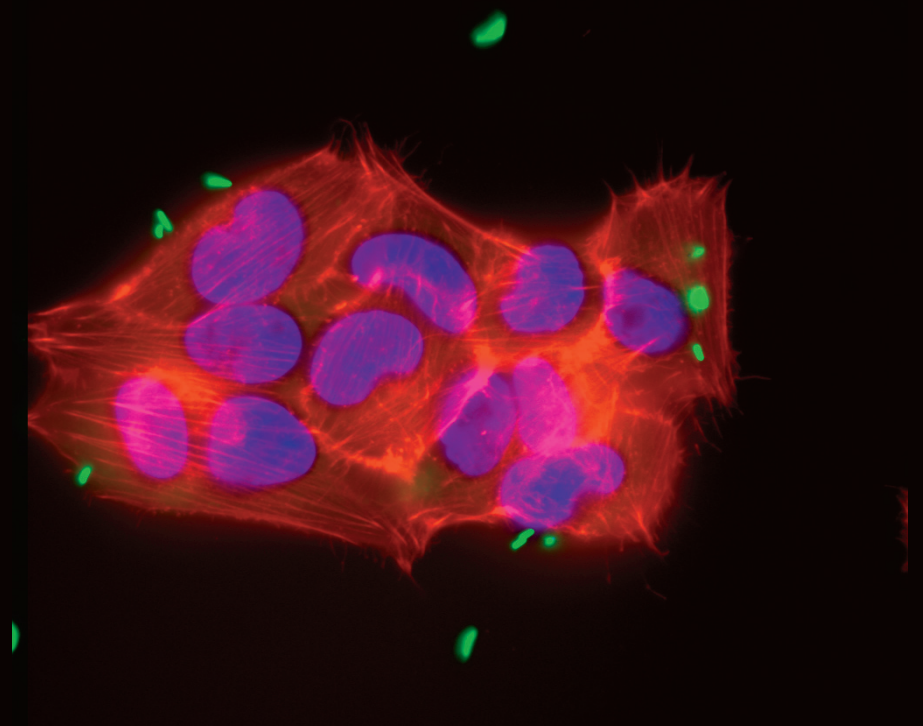


Thesis for doctoral degree (Ph.D.)
2011

Regulation of internalization and replication of intracellular bacterial pathogens



Eugénie Bassères

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**Karolinska
Institutet**



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Cover illustration: HEK293 cells with UCH-L1 knock down infected with GFP-*Listeria* (green). Nucleus (blue), actin (red).

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“Plonge dans l’étonnement et la stupefaction sans limites, ainsi tu peux être sans limites, ainsi tu peux être infiniment.”

Eugène Ionesco

ABSTRACT

The capacity of intracellular bacteria to cause disease depends on their ability to invade and replicate within eukaryotic host cells. These characteristics also allow preferential invasion and replication by facultative anaerobic bacteria in solid tumours, which can be exploited to design delivery vectors for cancer therapy.

The aim of this thesis is to study the molecular mechanisms that regulate two parameters of bacterial invasion: internalization and escape from the host innate immune response.

We show that the deubiquitinating enzyme UCH-L1 promotes internalization of *Listeria monocytogenes* and *Salmonella enterica* in epithelial cells. Knockdown of UCH-L1 reduces the uptake of both bacteria in UCH-L1-positive epithelial cells, while expression of the catalytically active enzyme promotes internalization in the UCH-L1-negative HeLa cell line. This effect is dependent on modulation of the actin cytoskeleton dynamics, alteration of clustering and activation of the *L. monocytogenes* receptor Met, a receptor tyrosine kinase (RTK). Actin cytoskeleton re-arrangement and RTK signalling share a common effector protein: the focal adhesion kinase (FAK), a key regulator of focal adhesion complexes. We found that UCH-L1 interacts with components of the focal adhesion and cadherin complexes: FAK, paxillin, vinculin, β -catenin and p120, and further regulates the activation of FAK and the formation of focal adhesion complexes, leading to an increase of adhesive capacity and motility of the cells.

These findings highlight an unrecognized involvement of the ubiquitin cycle in bacterial entry. Considering that UCH-L1 is highly expressed in malignant cells, this may represent one of the mechanisms by which intracellular facultative anaerobic bacteria preferentially localize within solid tumours.

Intracellular bacteria replication is controlled by the activation of a broad array of defensive mechanisms, but mainly relies on compartmentalization followed by lysosomal destruction of the invading microorganisms in professional phagocytic cells, macrophages and neutrophils. Several pro-inflammatory cytokines enhance the bactericidal capacity of the host cells. We demonstrated that the *bona fide* cytokine Thioredoxin (Trx) 80, a truncated form of Thioredoxin 1, induces monocytes activation and inhibits replication of intracellular pathogens by trapping the bacteria into the lysosomal compartment, thus promoting their destruction. Our results show that Trx80 potentiates the bactericidal activities of professional phagocytes, and contributes to the first line of defense against intracellular pathogens.

LIST OF PUBLICATIONS

This thesis is based on the following papers and manuscript. They will be referred in the text by their roman numeral.

- I. The ubiquitin C-terminal hydrolase UCH-L1 promotes bacterial invasion by altering the dynamics of the actin cytoskeleton.

Bassères E, Coppotelli G, Pfirrmann T, Andersen JB, Masucci M, Frisan T.
Cell Microbiol. 2010 Nov;12(11):1622-33.

- II. The ubiquitin C-terminal hydrolase UCH-L1 protects the focal adhesion kinase from degradation and promotes formation of focal adhesion complexes.

Rikard Dryselius*, **Eugénie Bassères***, Teresa Frisan and Maria G Masucci.
Manuscript

* These authors equally contributed to the work.

- III. Thioredoxin 80-activated monocytes (TAMs) inhibit the replication of intracellular bacterial pathogens.

Cortes-Bratti X, **Bassères E**, Herrera-Rodriguez F, Botero-Kleiven S, Coppotelli G, Andersen JB, Masucci MG, Holmgren A, Chaves-Olarte E, Frisan T, Avila-Cariño J.
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LIST OF ABBREVIATIONS

5FC	5-fluorocytosine
BCV	<i>Brucella</i> containing vacuole
CD	Cytosine deaminase
ClyA	Cytolysin A
DNA	Deoxyribonucleic acid
DUB	Deubiquitinating enzyme
EEA1	Early endosome antigen 1
EMT	Epithelial to mesenchymal transition
ECEF	Eosinophil cytotoxicity enhancing factor
<i>E. coli</i>	<i>Escherichia coli</i>
ER	Endoplasmic reticulum
FAK	Focal adhesion kinase
Gab-1	GRB2-associated-binding protein 1
GTPase	Guanosine triphosphatase
GEF	Guanine nucleotide exchange factor
GFP	Green fluorescent protein
HEK293	Human embryonic kidney 293 cell line
HGF	Hepatocyte growth factor
iNOS	Inducible nitric oxide synthase
iRNA	Interference Ribonucleic acid
IFN- γ	Interferon gamma
IL	Interleukin
Inl	Internalin
JAMM	Josephins and JAB1/MPN/MOV34 metalloenzymes
K	Lysine
Lamp	Lysosomal-associated membrane protein
LFA-1	Lymphocyte function-associated antigen 1
<i>L. monocytogenes</i>	<i>Listeria monocytogenes</i>
LLO	Listeriolysin O
LPS	lipopolysaccharide
MAP	Mitogen activated protein
MHC	Major histocompatibility complex

MOI	Multiplicity of infection
NADPH	Nicotinamide adenine dinucleotide phosphate
NEL	Novel E3 ligase
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer cell
NLR	NOD-like receptor
NOD	Nucleotide-binding oligomerization domain
NR	Nitroreductase
OTU	Ovarian tumor proteases
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
PBMCs	Peripheral blood mononuclear cells
pFAK	Phosphorylated FAK
PI3K	Phosphatidylinositol 3-kinase
PLC	Phospholipase
PRR	Pattern recognition receptor
RNA	Ribonucleic acid
RTK	Receptor tyrosine kinase
qPCR	Quantitative polymerase chain reaction
SCV	<i>Salmonella</i> containing vacuole
<i>S. flexneri</i>	<i>Shigella flexneri</i>
<i>S. choleraesuis</i>	<i>Salmonella enterica</i> serovar choleraesuis
<i>S. typhimurium</i>	<i>Salmonella enterica</i> serovar typhimurium
TAMs	Trx80-activated-monocytes
TGF-β	Transforming growth factor beta
Th	T helper cell
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor alpha
Tr	Regulatory T cell
Trx	Thioredoxin
TTSS	Type III secretion system
Tyr	Tyrosine
UBD	Ubiquitin binding domain
Ub	Ubiquitin
UCH	Ubiquitin C-terminal hydrolase

UPS	Ubiquitin proteasome system
USP	Ubiquitin specific proteases
<i>V. cholerae</i>	<i>Vibrio cholerae</i>
WASP	Wiskott-Aldrich Syndrome protein
Y	Tyrosine

1. PRINCIPLES OF BACTERIAL PATHOGENESIS

Bacterial pathogenesis is a multi-step process that is modulated by several factors: the bacterial strain and its toxicity (defined by the presence of virulence factors), the amount of bacteria at the inoculation site and the efficiency of the host immune system. Here we will review some of the mechanisms that allow bacteria to establish efficient infection.

The first step of bacterial infection is cell adhesion. Indeed, most bacteria need to initiate attachment for a successful infection. Pathogens usually colonize host tissues that are in contact with the environment: conjunctive, respiratory, urinary or digestive tract. Bacteria attach to these mucosal surfaces via diverse adherence factors called adhesins, often in a specific manner. The adhesin is any bacterial ligand, such as a capsule component, cell wall, pili or fimbriae, that binds to a host cell surface receptor: glycoprotein such as integrins, cadherins, selectins. Commonly, one bacterium expresses and uses more than one adhesin [1].

Fimbriae are hair-like structures that extend from the bacterial surface. Particularly Gram-negative pathogens such as *Escherichia coli* (*E. coli*), *Vibrio cholerae* (*V. cholerae*), *Pseudomonas aeruginosa* (*P. aeruginosa*) or *Neisseria* species use fimbriae to adhere [2,3,4]. Afimbrial adhesins do not form the long, polymeric fimbrial structure leading to closer contact between the bacteria and the host cell. These are produced by Gram negative: *Yersinia pseudotuberculosis*, enteropathogenic *E.coli*, *Neisseria* spp., Gram positive: *Staphylococcus* spp. *Streptococcus* spp., and mycobacterial pathogens [2] [5] [6] [7] [8].

After adherence, pathogens need to reach niches in tissues to allow them to proliferate and disseminate. This is termed invasion. To invade tissues, bacteria produce extracellular proteins called invasins. Invasins are enzymes that damage the extracellular matrix and host cells, facilitating the growth and spreading of the bacteria.

Spreading factors such as hyaluronidase (which cleaves proteoglycans in connective tissue), streptokinase and staphylokinase (which break down fibrin clots), collagenase and neuraminidase affect the physical properties of the extracellular matrix to facilitate the diffusion of the bacteria. Other enzymes as phospholipase, lecithinase and hemolysin are able to lyse cell membranes leading to phagosome or cell lysis [1, 2].

Some digestion factors like proteases, lipases (which degrade accumulated host oils) or nucleases (which digest released DNA or RNA) may help to break down host barriers by degrading host cell molecules. Coagulase converts fibronectin into fibrin, causing clotting. *P. aeruginosa* secretes elastase, which degrades extracellular molecules and helps tissue invasion associated with keratitis, burnt tissue necrosis and cystic fibrosis [9].

The first line of defence against bacteria during the infectious process is inflammation with the recruitment of neutrophils and macrophages. Their function is to engulf, kill and digest bacteria. In order to survive, the most successful pathogens possess additional structural or biochemical implements and have elaborated different strategies to evade the host immune system.

The simplest strategy is to avoid encountering phagocytes. Some pathogens invade tissues without macrophages like in the lumen of glands (*Leptospira*). Certain agents induce minimal inflammation to not attract the phagocytes (*bacteroides* in mice). A few species inhibit chemotaxis towards phagocytes or leukocytes (*Staphylococcus aureus*).

Some bacteria hide from the immune system by covering their surface with a host component (*S. aureus*, *E. coli*).

An important component used by bacterial pathogens to evade clearance is the capsule, which protects bacteria from phagocytosis as well as antibiotics. The capsule is a coat made of excreted high molecular weight polysaccharides. *Streptococcus pneumoniae*, *Neisseria meningitidis* and *P. aeruginosa* produce capsules with different chemical composition and immunomodulatory effects. The incapacity of macrophages to engulf encapsulated bacteria leads to an enhanced inflammatory response, which in turn tends to increase tissue damage [10,11].

Finally, some bacteria produce extracellular enzymes or toxins called aggressins that are able to kill the phagocyte before or after ingestion. This is the case for the hemolysins produced by many Gram-positive bacteria or the exotoxin A produced by *P. aeruginosa*.

Intracellular bacteria have the ability to survive and multiply inside phagosomes after engulfment. Intracellular lifestyle permits the evasion of humoral immune response. However, inside infected cells, bacteria have to face intracellular defences and a particularly hostile and evolving environment inside phagosomes, characterized by poor nutrient contents, low pH, and presence of bactericidal compounds or enzymes added via lysosomal fusion. To stay alive, bacteria are able, through protein production, to modify their surroundings, creating specialized niches to replicate [1] [2] [12].

Once inside a host cell, a *Salmonella* containing vacuole (SCV) undergoes a maturation process. The vacuolar membrane rapidly acquires markers such as early endosome antigen 1 (EEA1) and the transferrin receptor, suggesting a direct fusion with early endosomes. Subsequently, proteins associated with later stages of the endosomal pathway such as lysosomal glycoproteins (lgps), lysosomal membrane associated protein (Lamp)1, Lamp2 and cd63 are acquired. However, unlike early

endosome, there is no direct interaction between SCV and late endosomal compartment. A selective delivery of lysosomal glycoproteins but not soluble enzymes occurs via a novel type of rab7- and lgps-containing vesicles. In addition, two Type III secretion systems (TTSS) and their effectors are involved in this maturation process which is yet poorly characterized [13].

Another example of persistence in the host, *Mycobacterium tuberculosis* inhibits the maturation of the phagosome. This arrest is associated with an aberrant retention of Rab5, an early endosome marker and a deficiency of Rab7, involved in late endosome trafficking and phagosome maturation. *Mycobacterium* also interacts with the host protein, coronin1 and the retention of this protein on the surface of the phagosome may avoid its fusion with phagolysosomes. This indicates that *Mycobacterium* might interfere at various stages of the maturation process of the phagosome in order to inhibit its fusion with lysosomes [14].

Some other bacteria such as *Listeria monocytogenes* and *Shigella flexneri* are able to escape the phagosome and replicate in the cytosol [9] [15]. The escape of *L. monocytogenes* from the phagosome is mediated by listeriolysin O (LLO) and phospholipases C (PLC). LLO production is tightly regulated: it is fully effective at low pH to protect infected cells from damage and is activated only in the vacuole, by the γ -interferon (IFN- γ) inducible lysosomal thiol reductase, a host factor. LLO forms pores into the vacuolar membrane by binding to cholesterol. Once in the cytosol, *Listeria* can replicate and hijacks the actin cell machinery to move to the membrane, spread to a neighbouring cell and start a new cycle of infection.

S. flexneri possesses a TTSS that allows the injection of effectors into the host cytosol. One effector, IpaB, has haemolytic activity and similarly to LLO, IpaB as a complex with another effector, IpaC, binds to cholesterol and creates pores in the plasma membrane of the cell during invasion. However, the role of this complex during

vacuolar escape is not confirmed: other genes from a large virulence plasmid could be involved.

In addition to adhesins and invasins, bacteria produce a broad array of toxins, which can be distinguished as endotoxins and exotoxins. Endotoxins are components of the cell wall (the non-proteinaceous toxins) such as lipopolysaccharide (LPS) from Gram-negative bacteria and teichoic acid from Gram-positive bacteria. Exotoxins are proteins, which are released from the bacteria and act at tissue sites removed from the site of bacterial growth (botulinum from *Clostridium botulinum*, LLO from *L. monocytogenes* or elastase from *P. aeruginosa* for example) [1].

2. BACTERIA AS VECTORS FOR ANTICANCER THERAPY

Knowledge about infection pathways is important for both treatment and the development of new antibacterial drugs. Moreover, an interesting twist in the ability of bacteria to invade eukaryotic cells is the possibility to use them as delivery vectors for anti-cancer therapy.

Bacteria possess unique properties that allow them to be used as original tools for cancer treatment. A number of bacteria specifically target tumour cells, actively penetrate tissues, and, once genetically modified, can induce toxicity in a controlled way. *Salmonella*, *Listeria* and *Clostridium*, for example, have been extensively studied the past decades and have been shown to control tumour growth and increase survival in animal models.

One of the major hassles in cancer therapy is the lack of specificity for tumour cells and the possibility to reach all the cells within the tumour mass. Bacteria have inherent biological properties to overcome these problems. Many bacteria such as *Salmonella*, *Listeria*, *Clostridium*, *Escherichia* and *Bifidobacterium* have been shown to accumulate specifically in tumours. To reach the tumour, bacteria possess characteristics like flagella or quorum sensing [16,17] [18,19,20].

The main problem that appears while using bacteria as anti-cancer agents is to balance their own toxicity and the toxicity needed for therapeutic efficacy. To increase their efficiency, genetically modified bacteria have been engineered to express a specific therapeutic gene. By producing the protein of interest specifically at the tumour site, bacteria may serve as vector or vehicles for delivering cytotoxic agents that are killing cancer cells. These agents can be bacterial toxins, cytokines or tumour antigens stimulating the immune system, angiogenic components or pro-drugs. Moreover, bacteria can be detected using different imaging techniques.

All of these qualities allow a tight regulation of the treatment delivery compared to intravenous administration. Intratumoral drug activity would be more efficient in killing cancer cells and therefore be less toxic to normal tissue. Many strategies have been already tested in animal models leading to several phase I clinical trials (Table 1).

Table 1: Human clinical trials using bacterial cancer therapies modified from [21]

Bacterial strain	Cancer type	Response /Clinical phase
<i>Clostridium butyricum</i> M-55	Squamous cell carcinoma Metastatic, malignant neuroma Leiomyosarcoma Melanoma Sinus carcinoma Vascular glioblastoma	Oncolysis Phase I complete
<i>Salmonella typhimurium</i> VNP20009	Metastatic melanoma Renal cell carcinoma	Focal tumour colonization Phase I complete
<i>Salmonella typhimurium</i> VNP20009	Metastatic melanoma	Tumour biopsy culture positive for VNP20009 Phase I complete
<i>Salmonella typhimurium</i> VNP20009	Squamous cell carcinoma Adenocarcinoma	Intratumoral bacterial colonization Phase I complete

The drawback of using bacteria as anti-cancer agents is to balance their own toxicity and the toxicity needed for therapeutic efficacy. To increase their efficiency, genetically modified bacteria have been engineered to express a specific therapeutic gene. Here are some examples of strategies already tested.

Cytolysin A (ClyA) is a bacterial toxin that forms pores in mammalian cell membranes inducing apoptosis. Several groups have shown that treating mice with *E. coli* or *S. typhimurium* expressing ClyA reduces tumour growth [22,23,24].

The pro-drug strategy uses anaerobic bacteria transformed with an enzyme that can convert a non-toxic pro-drug into a toxic agent. Anaerobic bacteria proliferate specifically in the necrotic and hypoxic areas of the tumour, allowing the enzyme to be expressed at the tumour site. Several enzyme/pro-drug systems are available. Cytosine deaminase (CD) converts 5-fluorocytosine (5FC) into 5-fluorouracil and nitroreductase (NR), which in turn converts the pro-drug CB1954 to a DNA cross-linking agent. Clinical trials are taking place using a highly attenuated strain of *Salmonella*, VPN20009, as a vector combined with CD and NR [25]. Its low pathogenicity is due in part to the purine auxotrophy of the strain (the bacteria cannot survive in the absence of this compound) and also to the disruption of the *msbB* gene, which alters the lipid A, decreasing the potential to activate tumour necrosis factor- α (TNF- α) and cause septic shock. The low pathogenicity, the lack of antibiotic resistance and the intrinsic properties of *Salmonella* make this strain a putative safe and effective vector.

Targeting angiogenesis is another strategy tested to kill tumour cells. Pre-clinical studies have shown that endostatin, a C-terminal fragment of collagen VIII that exhibits anti-angiogenic activity, inhibits tumour growth when they lack acquired tumour resistance. Indeed, the use of a *S. choleraesuis* attenuated strain carrying an eukaryotic vector expressing endostatin in a mice tumour model showed an inhibition of the tumour growth and higher survival [26].

Finally, an alternative anti-cancer approach is anti-tumour vaccination therapy. Preclinical studies are ongoing to use an attenuated strain of *S. typhimurium* carrying plasmid DNA encoding tumour-associated antigens against hepatocellular carcinoma (HCC) in mice. It has been shown that orally delivered attenuated *S. typhimurium* carrying HCC self-antigen α -fetoprotein (mAFP) protects mice against mAFP expressing tumours [27].

Extensive studies have led to the development of promising strategies for anti-cancer treatments. Yet to improve these tools, we need to understand the still poorly characterized mechanisms by which intracellular bacteria such as *L. monocytogenes* and *S. enterica* invade and replicate preferentially solid tumours.

3. AIMS

The work included in this thesis aimed to dissect two aspects that are relevant for a better understanding of bacterial pathogenesis and for the development of efficient bacteria based delivery vectors:

A. Regulation of bacterial entry

B. Bacterial escape from the host innate immune response

Specifically we asked:

1. Do components of the ubiquitin proteasome system regulate bacterial entry?

We demonstrated that the ubiquitin C-terminal hydrolase UCH-L1 promotes bacterial invasion by altering the dynamics of the actin cytoskeleton and activation of the *L. monocytogenes* cellular receptor Met.

2. Does Thioredoxin 80 prevent replication of intracellular bacteria?

We demonstrated the *bona fide* cytokine Thioredoxin 80, a truncated form of Thioredoxin 1, prevents replication of intracellular bacteria pathogens by promoting their lysosomal degradation in monocytes.

4. MODELS

As models we have chosen two intracellular bacterial pathogens: *Listeria monocytogenes* and *Salmonella enterica* serovar Typhimurium. These bacteria efficiently invade, survive and replicate within the host cells [1], and have been used as delivery vectors in phase I clinical trials [21].

Listeria monocytogenes

L. monocytogenes is an intracellular Gram-positive bacterium that is the causative agent of a severe food-borne disease, listeriosis. The ability of the bacteria to cross three host barriers: intestinal, blood-brain and materno-foetal barrier, lead to different types of syndromes such as gastroenteritis, meningitis, materno-foetal or perinatal infections, abortion or sepsis. The incidence of the disease is relatively low due to strict food control. However, the lethality rate reaches 30% because of a very high susceptibility in immunocompromised individuals, new borns and foetuses [28].

L. monocytogenes takes advantage of host cell signalling pathways at different stages of the infection by mimicking host proteins. *Listeria* invade a wide variety of cells through direct phagocytosis or by binding via virulence factors: Internalins (Inl) A and/or B. InlA binds to the adherent junction protein E-cadherin and InlB to the tyrosin kinase receptor Met. InlA and B mimic E-cadherin and Met respective ligands E-cadherin and hepatocyte growth factor (HGF) [29,30]. The binding of InlA and/or B to their receptor induces receptor ubiquitination, recruitment of clathrin, reorganization of the cytoskeleton, and then the uptake of the bacteria by a mechanism that is defined as “zipper” (Figure 1) [31,32,33,34]. Once internalized into a phagosome that has a mildly acidic pH (5,7 to 5,9), *Listeria* secrete other virulence factors: the pore forming toxin Listeriolysine O (LLO) and two phospholipases (PC-PLC and PI-PLC) that destabilize

the phagosolysosomal membrane, thus preventing its fusion with lysosomal compartments. *Listeria* rapidly escape into the cytoplasm of the cell, move to the cell membrane via an actin polymerase (ActA) that hijacks the host actin polymerization machinery, and replicate. Here again *Listeria* mimic structurally a host protein: the Wiskott-Aldrich Syndrome protein (WASP), to recruit the Arp2/3 complex and actin. The “comet tail” is then formed at the pole of the bacteria where ActA is accumulated, allowing *Listeria* to move unidirectionally in the cytosol (Figure 1). To adapt their metabolism to the cytosol environment, *Listeria* use glucose-1-phosphate available in high quantities there and express genes such as hexose phosphate transporter. At the cell membrane, *Listeria* induce protrusions to spread to a neighbouring cell, forming a double-membrane vacuole. Again, *Listeria* can escape this vacuole via LLO and PLC, replicate into the cytosol and spread [15].

Salmonella enterica

In humans, *Salmonella* spp. is believed to cause over one billion infections annually, with consequences ranging from acute gastroenteritis (food poisoning) to systemic, often fatal, typhoid fever. *Salmonella enterica* serovar Typhi is the causative agent of typhoid fever. The genus *Salmonella* has the particularity to invade different hosts except for *Salmonella typhi* and *Salmonella paratyphi* that are restricted to humans and cause enteric fever. *Salmonella typhimurium* is one of the main causes of food-borne gastroenteritis in humans and is also responsible of food poisoning in animals such as cattle, pigs and chickens. *Salmonella typhimurium* infection in mice mimics the typhoid fever symptoms occurring with *Salmonella typhi* infection in humans [129].

Salmonella are facultative anaerobic Gram-negative bacteria that have acquired a specialized protein secretion system, termed type III secretion system (TTSS) encoded by the *Salmonella* pathogenicity island-1 (SPI1) and -2 (SPI2) [35]. The TTSS1

delivers several bacterial effectors proteins directly into the host cell that induce a profound rearrangement of the actin cytoskeleton at the site of entry. This cytoskeletal remodeling drives localized membrane ruffling and lamellipodial extensions that envelop bacteria and trigger their internalization into membrane bound vacuoles (“trigger mechanism”) (Figure 1). At least five distinct effectors are known to contribute to efficient *Salmonella* entry: SipA, SopA, B, D and E2. SipA is involved in actin rearrangement. SopB, E and E2 activate the Rho family GTPases Rac1 and Cdc42 which in turn lead to the recruitment of other protein complexes involved in actin polymerization such as WASP, Scar/WAVE family proteins and the Arp2/3 complex. SptP, another effector protein acts as a negative feed back on actin rearrangement after invasion. Following internalization, *Salmonella* survive and replicate within a modified phagosome known as the *Salmonella*-containing vacuole (SCV), which initially is marked by the accumulation of early endosome markers. These early markers are then rapidly removed and within 60–90 min post invasion SCVs become highly enriched in markers of late endosomes and lysosomes (Figure 1). In addition *Salmonella* have other factors such as fimbriae, flagella and ion transporters that have important roles in establishing and maintaining the intracellular niche. Invasion and early post-invasion processes are modulated by TTSS1. Subsequently the TTSS2 effectors, involved in nutrient acquisition and avoidance of antibacterial mechanisms, are required for survival within the host cells [36].

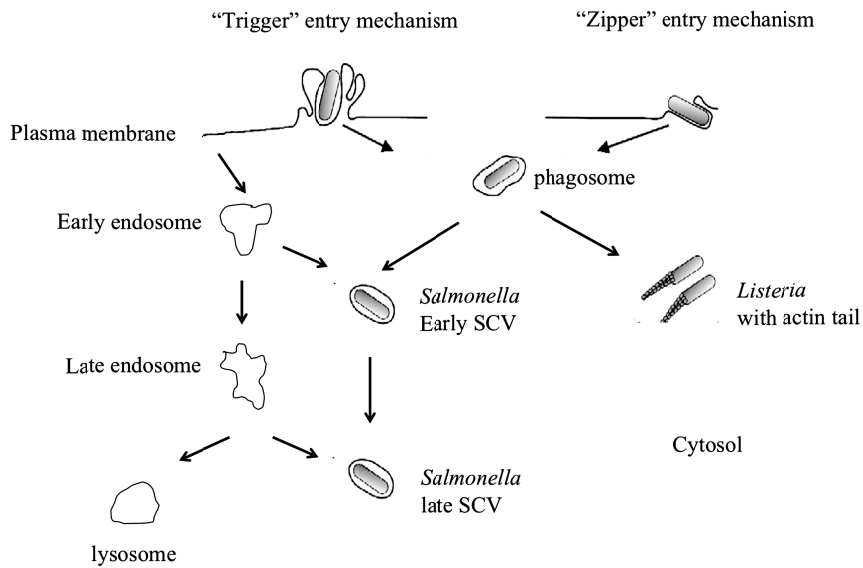


Figure 1: Lifestyles of *Listeria monocytogenes* and *Salmonella enterica*.

Adapted from [37]. *Listeria* enters into cells via the "zipper" mechanism and escapes phagosomes to replicate in the cytosol. *Listeria* moves in the cytosol by hijacking the host actin machinery. *Salmonella* is internalized through the "trigger" mechanism and replicates into early and late *Salmonella* containing vacuoles (SCV), avoiding fusion with lysosomes.

5. THE UBIQUITIN PROTEASOME SYSTEM (UPS) AND ITS ROLE IN REGULATION OF BACTERIA ENTRY

5.1 The UPS at glance

The ubiquitin (Ub) system, in an addition to a signal for protein degradation, is a main regulator of biological processes (Figure 2). Since the 1970s, protein degradation after ubiquitination has been extensively studied and revealed that Ub-mediated destruction plays a crucial role in cell cycle regulation, cell growth, DNA repair and immune functions. More recently, it has been shown that Ub is involved in non-proteolytic functions such as protein-protein interactions, vesicular trafficking, regulation of histone modification and viral budding. As Ub is implicated in so many cellular processes, any alteration in the Ub system can lead to diseases, such as cancer, neurodegenerative or immune disorders. It is therefore a challenge to understand the ubiquitin system in order to develop novel treatments for such diseases.

Ubiquitination is the covalent attachment of a small molecule, Ub, to a specific protein substrate through a three-steps process involving three enzymes. E1, the Ub -activating enzyme, forms a thioester between the catalytic cysteine of the E1 and the C-terminal glycine of Ub. Activated Ub is then transferred to an E2 enzyme forming a new thioester. Several E2, Ub conjugating enzymes, act as carrier proteins. In the last step, an E3 Ub ligase transfers the Ub to a lysine residue on the target protein. There are more than six hundred ligand specific E3 ligases. In a few cases, protein substrates containing an Ub binding domain (UBD) interact with the Ub-loaded E2 enzyme to directly ubiquitinate themselves [38,39,40].

Proteins are commonly ubiquitinated by the formation of an isopeptide bond between the C-terminal glycine of Ub and the ϵ -amino group of a lysine (K) of the targeted protein [41]. Some proteins may also be ubiquitinated on their N-terminal amino group

by N-terminal ubiquitination, like, for example, the Epstein-Barr virus latent membrane protein 1. Ubiquitination may also occur on amino acids other than lysine, such as cysteine, serine or threonine [42].

Each of the seven lysine residues present in the Ub molecule can also be covalently linked to another Ub moiety, leading to the formation of polyubiquitin chains. The fate of the targeted protein depends on which residues are ubiquitinated. Polyubiquitination via K48 is the signal for degradation by the proteasome while single, multiple or polyubiquitination control other pathways, such as endocytosis, DNA repair or transcription (Table 2) [43].

As all tightly regulated cellular processes, ubiquitination is reversible. The molecules responsible for the cleavage of Ub from its ligand are called deubiquitinating enzymes (DUBs). About 100 DUBs have been described and classified into five groups: ubiquitin C-terminal hydrolases (UCHs), Ub-specific proteases (USPs), ovarian tumour proteases (OTUs), Josephins and JAB1/MPN/MOV34 metalloenzymes (JAMMs). The UCH, USP, OTU and Josephin families are Cys proteases, whereas the JAMM/MPN+ family members are zinc metalloproteases. It is thought that each DUB has a limited number of substrates [44].

Table2: Function of ubiquitin chains

Chain structure	Lysine	Function
Monoubiquitination Multi-monoubiquitination		Protein interactions Protein localization Modulation of protein activity
Polyubiquitination	11	Targeting to the 26S proteasome
	48	
	63	NFκB activation DNA repair Targeting to the lysosome
	Amino acid terminus	NFκB activation
	6	Protein degradation? DNA repair?
	27	
	29	
	33	
Branched or forked ubiquitin chains		unknown

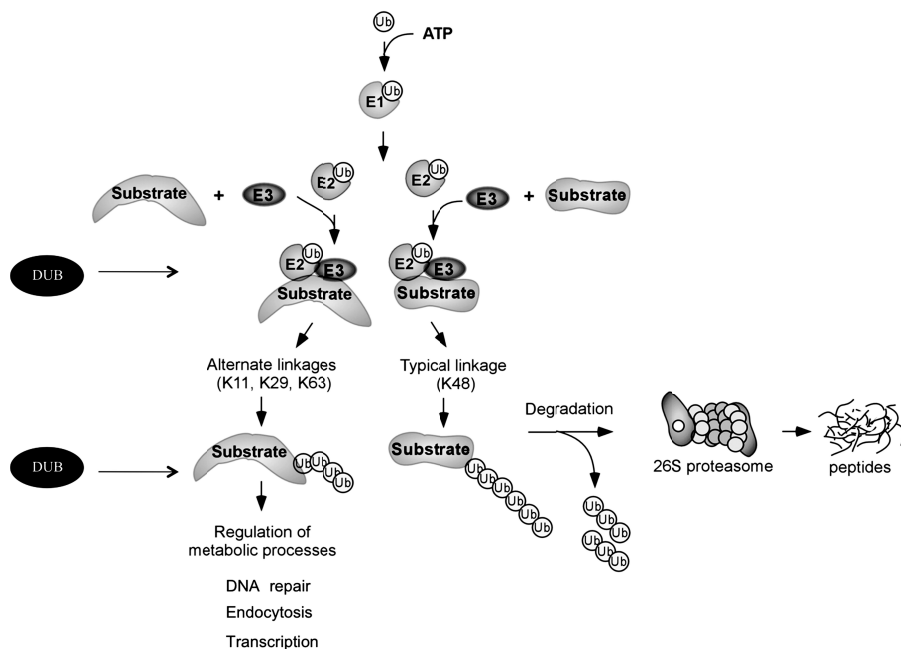


Figure 2: The Ubiquitin proteasome system adapted from [45]

Ubiquitination occurs through a multistep process involving E1, E2 and E3 enzymes leading to the attachment of Ub chains to target proteins. The type of chain modulates the fate of the protein. This process can be reversed at different levels by deubiquitinating enzymes (DUB). (K: lysine)

5.2 UCH-L1

The Ubiquitin C-terminal Hydrolase (UCH)-L1, also known as PGP9.5, is a DUB responsible for hydrolyzing Ub carboxyl terminal esters and amides, thus leading to Ub removal from protein substrates and Ub precursor proteins [46]. The enzyme is highly expressed in testis, ovary and neuronal cells. The *gad* mice, which present a spontaneous autosomal recessive deletion mutant, display axonal dystrophy and altered spermatogenesis [47]. In humans, mutations at or around the catalytic site are associated with familial Parkinson's disease [48] and other neurodegenerative disorders, characterized by the formation of protein aggregates, such as spinocerebellar

ataxia [49] and Huntington's disease [50]. On the other hand, high levels of UCH-L1 are associated with the more invasive variants of neuroblastoma [51], colon carcinoma [52], non small-cell-lung carcinoma [53], pancreatic carcinoma [54], prostate and breast carcinomas [55,56] and renal carcinoma [57]. Recent data indicate that UCH-L1 may contribute to the malignant phenotype by regulating epithelial-to-mesenchymal transition (EMT) [58], a process that allows polarized, generally immotile epithelial cells to acquire a motile mesenchymal phenotype [59]. Many signaling pathways, including the TGF- β , Wnt/ β -catenin, Notch, tyrosine kinase receptors and PI3K/AKT pathways regulate and induce EMT but the critical molecular event is the down-regulation of E-cadherin, a cell-cell adhesion molecule that is highly expressed in most epithelial cells.

Increasing evidence implicates UCH-L1 in the regulation of membrane proximal events, such as vesicular trafficking in pre-synaptic nerve terminals [60], cell motility and invasion [61] [62], the LFA-1 dependent homotypic adhesion of lymphoid cells [63], which is dependent on the clustering of specific receptors. Furthermore, UCH-L1 was recently shown to mediate the editing of tubulin ubiquitination, thereby regulating microtubule dynamics in a variety of transformed cells [65]. However, the lack of information on its physiological substrates has been a major obstacle in dissecting the mechanism of UCH-L1 action. The capacity to hydrolyze polyubiquitin precursors and small Ub adducts is the only experimentally confirmed enzymatic activity of UCH-L1 [66]. High expression of the enzyme was shown to be associated with increased levels of free Ub in neurons but a catalytically inactive mutant had a similar effect [67]. The failure to identify putative UCH-L1 substrates might be explained by the requirement of specific modifications or co-factors regulating its activity. Indeed, the crystal structure of UCH-L1 has revealed a distorted and inaccessible catalytic site, suggesting

that a major conformational rearrangement is required to activate the enzyme [68]. A possible mechanism of activation was recently proposed in a study where monoubiquitination and auto-deubiquitination were shown to affect UCH-L1 activity, possibly by regulating protein-protein interaction [69]. In addition, the activity of UCH-L1 is also regulated by post-translational modifications, such as O-glycosylation [70], and oxidation that appears to be a major cause of UCH-L1 inactivation in the brain of patients suffering from Alzheimer and Parkinson diseases [71].

5.3 UPS and bacteria internalization

Although Ub is not present in bacteria, many bacterial pathogens target the host cell Ub pathway during infection. I will focus here on the internalization step of *Listeria monocytogenes* and *Salmonella enterica*.

Listeria enters into cells by subverting host cell receptors to mediate its own uptake. Two invasion proteins, InlA and InlB, mediate *Listeria* entry into mammalian cells. InlA interacts with the cell adhesion molecule E-cadherin, whereas InlB interacts most importantly with the hepatocyte growth factor receptor (Met/HGF-R) [29,30]. In epithelial cells, internalized E-cadherin can go through the endosomal pathway in order to be degraded by lysosomes or recycled to the cell membrane [72] [73]. E-cadherin internalization depends on its ubiquitination by the E3 ligase Hakai, upon phosphorylation by Src. Ubiquitination and internalization is inhibited by p120, which displaces Hakai from E-cadherin [74].

Concerning the InlB pathway, several studies have shown that InlB mimics functionally but not structurally the Met natural ligand: the hepatocyte growth factor (HGF). Met is a tyrosin kinase receptor, its activation by the binding of InlB via its N-terminal leucin-rich repeats induces Rac-mediated actin rearrangements, namely

“zipper mechanism”[75]. Upon binding, Met dimerizes, autophosphorylates, and is ubiquitinated by the E3 ligase c-Cbl. These modifications trigger the recruitment and/or phosphorylation of several proteins adaptors such as Gab1, Shc and CrkII [76]. These adaptors will then recruit phosphatidylinositol 3-kinase type 1 (PI3K) at the site of bacterial entry, which is involved in the activation of Rac1 [77]. How this pathway then activates the small G proteins Rac and Cdc42 is unknown. Rac and Cdc42 activate Wave and/or N-Wasp, which in turn activate the Arp2/3 complex leading to actin polymerization. Clathrin, which has recently been shown to be involved in internalization of large cargos, is also necessary for Arp2/3 activation [34]. Other PI3K independent partners linked to InlB/Met signaling such as phosphatidylinositol 4-kinases and septin family proteins have also been recently described [78], [79].

Salmonella typhimurium invade non-phagocytic cells via a TTSS [36]. Translocation of effectors into the cytosol of the host cell induce a dramatic rearrangement of the actin cytoskeleton leading to membrane ruffles formation and engulfment of the bacteria, an internalization mechanism known as “trigger” [35]. *Salmonella* hijacks the host cell ubiquitin system to permit a sequential activity of the TTSS1 effector proteins, such as SopE and SptP. SopE acts as a guanine nucleotide exchange factor (GEF) to activate Rho GTPases and induces actin polymerization [80]. In contrast, SptP catalyzes the hydrolysis of GTP inactivating GTPases reversing the pathogen-induced cytoskeletal changes. SopE and SptP are translocated at the same time but have to work sequentially. This is achieved by cellular mediated ubiquitination of SopE, leading to its rapid degradation and therefore fast inactivation [81].

SopB, another TTSS1 effector is associated with the plasma membrane and activates SH3-containing GEF, an exchange factor for RhoG, leading to actin remodelling. After bacterial internalization, SopB is translocated through the SCV membrane where it

plays a role in compartment maturation by reducing the level of negatively charged lipids [82]. Ubiquitination downregulates SopB activity at the membrane and increases its retention in the SCV. The mechanism is yet unknown but does not require any *Salmonella* E3 ubiquitin ligases [83,84].

SopA is a TTSS1 effector associated with the entry of the bacteria into the host [83]. Structural analyses have shown that SopA is a novel HECT-like E3 ligase. No ligand has been identified so far [85].

SlrP, SspH1 and SspH2 effectors are novel E3 ligase (NEL) family members. SspH2 is secreted by TTSS1 and therefore involved in invasion, whereas SlrP and SspH1 are translocated by TTSS1 and 2, indicating a role at different stages of the infection [86,87,88,89]. The catalytic mechanism is distinct from SopA. They have a strong activity when associated *in vitro* with the human E2 ligase UbcH5 [90]. The significance with *Salmonella* pathogenesis is not clear.

SseL and AvrA effectors have deubiquitinase activity and may be involved in downregulating immune response. SseL is translocated by TTSS2 whereas AvrA is secreted by TTSS1 but their roles remain elusive [91,92].

UCH-L1 has been shown to regulate receptor clustering and activation of small GTPases involved in the regulation of actin cytoskeleton, such as Rac1. Both of these processes are connected to bacteria internalization. Therefore in **paper I** “The ubiquitin C-terminal hydrolase UCH-L1 promotes bacterial invasion by altering the dynamics of the actin cytoskeleton”, we investigated the role of this DUB in bacteria entry. We demonstrated that UCH-L1 promotes *L.monocytogenes* entry into HEK 293 and CasKi cell lines. We have generated UCH-L1 knock down cell lines by transduction of UCH-L1 specific shRNA into these cell lines that led to 90% reduction of the endogenous protein level. A gentamicin assay, consisting of infecting cells with the appropriate

multiplicity of infection (MOI) during 45min followed by 1h incubation with gentamicin to kill extracellular bacteria, was assessed to determine the bacterial recovery at the given time after the assay. A significant reduction was observed in UCH-L1 knock down cells. These results were further confirmed by confocal microscopy analyses using GFP tagged-*L. monocytogenes* in HEK 293 cells. The reverse experiment was done in HeLa cells (UCH-L1 negative) transfected with a control vector, HA-UCH-L1 or the catalytic mutant HA-UCH-L1_{C90S} plasmids. The gentamicin assay showed a two-fold increase of bacterial uptake in UCH-L1 expressing cells compared to vector control and no difference between control and catalytic mutant, suggesting that the DUB catalytic activity is responsible for the phenotype observed. As *L. monocytogenes* binding to Met mimics the activation of the receptor by HGF, we studied whether UCH-L1 knock down alters the Met-signaling pathway in HEK 293 cells upon HGF stimulation. We observed a higher expression of Met in UCH-L1 knock down cells, which was not due to a higher transcription of mRNA (showed by qPCR). Decreased levels of UCH-L1 expression induced by iRNA were associated with a significant reduction of ubiquitinated Met after HGF stimulation. No differences were observed in the levels of Cbl-b and c, the Met E3 ligases. However, the levels of phosphorylation of Tyr1003 (where Cbl-c is recruited) were constitutively higher but did not increase in UCH-L1 knock down cells upon HGF stimulation. We further showed that the activation of the MAPK Erk1/2 and Akt pathways was altered upon UCH-L1 knock down in HGF stimulated cells.

The selective effect on Met Tyr1003 phosphorylation suggests that UCH-L1 may regulate early events in receptor triggering, such as receptor clustering, that are controlled by the actin cytoskeleton dynamics [93,94]. To test this possibility we used a different bacterium, *S. enterica* serovar Typhimurium, whose entry does not involve a specific receptor but is dependent on extensive remodeling to the actin cytoskeleton

[95]. We observed a 60% reduction in *S. enterica* entry in HEK 293 cells transduced with lentivirus expressing the UCH-L1 specific shRNA compared to control cells, while expression of a functional UCH-L1 induced a 7-fold increase in bacteria uptake in HeLa cells compared to the UCH-L1 negative control cells. This effect was dependent on the enzymatic activity of UCH-L1, since the internalization of *S. enterica* in cells transfected with the UCH-L1_{C90S} mutant was similar to that observed in control cells. The effect of UCH-L1 on the regulation of the actin cytoskeleton dynamics was further established by visualization of polymeric actin in cells stained with TRITC phalloidin. Expression of a functional UCH-L1 was associated with a 4-fold increase in the spontaneous formation of actin stress fibers compared to cells transfected with the vector control or the UCH-L1_{C90S} mutant. A small but reproducible increase of actin stress fibers was also observed upon *L. monocytogenes* or *S. enterica* infection of HeLa cells expressing the wild type UCH-L1 but not in control cells or cells expressing the UCH-L1_{C90S} mutant.

This study identifies UCH-L1 as the first DUB that regulates bacteria entry and highlights the complex interplay between bacteria internalization and ubiquitin-dependent signaling. The capacity of enteropathogenic bacteria to invade epithelial cells is an essential feature of their virulence. Thus, elucidation of the mechanisms that regulate entry will provide important information towards the development of new strategies for limiting bacteria infection and spread. In addition, attenuated strains of *L. monocytogenes* and *S. enterica* serovar Typhimurium have been used as delivery vectors for immunogenic antigens (HPV E7) or enzymes, such as cytosine deaminase, that convert pro-drug to toxic drugs in tumour cells [96,97] [98,99]. Our findings predict that the success of this tumour targeting strategy will be at least in part dependent on the expression of UCH-L1, suggesting that it may be particularly

indicated for tumour types, such as neuroblastoma [51], colon carcinoma [52], prostate and breast carcinomas [55,56] where UCH-L1 is expressed at very high levels.

A question that still remains open is the molecular mechanisms by which UCH-L1 regulates Met signaling and actin cytoskeleton dynamics and the identification of its cellular target(s). The data presented in **paper I** demonstrated that UCH-L1 knockdown is associated with altered signaling capacity of the Met receptor that acts as docking site for *L.monocytogenes*. Positive and, in some instances negative regulation of signaling is achieved by clustering of the receptor in the plasma membrane [94]. It is now well established that clustering of tyrosine receptor kinases (RTK) can be regulated by integrin activation. The formation of direct or indirect complexes between the RTKs and the integrins has been shown to increase RTKs dimerization and cross-phosphorylation. Integrin-associated cytoskeletal components may be involved as well in these putative complexes [100]. Since UCH-L1 has been previously shown to regulate activation of the integrin LFA in B cells [63], we assessed whether UCH-L1 interacts with components of the focal adhesion and adherent junction complexes in **manuscript II** “The Ubiquitin C-terminal Hydrolase UCH-L1 protects the focal adhesion kinase from degradation and promotes formation of focal adhesion complexes”. This analysis was performed by co-immunoprecipitation experiments using as model HeLa cell lines transfected with vector control, HA-UCH-L1 or HA-UCH-L1_{C90S}. We demonstrated that the catalytic active form of UCH-L1 interacts with focal adhesion kinase (FAK), paxillin, vinculin from the focal adhesion complexes, β -catenin and p120 from the adherent junction complexes. As we observed no differences in the steady state levels of these proteins in the HeLa cell lines, we focused on FAK as a central actor of the integrin signaling pathway that leads to actin rearrangement. We looked at the FAK turnover by cycloheximide chase experiments in the presence or

absence of the proteasome and cysteine protease inhibitor MG132. We observed an accumulation of a cleavage product of FAK in control cells and in cells expressing the catalytic mutant UCH-L1_{C90S}. This cleavage was inhibited by the protease inhibitor MG132. A significant reduction of the FAK processing was observed in UCH-L1 expressing HeLa cells, where the enhanced stability of this protein correlated with an increased activation of FAK, phosphorylated on the tyrosine residue 397 (Y397). As activated FAK acts as a scaffold protein in focal adhesion complexes we tested whether pFAK_{Y397} stabilization would enhance paxillin recruitment. We observed a twofold increase of paxillin recruitment via FAK in cells expressing active UCH-L1 compared to controls. To further investigate the role of UCH-L1 in the formation of focal adhesion complexes, we looked at the integrin dependent adherence of the HeLa cell lines to fibronectin coated plates. After letting the cells adhere for 30min, a higher number of cells expressing the active UCH-L1 were recovered. In agreement with these results, immunofluorescence analysis showed a greater spreading of active UCH-L1 expressing cells compared to controls 4h after seeding on fibronectin-coated coverslips. The number of focal adhesion containing integrin β 1, FAK or paxillin were quantified by immunostaining under the same conditions. A higher number of focal adhesion complexes were observed in cells expressing the catalytically active UCH-L1 compared to controls. As the integrin signaling pathway regulates cell adhesion and motility, we additionally checked whether UCH-L1 also played a role in this process. Wound healing assay showed that cells expressing active UCH-L1 were more motile than control cells.

Since UCH-L1 contributes both to the regulation of FAK stability and sustained phosphorylation of the key residue Y397, it is likely that the UCH-L1-dependent stabilization and activation of FAK are the molecular mechanisms that coordinate all

the phenotypes observed in UCH-L1 expressing cells: enhanced motility and invasion, actin cytoskeleton remodeling, and proliferation.

Indeed, activation of FAK has been shown to promote all the effects that have been associated with UCH-L1 expression.

UCH-L1 has been shown to promote cancer cell invasion and migration [61] and **manuscript II**. Migration is dependent on a rapid turnover of focal adhesion complexes [101] [102] 2010 [103], which is regulated by FAK [104] [103].

Expression of wild type UCH-L1 in the prostate epithelial cell line RWPE1 induces EMT through the downregulation of E-cadherin and β -catenin expression and the upregulation of vimentin eventually resulting in cell scattering and decreased cell-cell junction [58]. In addition, we showed that expression of UCH-L1 is required to regulate signaling via the tyrosine kinase receptor Met [64], a promoter of EMT (reviewed in [59]), and induce cell scattering upon triggering of the receptor with its natural ligand, the hepatocyte growth factor (HGF) [62]. There are several evidences demonstrating a role of FAK in induction of EMT, via diverse mechanisms. Expression of a constitutively activated Src in the KM12C colon cancer cells promotes ETM, characterized by enhanced assembly of integrin-mediated focal adhesion complexes, induction of vimentin expression and down-regulation of cell-cell junction and endocytosis of E-cadherin, which is required for signaling via the $\alpha v/\beta 1$ integrin and Src-dependent phosphorylation of FAK [105,106]. Similarly, inhibition of FAK using a dominant negative mutant seems to impair loss of E-cadherin in a TGF- β -induced model of EMT in the hepatocytic cell line MMH [127].

UCH-L1 is highly expressed in Burkitt's lymphoma derived cell lines where it promotes cell proliferation and anchorage-independent growth associated with an UCH-L1 dependent, decreased expression of the the cyclin-dependent kinase inhibitor p27^{Kip1}. Overexpression of wild-type FAK exerts similar effects in the U-251MG

glioblastoma cell line. These events also require FAK phosphorylation on Y397, because the expression of a mutant FAK, where the tyrosine in position 397 is substituted with phenylalanine (Y397F) does not down-regulate expression of p27^{Kip1}, promotes exit from the G1 phase of the cell cycle and inhibits soft agar growth [128].

The precise dissection of the molecular events involved in the stabilization of activated FAK are still unknown. Since only the catalytically active enzyme mediates these effects, it is tempting to speculate that UCH-L1 may regulate the ubiquitination of FAK, which could affect the accessibility of FAK to cleavage by proteases. Very little information is available regarding the regulation of FAK by the ubiquitin proteasome system, and we are currently assessing whether expression of UCH-L1 modifies the ubiquitination of FAK or any other component present in the complex with the active enzyme.

6. CONTROL OF INTRACELLULAR BACTERIAL REPLICATION BY THE HOST IMMUNE SYSTEM

Intracellular bacteria replication is controlled by activation of a broad array of defensive mechanisms, mainly relying on compartmentalization followed by lysosomal destruction of the invading microorganisms. Several pro-inflammatory cytokines, such as interferon gamma (IFN- γ), can enhance the bactericidal capacity of the host cells.

The innate immunity is the first line of defence against invading organisms. Innate immunity is involved in multiple aspects: pathogen recognition, antimicrobial defence and initiation of the adaptive immune response [107]. This reaction involves the recruitment of neutrophils and macrophages (professional phagocytes) at the site of infection to release pro-inflammatory cytokines such as interleukin 1 (IL-1), IL-6, IL-12 or tumour necrosis factor- α (TNF- α). The response is adapted to the pathogen, however cytokines have significant redundancy. Phagocytes recognize pathogens via innate immune receptors so-called pattern recognition receptors (PRRs), which can distinguish bacterial surface molecules. PRRs include Toll-like receptors (TLRs), nucleotide-binding oligomerization domain protein (NOD)-like receptors (NLRs), scavenger receptors, C-type lectin receptors among others [108].

TLRs are transmembrane receptors present on the cell surface or inside endosomes. They are highly conserved among species. Thirteen have been identified so far in mice and ten in humans. They recognize a broad range of microbial products such as flagellin, LPS or microbial DNA [109].

PRR signalling induces pro-inflammatory cytokines and chemokines production, attracting more immune cells to the site of infection. This occurs through the activation

of nuclear factor κ enhancer binding protein (NF- κ B) and mitogen activated protein (MAP) kinases downstream pathways. Inflammation induces killing of bacteria and repair of the tissue [110].

Interferon- γ (IFN- γ) is one the most important cytokines in the control of replication of intracellular bacteria during the early stage of infection, and it acts in synergy with signals triggered by PRRs to enhance bactericidal mechanisms. T lymphocytes expressing CD4, also known as T helper (Th) cells, are the most prolific cytokines producers. This cell subset is further divided into Th1 and Th2 cells producing Th1- or Th2-cytokines respectively. IFN- γ is the main Th1 cytokine. Th2-cytokines include IL-10, with an anti-inflammatory effect to counteract Th1 response [111].

Natural killer (NK) and T cells are the main producers of IFN- γ . Infected macrophages secrete IL-12, stimulating NK and T cells to release IFN- γ , which further activates macrophages to secrete more IL-12 and so on. As well, macrophages activated with live bacteria, LPS, IL-12 or IL-12 combined with IL-18 produce IFN- γ , adding another positive feedback loop.

Interaction of IFN- γ with its receptor (IFN- γ R) induces more than thousand genes through activation of different signalling pathways. Some of the most important proteins encoded to kill bacteria are: the inducible nitric oxide synthase (iNOS) and phagocyte oxidase (NADPH oxidase). iNOS produces nitric oxide and NADPH oxidase catalyse the oxidative burst. IFN- γ also induces the expression of Fc and complement receptors to enhance phagocytosis and expression of molecules that are associated with interactions of Th1 cells, such as MHC class II, CD40, adhesion and co-stimulatory molecules [112].

6.1 Thioredoxin80 (Trx80)

Trx80 is composed of the 1-80 or 1-84 N-terminal amino-acid residues of Thioredoxin (TRx) 1 obtained by cleavage through an unknown mechanism. Produced mainly by monocytes, it is functionally different from Trx, since it does not possess redox activity; is present as a dimer in solution is localized at the cell membrane rather than in the cytosol. The biological functions of Trx and Trx80 are also different. Trx protects cells from oxidative stress by redox control instead Trx80 is a cytokine that activates monocytes. Interestingly, the levels of Trx80 in the plasma of healthy donors vary widely: from 1 to 171ng/ml without any correlation with the levels of Trx. Trx80 was first purified and cloned as an eosinophil cytotoxicity enhancing factor (ECEf) [113,114]. Later, Trx80 has been shown to activate human CD14⁺ monocytes into a cell type designated as Trx80-activated-monocytes (TAMs). TAMs display surface markers involved in the innate defense immunity and activation of T cells, e.g. CD14, CD40, CD54 and CD86. TAMs exhibit a high pinocytic capacity; release significantly high amounts of the pro-inflammatory cytokines TNF- α , IL-1 β and IL-6; express high levels of CD14 and the mannose receptor and induce a significantly lower proliferative response in allogeneic peripheral blood mononuclear cells. CD14 is a surface receptor that mainly binds LPS in association with LPS-binding protein. The upregulation of CD14 may stimulate microbes as well as phagocytosis of apoptotic cells. However, the removal of apoptotic cells by CD14 does not lead to an inflammatory response. CD40 upregulation induces interactions between monocytes and T cells via CD40-CD154 interactions, which will then lead to IL-12 production and CD86 expression. CD86 is a costimulatory molecule that interacts with CD28 on T cells leading to IL-2 secretion and polarizing T cells to Th1 type cells. Moreover, Trx80 has been shown to induce

interferon gamma (IFN- γ) in peripheral blood mononuclear cells (PBMCs) (Figure 4) [115,116,117].

Interestingly, Trx80 is the first cytokine to induce the production of the regulatory and anti-inflammatory cytokines IL-12 and IL-10 respectively.

As the characteristics of TAMs resemble those of activated macrophages, we suggested that TAMs play a major role in the defense against intracellular pathogens.

We chose to focus on two intracellular pathogens known to survive and replicate inside monocytes: *Brucella abortus* and *Listeria monocytogenes*. To achieve their goal, they must interfere with the different stages of phagosomal maturation to prevent their killing. Phagocytosis of *Brucella* and *Listeria* is mediated by scavenger receptors and functional lipid rafts [118] [119].

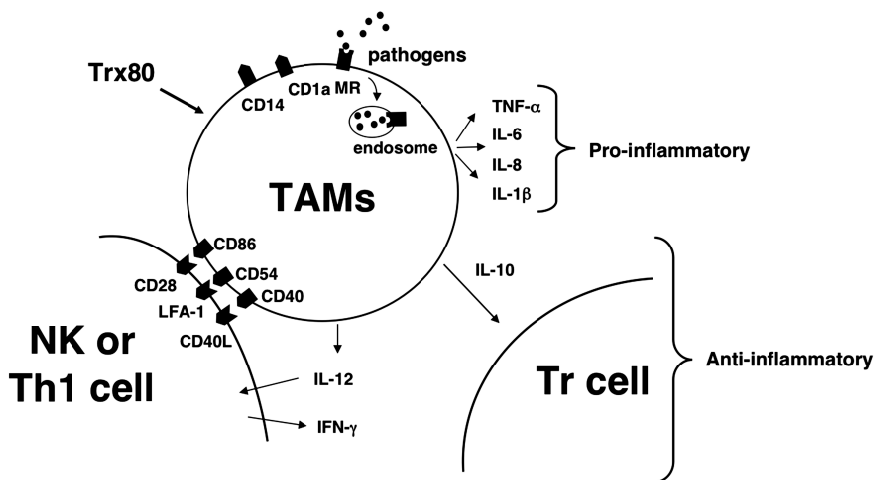


Figure 4: Monocyte response to Trx80 stimulation modified from [116].

TAMs: Trx80 activated monocytes

6.2 Models

Listeria monocytogenes have been previously described (Figure 5), therefore in this section, the focus will be on *Brucella abortus*.

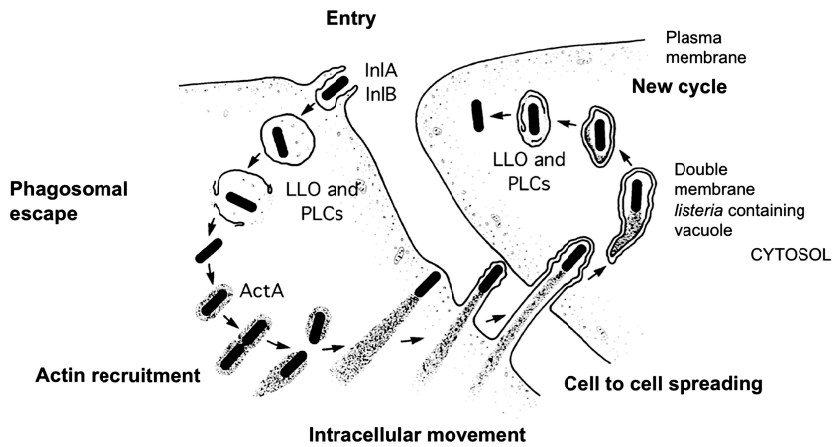


Figure 5: *Listeria monocytogenes* intracellular cycle modified from [122].

Brucella is a small Gram-negative α -Proteobacterium that is the causative agent of brucellosis. Pathogenic *Brucella* causes abortion in female animals by colonization of placental trophoblasts and cause sterility in male animal. Human infections with *B. abortus* may present various forms and often become chronic. Although the majority of patients exhibit undulant fever, malaise, sweats, and lymphadenopathy/hepatosplenomegaly, other complications may also occur i.e. arthritis or epididymo-orchitis. More serious and hard-to-treat complications include spondylitis, neurobrucellosis, liver abscess formation, and endocarditis, the latter potentially fatal. *Brucella* is able to enter and replicate efficiently in a variety of cells, including epithelial cells, placental trophoblasts, dendritic cells and macrophages [120]. Despite their importance in the initial steps for virulence, the molecular determinants and mechanisms of the internalization process remain poorly understood. Several important virulence factors for persistence of *Brucella* in infected cells have been

identified. These molecules allow *Brucella* to survive and proliferate within a membrane compartment so-called the *Brucella*-containing vacuole (BCV). After undergoing transient interactions with endosomes, BCV fuse with the endoplasmic reticulum (ER) membrane to establish a replicative compartment. During intracellular replication, the BCV contains ribosomes and numerous ER markers. Association with the ER is a way for *Brucella* to avoid fusion of the BCV with lysosomes (Figure 6) [121].

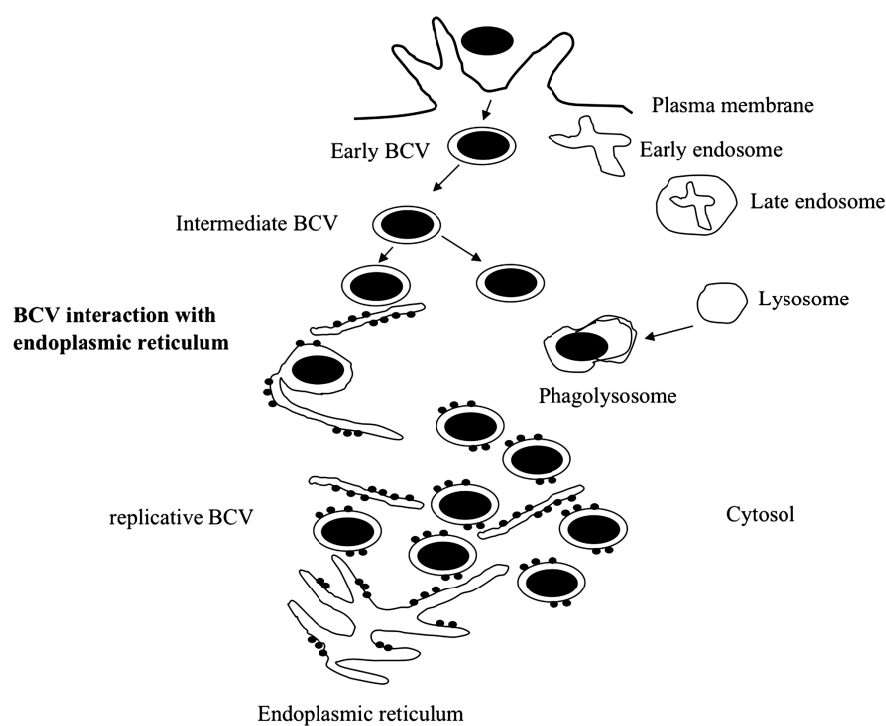


Figure 6: *Brucella* intracellular cycle modified from [121].

BCV: *Brucella* containing vacuole

6.3 Role of Trx80-activated monocytes in the control of replication of *L. monocytogenes* and *B. abortus*

In **paper III**, “Thioredoxin 80-activated-monocytes (TAMs) inhibit the replication of intracellular pathogens”, we observed a reduced recovery of *Listeria monocytogenes* at 48h and 24h post-infection in TAMs compared to control cells. To discriminate between reduced internalization and replication we assessed bacteria uptake using either gentamicin assays or immunofluorescence analysis. Both techniques showed a higher uptake of bacteria in TAMs compared to control cells meaning that the lower bacteria recovery observed in infected TAMs was due to the control of *Listeria* replication after the internalization step. Since *Listeria* escape the phagolysosome in order to survive inside the cells, we used a *L. monocytogenes* strain (NF-L327) with the ability to turn green only when it is present in the cytosol. At 8h post-infection, we observed 3 to 4 times more GFP-bacteria in control cells compared to TAMs, while bacterial uptake was not altered. These results indicate that Trx80 activation prevents the escape from the phagolysosomal compartment and enhances bacteria clearance mediated by the lysosomes. To test this hypothesis, monocytes and TAMs were infected with a GFP-tagged *Listeria* and localization of the bacteria was assessed 4h post-infection using LysoTracker Red to visualize acidified vacuoles. A 2 to 3-fold increase in the number of bacteria within acidified compartments was observed in TAMs compared to non-stimulated monocytes 4 hours post-infection. In agreement with these data, inhibition of the lysosomal function by chloroquine enhanced bacteria recovery in TAMs compared to that observed in non-stimulated cells. Our data indicate that lysosomal-dependent degradation is a key bactericidal mechanism induced by Trx80 stimulation.

Listeria and *Brucella* have elaborated strategies to avoid phagosome maturation and/or fusion with lysosomes. *Listeria* escape the phagosome before it matures and fuses with the lysosome. This step is very rapid, occurring within five minutes from the bacterial engulfment into the host cell and is mediated by the production and secretion of listeriolysin O (LLO) and two phospholipases, PI-PLC and PC-PLC. These enzymes cause the breakdown of the membrane of the *Listeria*-containing phagosome and thereby enable the bacteria to escape to the cytosol where they replicate [123]. The data presented in Figures 4 and 5 indicate that more bacteria are localized within acidified vesicles 4 hours after infection, resulting in 3 to 4-fold decreased recovery of *Listeria* in the cytosol of infected cells. Trx80-dependent activation may alter the environment of the phagosome preventing activation of LLO and secretion of phospholipases. Maturation of the phagolysosome *per se* may be accelerated upon cell activation and bacteria can be killed with a faster kinetic. Alternatively, Trx80 activation may enhance the autophagy process, resulting in formation of autophagosomes [124]. Similar results have been reported in macrophages activated with IFN- γ or LPS [125], indicating that at least for the control of *Listeria* replication, Trx80 activation has similar effects as other well-known activators of the macrophage function.

Survival of *Brucella* inside the cell relies on avoiding fusion of the *Brucella*-containing vacuole (BCV) with lysosomes. For its intracellular survival, *Brucella* produces cyclic glucans and requires the type IV secretion system VirB. Cyclic glucans modulate maturation of BCV to avoid fusion with lysosomes [126], while VirB is required for the late BCV maturation events corresponding to sustained interaction and fusion with the ER [121]. TAMs could act either by neutralizing the cyclic glucans at the early stage of the infection or interfering with the VirB system to prevent fusion of the BCV with the ER.

Collectively, we have demonstrated that Trx80 possesses an intrinsic capacity to control intracellular infections, and that TAMs are effective against bacteria, such as *B. abortus* and *L. monocytogenes*. The effects of Trx80 on monocytic cells are similar to those induced by IFN- γ , which plays a mandatory role in protection against intracellular pathogens. These data suggest that TAMs are efficient effectors of the innate immune response and represents a first line of defence against intracellular infections, before the immune system can mount a proper T-cell response.

7. CONCLUDING REMARKS

By looking at some mechanisms affecting the capacity of pathogenic bacteria to invade and replicate in eukaryotic cells, we aimed at finding new properties that may be exploited for therapeutic purposes.

Previous studies from our group and others led us to further investigate the role of UCH-L1 in bacterial entry. This thesis demonstrates, in **paper I**, that cytoskeleton dynamics are regulated by UCH-L1, which highlights a previously unrecognized involvement of UCH-L1 and more generally for a deubiquitinating enzyme in bacterial entry. Importantly, these findings not only improve the understanding of bacterial pathogenesis but may also be important for anti-cancer therapy. Indeed, malignant and metastatic cells highly expressing UCH-L1 may be particularly susceptible to invasion by bacterial-based drug delivery systems. Additionally, our results open new perspectives in the field of deubiquitinating enzymes. As most of these enzymes remain poorly characterized, it is of interest to study their potential role in bacterial internalization and cytoskeleton dynamics.

To further understand the mechanisms involved in the interplay between UCH-L1 and actin cytoskeleton, we looked for interacting partners and showed, in **paper II**, that UCH-L1 is a key regulator of focal adhesion complexes. UCH-L1 interacts with FAK, a common effector between actin cytoskeleton dynamics and adhesion, by protecting its degradation. Further investigations are needed to understand the molecular mechanism underlying the observed protection. Moreover, as the focal adhesions are complexes involving many proteins, determining whether UCH-L1 can stabilize other partners would help to identify new molecular mechanisms and better understand the biological function of UCH-L1.

In the second part of the thesis, we investigated the role of Trx80 in intracellular bacterial replication based on previous studies describing Trx80 as a cytokine. As Trx80 activated monocytes resembled macrophages, we investigated their response to intracellular bacteria infection. In **paper III** we show that intracellular bacteria such as *Listeria* and *Brucella* are trapped and killed in acidic vesicles, shedding light on a new role of Trx80 as a *bona fide* cytokine that is involved in the first line of defence against intracellular bacterial pathogens. However, further characterization of the molecular mechanisms is still needed to understand the bactericidal activity of Trx80 and its role on the bacteria containing vacuoles.

Collectively, these findings contribute to the comprehension of several aspects of bacterial pathogenesis that may also be important to elaborate novel therapeutic approaches such as bacterial delivery vectors to treat cancer.

8. REFERENCES

1. Finlay BB, Falkow S (1997) Common themes in microbial pathogenicity revisited. *Microbiol Mol Biol Rev* 61: 136-169.
2. Donnenberg MS (2000) Pathogenic strategies of enteric bacteria. *Nature* 406: 768-774.
3. Merz AJ, So M (2000) Interactions of pathogenic neisseriae with epithelial cell membranes. *Annu Rev Cell Dev Biol* 16: 423-457.
4. Hahn HP (1997) The type-4 pilus is the major virulence-associated adhesin of *Pseudomonas aeruginosa*--a review. *Gene* 192: 99-108.
5. Joh D, Wann ER, Kreikemeyer B, Speziale P, Hook M (1999) Role of fibronectin-binding MSCRAMMs in bacterial adherence and entry into mammalian cells. *Matrix Biol* 18: 211-223.
6. Novak R, Tuomanen E (1999) Pathogenesis of pneumococcal pneumonia. *Semin Respir Infect* 14: 209-217.
7. Cleary PP, Cue D (2000) High frequency invasion of mammalian cells by beta hemolytic streptococci. *Subcell Biochem* 33: 137-166.
8. Bermudez LE, Sangari FJ (2000) Mycobacterial invasion of epithelial cells. *Subcell Biochem* 33: 231-249.
9. Walker (1998) *Microbiology*; Company PWS, editor.
10. Garcia E, Llull D, Lopez R (1999) Functional organization of the gene cluster involved in the synthesis of the pneumococcal capsule. *Int Microbiol* 2: 169-176.
11. Bhattacharjee AK, Jennings HJ, Kenny CP, Martin A, Smith IC (1976) Structural determination of the polysaccharide antigens of *Neisseria meningitidis* serogroups Y, W-135, and BO1. *Can J Biochem* 54: 1-8.
12. Russell (2000) Where to stay inside the cell: a homesteader's guide to intracellular parasitism. *Cellular microbiology*. Washington DC: ASM Press.
13. Ibarra JA, Steele-Mortimer O (2009) *Salmonella*--the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Cell Microbiol* 11: 1579-1586.
14. Vergne I, Chua J, Singh SB, Deretic V (2004) Cell biology of mycobacterium tuberculosis phagosome. *Annu Rev Cell Dev Biol* 20: 367-394.
15. Ray K, Marteyn B, Sansonetti PJ, Tang CM (2009) Life on the inside: the intracellular lifestyle of cytosolic bacteria. *Nat Rev Microbiol* 7: 333-340.
16. Pawelek JM, Low KB, Bermudes D (1997) Tumor-targeted *Salmonella* as a novel anticancer vector. *Cancer Res* 57: 4537-4544.
17. Yu YA, Shabahang S, Timiryasova TM, Zhang Q, Beltz R, et al. (2004) Visualization of tumors and metastases in live animals with bacteria and vaccinia virus encoding light-emitting proteins. *Nat Biotechnol* 22: 313-320.
18. Parker RC, Plummer HC, et al. (1947) Effect of histolytic infection and toxin on transplantable mouse tumors. *Proc Soc Exp Biol Med* 66: 461-467.
19. Malmgren RA, Flanigan CC (1955) Localization of the vegetative form of *Clostridium tetani* in mouse tumors following intravenous spore administration. *Cancer Res* 15: 473-478.
20. Kohwi Y, Imai K, Tamura Z, Hashimoto Y (1978) Antitumor effect of *Bifidobacterium infantis* in mice. *Gann* 69: 613-618.

21. Forbes NS (2010) Engineering the perfect (bacterial) cancer therapy. *Nat Rev Cancer* 10: 785-794.
22. Nguyen VH, Kim HS, Ha JM, Hong Y, Choy HE, et al. (2010) Genetically engineered *Salmonella typhimurium* as an imageable therapeutic probe for cancer. *Cancer Res* 70: 18-23.
23. Jiang SN, Phan TX, Nam TK, Nguyen VH, Kim HS, et al. (2010) Inhibition of tumor growth and metastasis by a combination of *Escherichia coli*-mediated cytolytic therapy and radiotherapy. *Mol Ther* 18: 635-642.
24. Ryan RM, Green J, Williams PJ, Tazzyman S, Hunt S, et al. (2009) Bacterial delivery of a novel cytolysin to hypoxic areas of solid tumors. *Gene Ther* 16: 329-339.
25. Patyar S, Joshi R, Byrav DS, Prakash A, Medhi B, et al. (2010) Bacteria in cancer therapy: a novel experimental strategy. *J Biomed Sci* 17: 21.
26. Lee CH, Wu CL, Shiau AL (2004) Endostatin gene therapy delivered by *Salmonella choleraesuis* in murine tumor models. *J Gene Med* 6: 1382-1393.
27. Chou CK, Hung JY, Liu JC, Chen CT, Hung MC (2006) An attenuated *Salmonella* oral DNA vaccine prevents the growth of hepatocellular carcinoma and colon cancer that express alpha-fetoprotein. *Cancer Gene Ther* 13: 746-752.
28. Bonazzi M, Lecuit M, Cossart P (2009) *Listeria monocytogenes* internalin and E-cadherin: from structure to pathogenesis. *Cell Microbiol* 11: 693-702.
29. Lecuit M, Dramsi S, Gottardi C, Fedor-Chaiken M, Gumbiner B, et al. (1999) A single amino acid in E-cadherin responsible for host specificity towards the human pathogen *Listeria monocytogenes*. *EMBO J* 18: 3956-3963.
30. Braun L, Ohayon H, Cossart P (1998) The InIB protein of *Listeria monocytogenes* is sufficient to promote entry into mammalian cells. *Mol Microbiol* 27: 1077-1087.
31. Bonazzi M, Veiga E, Pizarro-Cerda J, Cossart P (2008) Successive post-translational modifications of E-cadherin are required for InlA-mediated internalization of *Listeria monocytogenes*. *Cell Microbiol* 10: 2208-2222.
32. Cossart P (1998) Interactions of the bacterial pathogen *Listeria monocytogenes* with mammalian cells: bacterial factors, cellular ligands, and signaling. *Folia Microbiol (Praha)* 43: 291-303.
33. Veiga E, Cossart P (2005) *Listeria* hijacks the clathrin-dependent endocytic machinery to invade mammalian cells. *Nat Cell Biol* 7: 894-900.
34. Veiga E, Guttman JA, Bonazzi M, Boucrot E, Toledo-Arana A, et al. (2007) Invasive and adherent bacterial pathogens co-Opt host clathrin for infection. *Cell Host Microbe* 2: 340-351.
35. Schlumberger MC, Hardt WD (2006) *Salmonella* type III secretion effectors: pulling the host cell's strings. *Curr Opin Microbiol* 9: 46-54.
36. Steele-Mortimer O (2008) The *Salmonella*-containing vacuole: moving with the times. *Curr Opin Microbiol* 11: 38-45.
37. Alonso A, Garcia-del Portillo F (2004) Hijacking of eukaryotic functions by intracellular bacterial pathogens. *Int Microbiol* 7: 181-191.
38. Jin J, Li X, Gygi SP, Harper JW (2007) Dual E1 activation systems for ubiquitin differentially regulate E2 enzyme charging. *Nature* 447: 1135-1138.
39. Pelzer C, Kassner I, Matentzoglou K, Singh RK, Wollscheid HP, et al. (2007) UBE1L2, a novel E1 enzyme specific for ubiquitin. *J Biol Chem* 282: 23010-23014.

40. Ciechanover A (2005) Intracellular protein degradation: from a vague idea, through the lysosome and the ubiquitin-proteasome system, and onto human diseases and drug targeting (Nobel lecture). *Angew Chem Int Ed Engl* 44: 5944-5967.
41. Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. *Physiol Rev* 82: 373-428.
42. Ciechanover A (2005) N-terminal ubiquitination. *Methods Mol Biol* 301: 255-270.
43. Ye Y, Rape M (2009) Building ubiquitin chains: E2 enzymes at work. *Nat Rev Mol Cell Biol* 10: 755-764.
44. Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, et al. (2005) A genomic and functional inventory of deubiquitinating enzymes. *Cell* 123: 773-786.
45. Debigare R, Price SR (2003) Proteolysis, the ubiquitin-proteasome system, and renal diseases. *Am J Physiol Renal Physiol* 285: F1-8.
46. Wilkinson KD, Lee KM, Deshpande S, Duerksen-Hughes P, Boss JM, et al. (1989) The neuron-specific protein PGP 9.5 is a ubiquitin carboxyl-terminal hydrolase. *Science* 246: 670-673.
47. Saigoh K, Wang YL, Suh JG, Yamanishi T, Sakai Y, et al. (1999) Intragenic deletion in the gene encoding ubiquitin carboxy-terminal hydrolase in gad mice. *Nat Genet* 23: 47-51.
48. Liu Y, Fallon L, Lashuel HA, Liu Z, Lansbury PT, Jr. (2002) The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. *Cell* 111: 209-218.
49. Fernandez-Funez P, Nino-Rosales ML, de Gouyon B, She WC, Luchak JM, et al. (2000) Identification of genes that modify ataxin-1-induced neurodegeneration. *Nature* 408: 101-106.
50. Naze P, Vuillaume I, Destee A, Pasquier F, Sablonniere B (2002) Mutation analysis and association studies of the ubiquitin carboxy-terminal hydrolase L1 gene in Huntington's disease. *Neurosci Lett* 328: 1-4.
51. Yanagisawa TY, Sasahara Y, Fujie H, Ohashi Y, Minegishi M, et al. (1998) Detection of the PGP9.5 and tyrosine hydroxylase mRNAs for minimal residual neuroblastoma cells in bone marrow and peripheral blood. *Tohoku J Exp Med* 184: 229-240.
52. Yamazaki T, Hibi K, Takase T, Tezel E, Nakayama H, et al. (2002) PGP9.5 as a marker for invasive colorectal cancer. *Clin Cancer Res* 8: 192-195.
53. Sasaki H, Yukiue H, Moriyama S, Kobayashi Y, Nakashima Y, et al. (2001) Expression of the protein gene product 9.5, PGP9.5, is correlated with T-status in non-small cell lung cancer. *Jpn J Clin Oncol* 31: 532-535.
54. Tezel E, Hibi K, Nagasaka T, Nakao A (2000) PGP9.5 as a prognostic factor in pancreatic cancer. *Clin Cancer Res* 6: 4764-4767.
55. Aumuller G, Renneberg H, Leonhardt M, Lilja H, Abrahamsson PA (1999) Localization of protein gene product 9.5 immunoreactivity in derivatives of the human Wolffian duct and in prostate cancer. *Prostate* 38: 261-267.
56. Schumacher U, Mitchell BS, Kaiserling E (1994) The neuronal marker protein gene product 9.5 (PGP 9.5) is phenotypically expressed in human breast epithelium, in milk, and in benign and malignant breast tumors. *DNA Cell Biol* 13: 839-843.

57. D'Andrea V, Malinovsky L, Berni A, Biancari F, Biassoni L, et al. (1997) The immunolocalization of PGP 9.5 in normal human kidney and renal cell carcinoma. *G Chir* 18: 521-524.
58. Jang MJ, Baek SH, Kim JH (2011) UCH-L1 promotes cancer metastasis in prostate cancer cells through EMT induction. *Cancer Lett* 302: 128-135.
59. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. *J Clin Invest* 119: 1420-1428.
60. Chen F, Sugiura Y, Myers KG, Liu Y, Lin W (2010) Ubiquitin carboxyl-terminal hydrolase L1 is required for maintaining the structure and function of the neuromuscular junction. *Proc Natl Acad Sci U S A* 107: 1636-1641.
61. Kim HJ, Kim YM, Lim S, Nam YK, Jeong J, et al. (2009) Ubiquitin C-terminal hydrolase-L1 is a key regulator of tumor cell invasion and metastasis. *Oncogene* 28: 117-127.
62. Buus R, Faronato M, Hammond DE, Urbe S, Clague MJ (2009) Deubiquitinase Activities Required for Hepatocyte Growth Factor-Induced Scattering of Epithelial Cells. *Curr Biol*.
63. Rolen U, Freda E, Xie J, Pfirrmann T, Frisan T, et al. (2009) The ubiquitin C-terminal hydrolase UCH-L1 regulates B-cell proliferation and integrin activation. *J Cell Mol Med* 13: 1666-1678.
65. Bheda A, Gullapalli A, Caplow M, Pagano JS, Shackelford J (2010) Ubiquitin editing enzyme UCH L1 and microtubule dynamics: Implication in mitosis. *Cell Cycle* 9.
66. Larsen CN, Price JS, Wilkinson KD (1996) Substrate binding and catalysis by ubiquitin C-terminal hydrolases: identification of two active site residues. *Biochemistry* 35: 6735-6744.
67. Osaka H, Wang YL, Takada K, Takizawa S, Setsuie R, et al. (2003) Ubiquitin carboxy-terminal hydrolase L1 binds to and stabilizes monoubiquitin in neuron. *Hum Mol Genet* 12: 1945-1958.
68. Das C, Hoang QQ, Kreinbring CA, Luchansky SJ, Meray RK, et al. (2006) Structural basis for conformational plasticity of the Parkinson's disease-associated ubiquitin hydrolase UCH-L1. *Proc Natl Acad Sci U S A* 103: 4675-4680.
69. Meray RK, Lansbury PT, Jr. (2007) Reversible monoubiquitination regulates the Parkinson disease-associated ubiquitin hydrolase UCH-L1. *J Biol Chem* 282: 10567-10575.
70. Cole RN, Hart GW (2001) Cytosolic O-glycosylation is abundant in nerve terminals. *Journal of Neurochemistry* 79: 1080-1089.
71. Choi J, Levey AI, Weintraub ST, Rees HD, Gearing M, et al. (2004) Oxidative modifications and down-regulation of ubiquitin carboxyl-terminal hydrolase L1 associated with idiopathic Parkinson's and Alzheimer's diseases. *J Biol Chem* 279: 13256-13264.
72. Palacios F, Tushir JS, Fujita Y, D'Souza-Schorey C (2005) Lysosomal targeting of E-cadherin: a unique mechanism for the down-regulation of cell-cell adhesion during epithelial to mesenchymal transitions. *Mol Cell Biol* 25: 389-402.
73. Langevin J, Morgan MJ, Sibarita JB, Aresta S, Murthy M, et al. (2005) Drosophila exocyst components Sec5, Sec6, and Sec15 regulate DE-Cadherin trafficking from recycling endosomes to the plasma membrane. *Dev Cell* 9: 365-376.

74. Fujita Y, Krause G, Scheffner M, Zechner D, Leddy HE, et al. (2002) Hakai, a c-Cbl-like protein, ubiquitinates and induces endocytosis of the E-cadherin complex. *Nat Cell Biol* 4: 222-231.
75. Banerjee M, Copp J, Vuga D, Marino M, Chapman T, et al. (2004) GW domains of the *Listeria monocytogenes* invasion protein InlB are required for potentiation of Met activation. *Mol Microbiol* 52: 257-271.
76. Reinl T, Nimtz M, Hundertmark C, Johl T, Keri G, et al. (2009) Quantitative phosphokinome analysis of the Met pathway activated by the invasin internalin B from *Listeria monocytogenes*. *Mol Cell Proteomics* 8: 2778-2795.
77. Ireton K, Payraastre B, Chap H, Ogawa W, Sakaue H, et al. (1996) A role for phosphoinositide 3-kinase in bacterial invasion. *Science (Wash DC)* 274: 780-782.
78. Pizarro-Cerda J, Payraastre B, Wang YJ, Veiga E, Yin HL, et al. (2007) Type II phosphatidylinositol 4-kinases promote *Listeria monocytogenes* entry into target cells. *Cell Microbiol* 9: 2381-2390.
79. Mostowy S, Janel S, Forestier C, Roduit C, Kasas S, et al. (2011) A Role for Septins in the Interaction between the *Listeria monocytogenes* Invasion Protein InlB and the Met Receptor. *Biophys J* 100: 1949-1959.
80. Hardt WD, Chen LM, Schuebel KE, Bustelo XR, Galan JE (1998) *S. typhimurium* encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. *Cell* 93: 815-826.
81. Kubori T, Galan JE (2003) Temporal regulation of salmonella virulence effector function by proteasome-dependent protein degradation. *Cell* 115: 333-342.
82. Bakowski MA, Braun V, Lam GY, Yeung T, Heo WD, et al. (2010) The phosphoinositide phosphatase SopB manipulates membrane surface charge and trafficking of the Salmonella-containing vacuole. *Cell Host Microbe* 7: 453-462.
83. Patel JC, Hueffer K, Lam TT, Galan JE (2009) Diversification of a Salmonella virulence protein function by ubiquitin-dependent differential localization. *Cell* 137: 283-294.
84. Knodler LA, Winfree S, Drecktrah D, Ireland R, Steele-Mortimer O (2009) Ubiquitination of the bacterial inositol phosphatase, SopB, regulates its biological activity at the plasma membrane. *Cell Microbiol* 11: 1652-1670.
85. Diao J, Zhang Y, Huibregtse JM, Zhou D, Chen J (2008) Crystal structure of SopA, a Salmonella effector protein mimicking a eukaryotic ubiquitin ligase. *Nat Struct Mol Biol* 15: 65-70.
86. Miao EA, Scherer CA, Tsolis RM, Kingsley RA, Adams LG, et al. (1999) Salmonella typhimurium leucine-rich repeat proteins are targeted to the SPI1 and SPI2 type III secretion systems. *Mol Microbiol* 34: 850-864.
87. Tsolis RM, Townsend SM, Miao EA, Miller SI, Ficht TA, et al. (1999) Identification of a putative Salmonella enterica serotype typhimurium host range factor with homology to IpaH and YopM by signature-tagged mutagenesis. *Infect Immun* 67: 6385-6393.
88. Haraga A, Miller SI (2006) A Salmonella type III secretion effector interacts with the mammalian serine/threonine protein kinase PKN1. *Cell Microbiol* 8: 837-846.

89. Miao EA, Miller SI (2000) A conserved amino acid sequence directing intracellular type III secretion by *Salmonella typhimurium*. *Proc Natl Acad Sci U S A* 97: 7539-7544.
90. Quezada CM, Hicks SW, Galan JE, Stebbins CE (2009) A family of *Salmonella* virulence factors functions as a distinct class of autoregulated E3 ubiquitin ligases. *Proc Natl Acad Sci U S A* 106: 4864-4869.
91. Le Negrate G, Faustin B, Welsh K, Loeffler M, Krajewska M, et al. (2008) *Salmonella* secreted factor L deubiquitinase of *Salmonella typhimurium* inhibits NF-kappaB, suppresses IkappaBalpha ubiquitination and modulates innate immune responses. *J Immunol* 180: 5045-5056.
92. Lu R, Wu S, Liu X, Xia Y, Zhang YG, et al. (2010) Chronic effects of a *Salmonella* type III secretion effector protein AvrA in vivo. *PLoS One* 5: e10505.
93. Treanor B, Harwood NE, Batista FD (2009) Microsignalosomes: spatially resolved receptor signalling. *Biochem Soc Trans* 37: 1014-1018.
94. McClatchey AI, Fehon RG (2009) Merlin and the ERM proteins--regulators of receptor distribution and signaling at the cell cortex. *Trends Cell Biol* 19: 198-206.
95. Veiga E, Cossart P (2006) The role of clathrin-dependent endocytosis in bacterial internalization. *Trends Cell Biol* 16: 499-504.
96. Cunningham C, Nemunaitis J (2001) A phase I trial of genetically modified *Salmonella typhimurium* expressing cytosine deaminase (TAPET-CD, VNP20029) administered by intratumoral injection in combination with 5-fluorocytosine for patients with advanced or metastatic cancer. Protocol no: CL-017. Version: April 9, 2001. *Hum Gene Ther* 12: 1594-1596.
97. Nemunaitis J, Cunningham C, Senzer N, Kuhn J, Cramm J, et al. (2003) Pilot trial of genetically modified, attenuated *Salmonella* expressing the *E. coli* cytosine deaminase gene in refractory cancer patients. *Cancer Gene Ther* 10: 737-744.
98. Maciag PC, Radulovic S, Rothman J (2009) The first clinical use of a live-attenuated *Listeria monocytogenes* vaccine: a Phase I safety study of Lm-LLO-E7 in patients with advanced carcinoma of the cervix. *Vaccine* 27: 3975-3983.
99. Brockstedt DG, Dubensky TW (2008) Promises and challenges for the development of *Listeria monocytogenes*-based immunotherapies. *Expert Rev Vaccines* 7: 1069-1084.
100. Sieg DJ, Hauck CR, Ilic D, Klingbeil CK, Schaefer E, et al. (2000) FAK integrates growth-factor and integrin signals to promote cell migration. *Nat Cell Biol* 2: 249-256.
101. Carragher NO, Fincham VJ, Riley D, Frame MC (2001) Cleavage of focal adhesion kinase by different proteases during SRC-regulated transformation and apoptosis. Distinct roles for calpain and caspases. *J Biol Chem* 276: 4270-4275.
102. Carragher NO, Frame MC (2002) Calpain: a role in cell transformation and migration. *Int J Biochem Cell Biol* 34: 1539-1543.
103. Chan KT, Bennin DA, Huttenlocher A (2010) Regulation of adhesion dynamics by calpain-mediated proteolysis of focal adhesion kinase (FAK). *J Biol Chem* 285: 11418-11426.
104. Owen KA, Thomas KS, Bouton AH (2007) The differential expression of *Yersinia pseudotuberculosis* adhesins determines the requirement for FAK and/or Pyk2 during bacterial phagocytosis by macrophages. *Cell Microbiol* 9: 596-609.

105. Avizienyte E, Fincham VJ, Brunton VG, Frame MC (2004) Src SH3/2 domain-mediated peripheral accumulation of Src and phospho-myosin is linked to deregulation of E-cadherin and the epithelial-mesenchymal transition. *Mol Biol Cell* 15: 2794-2803.
106. Avizienyte E, Wyke AW, Jones RJ, McLean GW, Westhoff MA, et al. (2002) Src-induced de-regulation of E-cadherin in colon cancer cells requires integrin signalling. *Nat Cell Biol* 4: 632-638.
107. Janeway CA, Jr., Medzhitov R (2002) Innate immune recognition. *Annu Rev Immunol* 20: 197-216.
108. Delbridge LM, O'Riordan MX (2007) Innate recognition of intracellular bacteria. *Curr Opin Immunol* 19: 10-16.
109. Akira S, Uematsu S, Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* 124: 783-801.
110. Ismail N, Olano JP, Feng HM, Walker DH (2002) Current status of immune mechanisms of killing of intracellular microorganisms. *FEMS Microbiol Lett* 207: 111-120.
111. Boehm U, Klamp T, Groot M, Howard JC (1997) Cellular responses to interferon-gamma. *Annu Rev Immunol* 15: 749-795.
112. Frucht DM, Fukao T, Bogdan C, Schindler H, O'Shea JJ, et al. (2001) IFN-gamma production by antigen-presenting cells: mechanisms emerge. *Trends Immunol* 22: 556-560.
113. Dessein AJ, Lenzi HL, Bina JC, Carvalho EM, Weiser WY, et al. (1984) Modulation of eosinophil cytotoxicity by blood mononuclear cells from healthy subjects and patients with chronic schistosomiasis mansoni. *Cell Immunol* 85: 100-113.
114. Silberstein DS, Ali MH, Baker SL, David JR (1989) Human eosinophil cytotoxicity-enhancing factor. Purification, physical characteristics, and partial amino acid sequence of an active polypeptide. *J Immunol* 143: 979-983.
115. Pekkari K, Avila-Carino J, Bengtsson A, Gurunath R, Scheynius A, et al. (2001) Truncated thioredoxin (Trx80) induces production of interleukin-12 and enhances CD14 expression in human monocytes. *Blood* 97: 3184-3190.
116. Pekkari K, Goodarzi MT, Scheynius A, Holmgren A, Avila-Carino J (2005) Truncated thioredoxin (Trx80) induces differentiation of human CD14+ monocytes into a novel cell type (TAMs) via activation of the MAP kinases p38, ERK, and JNK. *Blood* 105: 1598-1605.
117. Pekkari K, Avila-Carino J, Gurunath R, Bengtsson A, Scheynius A, et al. (2003) Truncated thioredoxin (Trx80) exerts unique mitogenic cytokine effects via a mechanism independent of thiol oxidoreductase activity. *FEBS Lett* 539: 143-148.
118. Dunne DW, Resnick D, Greenberg J, Krieger M, Joiner KA (1994) The type I macrophage scavenger receptor binds to gram-positive bacteria and recognizes lipoteichoic acid. *Proc Natl Acad Sci U S A* 91: 1863-1867.
119. Kim S, Watarai M, Suzuki H, Makino S, Kodama T, et al. (2004) Lipid raft microdomains mediate class A scavenger receptor-dependent infection of *Brucella abortus*. *Microb Pathog* 37: 11-19.
120. Seleem MN, Boyle SM, Sriranganathan N (2010) Brucellosis: a re-emerging zoonosis. *Vet Microbiol* 140: 392-398.

121. Celli J (2006) Surviving inside a macrophage: the many ways of *Brucella*. *Res Microbiol* 157: 93-98.
122. Pizarro-Cerda J, Cossart P (2009) *Listeria monocytogenes* membrane trafficking and lifestyle: the exception or the rule? *Annu Rev Cell Dev Biol* 25: 649-670.
123. Flannagan RS, Cosio G, Grinstein S (2009) Antimicrobial mechanisms of phagocytes and bacterial evasion strategies. *Nat Rev Microbiol* 7: 355-366.
124. Orvedahl A, Levine B (2009) Eating the enemy within: autophagy in infectious diseases. *Cell Death Differ* 16: 57-69.
125. Pamer EG (2004) Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol* 4: 812-823.
126. Arellano-Reynoso B, Lapaque N, Salcedo S, Briones G, Ciocchini AE, et al. (2005) Cyclic beta-1,2-glucan is a *Brucella* virulence factor required for intracellular survival. *Nat Immunol* 6: 618-625.
127. Cicchini C, Laudadio I, Citarella F, Corazzari M, Steinder C, et al. (2008) TGFbeta-induced EMT requires focal adhesion kinase (FAK) signaling. *Exp Cell Res* 1: 143-152.
128. Hecker TP, Ding Q, Rege TA, Hanks SK, Gladson CL. (2004) Overexpression of FAK promotes Ras activity through the formation of a FAK/p120RasGAP complex in malignant astrocytoma cells. *Oncogene* 22: 3962-3971.
129. Ohl ME, Miller SI. (2001) *Salmonella*: a model for bacterial pathogenesis. *Annu Rev Med* 52: 259-274.

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