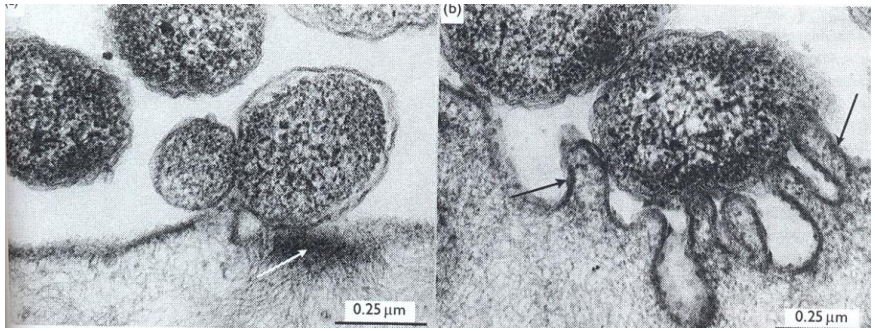
Chapter 7 Survival strategies of pathogens into the host

a.a.2020-21

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



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

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

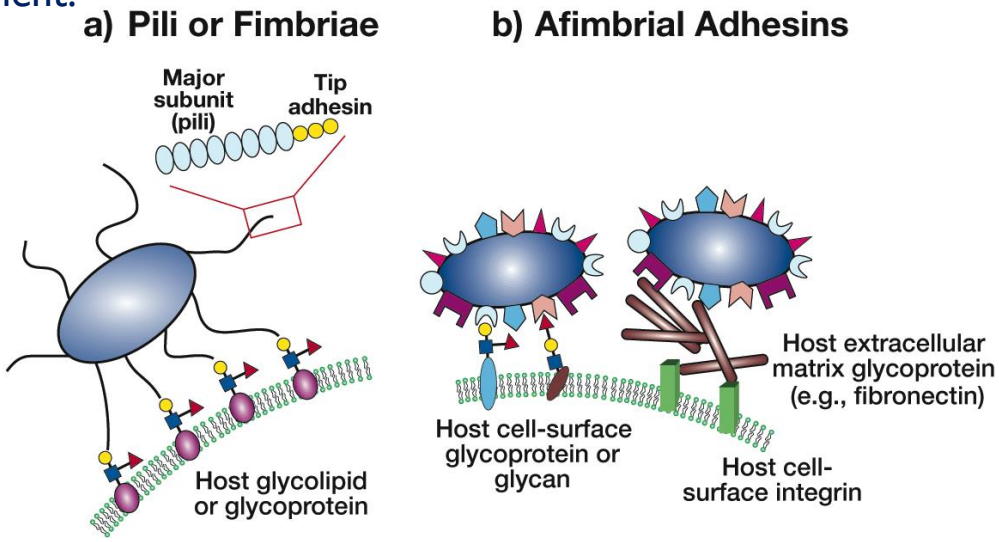
Bacterial structures involved in adherence to host cells

Adhesion of bacteria to host surfaces is a crucial aspect of host colonization as it prevents the mechanical clearing of pathogens.

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 **pili or fimbriae**a (scaffold-like structures on the bacterial surface)
b) non polymeric adhesins exposed to the bacterial surface (afimbrial 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.

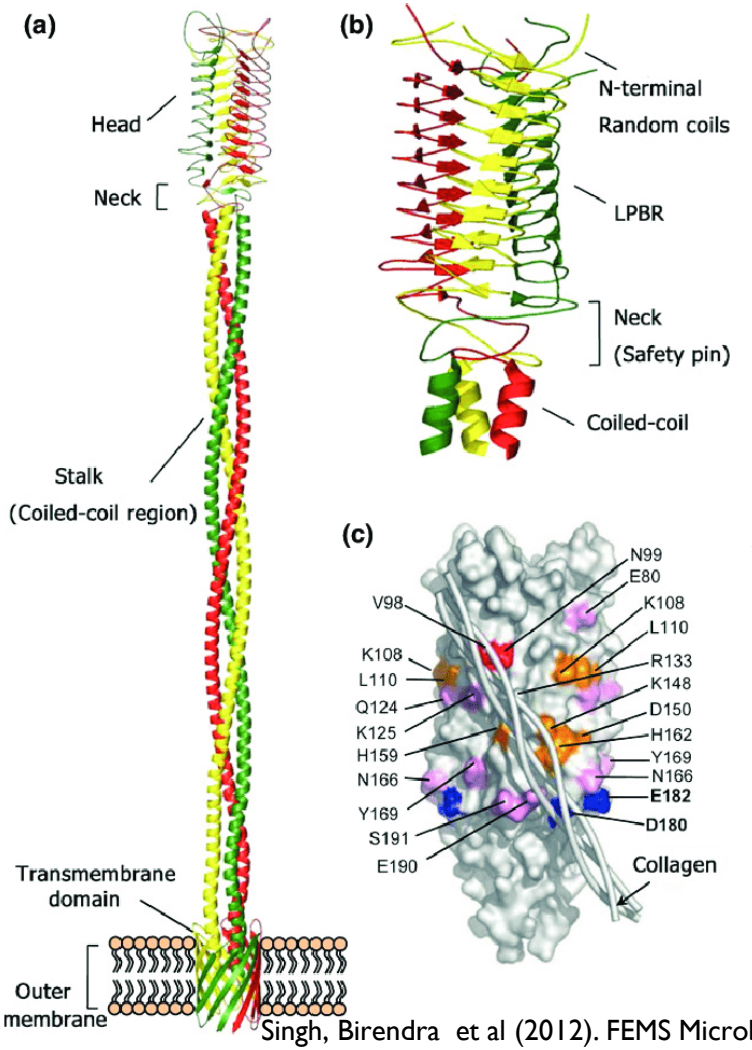
Cell adhesion molecules as receptors

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

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

Invasive pathogens as Listeria, Shigella and Yersinia: adhesion is the first step that precedes their internalization within host cells.

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



Singh, Birendra et al (2012). FEMS Microbiology Reviews.

Trimeric autotransporter YadA and collagen-binding model.

YAdA is an essential virulence factor of *Y. enterocolitica*, (the agents of enteric yersiniosis) 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.

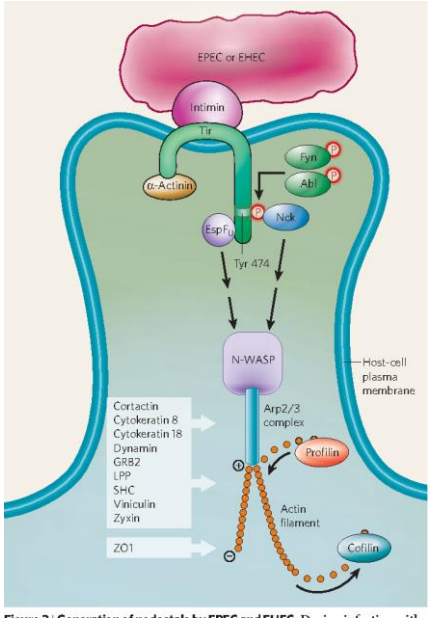
YadA shows a extended triple coiled coil stalk attached to the β-barrel anchor and an N-terminal head with adhesive properties.

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

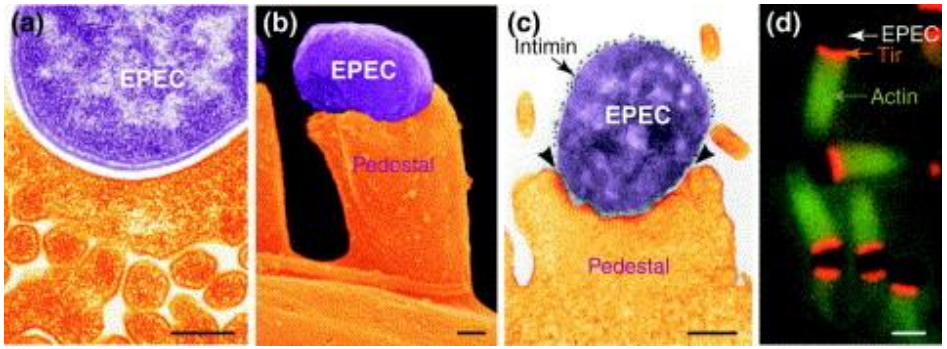
YadA head domain with a collagen triple helix

Bacterially-Encoded Cellular Receptor

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



A.P. Bhavsar, et al. Nature volume 449, 827–834(2007)



EPEC forming attaching and effacing lesions on epithelial cells in culture. G. Frankel et al. Trends in microbiology 2001

ThroughT3SS (coded by PAI LEE) the pathogen injects 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 after intimin binding 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.

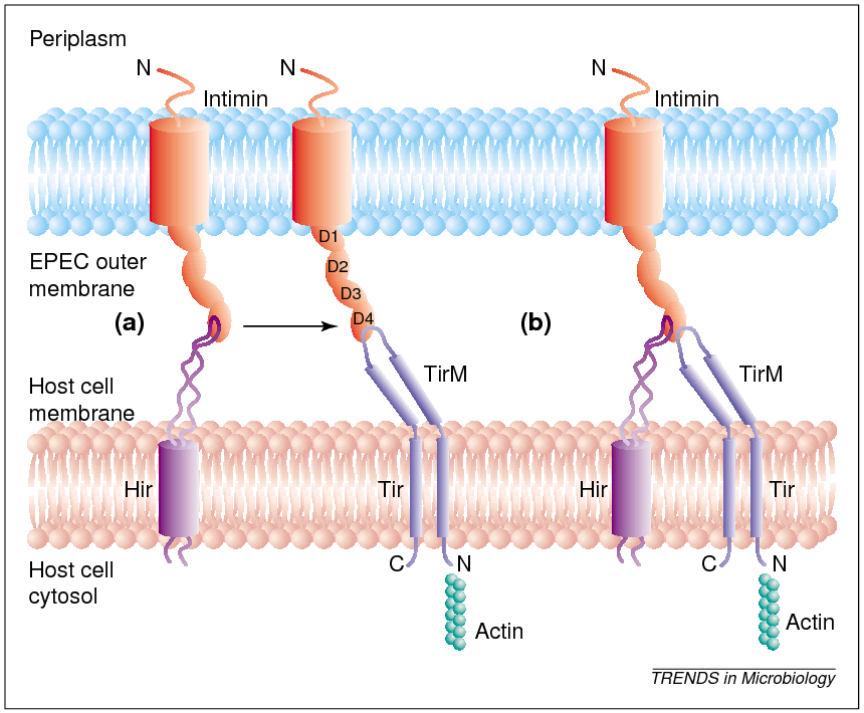
Model of the complex between intimin and Tir receptor

Tir (translocated intimin receptor) is injected into host target cells and then is inserted into the host-cell membrane, where it functions as a receptor for another LEE encoded molecule, the outer membrane protein **intimin**

G. Frankel et al. Trends in microbiology 2001

Model of the entero pathogenic Escherichia coli (EPEC)–host cell adhesion interface.

Intimin is shown in orange colour. The Ig-like domains are shown as D1-D3, and the lectin-like domain D4, which binds to the Tir intimin is shown. The N-terminal domain of Tir anchors host to cytoskeletal components.



Outer membrane of bacteria

Host cell membrane

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

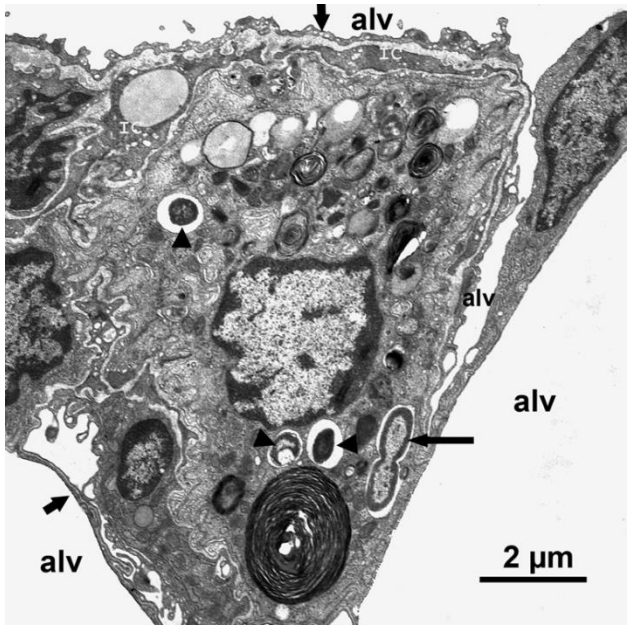
Bacterial invasion as a virulence mechanism

Invasive bacteria are pathogens able to **penetrate into host cells** by crossing the epithelium.

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.

Invasive bacteria 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.

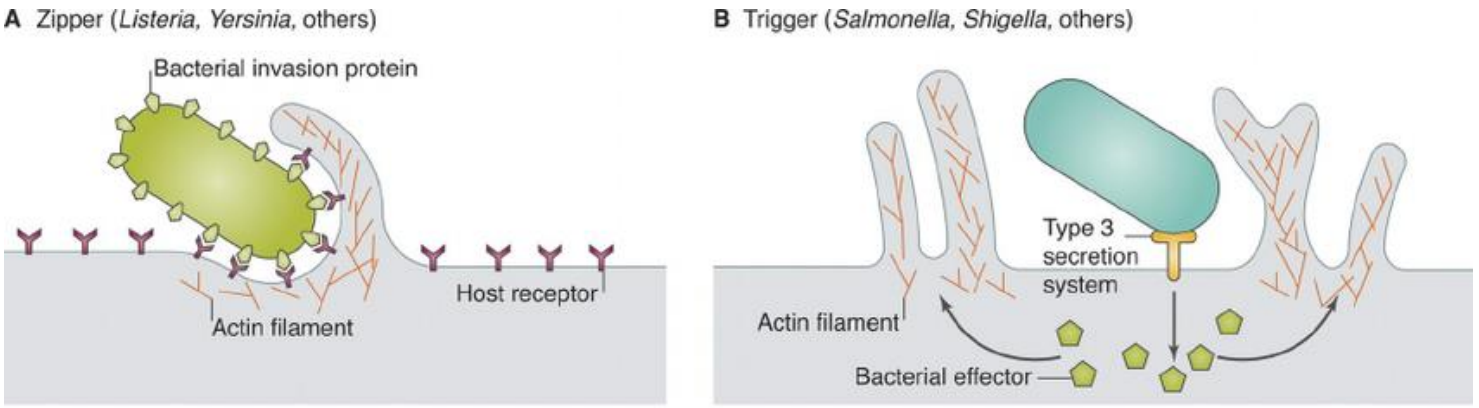
To enter non-phagocytic cells invasive bacteria express adhesins to bind eukaryotic cell adhesion molecules such as surface receptors involved in cell-matrix (integrins) or cell-cell adherence (cadherins).



Alveolar epithelial cell type II (AEII) with engulfed bacteria at 1 h after infection. Vacuoles with partially digested bacteria (arrowheads) lie in the epithelial cytoplasm.

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*).

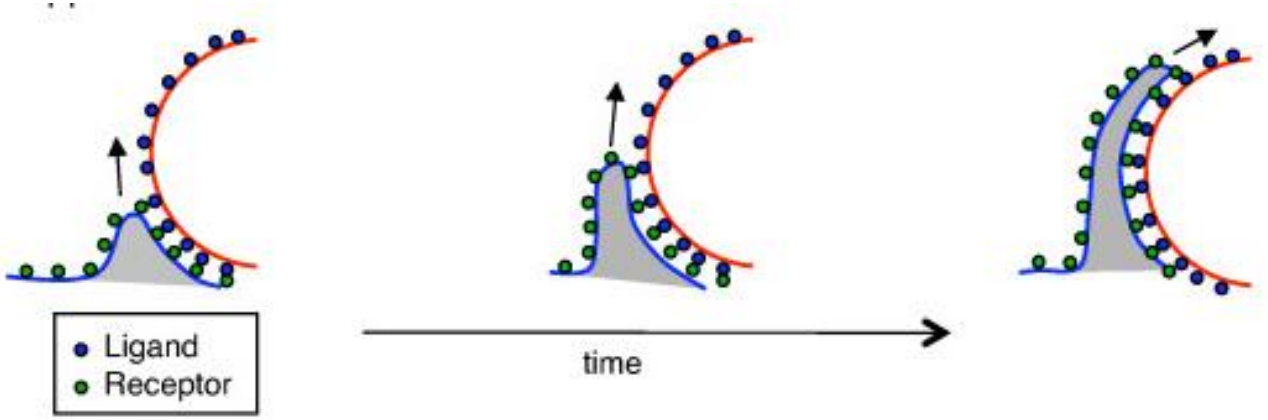


Zipper: uptake mechanism that involves bacterial surface molecules (invasion protein) that binds tightly to a cellular host receptor.

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.

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.



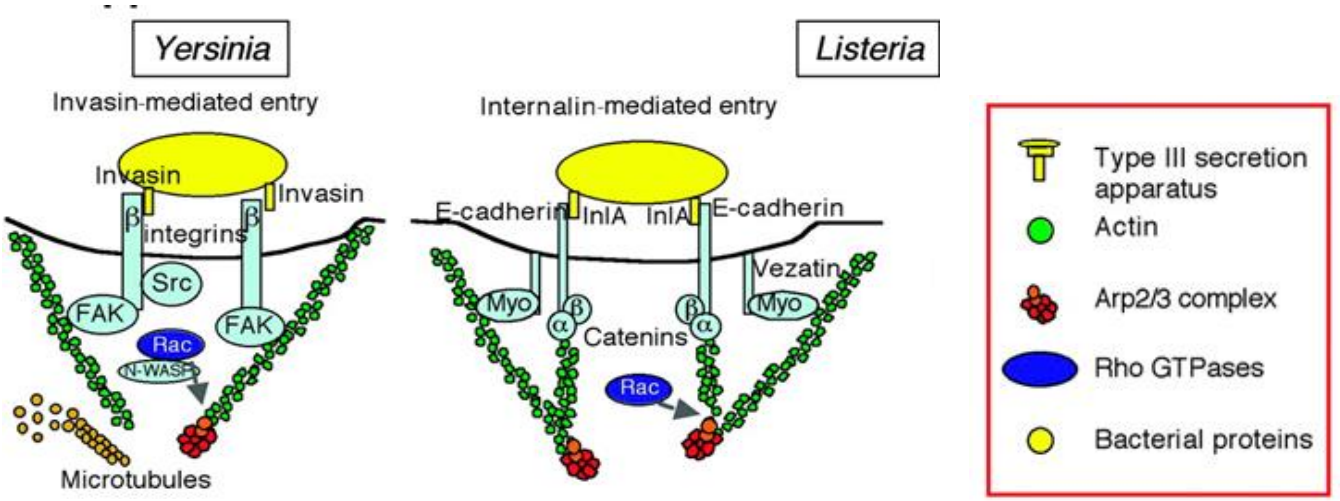
The host cell membrane then wrap the surface of the bacterium.

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 transduction in zipper mechanism of entry

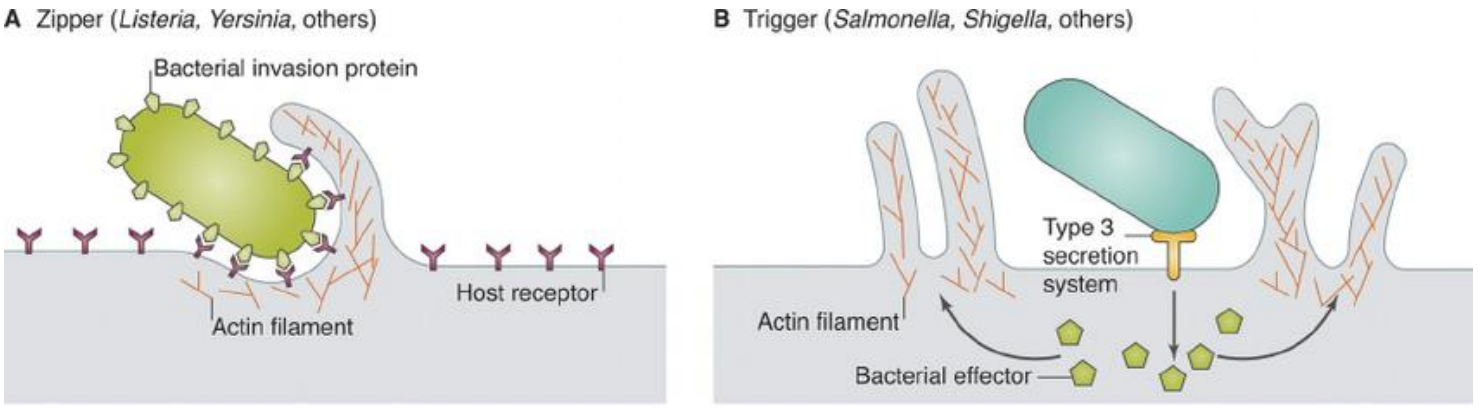
The gram+ food-borne pathogen *Listeria monocytogenes* uses a similar zipper mechanism of internalization. It is based on the expression of a specific adhesion protein (**Internalin,** InlA) that bind to the transmembrane cell-adhesion proteins (**E cadherins)** as receptors for entry into epithelial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



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

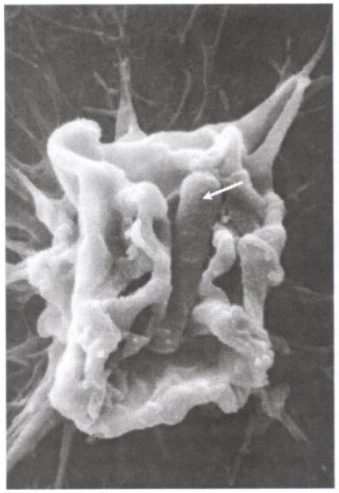
In trigger mechanisms, exemplified by *Salmonella Typhimurium* and *Shigella flexneri*, the pathogen induces its internalization into non-phagocytic cells by injecting **soluble effector proteins** across the host membrane, via the syringe-like T3SS, inducing a bloom of actin-rich membrane ruffles that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



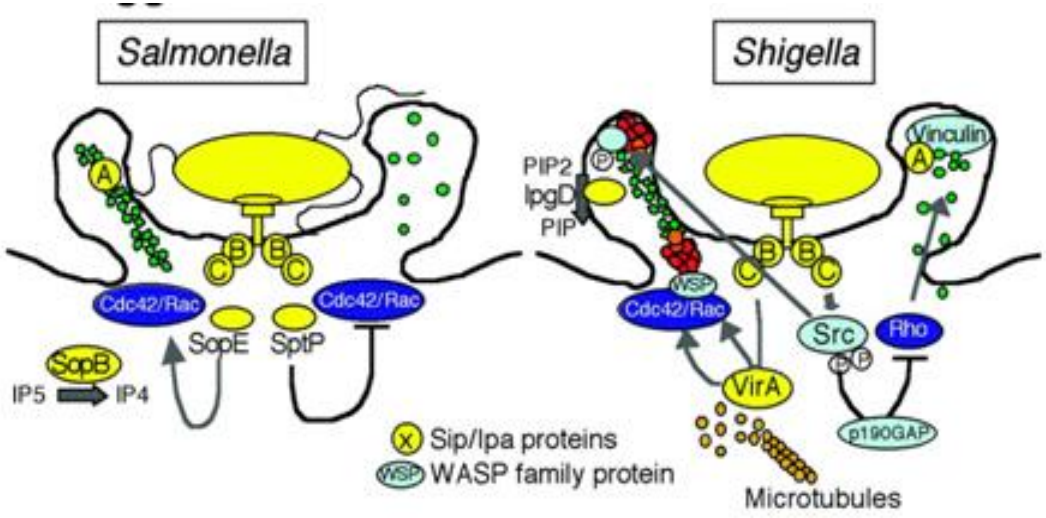
Ruffles directly mediate macropinocytosis, a process in which extracellular cargo is taken up non selectively.

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.

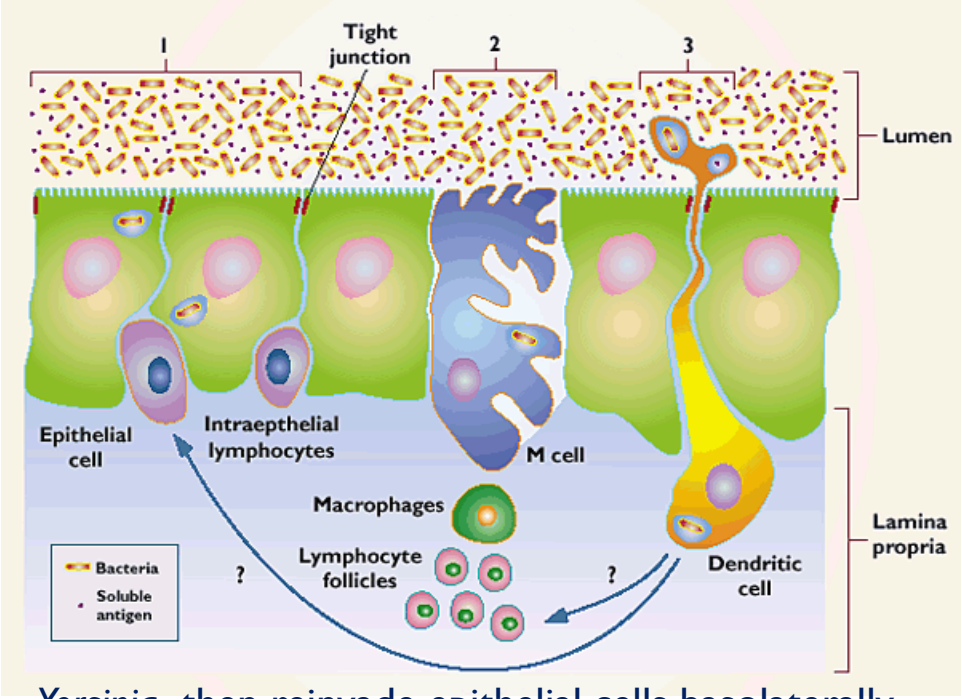


Formation of a macropinocytic pocket involves localized but **massive rearrangements** of the cell surface, characterized by the formation of intricate filopodial and lamellipodial structures that appear similar in Salmonella and Shigella.

Crossing of host barriers

Intestine M cells may constitute **entry portals for** invasive **pathogens** that exploit 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 from 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

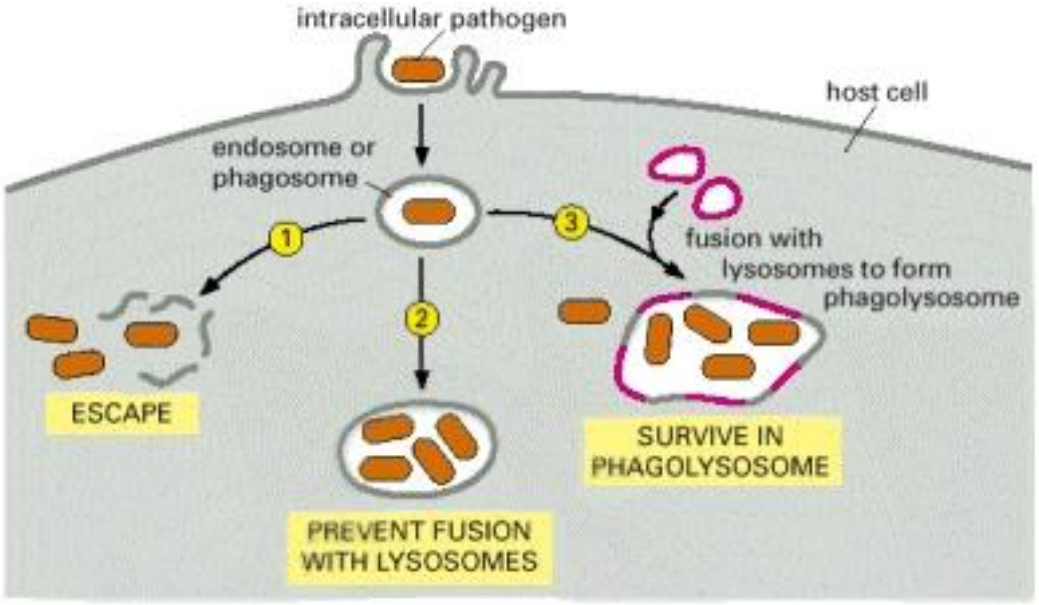
A pathogenic microbe that has been internalized by a eukaryotic host cell must either avoid delivery to a degradative lysosomal compartment or develop strategies for survival within this degradative organelle.

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).*

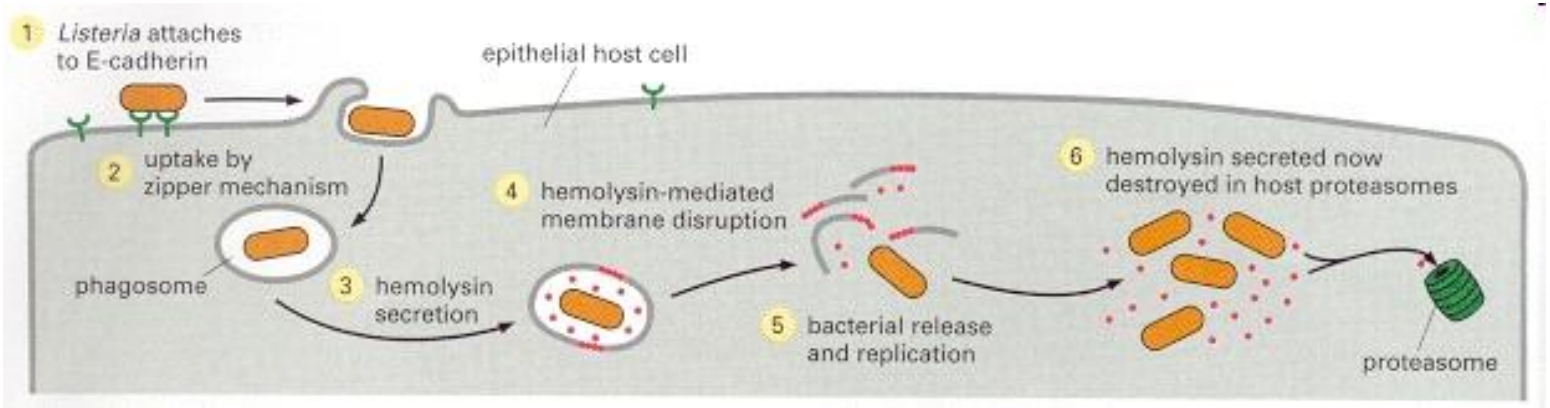
3) find ways to survive in the hostile environment of the **phagolysosome** (in professional phagosomes) (*Coxiella burnetii*)



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.

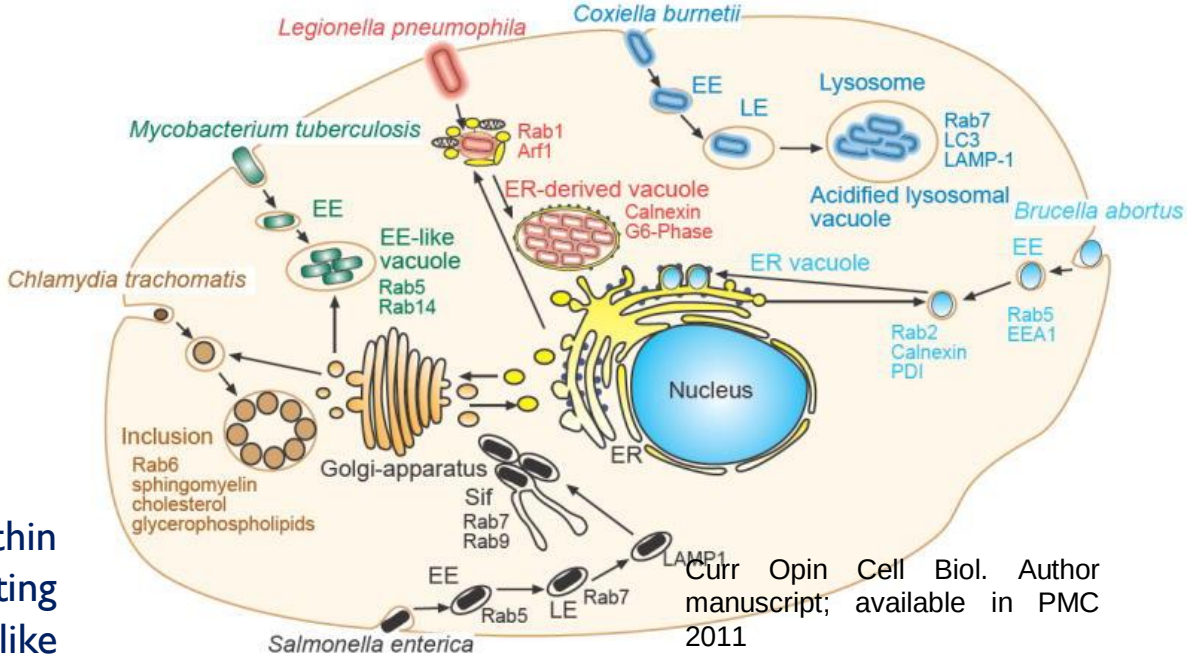
Survival by inhibition of phagosome maturation

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 transport pathways are utilized during infection.

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

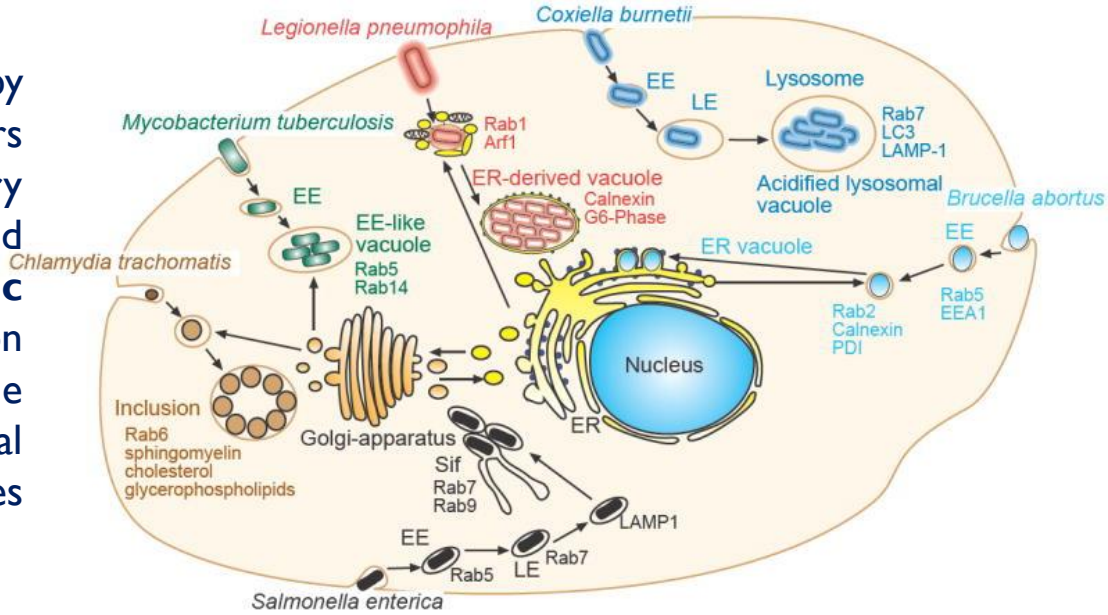


Curr Opin Cell Biol. Author manuscript; available in PMC 2011

Survival by Remodelled endosomal compartments

After internalization *S. enterica* reside in an atypical acidic compartment called **SCV** (*Salmonella* containing vacuole) which acquire markers of both late and early endosome (Rab5 Rab7) and shows typical Salmonella-induced filament (SIF) developed by bacterial effectors. Its maturation is arrested at a stage prior to lysosomal fusion.

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).



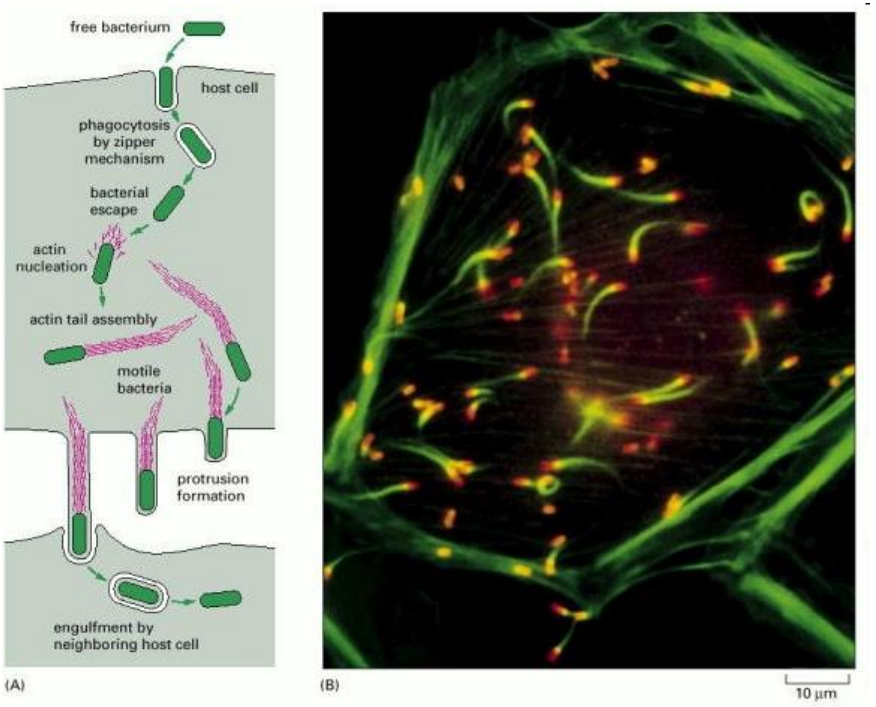
L. pneumophila, and other vacuolar pathogens encode SNARE mimics that directly modulate membrane transport.

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.

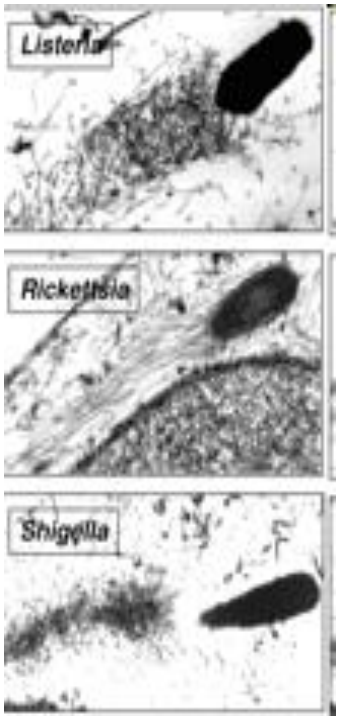


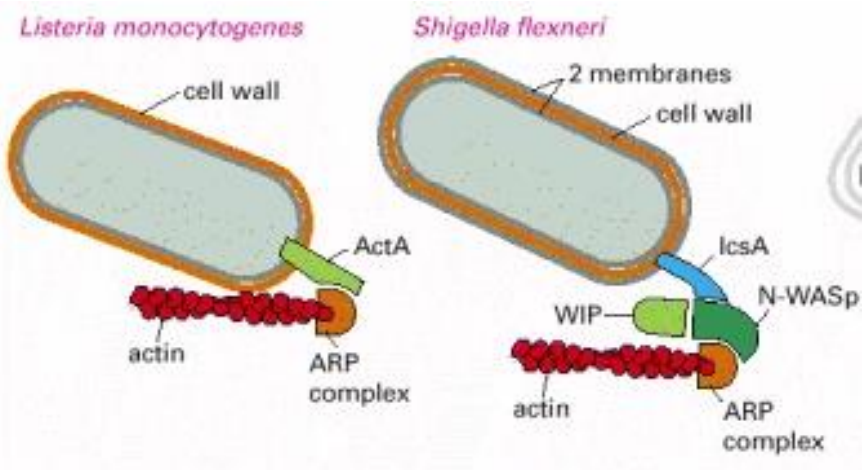
The actin-based movement of *Listeria monocytogenes* within and between host cells comet-like tail of actin filaments *(green)* behind each moving bacterium *(red)*.

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.





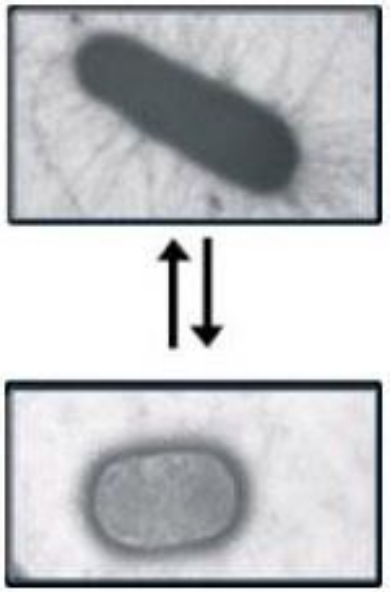
L. monocytogenes surface protein (ActA) directly binds to and activates the ARP2/3 complex to initiate the formation of an actin tail, while an unrelated surface protein on *S. flexneri* (IcsA) binds to and activates actin nucleating factors.

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

Phase and antigenic variations

Phase and antigenic variation: genetic mechanisms by which an infectious organism alters its surface proteins in order to evade host immune responses.

Pathogens that express these characteristics and undergo these genetic variations have a selective advantage over their more genetically stable counterparts.



By phase and/or antigenic variation microorganisms results in a **heterogenic phenotype** in which individual cells either express the phase-variable protein(s) or not (**phase variation**), or express one of alternative and multiple forms of the protein (**antigenic variation**).

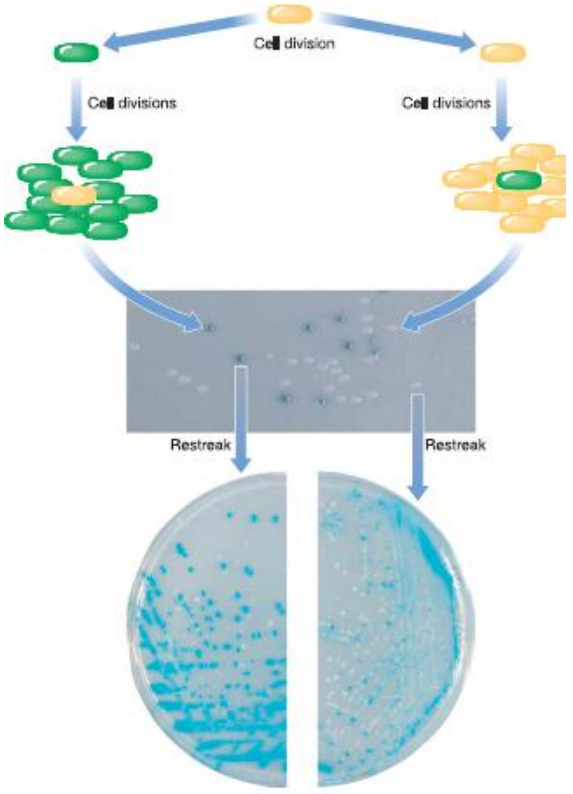
(expression) phase variation

A reversible switch between an "all-or-none" (on/off) expressing phase, resulting **in variation in expression** of one or more proteins between individual cells of a clonal population.

The switch is a stochastic event and the frequency and that of its reversion **exceed that of a random mutation**. (1/100-1/1000 per generation), but the switching frequency can be modulated by external factors.

Daughter cells will inherit the expression phase of the parent, and the phase of expression is reversible between generations.

Structures that were found to phase vary were **on the cell surface**, where they would be exposed to the immune system.



Microbe, 2008 vol 3 pp 21-26

Representative examples of phase and/or antigenic variations

The strategy is particularly important for organisms that target long-lived hosts, or repeatedly infect a single host.

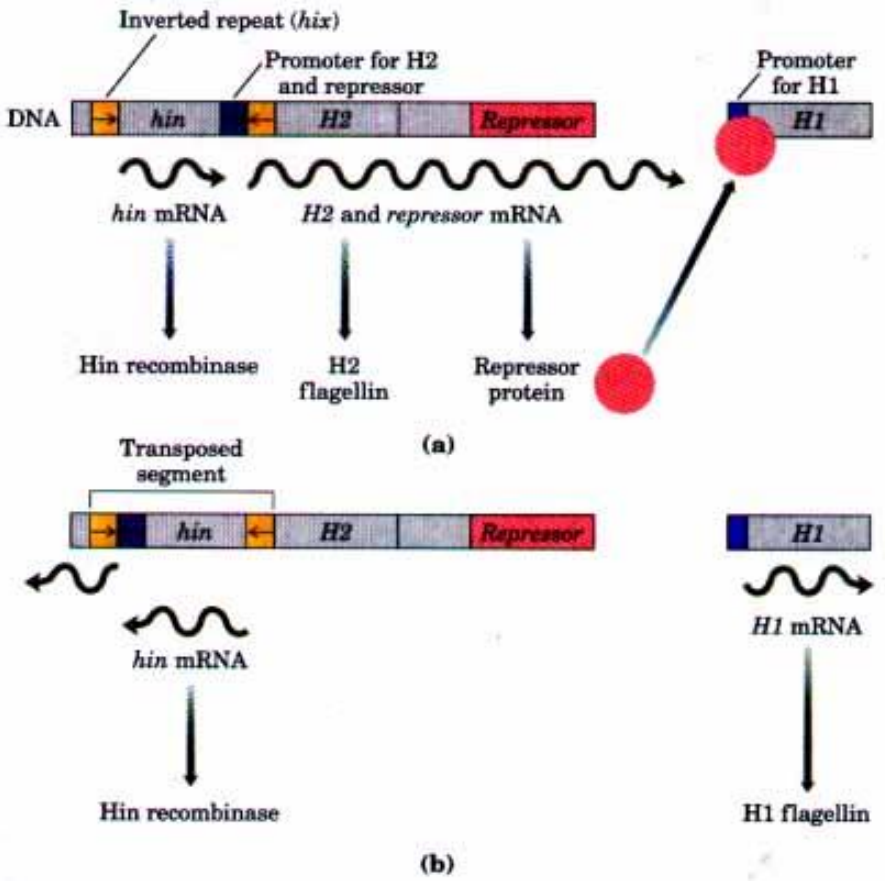
Bacterial species	*Affected moiety or phenotype*	*Gene(s) or operon regulated*	*Variation of regulated gene(s)*[c]	*Class(es) of regulated gene or operon*	*Molecular mechanism*
B. Pertussis	Fimbriae	*fim3*, *fim3*	Phase	Structural	SSM
	bvg+	*bvgS*	Phase	Regulatory	SSM
E. coli	type I fimbriae	fim operon	Phase	Structural	CSSR
	Pilus P	pap operon	Phase	Structural	epigenetic
S. Typhimurium	Flagella	*fljBA*, *fliC*	Phase	Structural	CSSR
N. Gonorrhoeae and N. meningitidis	Type IV pilin	pilE, pilS	Antigenic	Structural	Rec
S. pneumonieae	Capsule	*cap3A*	antigenic	Structural	Rec

Different molecular mechanisms involved: DNA inversion (recombination) such as CSSR= conservative site-specific recombination, Rec= recombination; or DNA mispairing, such as SSM= slipped strand mispairing, (appaiamento sfalsato di corte sequenze ripetute in tandem), or epigenetic (methylation)

Flagellar phase variation in Salmonella

Salmonella is able to switch between two distinct **flagellin** proteins once about every 1,000 cell generations which is accomplished by periodic inversion of a segment of DNA containing the promoter for a flagellin gene

DNA recombinase (hin) which promotes inversion of sequences at specific site of 14 base pairs (*hix* sequences)

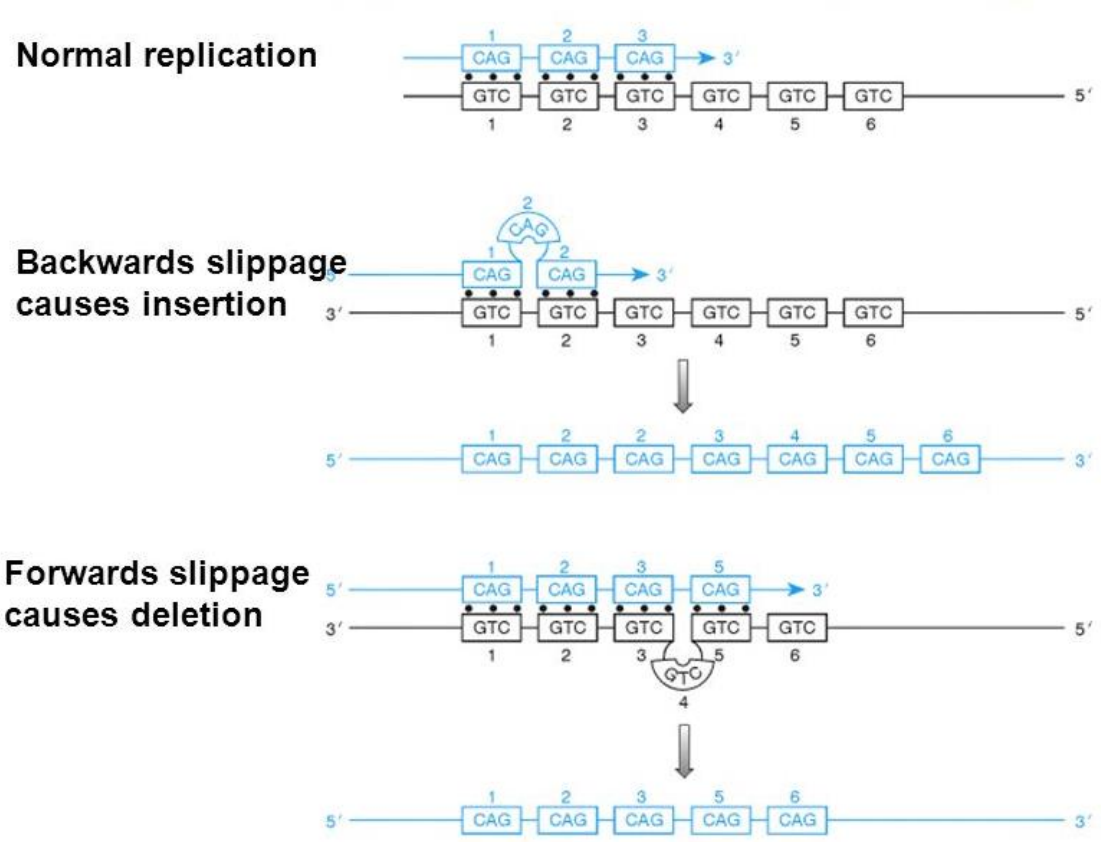


Regulation of flagellin genes in Salmonella. H1 (*fljA*) and H2 (*fljB*) are different flagellins. In one orientation H2 is expressed **(a)**; in the opposite orientation H1 is expressed **(b)**.

Mechanism of slipped-strand mispairing (SSM)

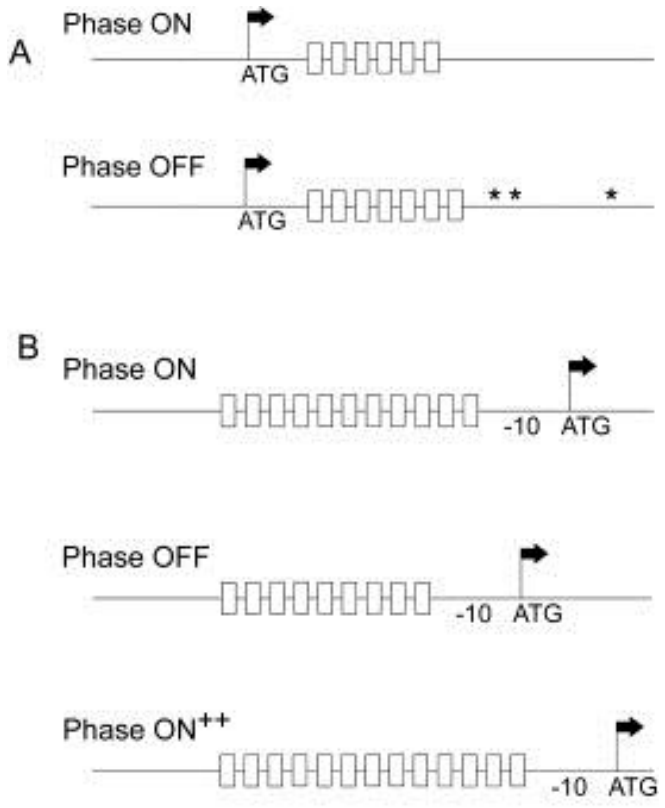
SSM is a common strategy for phase variation by bacterial, fungal and protozoan pathogens. It is a process that produces mispairing of **short repeat sequences** between the mother and daughter strand during DNA synthesis.

Illegitimate base pairing in regions of repetitive DNA during replication, can produce **deletions or insertions of repeat units**. Backwards slippage and forwards slippage gives rise to larger and smaller numbers of repeat units in the synthesized strand.



Levinson and Gutman, Nature **322**: 652-656, 1987

Model for phase variation via slipped-strand mispairing.

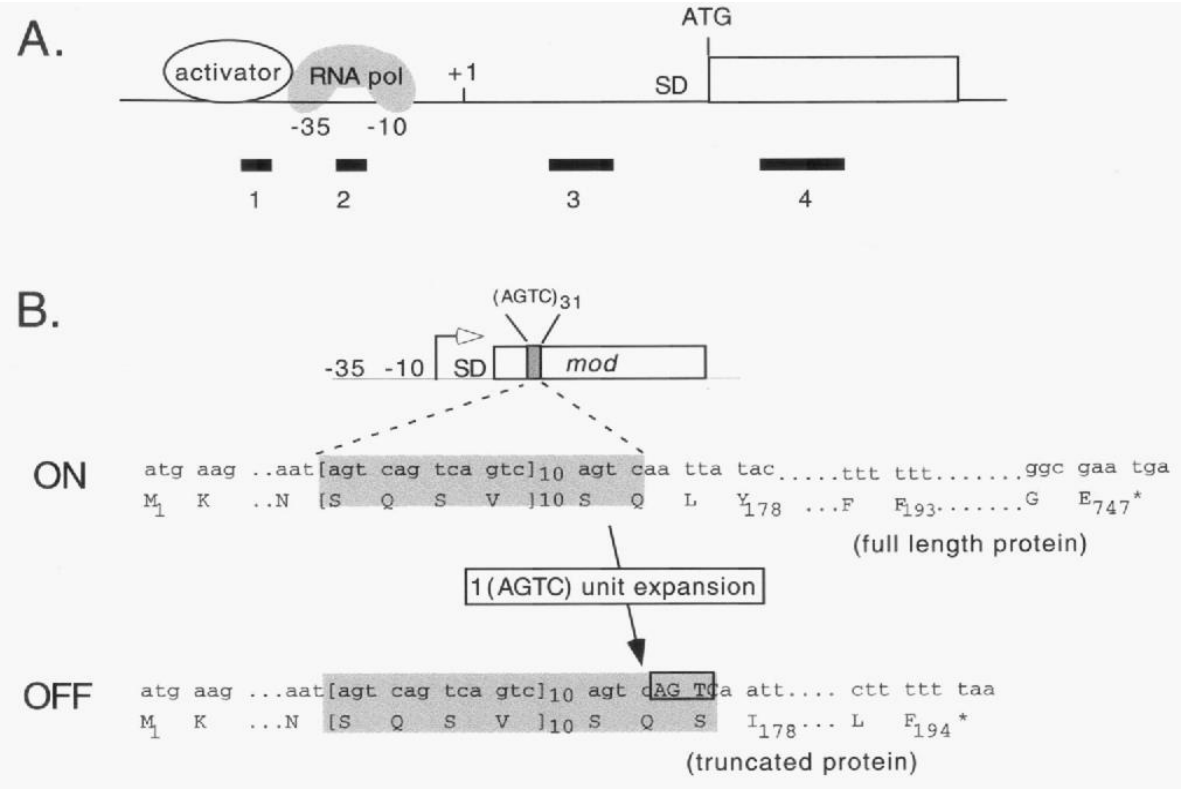


Variations in the number of repeats **within the coding region** of the gene results in a shift of reading frame in or out of frame. A shift out of frame will introduce premature stop codons (*).

Variations in the number of repeats **within the promoter region** of the gene will vary promoter −10 and −35 spacing, thereby increasing (ON++) or decreasing (ON or OFF) promoter efficiency

Phase variation in gene encoding a major fimbriae in *H. influenzae*

A tetranucleotide repeat sequence (AGTC) is present in the promoter and in the coding sequences of the *mod* gene for fimbriae protein in H. influenzae



A. Scheme of four positions, relative to a gene, at which tetranucleotide **AGTC** repeats are presents.

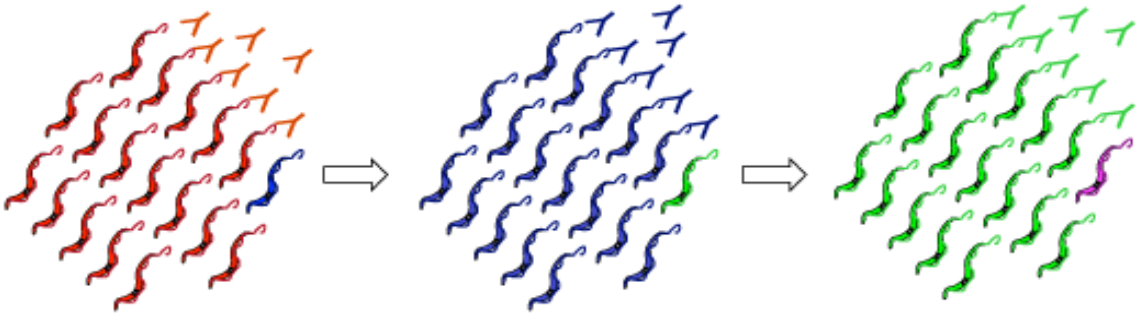
B. One-unit insertion of the repeat AGTC in the **coding sequence**. The reading frame changes leading to the formation of a premature stop codon (*).

A similar mechanism is present in bvgS regulatory gene of *B. pertussis* and Opa and Opc protein of *Neisseria* species. $(CTCTT)_n$

Antigenic variation

Antigenic variation refers to the expression of functionally conserved moieties within a clonal population **that are antigenically distinct**. The genetic information for producing a family of antigenic variants is available in the cell, but only one variant is expressed at a given time.

Antigenic variation is present also in eukaryotic pathogens, including *Plasmodium falciparum* and trypanosomes.

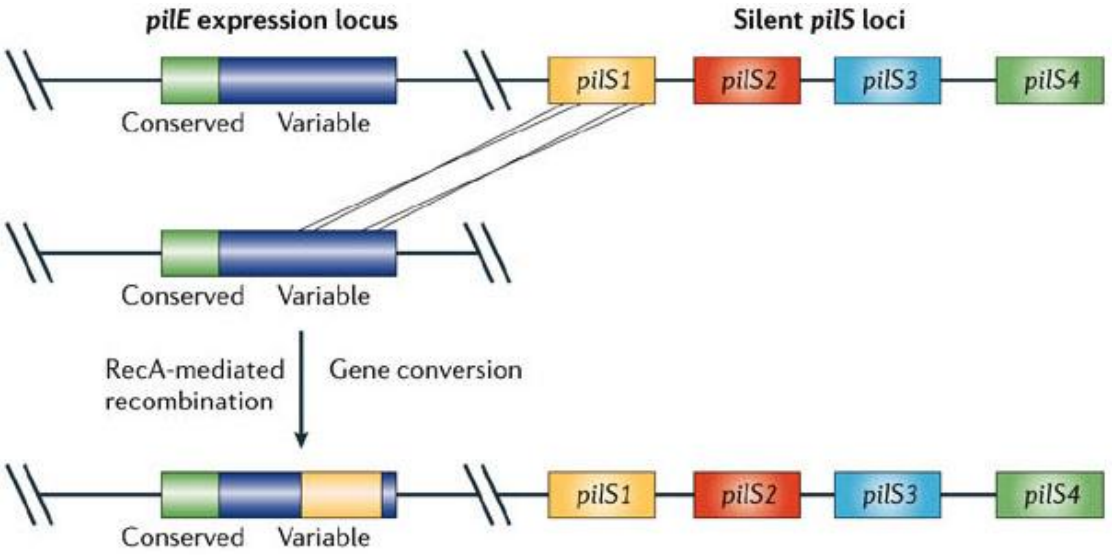


Trypanosomes multiply in the blood of the host until an antibody response results in lysis of recognized variants. Switched variants have a selective growth advantage until a host antibody response is mounted against these too.

Type IV pilin antigenic variation in *Neisseria gonorrhea*

The most remarkable feature of *N. gonorrhea* is its ability to evade the host's immune system through variation of its surface antigens (**type IV pili).** Recombination (>10^{-3}), between different variants (up to 1×10^{6}) of the same gene can occur.

It is a result of unidirectional transfer to the expression locus ***pilE*** of a sequence from one of the numerous **silent *pilS* loci** without altering the donor *pilS* sequence **(gene conversion).**



There can be 1-10 copies of the silent loci on the genome, and a even higher variability may occur by intergenic recombination after natural transformation with pilS genes of different bacteria.

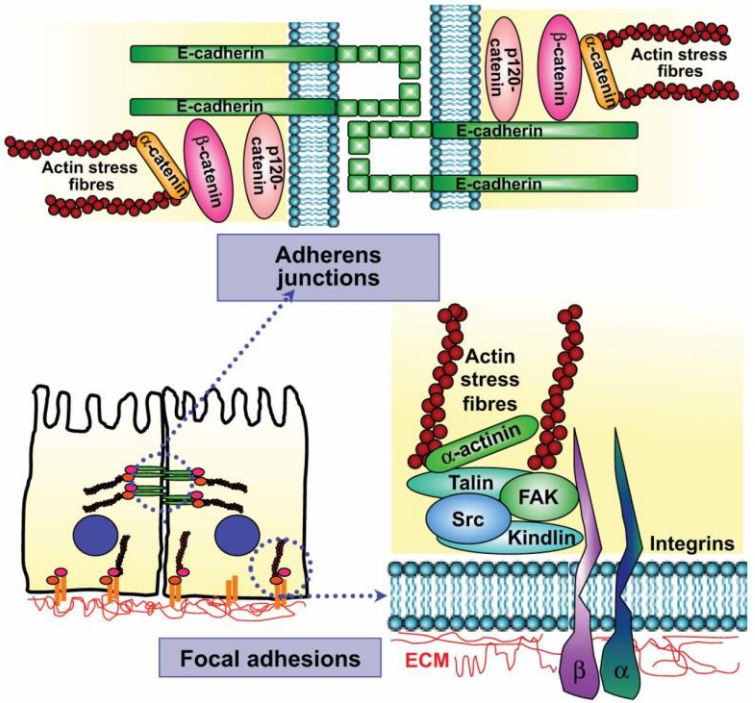
Summary of Anti-Immune Strategies of Bacteria

Strategy	mechanisms
Modulators on the pathogen surface	Self carbohydrates for the capsules Reducing the negative charges of the surface
Antigenic hypervariability	•Antigenic Variation in suface structures
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
Inhibit cytokines/ interferon/chemokines activities	•Secrete proteases to degrade cytokines
To impair T cells responses	superantigens

Slides aggiuntive di ripasso utili (non in programma d'esame) per comprendere gli argomenti descritti in questo capitolo

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.



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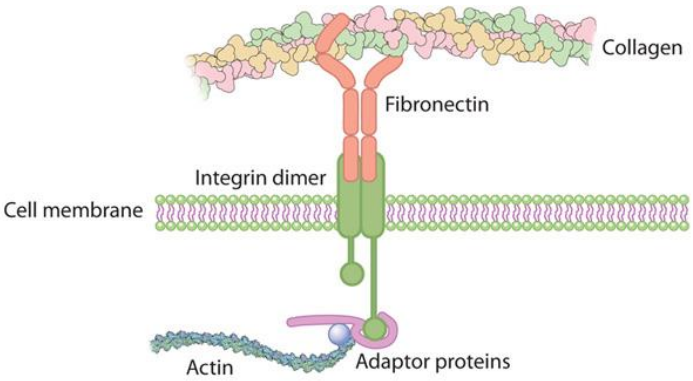
E-cadherin, single-pass transmembrane protein, whose extracellular domain, which is composed of five Ca^{2+}-binding repeats (green squares), mediates specific **homophilic interactions** with neighbouring cells (adherens junction).

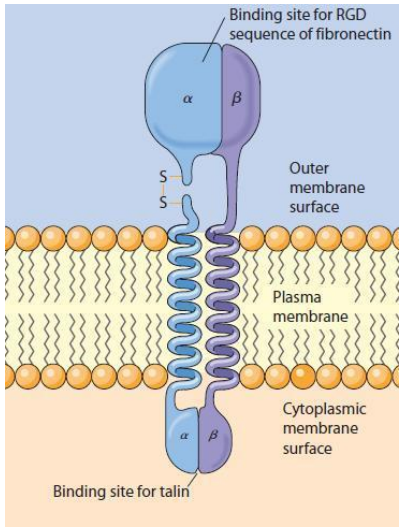
Focal adhesions are multi-protein complexes that mediate the contact of cells to the ECM (red lines); the membrane receptors for this type of adhesion are heterodimers of **α- and β-integrins.**
They form multi-protein complexes that are linked to the actin cytoskeleton

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.

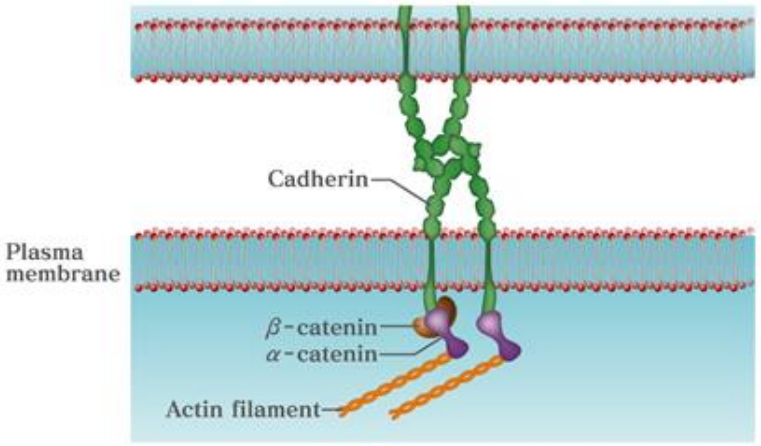
Cadherins are a class of membrane glycoproteins folded into polypeptide **chain repeats.** Adhesiveness of cadherins depends on the presence of extracellular Ca^{2+}, allowing interaction with similar domains on other cell (**homophilic interactions).**

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.

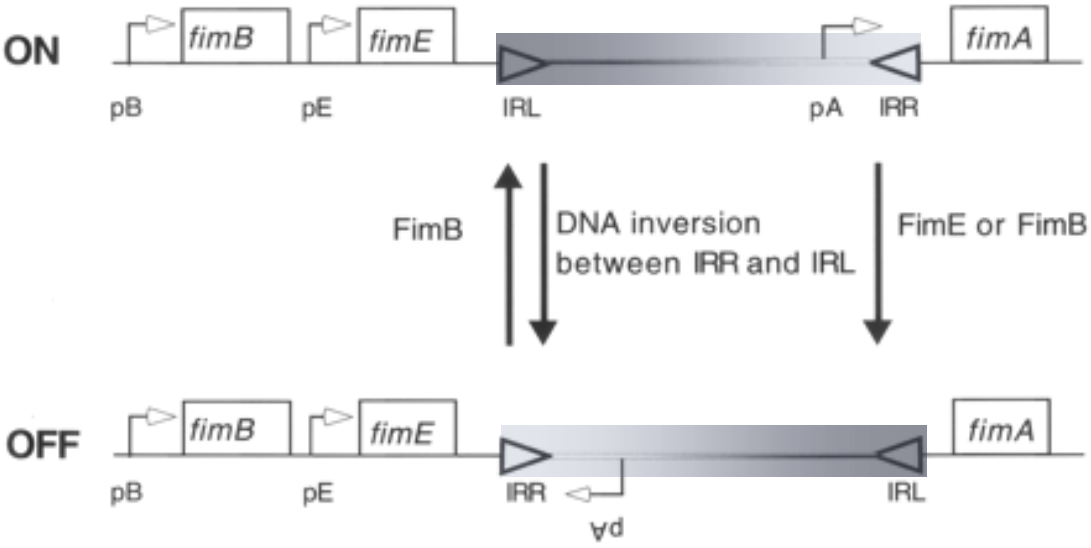


http://csls-text.c.u-tokyo.ac.jp/active/11_01.html

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.

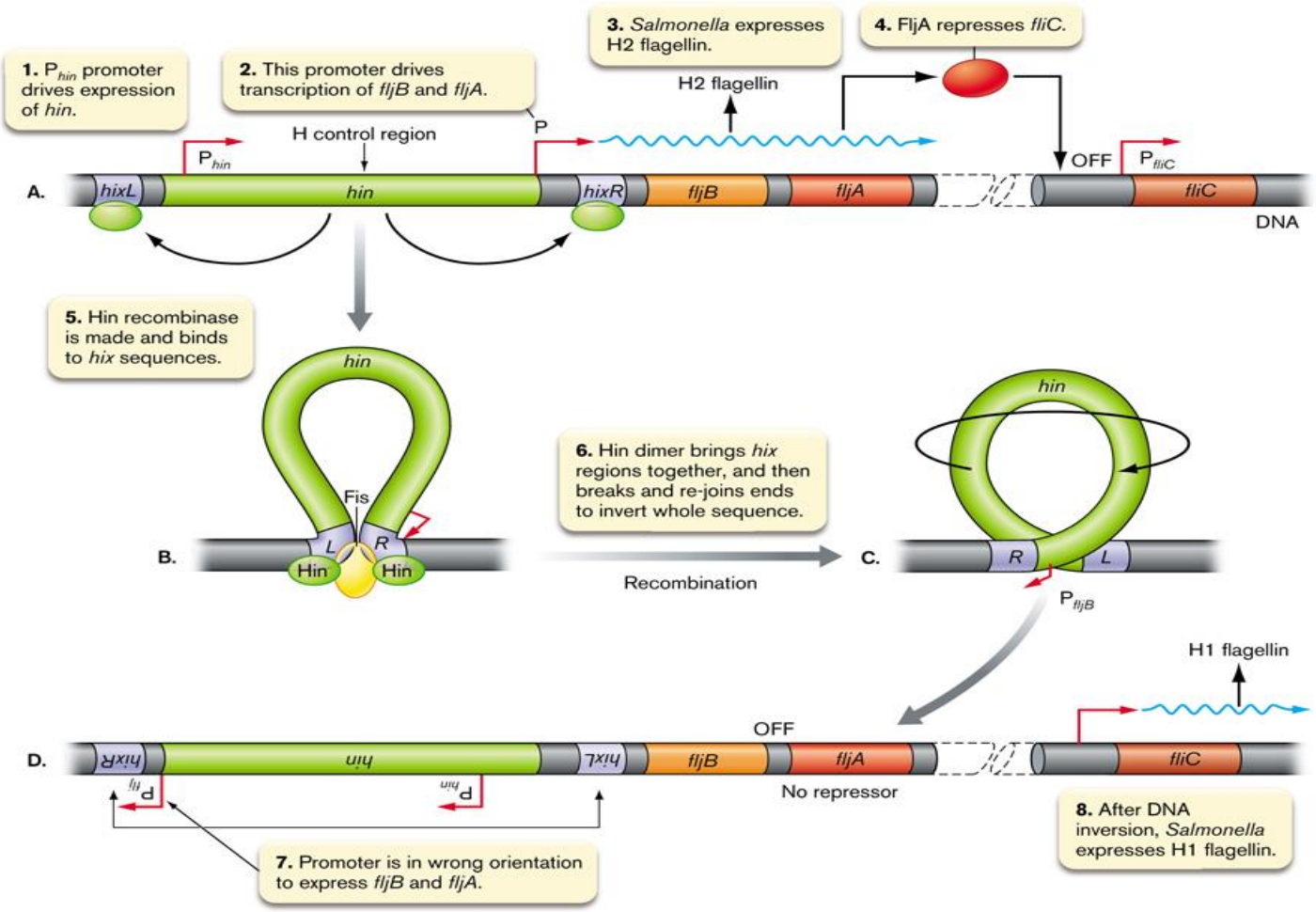
Phase variation by means of DNA inversion

Phase variation of **type I fimbrial expression**, encoded by the *fim* operon, in *E. coli* as a result of DNA inversion. The main subunit of the fimbriae is coded by *fimA* in ON orientation.



The mechanism of inversion is a **site-specific recombination**: the invertible element contains the promoter for *fimA* that is essential to transcribe the structural operon. The invertible element consists of 300 bp flanked by two 9-bp inverted repeats which are within the binding sites for the **recombinases** FimB and FimE.

Hin recombinase-mediated inversion



The mechanism of inversion is a **site-specific recombination**: the invertible element contains the promoter for the structural operon encoding *H2 flagellin and* H1 repressor.