Chapter 11 Survival strategies of pathogens in the host

a.a. 2018-19

Survival strategies of pathogens

In order to survive in a host a pathogen must be able to

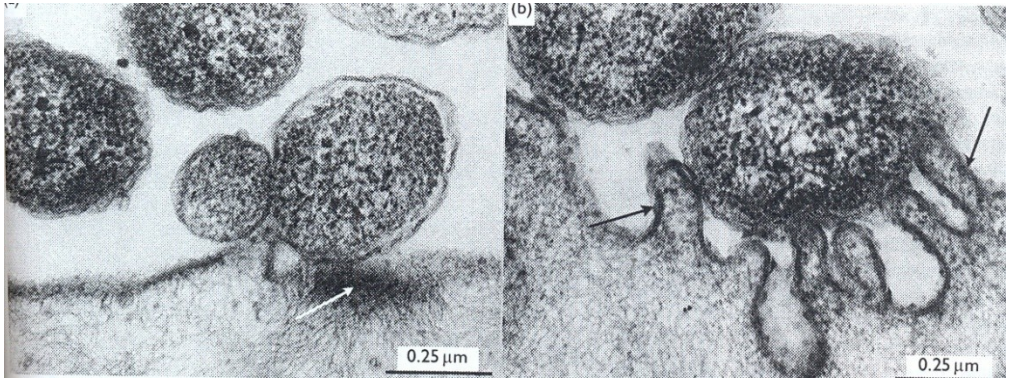
- Penetrate into the body
- Attach to host cells for colonization
- Obtain nutrients which my be limiting within the host in order to multiply
- Disseminate or spread within the host and to other hosts
- Evade the host's innate and adaptive immunity to persist in the host

Attach to host cells for colonization

An essential step in the successful infection and production of disease by microbial pathogens is their ability to interact with host cell components.

Physical attachment of bacteria to host tissues (**adhesion)** is accomplished by **specific molecular interaction** accompanied by changes in the phenotype of the bacterium and changes in the behavior of the host cell.

The ability to adhere enable a pathogen to target itself to a particular tissue (**tissue tropism**).



Adesion of H. influenzae to human oropharyngeal cells. M Wilson

- **Pathogens growing extracellularly** on the apical surface of an epithelium: adhesion needs to resist to mechanical clearing mechanisms or to overcome the normal microbiota;
- **Invasive pathogens:** adhesion is the first step that precedes their internalization within host cells. It needs to cross the epithelial layer and to penetrate deeper within host tissues where further interactions with the underlying **extracellular matrix (ECM).** Damaged ECM can be the basis for initiation and progression of bacteria infection.

Bacterial structures involved in adherence to host cells

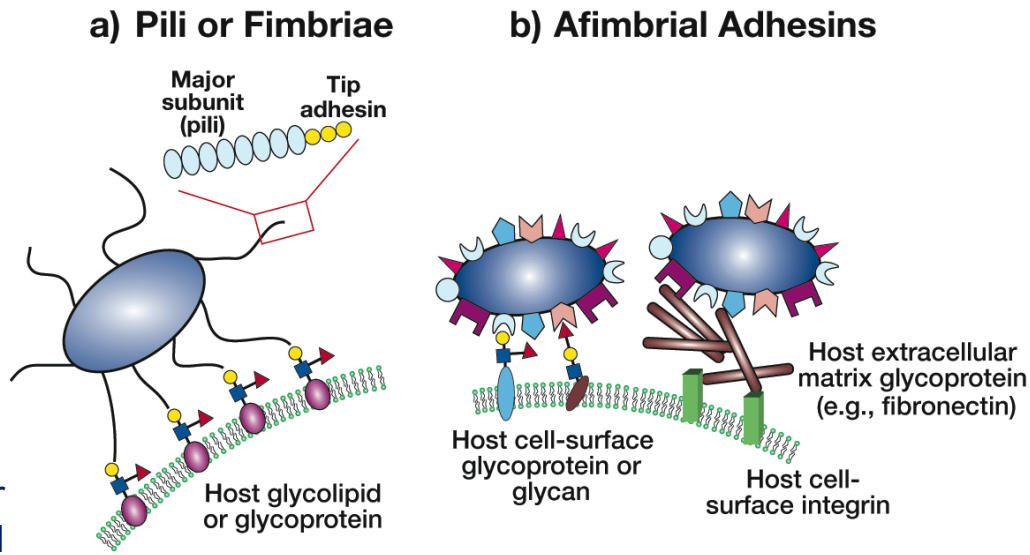
Bacteria have evolved a very large arsenal of molecular strategies allowing them to target and adhere to host cells. Often they are synergistic in their function, and their expression is regulated on the basis of different environment.

Different type of **adhesins**:

a) at the tip of a scaffold-like structure on the bacterial surface (**pili or fimbriae**)

b) anchored in the bacterial surface exposed (afimbrial or **non polymeric adhesins**).

Pathogens bind to host cell receptors, or to soluble proteins such as the ECM proteins or blood proteins (complements) that serve as a bridge between the bacterium and host cell surface.



Examples of mechanisms of bacterial adherence to host-cell surfaces

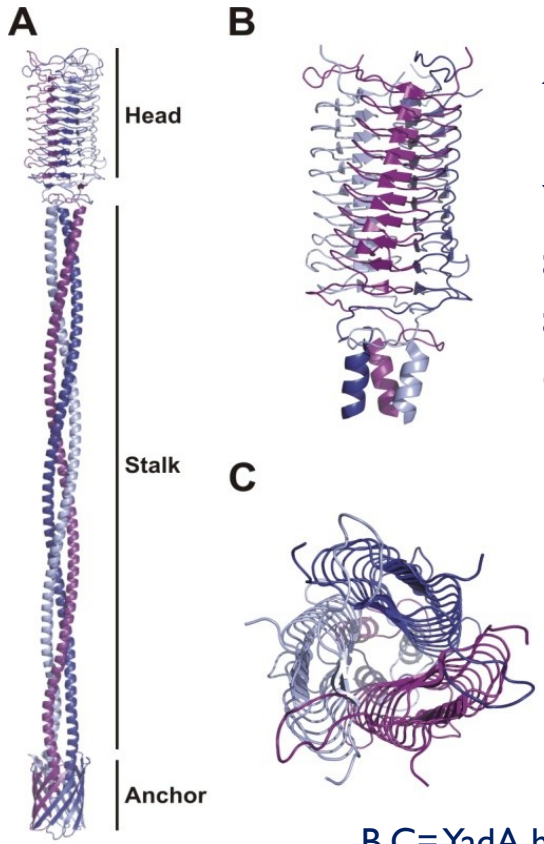
Cell adhesion molecules as receptors

Non polymeric surface proteins that mediate the tighter binding of bacteria to host cell. These adhesins are important components of the system that allow bacteria to attach to and, in same cases invade host cells.

Pathogen	Ligand (adhesin)	Counterligand (receptor)
B. pertussis	FHA (RGD domain)	integrins
N. gonorrhoeae/meningitidis	Opa proteins	integrins
Staphylococcus	FnBP, LTA,	Fibronectin
Streptococcus	LTA, M protein, FnBP	Fibronectin
Yersinia	YadA	Fibronectin, Collagens
N. gonorrhoeae	Opc	HS proteoglycans, Fibronectin,
E. coli EPEC EHEC	Intimin	intimin receptor (Tir)
Listeria	Internalin,	E- cadherin
Shigella	IpaB, IpaC	CD44, integrin $\alpha_5\beta_1$
Yersinia	Inv (Invasin)	$\alpha_{3\text{-}6}\beta_1$ Integrin

Receptors: integral host membrane receptors and components of extracellular matrix,

Non-polymeric adhesins: the trimeric autotransporter protein YadA of pathogenic Yersinia.



Structure of **YadA** of enteropathogenic species of *Yersinia*. It is an essential virulence factor of *Y. enterocolitica*, and removing this protein from the bacteria leads to avirulence.

YadA is the prototype of the subfamily of trimeric autotransporters, in which three autotransporter subunits associate to form the functional pore. YadA shows a extended triple α-helical coiled coil stalk attached to the β-barrel anchor and an N-terminal head with adhesive properties.

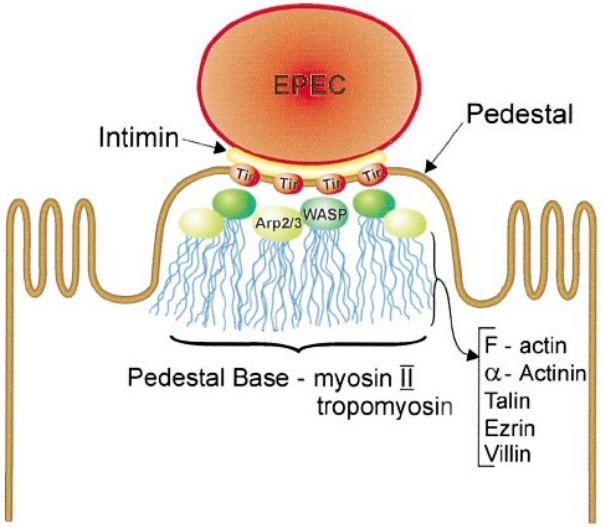
YadA head contain different binding site that mediates adhesion to collagens, laminin, and fibronectin.

B,C= YadA head domain in side and top views

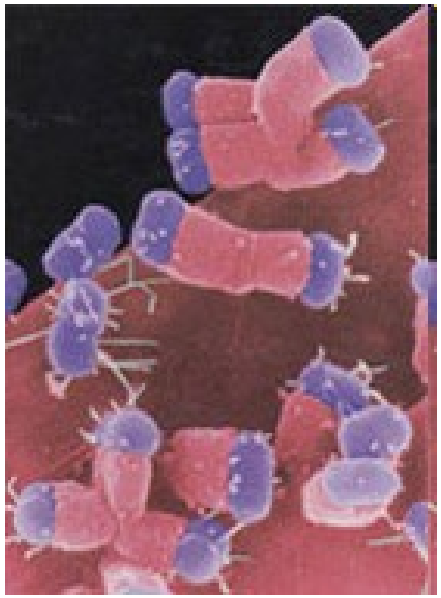
http://www.helsinki.fi/goldman/Pages/research3.html

Bacterially-Encoded Cellular Receptor

E. coli EPEC and EHEC have developed an original bacterial adhesion system to create an intimate contact with host cells.
These pathogens induce characteristic lesions known as the "**attaching and effacing**". After attachment to intestinal epithelial cells bacteria induce the local effacement of absorptive microvilli and the formation of pedestal-like structures on which bacteria seat.



EPEC forming attaching and effacing lesions on epithelial cells in culture. (Stuart Knutton, Imperial College, London)



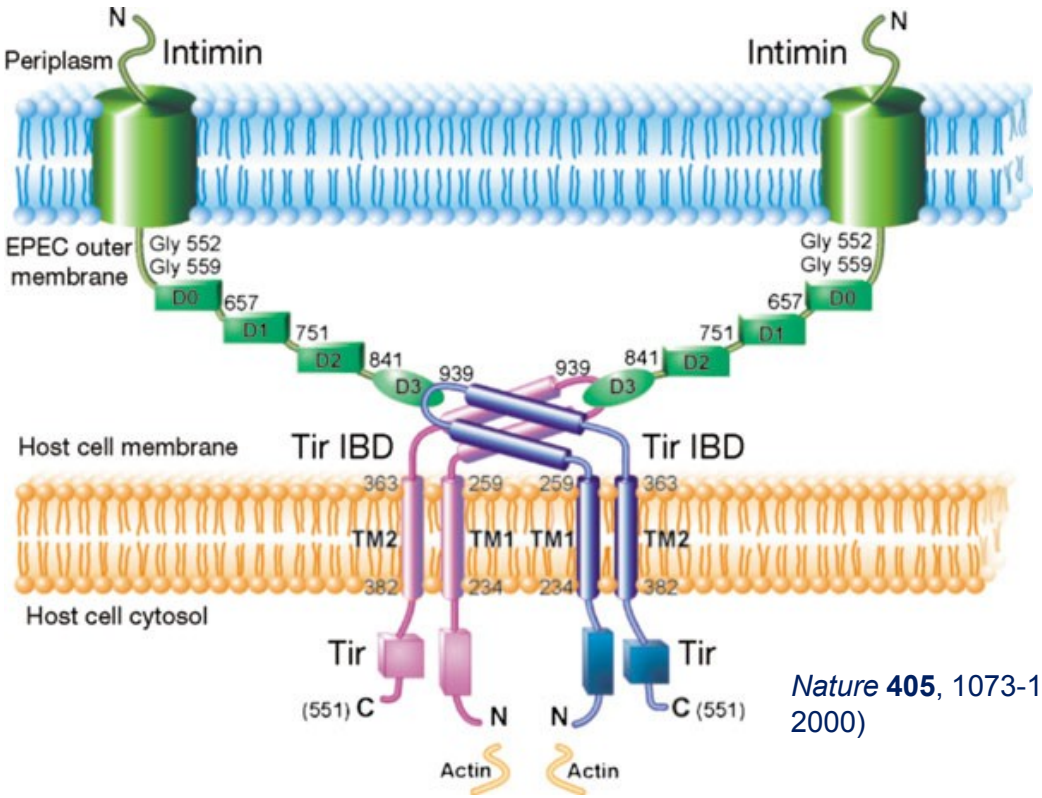
ThroughT3SS (coded by PAI LEE) they inject into the host **Tir** effector protein, that inserts into the host cell plasma membrane and serves as an "exogenous" receptor for the bacterial surface adhesin **intimin** into host target cells.

Tir is **phosphorylated** by host kinases and is involved in recruitment of host actin nucleators (WASP, Arp2/3) that in turn locally remodels **actin cytoskeleton** leading to the formation of bacterial-associated pedestals.

Model of the complex between intimin and Tir receptor

Outer membrane

Host cell membrane



Nature **405**, 1073-1077 (29 June 2000)

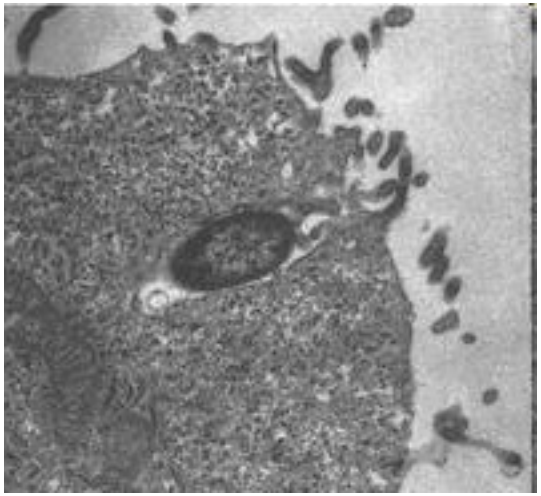
Intimin is shown in green colour. The Ig-like domains are shown as rectangles, and the lectin-like domain D3, which binds to the Tir intimin binding domain (IBD), as an oval. The N-terminal domain of Tir anchors host cytoskeletal components.

Bacterial invasion as a virulence mechanism

Some pathogens (**invasive bacteria**) are able to **penetrate into host cells** by crossing the epithelium. They induce their own phagocytosis into cells (epithelial and endothelial cells) that are **normally non-phagocytic** and that are not generally capable to engulfing particles as large as bacteria.

In bacterial-induced phagocytosis, **bacterium is an active player** in the complex interplay between the invading microbe and the host cell.

To enter non-phagocytic cells invasive bacteria express adhesins to bind eukaryotic cell adhesion molecules such as surface receptors involved in cell-matrix (integrins) or cell-cell adherence (cadherins).

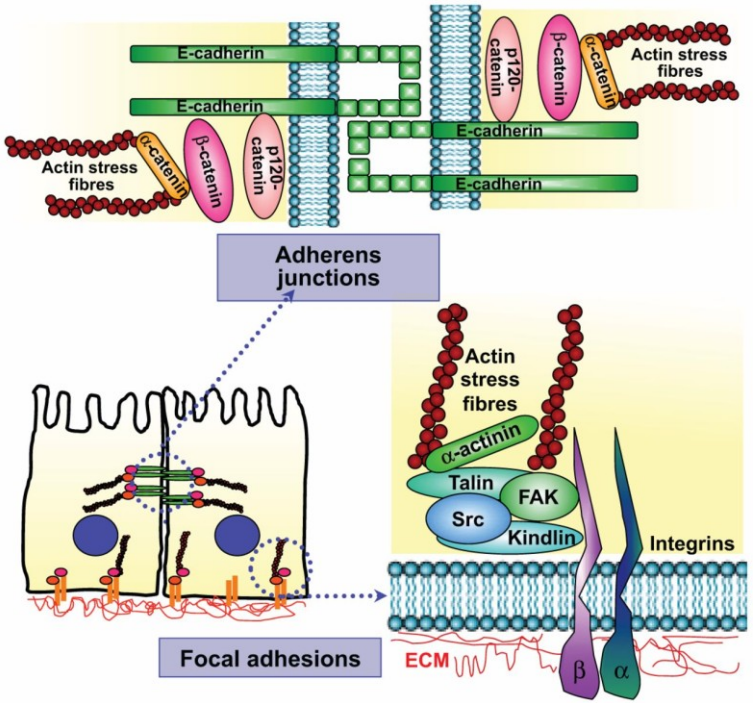


Adhesion and invasion of entero-pathogenic bacteria into human epithelial cells (Dersch/Kaulbars/Özel, RKI 2004)

An intracellular lifestyle provides advantages such as to become inaccessible to humoral and complement attack, to avoid shear stress-induced clearance, to get access to a wide range of nutrients.

Cell adhesion molecules:

Cell Adhesion Molecules are the molecules responsible for creating **cell junctions** that connect the epithelial and non epithelial cells each other and with ECM.



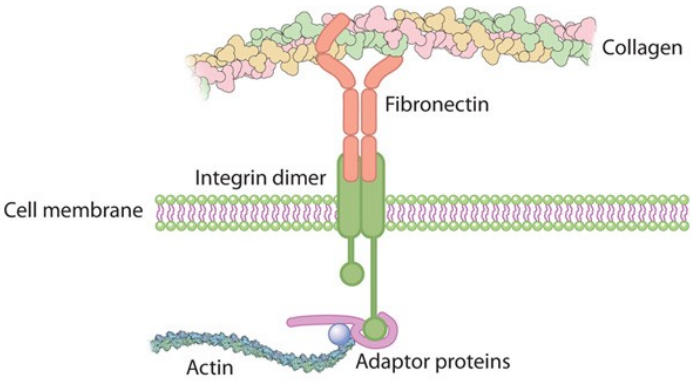
Marta Canel et al. J Cell Sci 2013;126:393-401

E-cadherin, single-pass transmembrane protein, whose extracellular domain, which is composed of five Ca^{2+}-binding repeats (green squares), mediates specific **homophilic interactions** with neighbouring cells (adherens junction).

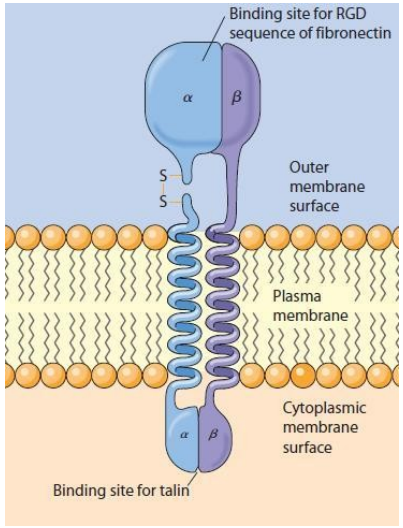
Focal adhesions are multi-protein complexes that mediate the contact of cells to the ECM (red lines); the membrane receptors for this type of adhesion are heterodimers of **α- and β-integrins.**
They form multi-protein complexes that are linked to the actin cytoskeleton

Cell surface receptors: integrins

More than 20 members of **heterodimeric transmembrane proteins.** Different subunits α and β let to obtain combinatorial diversity.



Integrins bind to **RGD motif** present in **fibronectin**, and other recognition sequences in collagen and laminin providing a physical linkage between the ECM and the internal cytoskeleton.



Integrins typically exhibit low affinities for their ligands: multiple weak interactions generated by the binding of hundreds or thousands of integrin molecules to their ligands on ECM allow a cell to remain firmly anchored to its ligand-expressing target. The weakness of individual integrin-mediated interactions facilitates cell migration.

Cell-Cell adhesion: cadherins

Key molecules in **cell–cell adhesion** and cell signaling. They play a critical role during tissue differentiation.

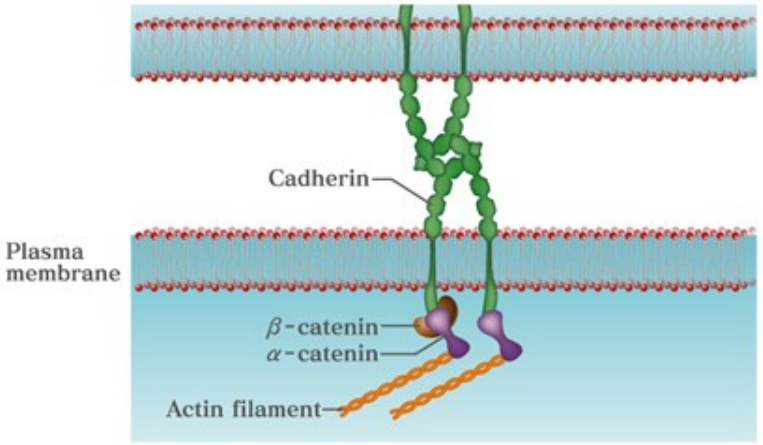
Cadherins are a class of membrane glycoproteins folded into polypeptide **chain repeats.** Adhesiveness of cadherins depends on the presence of extracellular Ca^{2+}, allowing interaction with similar domains on other cell (**homophilic interactions).**

Homophilic interactions (**adherens junction)** between E-cadherins lead to the selective adhesion of epithelial cells to one another.

Cadherins not only mediate cell-cell adhesion, but also influence the establishment of cytoskeletal networks.

The intracellular domain of cadherin is bound with **catenins** (signal transducer proteins) and **actin filaments**

Stable adhesion junctions involving the cytoskeletons of adjacent cells are mediated by cadherins.

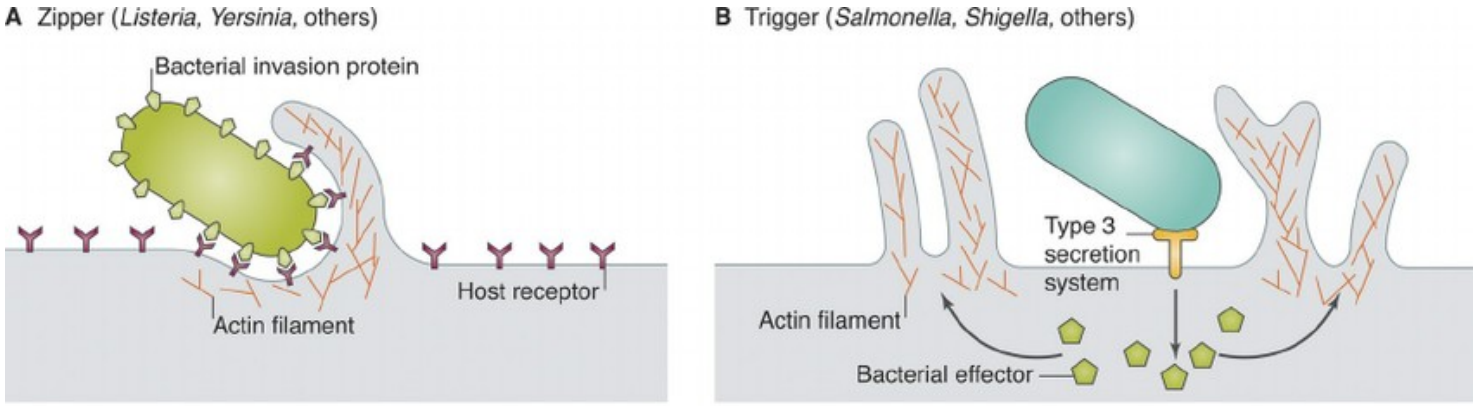


http://csls-text.c.u-tokyo.ac.jp/active/11_01.html

Chain repeats are alternated with Ca2+ ions which function to hold the chain together into a stiff structure, strong enough to link cadherins on one cell membrane interact with those on another cell in a zipper-like fashion forming a strong cell adhesion junction.

Main mechanisms of entry of invasive bacteria

Two main mechanisms of entry have been described: **zipper mechanism (***Yersinia* and *Listeria M.*) and **trigger mechanism (***Salmonella and Shigella*).



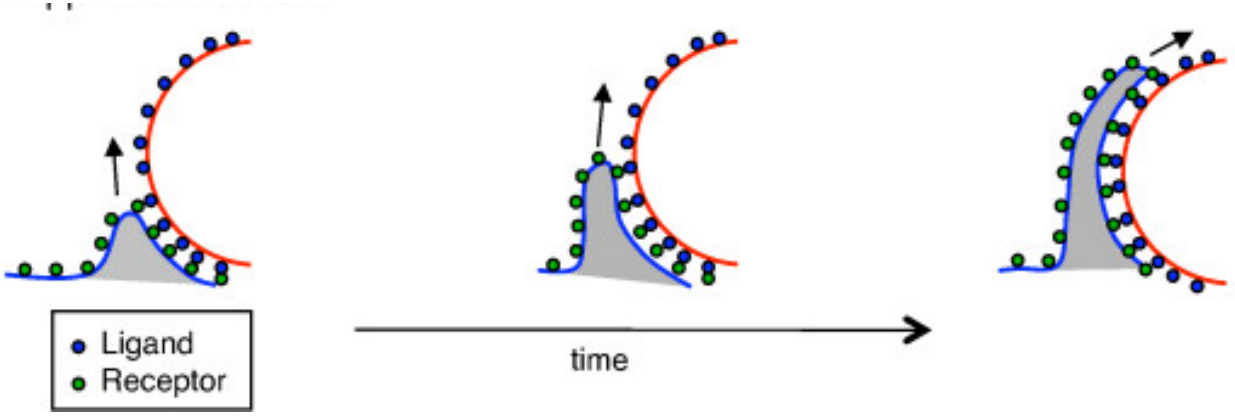
Zipper: uptake mechanism that involves bacterial surface molecules (invasion protein) that binds tightly to a cellular host receptor.

Trigger: pathogen induces its internalization into non-phagocytic cells by injecting soluble effector proteins across the host membrane, often via the syringe-like T3SS, inducing a bloom of actin-rich membrane ruffles that engulf the bacterium and nearby particles.

Both of them rely on the activation of signaling cascades, leading to the **reorganization of the actin cytoskeleton** at the level of the host plasma membrane.

Zipper-like uptake mechanism

Uptake mechanism of pathogenic *Yersinia spp.* involves the OM protein **invasin**, a bacterial surface molecule that binds tightly to a cellular host receptor. **Invasin**, resemble **fibronectin** and binds to host cell surface β1 integrins.



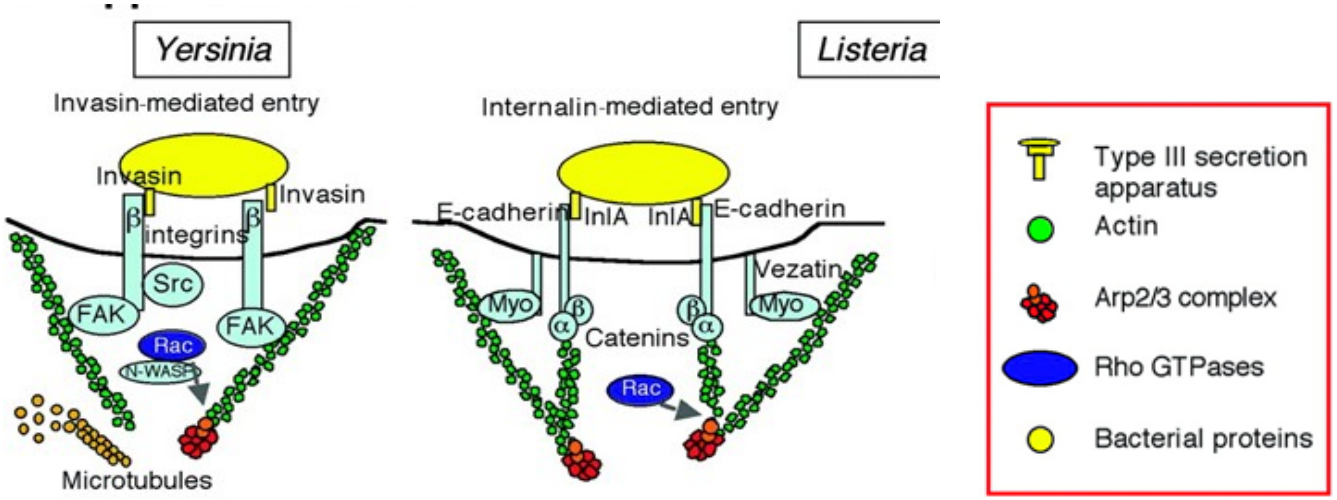
The host cell membrane then wrap the surface of the bacterium.

The high density of invasin expressed over the entire bacterial surface, and the density of β1 receptors on the host cells allow sequential binding of additional molecules to the host receptors and "zippering" the pathogen into the host cell.

The higher affinity for integrins combined with the ability to oligomerize leads to integrin receptor clustering. These signals induce actin polymerization in the cell and membrane extension. The host cell membrane then wrap the surface of the bacterium.

Signal tranduction in zipper mechanism of entry

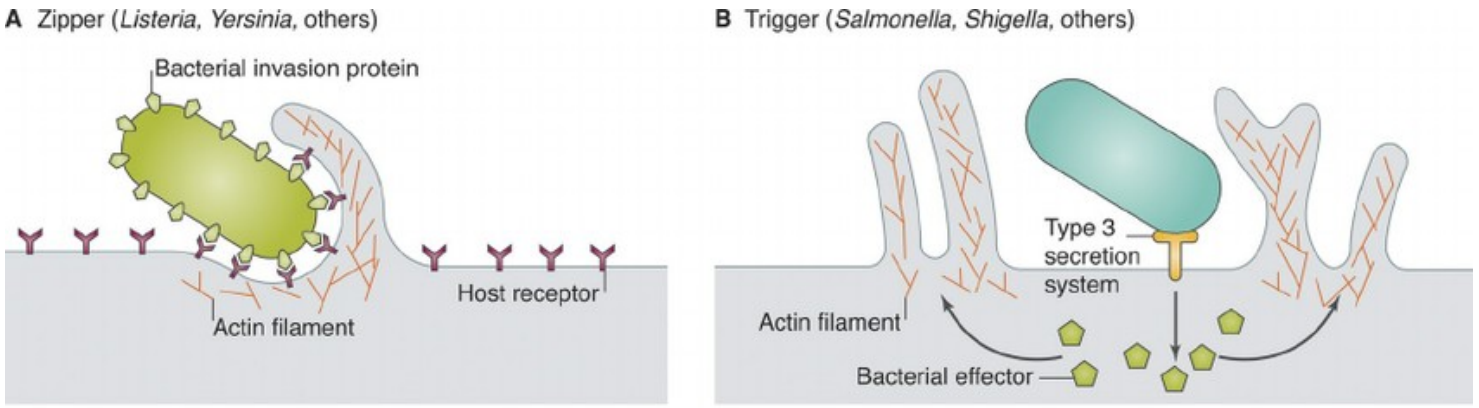
The gram+ food-borne pathogen *Listeria monocytogenes* uses a similar zipper mechanism of internalization based on transmembrane cell-adhesion proteins (**E cadherins)** as receptors for entry into epithelial cells the binding to a specific adhesion protein (**Internalin,** InlA).



The interactions between the bacterial adhesion proteins in Yersinia or Listeria and their receptors trigger a cascade of signals that involve **Rho GTPases** and activate actin nucleators **N-WASP** and **Arp2/3** responsible for synthesis of a local branched **actin network** that culminate in phagocytic cup closure and bacterial internalization.

The trigger mechanism of entry

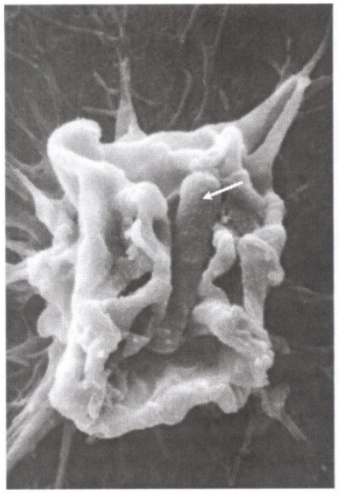
In trigger mechanisms, exemplified by *Salmonella Typhimurium* and *Shigella flexneri*, the pathogen induces its internalization into non-phagocytic cells by injecting **soluble effector proteins** across the host membrane, via the syringe-like T3SS, inducing a bloom of actin-rich membrane ruffles that engulf the bacterium and nearby particles.



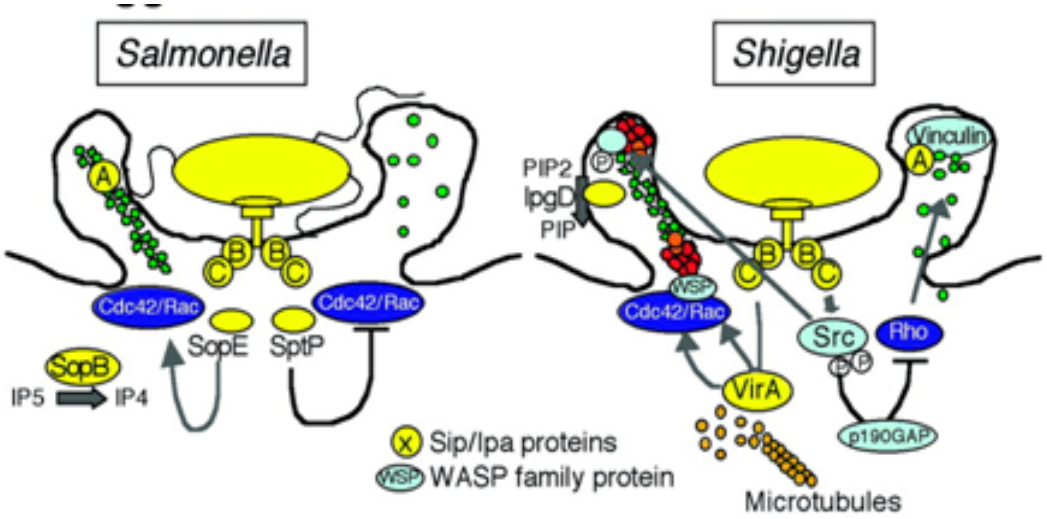
Ruffles directly mediate macropinocytosis, a process in which extracellular cargo is taken up non selectively.

The trigger mechanism induces membrane ruffling

Shigella: IpaC in Shigella initiates actin nucleation through their C-terminal domain, which is exposed to the cytoplasm of the eukaryotic cell, via the **IpaB/C** pore. IpaC activate host cell Rho GTPases (blue color) that stimulate actin cytoskeleton rearrangements and allow **membrane ruffling**.



SEM showing the membrane ruffling induced by Shigella, on contacting an epithelia cell.

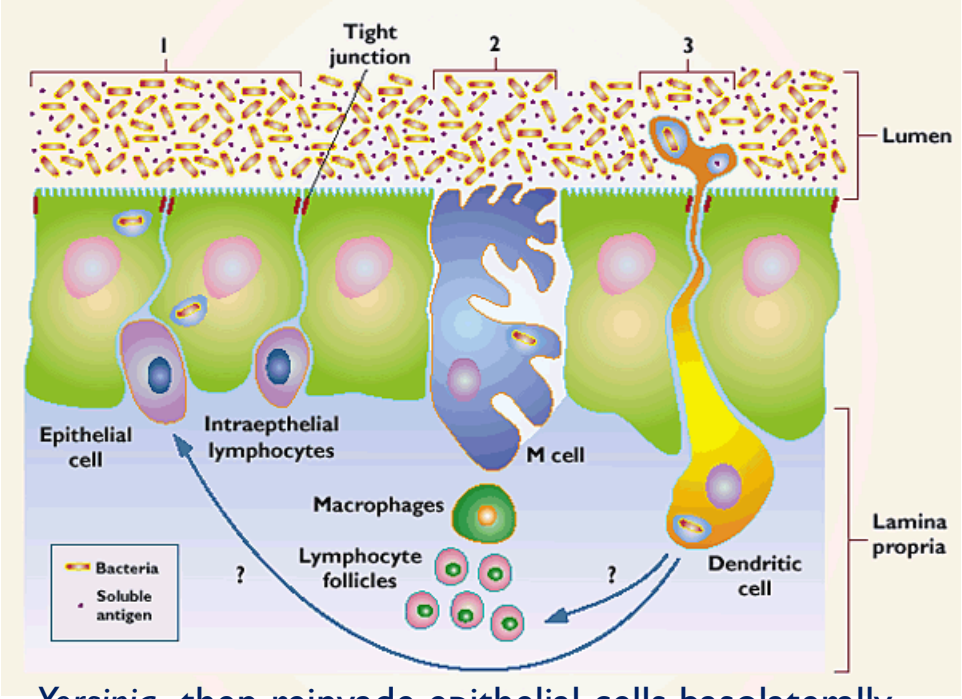


Formation of a macropinocytic pocket involves localized but **massive rearrangements** of the cell surface, characterized by the formation of intricate filopodial and lamellipodial structures that appear similar in Salmonella and Shigella.

Crossing of host barriers

Intestine M cells may constitute **entry portals for** invasive **pathogens** that expoit the transcytosis as a route of entry to deeper tissues of the host (2).

A second route across the epithelium uses uptake by the projections that **dendritic cells** extend into the intestinal lumen (3). Some pathogens escape into the cytoplasm or cause apoptosis of their host cells.



Yersinia then reinvade epithelial cells basolaterally

Nature Immunology **2**, 288 - 290 (2001)

Some pathogens, such as *Salmonella spp., L. monocytogenes*, and *Mycobacterium tuberculosis* are phagocitized by phagocytes and survive within macrophages and neutrophils.

Many Pathogens Alter Membrane Traffic in Host Cells

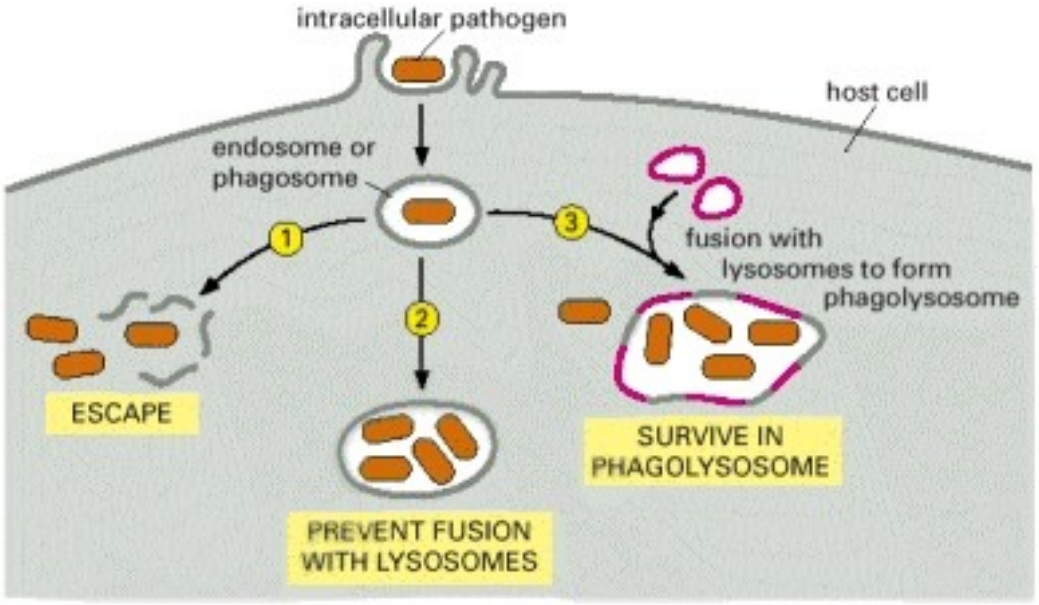
A pathogenic microbe that has been internalized by a eukaryotic host cell must either avoid delivery to a degradative lysosomal compartment or develop strategies for survival within this degradative organelle.

They therefore must follow one of these strategies to survive:

1) escape from the compartment before getting digested (*L. monocytogenes, and Shigella spp.*, viruses, and the protozoa *Trypanosoma cruzi)*;

2) modify the compartment to prevent its fusion (*M. tuberculosis, S. enterica, L. pneumophila).*

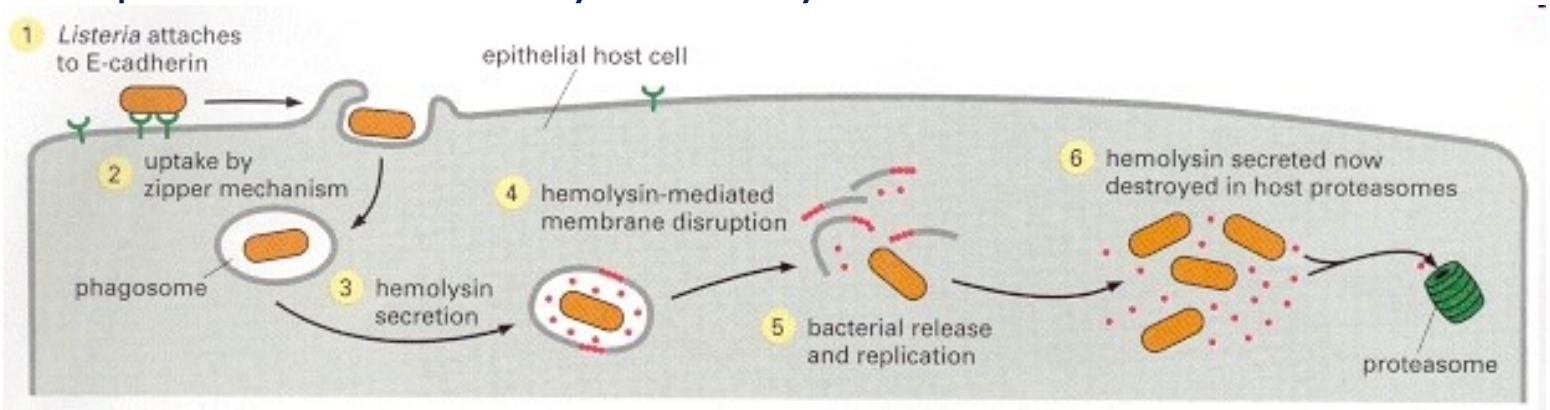
3) find ways to survive in the hostile environment of the **phagolysosome** (in professional phagosomes) (*Coxiella burnetii*)



Different strategies adopted by pathogens to survive in the phagosome of the host cells.

Selective destruction of the phagosomal membrane to escape

L. monocytogenes induces its own uptake and escapes from vacuole. Within the phagosome, the bacterium secretes **listeriolysin O** (hemolysin in the figure) a pore forming toxin which creates large pores and eventually disrupt the membrane.
Shigella escapes from the vacuole by similar way.



Once in the host cell cytosol, the bacteria begin to replicate. Because listeriolysis contains a **PEST sequence** is rapidly degraded by proteasomes, so that the host cell plasma membrane remains intact an the cell is nor damaged.

The LLO secreted by *L. monocytogenes* is closely related to hemolysins secreted by other bacteria that are not intracellular pathogens and all lack PEST sequences. It seems that the *L. monocytogenes* has acquired an essentially eukaryotic protein domain expressly to allow its activity to be regulated in the host cell.

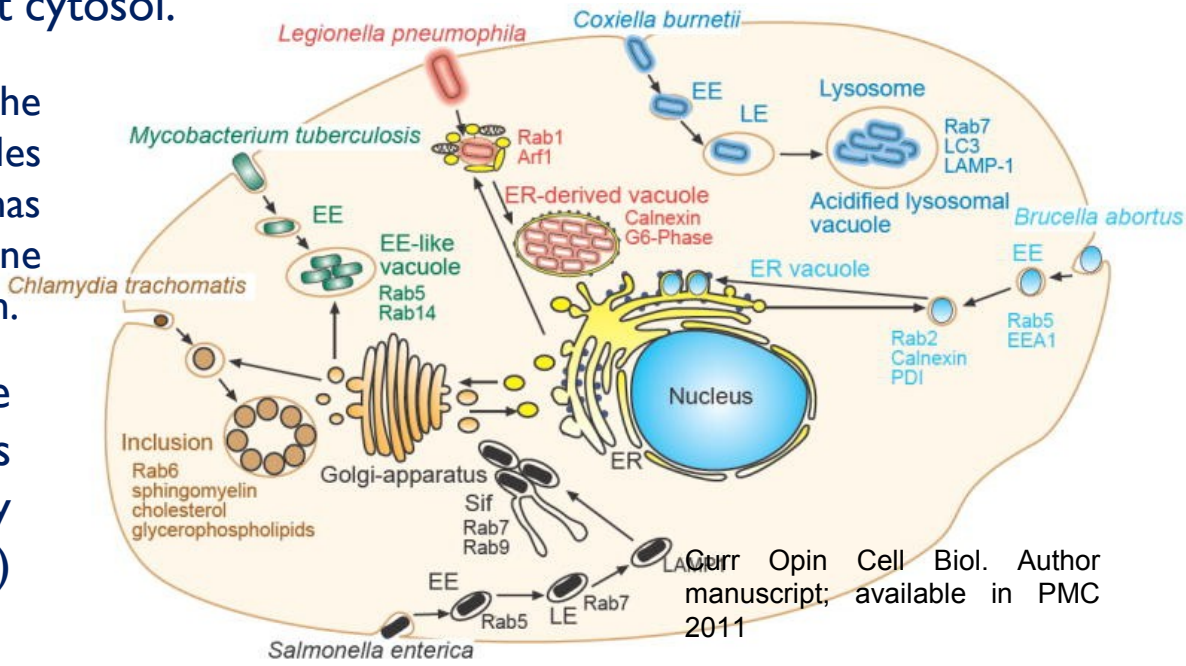
Survival by inhibition of phagosome maturation

Some invasive bacterial pathogens have a variety of strategies to **manipulate the vesicle trafficking** thus creating for themselves a less hostile niche that is permissive for their survival and growth.

They must prevent lysosomal fusion, and secondarily, they must provide a pathway for importing nutrients from the host cytosol.

Examining the association of the different Rab proteins on vacuoles containing bacterial pathogens has determined which host membrane pathways are utilized during infection.

M. tuberculosis, can survive within macrophage phagosomes inhibiting maturation of the early endoscope-like vacuole (rab 5) that contains it.



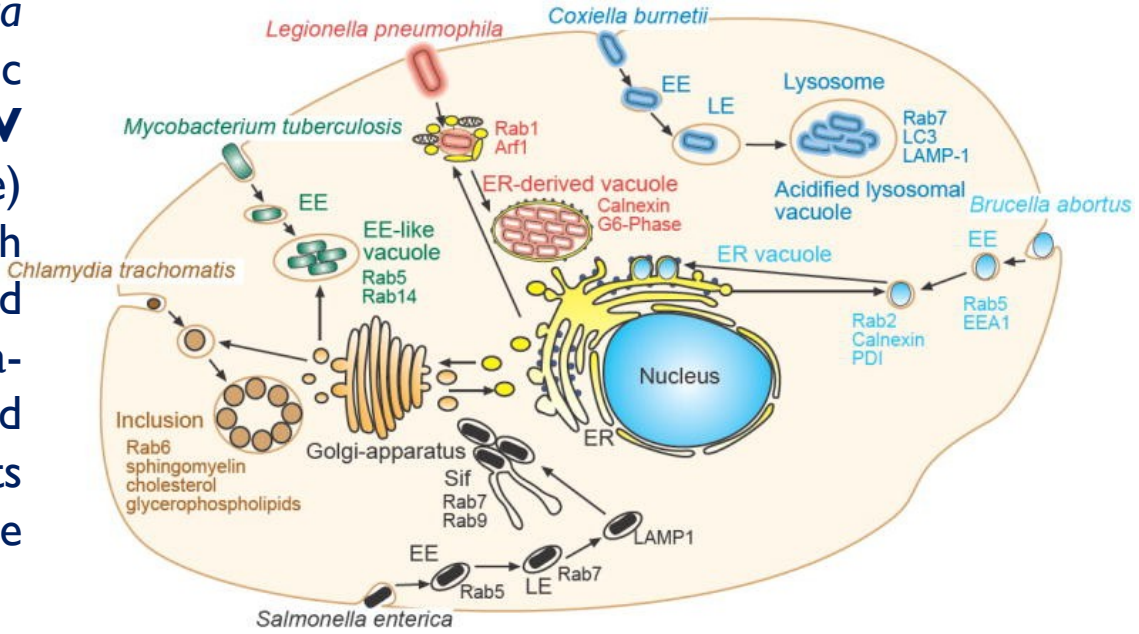
Curr Opin Cell Biol. Author manuscript; available in PMC 2011

Fusion is prevented by secreted proteins on type VII secretion system that interact with endosomal-sorting complex required for transport (ESCRT) and disrupts phagosome maturation. Other bacterial proteins avoid endosome acidification.

Survival by Remodelled endosomal compartments

Rab proteins revealed a novel pattern of Rab association on vacuoles containing virulent ***S. enterica***

After internalization *S. enterica* reside in an atypical acidic compartment called **SCV** (*Salmonella* containing vacuole) which acquire markers of both late and early endosome and shows typical Salmonella-induced filament (SIF) developed by bacterial effectors. Its maturation is arrested at a stage prior to lysosomal fusion.

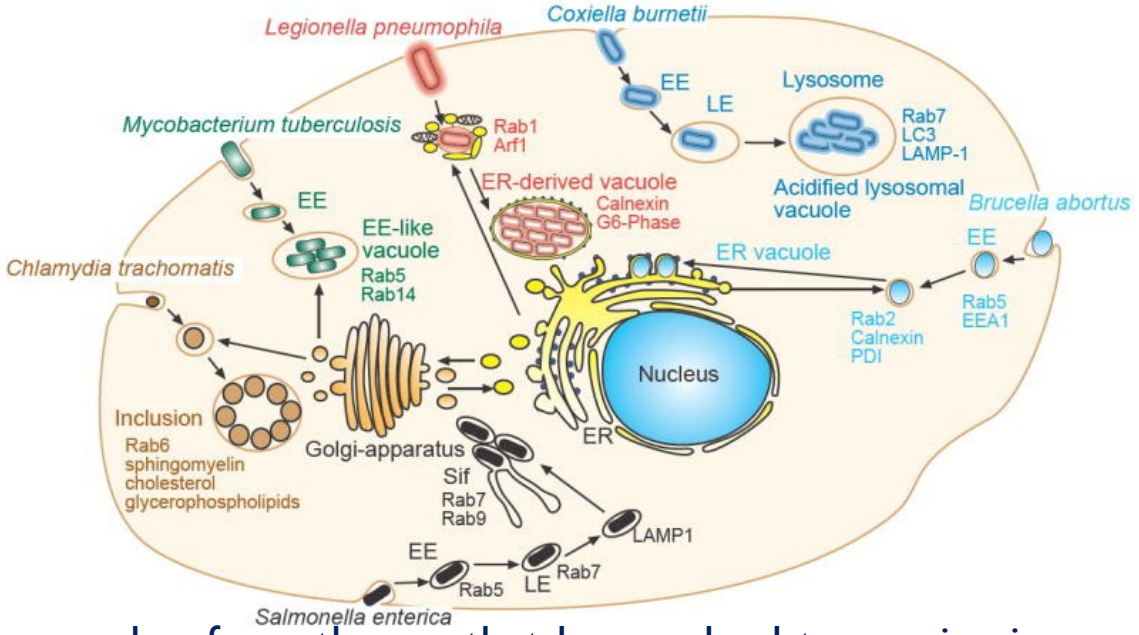


Numerous bacterial genes are required for survival and replication. A key role is played by the SPI2 effector SifA—a protein required for the formation of Sifs, filaments enriched in lysosomal glycoproteins

Survival by remodelled endosomal compartments

- *L. Pneumophila* shows mechanisms by which bacteria can subvert host factors involved in the transport of secretory vesicles to generate a vacuole derived from the **host endoplasmic reticulum**. Using a **type IV secretion system** it prevents fusion of the vacuole in which it resides with endosomal compartments and recruits vesicles derived from the ER (rab1) .

L. pneumophila, and other vacuolar pathogens such as *Chlamydia*, may encode SNARE mimics that directly modulate membrane transport

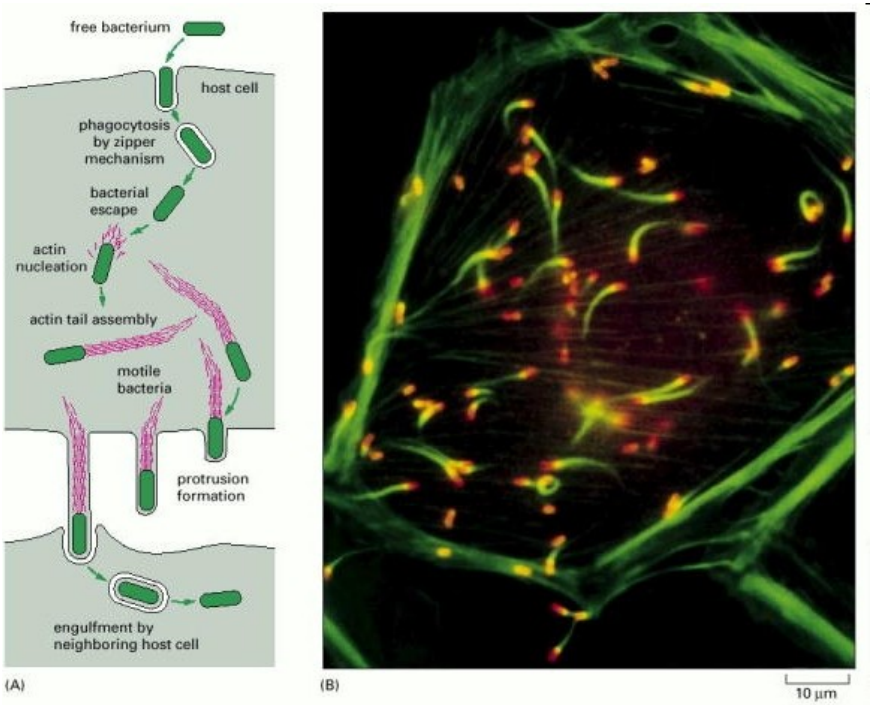


Coxiella burnetii provides an example of a pathogen that has evolved to survive in a lysosome-derived vacuole. This bacterium requires an acidic lysosomal environment for intracellular replication

Diverse pathogens "discovered" actin-based motility

Some invasive bacteria that replicate in the host cell cytosol (*Listeria monocytogenes*, *Shigella flexneri)* have adopted a remarkable **mechanism for moving between cells** very effectively, enabling them to evade the humoral immune response of the host.

They induce the nucleation and assembly of host cell actin filaments **at one pole of the bacterium.** The growing filaments generate force and push the bacteria through the cytoplasm at rates up to 1 µm/sec.

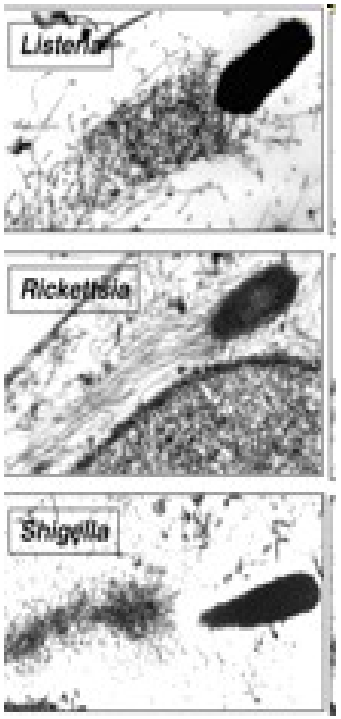


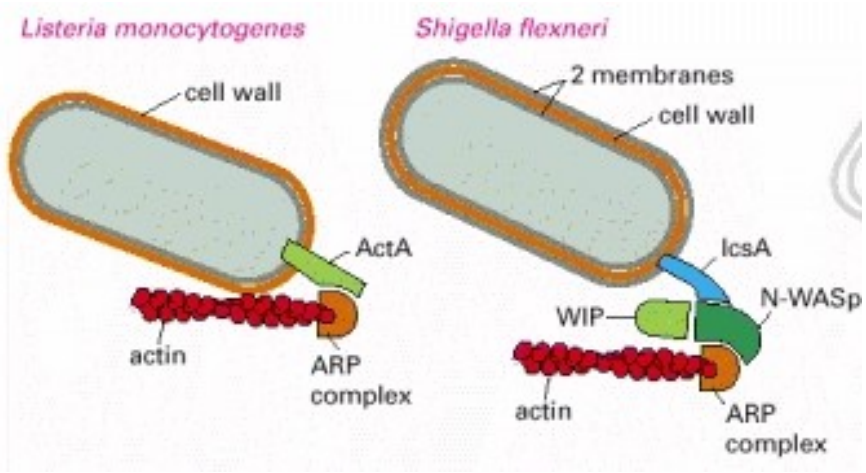
The actin-based movement of *Listeria monocytogenes* within and between host cells comet-like tail of actin filaments *(green)* behind each moving bacterium *(red)*.

When they reach the plasma membrane they continue to move outward, inducing the formation of a protrusion with the bacterium at its tip which is engulfed by a neighboring cell, allowing the bacterium to enter the cytoplasm without exposure to the extracellular environment.

Pathogens exploit the Host Cell Cytoskeleton for Intracellular Movement

Molecular mechanisms of different pathogen-induced actin assembly have been determined. All of them make use of the same host cell regulatory pathway that normally controls the nucleation of actin filaments, but they exploit different points in the pathway.



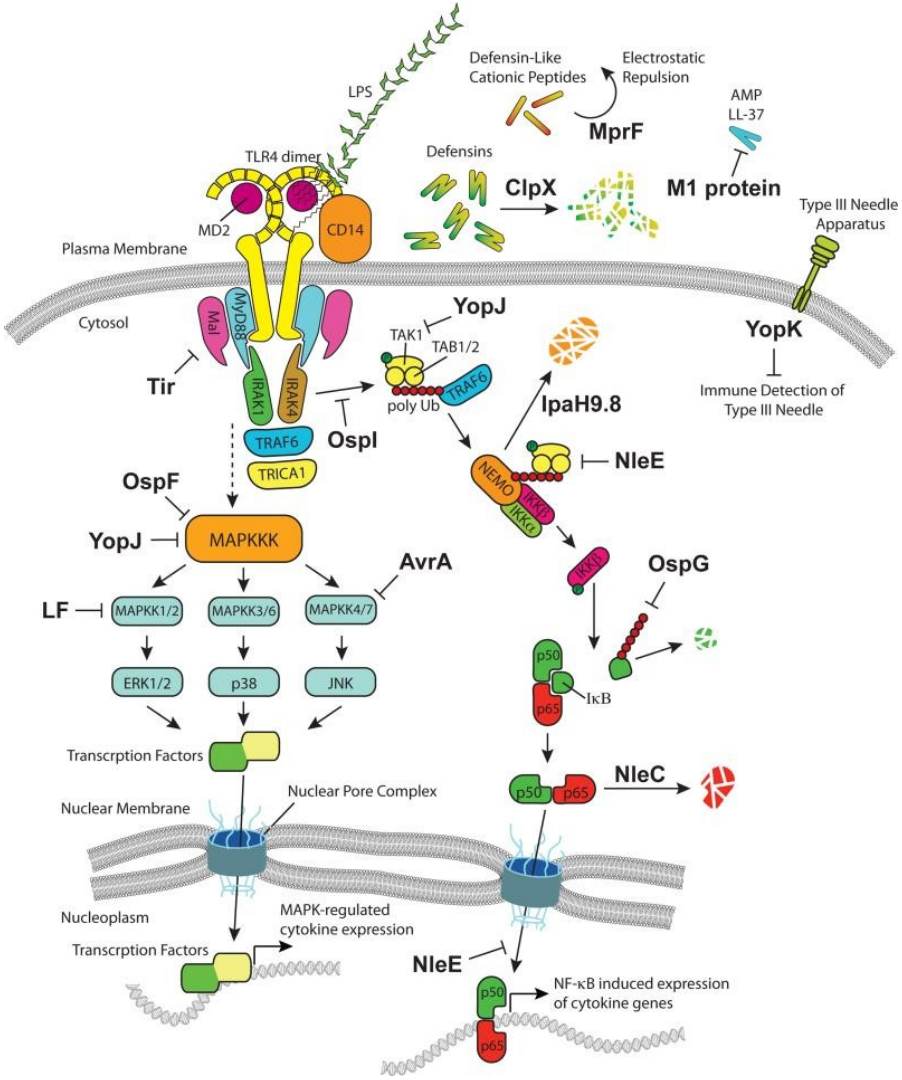


L. monocytogenes surface protein (ActA) directly binds to and activates the ARP2/3 complex to initiate the formation of an actin tail, while an unrelated surface protein on *S. flexneri* (IcsA) binds to and activates actin nucleating factors.

Video on L monocytogenes moving into host cell: https://www.youtube.com/watch?v=sF4BeU60yT8

Pathogens Target and Subvert the Host Innate Immune System

Despite the fast-acting intracellular signaling mechanisms induced by PRRs, microbial pathogens have evolved countermeasures to thwart innate immunity in order to promote their lifestyle in the infected host organism



Summary of Anti-Immune Strategies of Bacteria

Strategy	mechanisms	
Modulators on the pathogen surface	Self carbohydrates for the capsules Reducing the negative charges of the surface	Chapter 3
Antigenic hypervariability	•Antigenic Variation in suface structures	Chaper 7
To Interfere with TLRs	•Modification of lipid A to reduce TLR4 responses •inject effectors to inhibit downstream inflammation signaling (Downregulation of inflammatory pathways NFkB)	
Subvert or kill immune cells/phagocytes	•avoid phagosome fusion with lysosome •block inflammatory pathways by injecting effectors	Chapter 11
Inhibit cytokines/ interferon/chemokines activities	•Secrete proteases to degrade cytokines	Chapter 9
To impair T cells responses	superantigens	Chapter 12