Ubiquitin, SUMO, and NEDD8: Key Targets of Bacterial Pathogens
David Ribet, Pascale Cossart

To cite this version:

HAL Id: pasteur-01890548
https://hal-pasteur.archives-ouvertes.fr/pasteur-01890548v2
Submitted on 10 Oct 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers. L’archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d’enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.
Ubiquitin, SUMO, Nedd8:

privileged targets of bacterial pathogens

David Ribet¹ and Pascale Cossart²*

1. Normandie Université, Université de Rouen, Institute for Research and Innovation in Biomedicine, INSERM, UMR1073, Nutrition Inflammation and Dysfunction of the gut-brain axis, Rouen, France.

2. Institut Pasteur, INSERM, INRA, Unité des Interactions Bactéries-Cellules, Paris, France.

* Correspondence: pascale.cossart@pasteur.fr

Keywords (6 max): Ubiquitin, SUMO, ISG15, NEDD8, FAT10, host-pathogen interactions
Abstract (100-120 words)

Manipulation of host protein post-translational modifications is used by various pathogens to interfere with host cell functions. Among these modifications, Ubiquitin and Ubiquitin-like proteins constitute privileged targets as they represent regulators of pathways essential for the host cell. In particular, these post-translational modifiers control pathways that have been described as critical for infection such as pathogen entry, replication, propagation or detection by the host. Although bacterial pathogens lack Ubiquitin or Ubiquitin-like protein systems, many of them produce proteins that specifically interfere with these host post-translational modifications during infection. In this review, we will discuss the different mechanisms used by bacteria to interfere with host Ubiquitin and Ubiquitin-like proteins (UBLs), such as SUMO or NEDD8.
Highlights:

* Ubiquitin and UBLs regulate essential pathways of the host cell involved in critical steps of bacterial infections. Not surprisingly, bacterial pathogens have evolved numerous strategies to interfere with these host post-translational modifications.

* Besides Ubiquitin, Ubiquitin-like proteins such as SUMO and NEDD8 have recently emerged as privileged targets of bacterial pathogens.

* Strategies used by bacteria to interfere with host Ubi/UBL encompass the targeting of Ubi/UBL conjugation machineries, the modulation of the Ubi/UBL conjugation level of specific host factors and the direct targeting of Ubi/UBL proteins.

* Host proteins modified by Ubi/UBL and targeted by bacteria cluster into specific host cell functions such as gene regulation, cytoskeleton dynamics or cell-autonomous immunity.

* Bacteria hijack the host Ubi/UBL systems to modify their own proteins allowing a regulation of their intracellular localization, stability or interaction abilities.

Outstanding questions:

* Are the recently described non-canonical ubiquitination mechanisms (*i.e.* conjugation involving non-RING/non-HECT E3 ligases or E1/E2-independent ubiquitin conjugation) strictly restricted to bacteria? Or are there functional homologs of these bacterial enzymes encoded by human cells?

* Recent improvements in proteomic analyses now allow to thoroughly monitor changes in the host ubiquitinome/"UBL-ome" in response to infection. These approaches usually generate lists of thousands of protein and/or sites modified during infection. Which strategies researchers should use to cope with this complex set of data and identify the key players affecting the outcome of infection?

* What are the mutations in the human population affecting the Ubi/UBL systems that may confer higher susceptibility to bacterial pathogens?

* Would drugs targeting bacteria-specific enzymes interfering with host Ubi/UBL conjugation be efficient to treat infectious diseases?
Ubiquitin and Ubiquitin-like proteins constitute essential modifiers of host proteins

Post-translational modifications (PTM) of proteins encompass a wide range of chemical modifications. These PTMs include the cleavage of peptide bonds (proteolysis), the modification of specific amino acid side chains such as deamidation, elimininylation or the covalent addition of chemical moieties ranging from simple groups (such as phosphate, acetyl or methyl groups) to more complex groups such as sugar, lipids or even small polypeptides.

Ubiquitin is a small polypeptide of 76 amino acids that can be covalently linked, via its C-terminal glycine residue, to target proteins. Ubiquitination, *i.e.* the conjugation of Ubiquitin, usually occurs on lysine residues of target proteins although conjugation to other amino acids such as threonine, serine, tyrosine or cysteine may happen. Ubiquitin itself contains seven lysines (K6, K11, K27, K29, K33, K48 and K63) that can serve as sites for additional cycles of Ubiquitin attachment, resulting in the formation of Ubiquitin chains. The topology of these chains is very diverse, ranging from “homotypic” K48- or K63-linked chains, composed of only one type of Ubiquitin linkage, to “mixed” chains containing for example both K11 and K63 linkages [1,2]. An additional type of chains, called “linear” chains, is generated when Ubiquitin is attached to the N-terminus of a second Ubiquitin [3]. Targeting of a given protein by Ubiquitin may thus result in mono-ubiquitination, multi-mono-ubiquitination (*i.e.* several mono-ubiquitination on different amino acids) or poly-ubiquitination. Ubiquitin is attached to substrates by a three-step enzymatic cascade involving E1 (Ubiquitin activating enzyme), E2 (Ubiquitin-conjugating enzyme) and E3 (Ubiquitin ligase) enzymes [2]. Ubiquitin is first activated in an ATP-dependent manner by E1, which links the C-terminal glycine residue of Ubiquitin via a thioester bond to a cysteine residue within the E1 active site. This activated Ubiquitin is then transferred to the catalytic cysteine residue of an E2 enzyme. E3 ligases then finally mediate the transfer of ubiquitin from the E2 enzyme to specific substrates. There are
two major classes of E3s: the HECT (homologous to the E6-AP carboxyl terminus) type and the RING (really interesting new genes)/U-box type. HECT-type E3 Ubiquitin ligases form a reactive intermediate with ubiquitin before its transfer to the substrate protein whereas RING/U-box-type E3 ligases mediate transfer of ubiquitin from the E2 directly to the substrate protein, without formation of an E3-ubiquitin intermediate [4]. Conjugation of Ubiquitin is a reversible process as several cellular isopeptidases (called deubiquitinases or DUBs) can cleave the covalent bond between Ubiquitin and its targets and thereby remove ubiquitin [5].

Besides Ubiquitin, other polypeptides such as SUMO (Small Ubiquitin-like MOdifier) [6], NEDD8 (neural precursor cell expressed developmentally downregulated protein 8) [7], ISG15 (interferon-stimulated gene 15) [8] or FAT10 (HLA-F-adjacent transcript 10) [9] can be similarly conjugated to target proteins. These polypeptides are grouped in the so-called Ubiquitin-like proteins (UBL) family and share high structural homology with Ubiquitin [10].

The mechanisms of UBL conjugation on target substrates are very similar to the ones observed for ubiquitination. The enzymes required for all these modifications (i.e. E1 UBL activating enzymes, E2 UBL conjugating enzymes and E3 UBL ligases) share highly conserved domain structures [10]. Of note, the number of UBL specific E1, E2 and E3 enzymes is usually smaller than for Ubiquitin. For example, SUMO conjugation to thousands of cellular targets seem to rely only on one single SUMO E1 enzyme (SAE1/UBA2), one single SUMO E2 enzyme (UBC9) and a dozen of SUMO E3 ligases [6]. As for Ubiquitin, the formation of UBL chains (where UBLs are conjugated to internal lysines of other UBLs) has been reported for SUMO and NEDD8 [6,7]. Finally, as for Ubiquitin, the host cell encodes several ULPs (UBL-specific proteases) that guarantee the reversibility of UBLs conjugation [6-9].

The consequences of Ubi/UBL conjugation on the fate of the modified proteins are very diverse. Ubi/UBL can alter the half-life of the modified proteins, for example by targeting them to proteasome degradation. They can change the targets’ structure thereby changing their catalytic
activity. They can add new surfaces of interactions or mask internal binding domains and change the targets’ interactome. The cell encodes in particular many “receptors” containing Ubiquitin-binding domains (UBDs) or UBL binding domains (such as the SUMO interacting motifs [SIMs]), that interact with proteins once conjugated to Ubi/UBL and “decode” these modifications into biochemical cascades in the cell [6,11]. Besides the well-known example of K48-Ubiquitin chains conjugation that target modified proteins to proteasomal degradation, it is usually very difficult to anticipate the consequences of Ubiquitin or UBL conjugation of a given target.

Ubi/UBL are essential regulators of fundamental pathways in cell biology. Some of these pathways are critical for the outcome of infection by pathogens. For example, Ubiquitin is a major regulator of the NF-κB pathway, that triggers the expression of proinflammatory cytokines in response to pathogen detection [12]. SUMO is a central player in the regulation of type I interferon and in anti-viral gene expression programs [13]. ISG15 plays several independent roles in anti-viral defense and can restrict intracellular bacteria replication in vitro and in vivo [8,14,15]. FAT10 was reported to be involved in xenophagy and in antimicrobial defense [9,16]. It is thus not surprising that pathogens evolved strategies to target Ubi/UBL and interfere with these different cellular processes.

In this review, we will present how pathogens interfere with the host Ubi/UBL systems. Ubiquitin and UBL systems have been shown to be targeted by diverse pathogens such as viruses, bacteria or parasites, including *Plasmodium falciparum* or *Toxoplasma gondii* [17-24]. We will focus here on pathogenic bacteria as they display the widest variety of Ubi/UBL interfering strategies known to date. Although bacteria do not have their own Ubi/UBL systems, numerous species encode virulence factors that actually manipulate host Ubi/UBL systems. These factors can be toxins secreted in the extracellular space in the vicinity of the host cell, or effectors delivered directly into host cells via specialized secretion systems such as Type III
secretion systems (T3SS). We will discuss how bacterial pathogens (i) target Ubi/UBLs conjugation machineries, (ii) increase or decrease the Ubi/UBL conjugation on specific host factors, (iii) directly target Ubi/UBL polypeptides, or (iv) use host Ubi/UBL to modify their own proteins. We will enlighten how these mechanisms allow bacterial pathogens to manipulate specific host cellular pathways in order to promote infection.

Harnessing of host Ubiquitin and UBLs conjugation by bacterial pathogens

Targeting of host Ubiquitin and UBLs conjugation machinery enzymes

Targeting of host E1 or E2 ubiquitin enzymes is a conserved strategy used by pathogens to dampen ubiquitination (Fig. 1, Key figure). This strategy is used for example by Shigella flexneri, the etiological agent of bacillary dysentery. This bacterium secretes through its T3SS, an effector, named OspI, that deamidates the human E2 Ubiquitin enzyme UBC13 [25]. This deamidation inactivates UBC13 Ubiquitin-conjugating activity, leading to the dampening of the Ubiquitin-dependent TRAF6-mediated signaling pathways and to the inhibition of host inflammatory responses during infection [25]. Extracellular pathogens such as enteropathogenic Escherichia coli (EPEC) also targets the host Ubiquitin conjugation machinery. Adhesion of these bacteria to human cells leads to the degradation of UBE1 and UBA6, the two E1 Ubiquitin enzymes, and to a global decrease of host protein ubiquitination [26]. The SUMO conjugation machinery constitutes another target for bacterial pathogens. Listeria monocytogenes, the bacterium responsible for human listeriosis, dampens SUMOylation of specific host factors by triggering the degradation of UBC9, the unique host E2 SUMO enzyme [27-29]. This degradation of UBC9 is triggered by the formation of pores into the host plasma membrane by the bacterial toxin Listeriolysin O (LLO) [27]. As LLO pores are not reported to affect the activity of host deSUMOylases, UBC9 degradation ultimately results in a shift in the SUMOylation/deSUMOylation equilibrium in the cell and to the
deSUMOylation of host proteins such as transcription factors [28]. The deSUMOylation events triggered by LLO were shown to promote *Listeria* infection [27]. Of note, other toxins of the same family as LLO, and secreted by extracellular pathogens, were shown to downregulate UBC9, indicating that interference with host SUMOylation is a strategy conserved between different classes of pathogenic bacteria [27]. Inhibition of the SUMOylation machinery is also observed during infection with *Salmonella* Typhimurium, a bacterium responsible for gastroenteritis in humans, and with *Shigella flexneri* but the underlying mechanisms involved here do not rely on the production of bacterial toxins. In the case of *Salmonella* Typhimurium, infection leads to the upregulation in the host cell of two small noncoding RNAs (miR30c and miR30e) that downregulate UBC9 level [30]. In the case of *Shigella flexneri*, infection is associated with an influx of calcium into the host cell. This ion flux activates the host proteases calpains, which cleave UBA2, one of the two components of the E1 SUMO enzyme [31]. The resulting inhibition of SUMOylation is associated with an increase in *Shigella* entry [31,32].

**Secretion of bacterial effectors mimicking host Ubiquitin and UBL enzymes**

Besides interfering with Ubiquitin or UBL-conjugation machineries, bacterial pathogens produce proteins that can replace or act as components of these machineries (Fig. 1). In particular, several bacterial effectors possess Ubiquitin E3-like activity. Some of these bacterial effectors share structural homologies with the two major types of eukaryotic E3 ligases, *i.e.* the HECT type and the RING/U-box type E3 ligases [20-22]. These effectors may have been acquired by bacteria via horizontal transfer from diverse eukaryotic sources [33]. In addition to these types, three other classes of bacterial effectors display structures completely distinct from eukaryotic E3 ligases: NELs (for Novel E3 ligase) [33], XL-box-containing E3 ligases [34] and SidC ligase [35]. These ligases may represent structures evolved by pathogens to mimic the functions of these essential host enzymes. These different classes of E3 ligases enable bacteria...
to conjugate Ubiquitin on specific host factors, thereby altering their stability or function, subcellular localization or interaction with other cellular proteins. Bacterial E3 ligases may in particular conjugate K48-Ubiquitin chains to host proteins, thereby triggering their proteasome-dependent degradation. By re-routing host factors to one of the most efficient proteolytic system of the infected cell, bacteria manage to eliminate key host components that normally interfere with their replication and propagation. Finally, bacterial E3 ligases can also target other bacterial effectors, co-delivered during infection, allowing a tight restriction of their activity during a specific time frame[21,22,36] (see below).

In contrast to bacterial effectors mimicking host ubiquitin enzymes, a family of proteins secreted by the bacterial pathogen *Legionella pneumophila*, the causative agent of Legionnaires’ disease, was recently shown to catalyze the ubiquitination of host proteins without the need for E1 and E2 Ubiquitin enzymes [37-39]. The *Legionella* SdeA effector belongs to this family of enzymes: it conjugates Ubiquitin on endoplasmic reticulum (ER)-associated Rab GTPases and participate to bacteria virulence [37]. By acting independently of E1- and E2-Ubiquitin enzymes, SdeA extents the repertoire of proteins potentially modified by Ubiquitin. Conjugation of Ubiquitin on host targets by SdeA does not rely on ATP and does not occur on lysines. Ubiquitin is instead phosphoribosylated by SdeA on a specific arginine residue, before being conjugated to a serine residue of its host target through a phosphodiester bond [38]. In addition to ER-associated Rab GTPases, the *Legionella* effector SdeA and other members of the Sde family ubiquitinate the host protein reticulon 4 (Rtn4), leading to ER reorganization and promoting *Legionella*-containing vacuoles formation [39]. Unconventional Ubiquitin conjugation by Sde effectors is reversible as *L. pneumophila* codes for a specific deubiquitinase, SidJ, that removes phosphoribosylated Ubiquitin from its substrate [40]. Whether functional homologs of SdeA exist in eukaryotes and what roles they may play remain to be determined.
Deconjugation of Ubiquitin and UBL proteins from host targets catalyzed by bacterial effectors

Another strategy used by bacteria to interfere with Ubiquitin or UBL conjugation consists in the secretion into host cells of effectors with isopeptidase activity, which remove Ubiquitin or UBL from their targets (Fig. 1). XopD, for example, is a T3SS effector secreted by the plant pathogen Xanthomonas euvesicatoria, which possesses a SUMO-specific isopeptidase activity [41]. Upon infection of tomato cells, it deconjugates SUMO from the SIERF4 transcription factor to suppress host ethylene production, which constitutes an important pathway of plants anti-bacterial immunity [42]. Many other bacterial proteases targeting Ubiquitin or UBLs have been identified in bacterial pathogens including Salmonella, Shigella, Chlamydia, and Legionella, some of them being specific for one UBL while others display cross-reactivity between different UBLs [43,44]. Interestingly, several bacterial effectors possessing a deubiquitinase activity display a strong preference for K63-linked chains over K48 or K11 chains [44]. This may reveal a significant selection pressure for bacteria to interfere with this specific Ubiquitin-modification in order to promote infection.

Direct targeting of Ubiquitin and UBL polypeptides

Ubiquitin itself, as well as other UBLs, can be directly targeted and inactivated by bacterial effectors (Fig. 1). Phosphoribosylation of Ubiquitin for example, catalyzed by the Legionella SdeA effector, was reported to interfere with multiple steps of the ubiquitination cascade [38]. The presence of phosphoribosylated Ubiquitin in chains further confers resistance to various deubiquitinases [45]. SdeA, by both triggering E1 and E2-independent ubiquitination of specific host targets and by inhibiting ubiquitination of others, thus efficiently controls the host ubiquitinome.
Ubiquitin and NEDD8 are also targeted by a family of bacterial T3SS effectors called Cifs (for cycle inhibiting factors), produced by diverse pathogenic bacteria such as some EPEC or *Burkholderia pseudomallei* [46]. Cifs directly target NEDD8 and Ubiquitin and catalyse the deamidation of the Gln⁴⁰ residue of these polypeptides [47]. Deamidation of Ubiquitin interferes with Ubiquitin chain formation, whereas deamidation of NEDD8 blocks the activity of neddylated Cullin-RING E3 Ubiquitin ligases (CRLs) and impairs ubiquitination of several CRL substrates in EPEC-infected cells [47,48]. Cifs interfere in particular with the ubiquitination of Perforin-2/MPEG1 (Macrophage-expressed gene 1), an anti-microbial host protein forming pores on bacteria cells, thereby blocking its intracellular trafficking and its bactericidal activity [49].

**Main host pathways targeted by bacteria and regulated by Ubiquitin or UBLs**

During infection, bacterial pathogens alter the conjugation of Ubiquitin or UBLs on many different host proteins. These proteins belong to different pathways that are all essential for bacteria to efficiently enter into host cells and replicate therein, or to dampen host anti-bacterial responses. We will here detail some of the pathways tightly regulated by Ubi/UBL modifications and frequently targeted by bacterial pathogens.

**The NF-κB pathway**

The NF-κB pathway is an essential pillar of innate immunity and inflammation. Activation of this pathway, for example after the detection of bacteria-derived molecules by host sensors, triggers the expression of a wide range of proinflammatory chemokines and cytokines. Not surprisingly, many bacterial effectors target the NF-κB pathway to dampen the host innate immune response. One given pathogen may in particular produce several independent effectors targeting this pathway [12]. This apparent redundancy of effectors, that all target the same
One common strategy used by bacterial pathogens to dampen the NF-κB signaling cascade consists in conjugating K48-Ubiquitin chains to essential components of this pathway thereby triggering their proteasome-dependent degradation. *Shigella flexneri*, for example, uses at least five different effectors to inhibit essential branches of the NF-κB pathway: IpaH1.4 and IpaH2.5 ubiquitinate LUBAC, a complex involved in the activation of the NF-κB pathway that conjugates linear Ubiquitin chains to the NF-κB modulator NEMO [50]; IpaH0722 ubiquitates TRAF2, a factor involved in the NF-κB pathway activation following the detection of intracytosolic bacteria [51]; IpaH9.8 ubiquititates NEMO and thereby perturbs the NF-κB activation triggered by bacterial peptidoglycan detection [52].

Besides triggering proteasome-dependent degradation of components of the NF-κB pathway, bacterial pathogens also interfere with the endogenous Ubiquitination of critical NF-κB regulators: as mentioned above, the *Shigella* OspI effector inhibits the host E2 enzyme UBC13, thereby blocking TRAF6-mediated activation of the NF-κB pathway [25]; OspG, another *Shigella* effector, binds to and inhibits the host E2 Ubiquitin enzyme UBCH5, involved in IκBα ubiquitination [53]; the NleB effector, encoded by EPEC, blocks TRAF2 polyubiquitination, ultimately suppressing NF-κB activation [54] and NleE, another EPEC effector, inhibits IκBα phosphorylation, which is a prerequisite for its subsequent Ubiquitination and degradation [55].

The NF-κB pathway thereby constitutes a nice example of the diverse mechanisms evolved by bacteria to promote or inhibit ubiquitination of a large number of components in a coordinated fashion, resulting in the dampening of an essential arm of the host anti-bacterial response. Of course, these interfering strategies are not restricted to the NF-κB pathway and other important signaling cascades of the innate immune response, such as the IFN response or the activation of inflammasome, can be similarly targeted [21,56].
**Host cytoskeleton**

Remodeling of the host cytoskeleton is frequently used by intracellular bacterial pathogens to enter into the targeted cells, create a niche where they can efficiently replicate, and disseminate to neighboring cells. Several components of the host cytoskeleton are regulated by Ubiquitin. RhoGTPases, for example, which control the actin cytoskeleton dynamics, are degraded by the proteasome following Ubiquitin conjugation [57]. Interestingly, the ubiquitination level of RhoGTPases can be modulated during *Salmonella* infection, suggesting that this bacterium may modulate RhoGTPases turn-over [58]. SUMO can be conjugated to different components of the host cytoskeleton as well, including actin itself and actin regulatory proteins, septins or intermediate filaments such as keratins and lamins [59,60]. The role of Ubiquitin and UBL modifications in the regulation of the cytoskeleton is only in its infancy but one can anticipate that it may represent an important target for bacterial pathogens to manipulate the cell architecture.

**Transcription factors**

In order to exploit host functions, bacterial pathogens remodel the proteome of infected cells. This remodeling may result from deregulation of gene transcription by injection of bacterial proteins such as nucleomodulins that act directly on host nucleus [61], or by interference with host transcription factors, some of them being regulated by Ubiquitin or UBLs. *Listeria monocytogenes*, for example, dampens the SUMOylation of numerous transcription factors during infection [28]. As SUMO conjugation either increases or decreases transcription factors activity, this decrease in SUMOylation may modulate the expression of specific subset of genes and lead to a reprogramming of host gene expression. As mentioned above, decreasing the SUMOylation of host transcription factors is a strategy also used by the plant pathogen...
Xanthomonas euvesicatoria that specifically targets SUMO-SIERF4 to dampen the host ethylene-mediated antibacterial response [42]. Finally, the colibactin toxin, produced by some Escherichia coli strains in the intestine, induces a downregulation of the SUMO isopeptidase SENP1 and an increase in the SUMOylation of the transcription factor p53. This ultimately results in the emergence of senescent cells secreting growth factors that may promote colorectal carcinogenesis [62].

**PML Nuclear Bodies**

PML (Promyelocytic Luekemia Protein) is a protein that polymerizes in discrete nuclear assemblies known as PML nuclear bodies (NBs) and plays essential roles in many different cellular processes. Key to its function, PML can be post-translationally modified by SUMO. In addition to its role in anti-viral host defense [18], PML was recently identified as a sensor for bacteria producing pore-forming toxins [29]. Indeed, intoxication of human cells by the Listeriolysin O toxin, secreted by *L. monocytogenes*, triggers a massive deSUMOylation of PML. This deSUMOylation of PML, coupled to an oxidative stress-dependent multimerization of PML, initiates host cell anti-bacterial responses leading to a decrease in *Listeria* intracellular replication [29]. This example of PML highlights how SUMO alterations of some specific host proteins can constitute danger signals for the cells that triggers back adapted responses. The putative role of PML in other bacterial infections targeting host SUMOylation, such as *Shigella* or *Salmonella*, remains unknown but would deserves further investigation.

**Post-translational modifications of bacterial proteins during infection**

Besides interfering with host proteins post-translational modifications, bacteria can hijack host Ubiquitin or UBL-conjugation machineries to modify their own components (Fig. 1). As for eukaryotic proteins, conjugation of Ubiquitin or UBL have diverse effects on bacterial effectors
and may change their intracellular localization, their stability or their interaction with other bacterial or host factors. Post-translational modification of bacterial proteins couples their activity to their arrival into the host cell cytoplasm. Interestingly, post-translational modification of bacterial proteins can also be used by the host to tag exogenous proteins and target them for degradation.

Ubiquitination of *Salmonella* proteins constitutes a nice example illustrating the versatility of consequences of this post-translational modification on bacterial proteins activity. SopE and SptP are two *Salmonella* effectors that contribute to the transient remodeling of the host cell’s cytoskeleton. These two effectors, which are delivered simultaneously by *Salmonella*, exhibit different half-lives. SopE, which is involved in actin cytoskeleton rearrangement, membrane ruffling and bacteria uptake, is rapidly polyubiquitinilated and degraded by the host proteasome [63]. SptP, which displays an opposite activity to SopE, exhibits a much slower degradation kinetics, allowing recovery of the actin cytoskeleton’s normal architecture a few hours after infection [63]. Conjugation of Ubiquitin to SopB, a phosphoinositide phosphatase secreted by *Salmonella* via T3SS, modifies its cellular localization [64]. Upon delivery, SopB associates with the host plasma membrane where it participates to actin-mediated bacterial entry. Later on, Ubiquitination of SopB by TRAF6 leads to its translocation to the *Salmonella*-containing vacuoles, where it modulates vesicle trafficking and interferes with the delivery of these vacuoles to lysosomes [64,65]. Mass spectrometry-based large-scale analysis of the Ubiquitinome of cells infected by *Salmonella* recently provided additional examples of bacterial proteins modified by Ubiquitin [58]. In addition to the previosuly reported SopE and SopB, several effectors were identified as being ubiquitinated during infection. Interestingly, integral outer membrane proteins were reported to be conjugated to Ubiquitin and may represent the targets forming the Ubiquitin coat surrounding cytosolic bacteria and involved in host anti-bacterial autophagy [58,66]. Indeed, autophagy of invasive bacteria serves as a cellular
autonomous immune mechanism. During this process, a dense coat of poly-Ubiquitin chains is formed around bacteria, which serves as pathogen recognition receptor and directs intracellular bacteria for autophagic degradation [66,67].

In contrast to Ubiquitination, only few bacterial proteins were reported so far to be modified by SUMO and the biological consequences of these modifications during infection often remains elusive. These SUMO-modified bacterial proteins include two effectors, TRP120 and AmpA, secreted by two intracellular pathogens, *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* respectively [68,69]. OspF, an effector secreted by *Shigella flexneri*, constitutes another example for which SUMO conjugation is required for the translocation of this effector into the host nucleus where it modulates the expression of proinflammatory cytokines [70]. One can anticipate that recently developed techniques for large scale proteomic studies of UBL conjugation will increase the list of bacterial proteins modified by SUMO or other UBLs, and provide new insights in the role of these modifications during infection.

**Concluding Remarks and Future Perspectives**

Ubiquitin and UBL are essential post-translational modifiers of eukaryotic cells. Thousands of Ubi/UBL targets have been identified during these last years, suggesting that most proteins will be modified by this type of PTMs at some point in their cellular lifetime. It is thus not surprising that pathogens evolved so many strategies to interfere with these particular PTMs in order to manipulate host cell physiology. Harnessing of host Ubi/UBL systems is in particular observed both for intracellular pathogens, that tightly interact with host cell cytoplasmic components to create for example a protective niche where they can acquire nutrients from the host, and for extracellular pathogens, that manipulate host cells to favor their maintenance at the surface of the cells or dampen host immune responses.
Thanks to the continuous improvement in proteomic analyses, the list of proteins known to be modified by Ubiquitin or UBLs has greatly expanded during these last years. It is in particular now feasible to compare the variations of the ubiquitinome (or other “UBL-ome”) of cells during infection by a pathogen or after exposure to a bacterial toxin [28, 58]. Some of these techniques are furthermore compatible with *in vivo* analysis and the comparison of the content of proteins modified by Ubi/UBL in organs from infected or control animals is now possible [71, 72]. Interestingly, current proteomic-based approaches not only reveal the identity of the proteins modified by Ubi/UBL but also the modifications sites. These data are critical for further analysis of the role of these PTM in the function of the identified protein and hence, to decipher the consequences of bacterial alteration of these PTMs. Several recent studies on ubiquitin conjugation revealed that ubiquitination establishes a much more complex code than originally thought. Indeed, in addition to “mixed” ubiquitin chains involving different types of linkages between Ubiquitin monomers, chains mixing ubiquitin and other UBLs such as SUMO have also been reported [1, 2, 73]. In addition, Ubiquitin has recently been found to be itself post-translationally modified by acetylation or phosphorylation, which further expands the repertoire of ubiquitination [1, 2, 73]. We are only beginning to understand the tremendous diversity of Ubiquitin modifications and their roles in cell biology but it is very likely that bacterial pathogens have long learned how to break this so-called “Ubiquitin code” and efficiently use it for their own profit (see Outstanding Questions).

Finally, while this review focused on pathogenic bacteria, some non pathogenic bacteria such as commensals of the intestinal microbiota were also reported to interfere with host Ubi/UBL systems [74]. For example, production of butyrate by commensal bacteria leads to the inactivation of the E2 NEDD8 enzyme in intestinal epithelial cells and was proposed to participate to the inflammatory tolerance of gut bacteria [75, 76]. Some intestinal bacteria may even usurp host ubiquitin for their own purpose. Indeed, even though most bacteria lack
Ubiquitin or UBL genes, a Ubiquitin gene has been identified in the genome of some
*Bacteroides fragilis* strains [77,78]. Interestingly, this eukaryotic-like Ubiquitin, which was
probably acquired via horizontal gene transfer, does not seem to be involved in bacterial protein
modification since it lacks the critical terminal glycine residue. This protein is instead secreted
and acts as a bacterial toxin targeting and killing other intestinal bacteria [78]. Many other
surprises like this one are probably still awaiting to be discovered and, even though the first
report of a bacterium post-translationally modifying a host protein occurred almost 50 years
ago [79], the field of pathogen and host post-translational modifications is, without a doubt, still
very promising.
Figure 1: Main strategies used by bacterial pathogens to interfere with host Ubiquitin or Ubiquitin-like protein modifications.

During infection, bacteria may (1) inhibit Ubi/UBL conjugating enzymes, (2) secrete effectors possessing E3 ligase activity or (3) isopeptidase activity, or (4) directly inactivate Ubiquitin or UBLs. (5) Bacteria may also hijack the host Ubi/UBL systems to modify their own proteins during infection.
<table>
<thead>
<tr>
<th>Ubiquitin target</th>
<th>Bacteria</th>
<th>Extra/intracellular bacteria</th>
<th>Effector</th>
<th>Enzymatic activity</th>
<th>Effect</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ubiquitin</td>
<td><em>Salmonella Typhimurium</em></td>
<td>intracellular</td>
<td>SopA</td>
<td>E3 Ubi ligase (HECT)</td>
<td>Regulation of host inflammation</td>
<td>80</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>EPEC, EHEC</td>
<td>extracellular</td>
<td>NleL</td>
<td>E3 Ubi ligase (HECT)</td>
<td>Regulation of actin pedestal formation</td>
<td>81</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>EPEC, EHEC</td>
<td>extracellular</td>
<td>NleG</td>
<td>E3 Ubi ligase (RING)</td>
<td>?</td>
<td>82</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Pseudomonas syringae</em></td>
<td>extracellular</td>
<td>AvrPtoB</td>
<td>E3 Ubi ligase (U-box)</td>
<td>Inhibition of plant pattern-triggered immunity</td>
<td>83,84</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Shigella flexneri</em></td>
<td>Intracellular</td>
<td>OspI</td>
<td>Gln deamidase</td>
<td>Inactivation of UBE2N/UBC13 (E2 Ubi enzyme NF-κB pathway)</td>
<td>25</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Shigella flexneri</em></td>
<td>intracellular</td>
<td>IpaH1.4</td>
<td>E3 Ubi ligase (NEL)</td>
<td>Ubiquitination of LUBAC (NF-κB pathway)</td>
<td>50</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Shigella flexneri</em></td>
<td>intracellular</td>
<td>IpaH2.5</td>
<td>E3 Ubi ligase (NEL)</td>
<td>Ubiquitination of LUBAC (NF-κB pathway)</td>
<td>50</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Shigella flexneri</em></td>
<td>intracellular</td>
<td>IpaH0722</td>
<td>E3 Ubi ligase (NEL)</td>
<td>Ubiquitination of TRAF2 (NF-κB pathway)</td>
<td>51</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Shigella flexneri</em></td>
<td>intracellular</td>
<td>IpaH9.8</td>
<td>E3 Ubi ligase (NEL)</td>
<td>Ubiquitination of NEMO (NF-κB pathway)</td>
<td>52</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Legionella pneumophila</em></td>
<td>intracellular</td>
<td>SdeA</td>
<td>non eukaryotic Ubi ligase</td>
<td>E1/E2-independent ubiquitination of Rab GTPases and RTN4</td>
<td>37-39</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Shigella flexneri</em></td>
<td>intracellular</td>
<td>OspG</td>
<td>kinase</td>
<td>Inhibition of UBC5 (E2 Ubi enzyme; NF-κB pathway)</td>
<td>53</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>EPEC, EHEC</td>
<td>extracellular</td>
<td>NleB</td>
<td>Glycosyltransferase</td>
<td>Inhibition of TRAF2 ubiquitination (NF-κB pathway)</td>
<td>54</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>EPEC</td>
<td>extracellular</td>
<td>?</td>
<td>?</td>
<td>Downregulation of UBE1 and UBA6 (E1 Ubi enzymes)</td>
<td>26</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>EPEC</td>
<td>extracellular</td>
<td>NleE</td>
<td>Cys methyltransferase</td>
<td>Inactivation of TAB2 and TAB3 (NF-κB pathway)</td>
<td>55, 85</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Legionella pneumophila</em></td>
<td>intracellular</td>
<td>SidJ</td>
<td>deubiquitylase</td>
<td>?</td>
<td>40</td>
</tr>
<tr>
<td>Ubiquitin/ SUMO</td>
<td>Pathogen</td>
<td>Location</td>
<td>Enzyme</td>
<td>Activity</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>----------</td>
<td>----------</td>
<td>--------</td>
<td>----------</td>
<td>-----------</td>
<td></td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Shigella flexneri</em></td>
<td>intracellular</td>
<td>ShiCE deubiquitylase</td>
<td>?</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Chlamydia trachomatis</em></td>
<td>intracellular</td>
<td>ChlaDUB1 deubiquitylase</td>
<td>Inhibition of NF-κB pathway activation</td>
<td>86, 87</td>
<td></td>
</tr>
<tr>
<td>Ubiquitin</td>
<td><em>Burkholderia pseudomallei</em></td>
<td>extracellular</td>
<td>CHBP Gln deamidase</td>
<td>Deamidation of Ubiquitin</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>SUMO</td>
<td><em>Listeria monocytogenes</em></td>
<td>intracellular</td>
<td>LLO Pore-forming toxin</td>
<td>Downregulation of UBE2I/UBC9 (E2 SUMO enzyme)</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>SUMO</td>
<td><em>Clostridium perfringens</em></td>
<td>extracellular</td>
<td>PFO Pore-forming toxin</td>
<td>Downregulation of UBE2I/UBC9 (E2 SUMO enzyme)</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>SUMO</td>
<td><em>Streptococcus pneumoniae</em></td>
<td>extracellular</td>
<td>PLY Pore-forming toxin</td>
<td>Downregulation of UBE2I/UBC9 (E2 SUMO enzyme)</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>SUMO</td>
<td><em>Shigella flexneri</em></td>
<td>intracellular</td>
<td>? / Ca2+ influx</td>
<td>?</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>SUMO</td>
<td><em>Salmonella Typhimurium</em></td>
<td>intracellular</td>
<td>? / miRNAs</td>
<td>Downregulation of UBE2I/UBC9 (E2 SUMO enzyme)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>SUMO</td>
<td><em>Xanthomonas euvesicatoria</em></td>
<td>extracellular</td>
<td>XopD deSUMOylase</td>
<td>DeSUMOylation of SIERF4 (plant immune response)</td>
<td>41,42</td>
<td></td>
</tr>
<tr>
<td>NEDD8</td>
<td><em>EPEC</em></td>
<td>extracellular</td>
<td>CIF Gln deamidase</td>
<td>Deamidation of NEDD8</td>
<td>47,48</td>
<td></td>
</tr>
<tr>
<td>NEDD8</td>
<td><em>Chlamydia trachomatis</em></td>
<td>intracellular</td>
<td>ChlaDUB1 deNeddylase</td>
<td>Inhibition of NF-κB pathway activation</td>
<td>86, 87</td>
<td></td>
</tr>
</tbody>
</table>
Acknowledgments

We apologize to all colleagues whose work we were unable to include due to space constraints.

Authors received support from Institut Pasteur, INSERM, INRA, the French National Research Agency (ANR) (ERANET Infect-ERA PROANTILIS ANR-13-IFEC-0004-02), the French Government’s Investissement d’Avenir program, Laboratoire d’Excellence “Integrative Biology of Emerging Infectious Diseases” (ANR-10-LABX-62-IBEID), the European Research Council (ERC) (H2020-ERC-2014-ADG 670823-BacCellEpi), the Fondation le Roch les Mousquetaires, the Fondation Louis-Jeantet and the International Balzan Prize Fondation.

D.R. is a Research Associate from INSERM and P.C. is a Senior International Research Scholar of the Howard Hughes Medical Institute.

REFERENCES


42. Kim, J.G. et al. (2013) Xanthomonas type III effector XopD desumoylates tomato transcription factor SlERF4 to suppress ethylene responses and promote pathogen growth. Cell Host Microbe 13 (2), 143-54.


