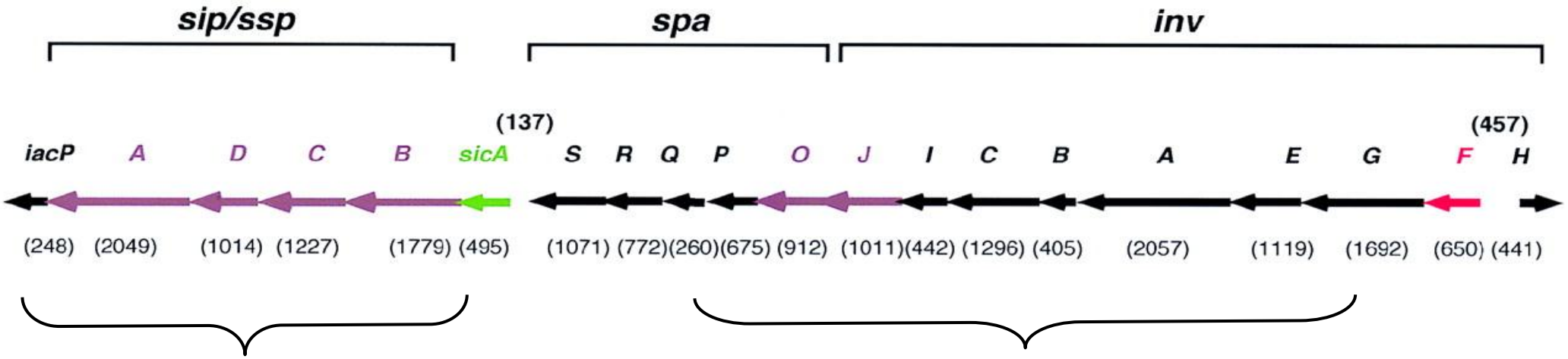
SPI-1 encoded Type III secretion system



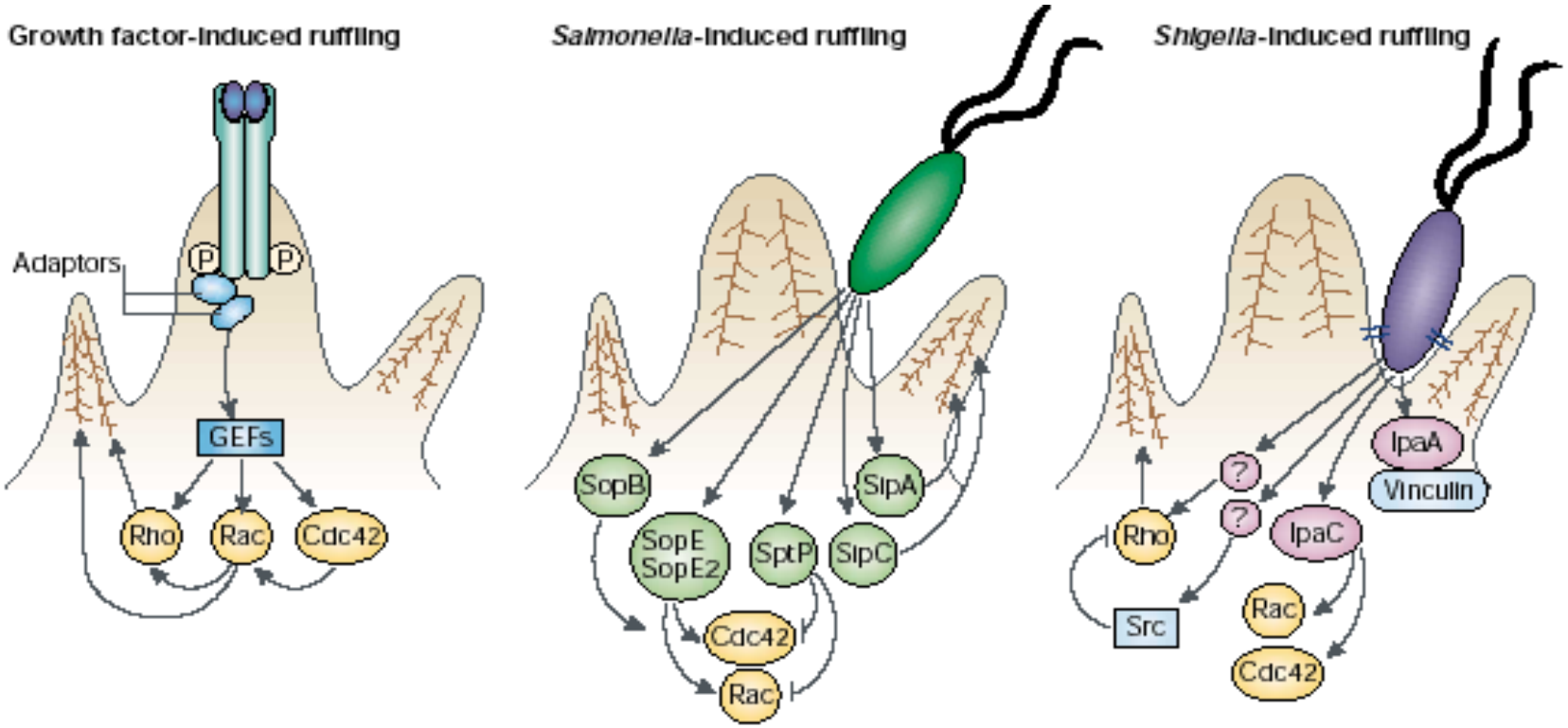
Kimbrough and Miller, 2002

Co-ordinate gene regulation: invasion

Membrane ruffling - requires SPI1



Invasion by *Salmonella* and *Shigella*

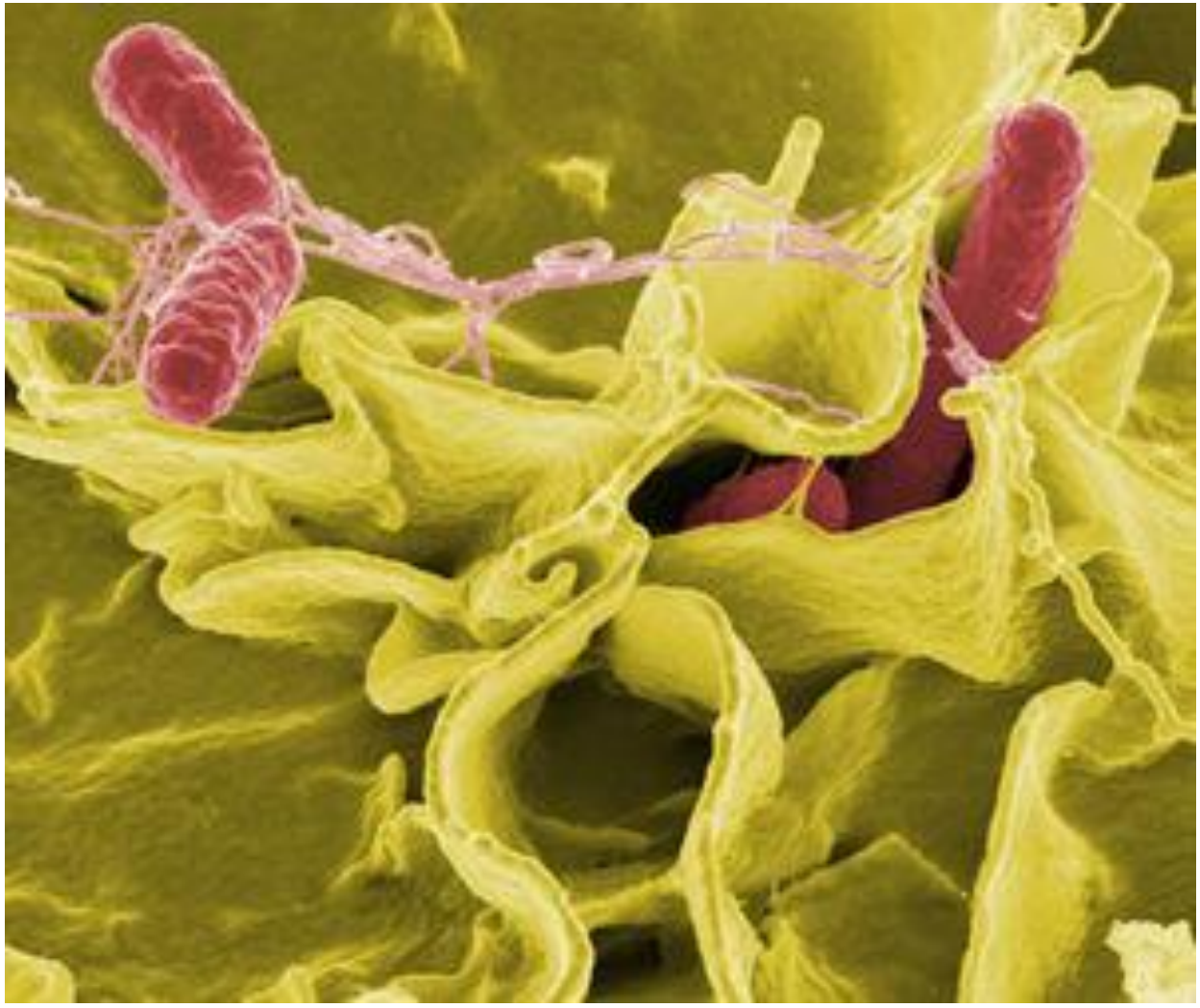


SipA, SipC and SipB homologous to Ipa A, IpaB and IpaC

presumed to have same function and mechanism of action

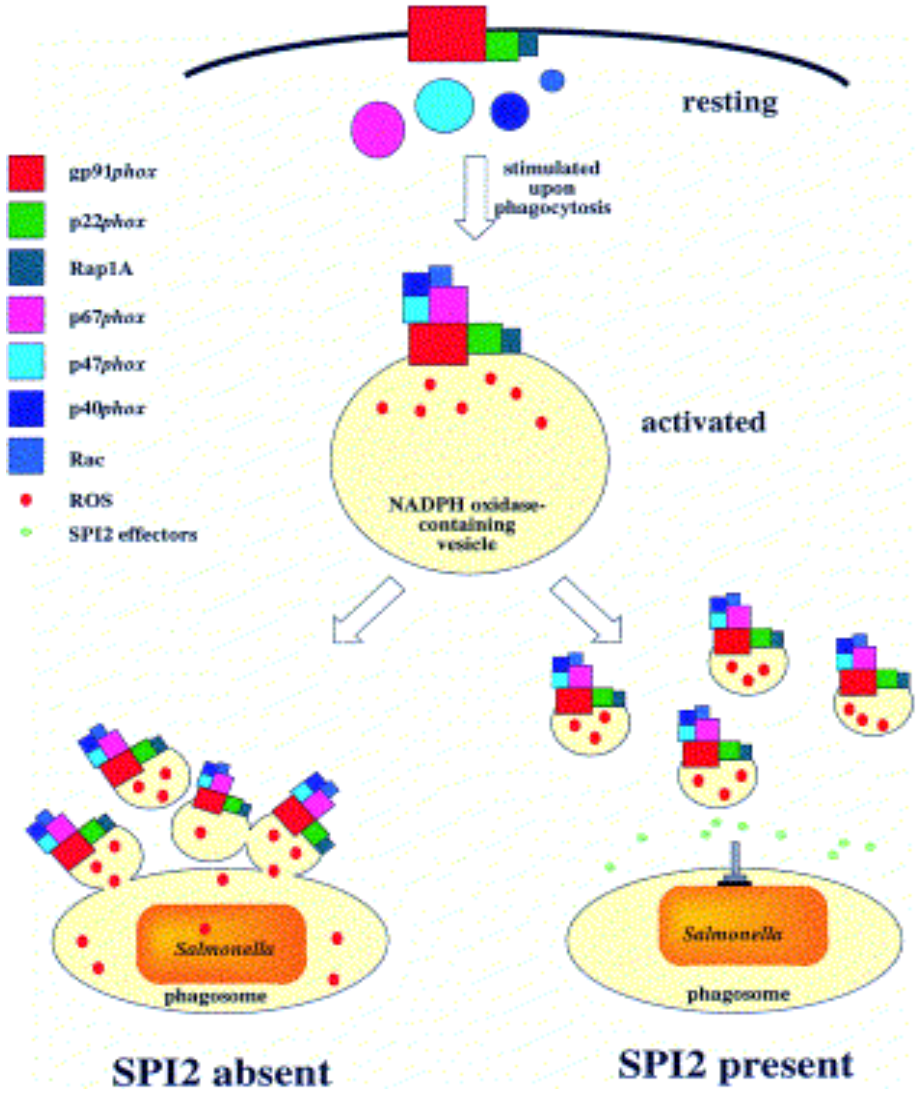
Knodler *et al*., 2001

Membrane ruffling



MicrobeWiki

Survival of *Salmonella* in SCVs

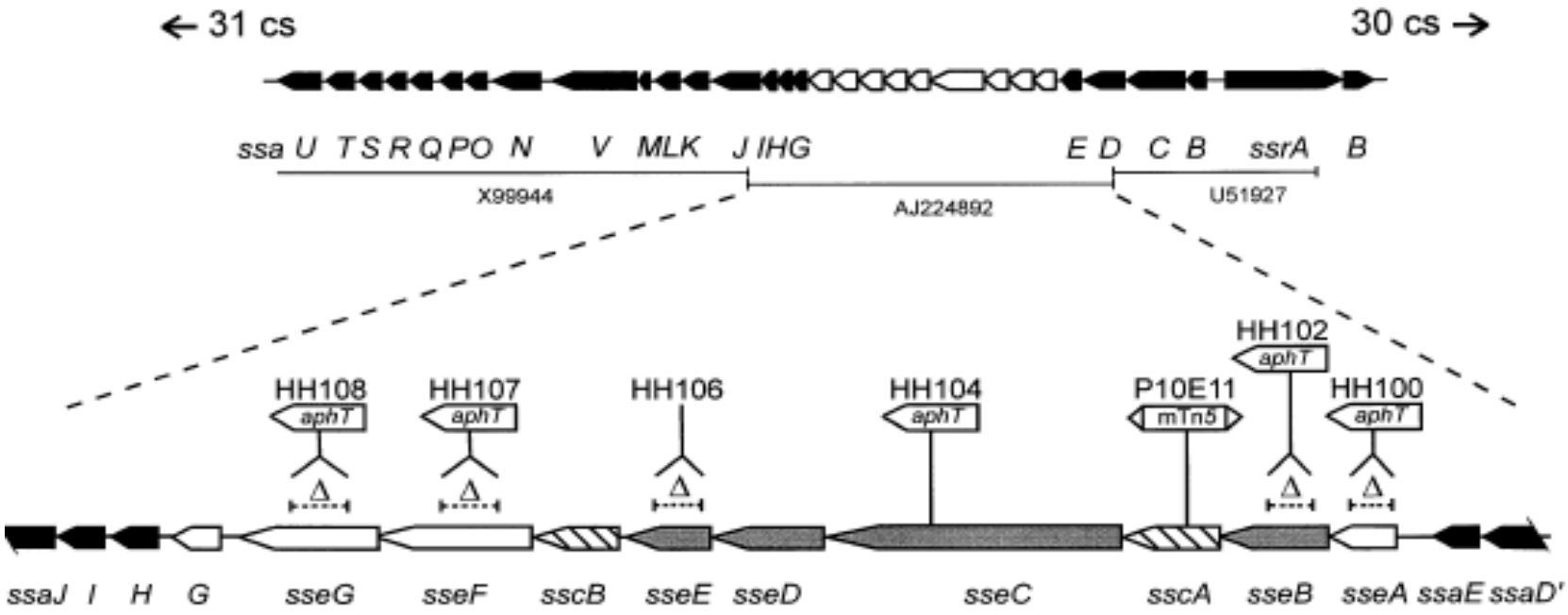


- SPI-2 proteins prevent fusion of NADPH oxidase-containing endosome to SCV
- Production of stress response proteins, enzymes, and scavengers that detoxify reactive oxygen species (ROS), also contribute to survival in phagosomes

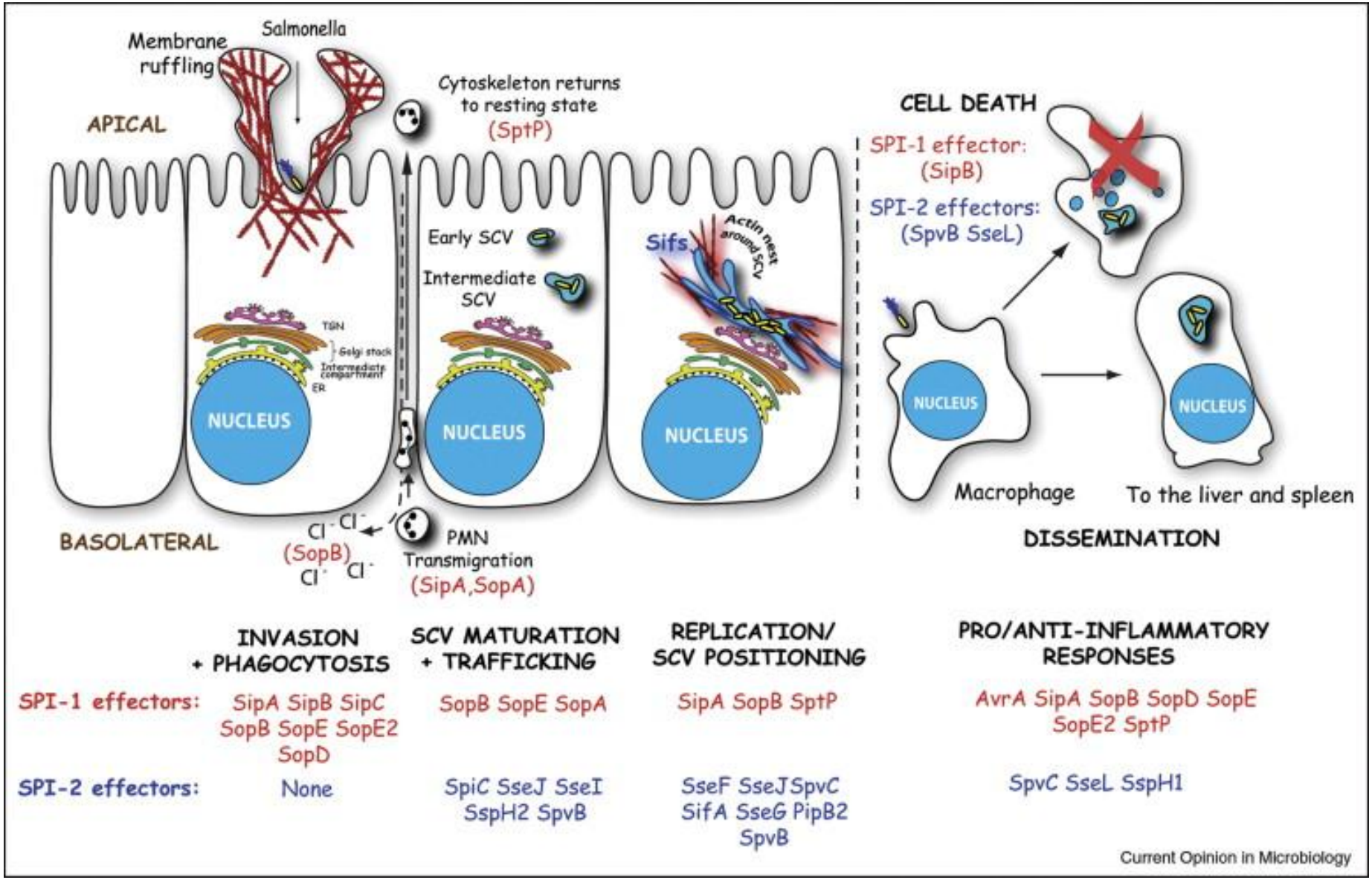
Vazquez-Torres and Fang, 2001

Co-ordinate gene regulation: survival

Requires SPI-2 encoded type III secretion system



Hensel *et al*., 1998



Koronakis., 2009

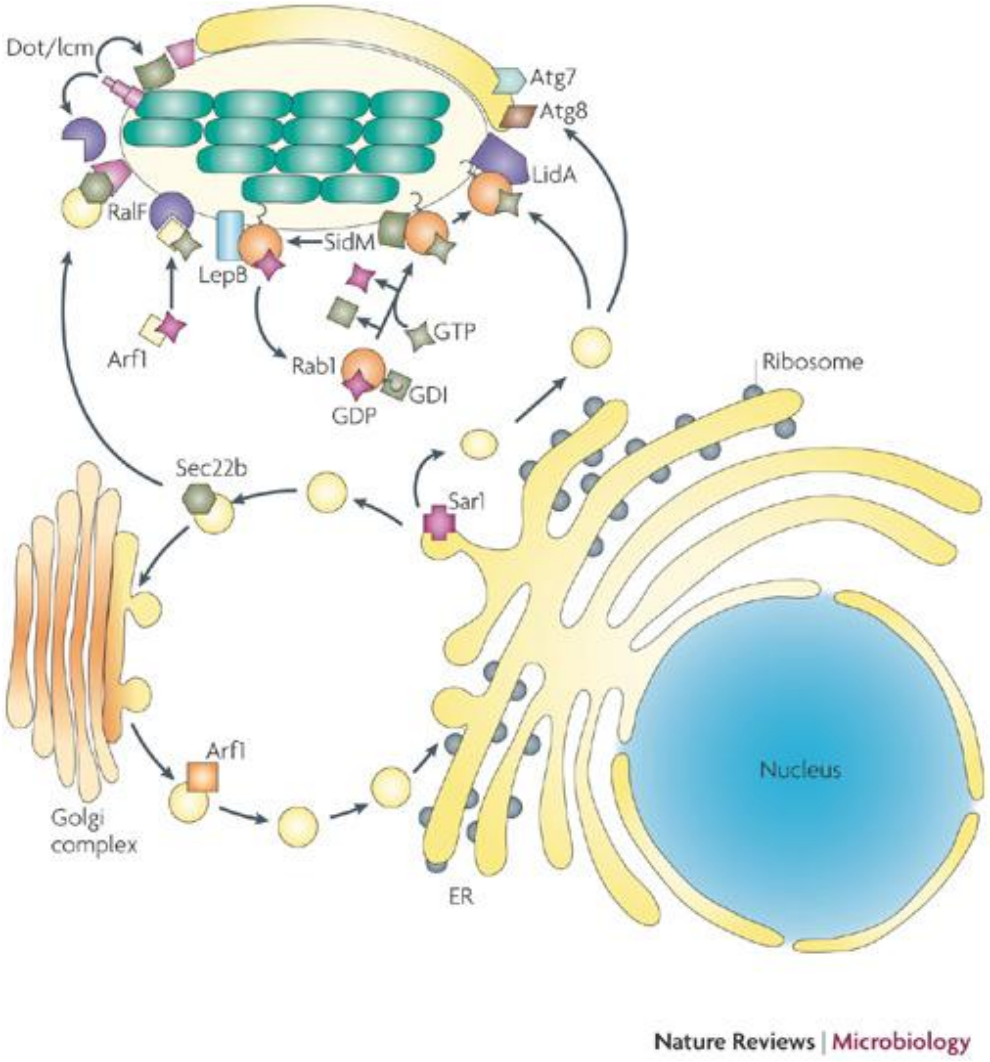
Legionella pneumophila

- *L. pneumophila*
  - Gm^- 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 injestion by alveolar macrophages (via coiling phagocytosis)
  - Phagosomes contaning 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

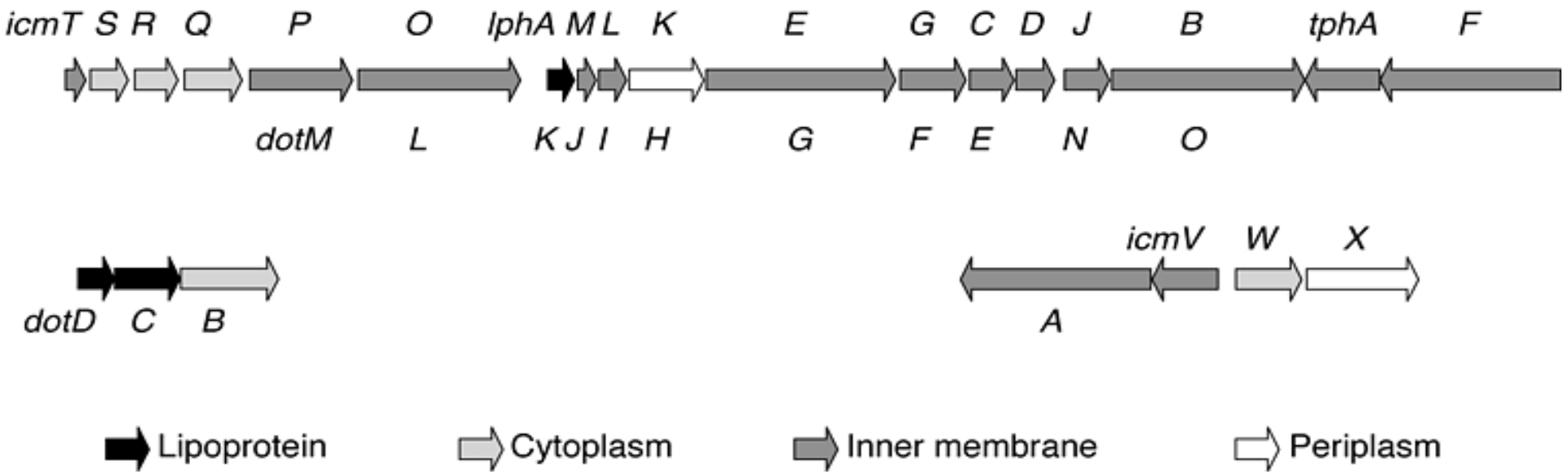
Type IV secretion system of *Legionella*



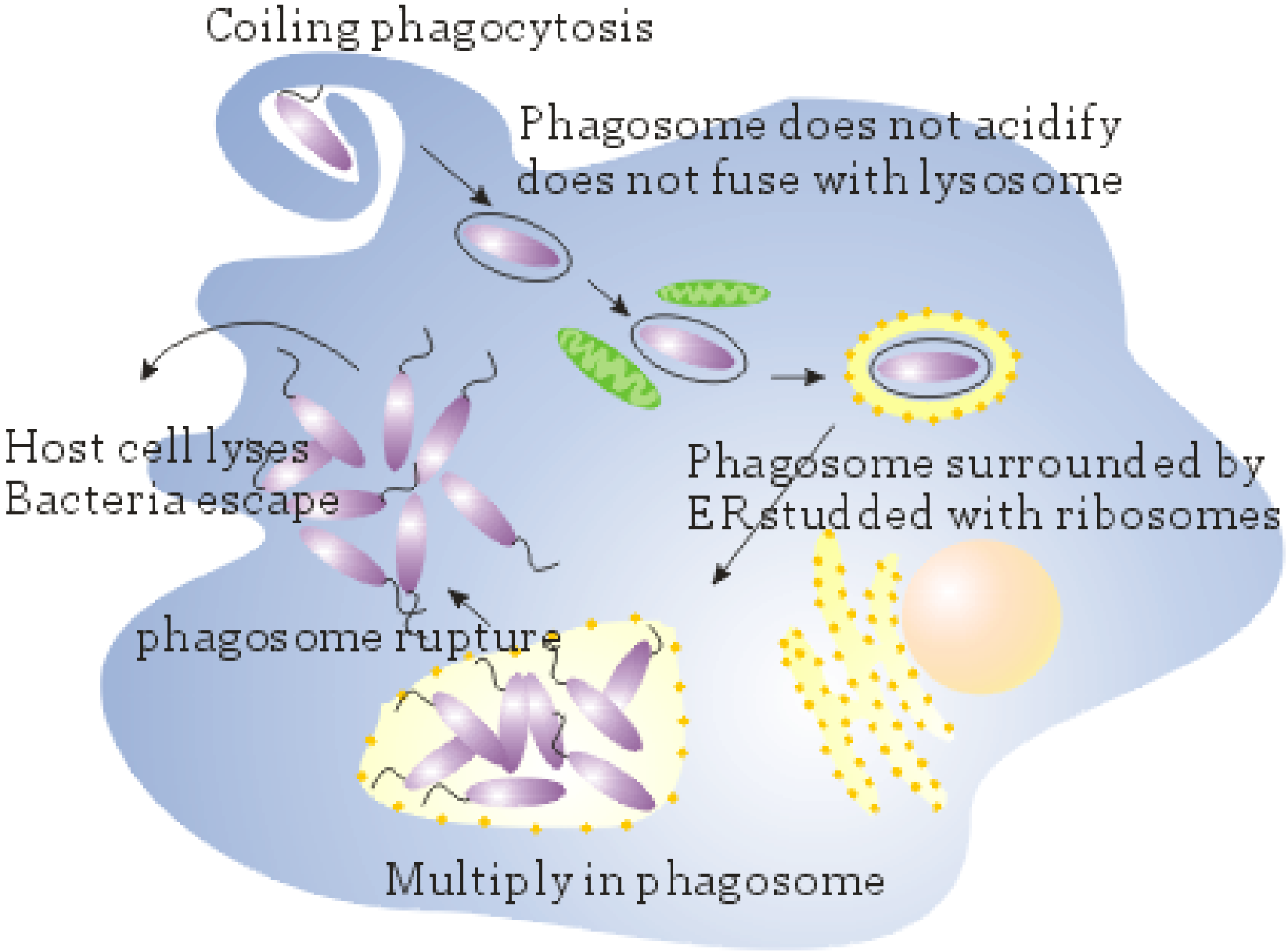
- Some Dot/Icm proteins form a channel through vacuole membrane
- Other Dot/Icm proteins are effectors that interact with host cell vesicle trafficking proteins
- Allows association with mitochondria and RER, avoiding normal targeting to lysosome
- Legionella replicate rapidly in the vacuole

Isberg et al., 2009

Co-ordinate gene regulation



Segal and Shuman, 1998



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