



Ubiquitin, SUMO, and NEDD8: Key Targets of Bacterial Pathogens

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15 **Abstract (100-120 words)**

16

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

89 two major classes of E3s: the HECT (homologous to the E6-AP carboxyl terminus) type and
90 the RING (really interesting new genes)/U-box type. HECT-type E3 Ubiquitin ligases form a
91 reactive intermediate with ubiquitin before its transfer to the substrate protein whereas
92 RING/U-box-type E3 ligases mediate transfer of ubiquitin from the E2 directly to the substrate
93 protein, without formation of an E3-ubiquitin intermediate [4]. Conjugation of Ubiquitin is a
94 reversible process as several cellular isopeptidases (called deubiquitinases or DUBs) can cleave
95 the covalent bond between Ubiquitin and its targets and thereby remove ubiquitin [5].
96 Besides Ubiquitin, other polypeptides such as SUMO (Small Ubiquitin-like MOdifier) [6],
97 NEDD8 (neural precursor cell expressed developmentally downregulated protein 8) [7], ISG15
98 (interferon-stimulated gene 15) [8] or FAT10 (HLA-F-adjacent transcript 10) [9] can be
99 similarly conjugated to target proteins. These polypeptides are grouped in the so-called
100 Ubiquitin-like proteins (UBL) family and share high structural homology with Ubiquitin [10].
101 The mechanisms of UBL conjugation on target substrates are very similar to the ones observed
102 for ubiquitination. The enzymes required for all these modifications (*i.e.* E1 UBL activating
103 enzymes, E2 UBL conjugating enzymes and E3 UBL ligases) share highly conserved domain
104 structures [10]. Of note, the number of UBL specific E1, E2 and E3 enzymes is usually smaller
105 than for Ubiquitin. For example, SUMO conjugation to thousands of cellular targets seem to
106 rely only on one single SUMO E1 enzyme (SAE1/UBA2), one single SUMO E2 enzyme
107 (UBC9) and a dozen of SUMO E3 ligases [6]. As for Ubiquitin, the formation of UBL chains
108 (where UBLs are conjugated to internal lysines of other UBLs) has been reported for SUMO
109 and NEDD8 [6,7]. Finally, as for Ubiquitin, the host cell encodes several ULPs (UBL-specific
110 proteases) that guarantee the reversibility of UBLs conjugation [6-9].
111 The consequences of Ubi/UBL conjugation on the fate of the modified proteins are very diverse.
112 Ubi/UBL can alter the half-life of the modified proteins, for example by targeting them to
113 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- κ B 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 extends 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.

214

215 *Deconjugation of Ubiquitin and UBL proteins from host targets catalyzed by bacterial effectors*

216 Another strategy used by bacteria to interfere with Ubiquitin or UBL conjugation consists in
217 the secretion into host cells of effectors with isopeptidase activity, which remove Ubiquitin or
218 UBL from their targets (Fig. 1). XopD, for example, is a T3SS effector secreted by the plant
219 pathogen *Xanthomonas euvesicatoria*, which possesses a SUMO-specific isopeptidase activity
220 [41]. Upon infection of tomato cells, it deconjugates SUMO from the SIERF4 transcription
221 factor to suppress host ethylene production, which constitutes an important pathway of plants
222 anti-bacterial immunity [42]. Many other bacterial proteases targeting Ubiquitin or UBLs have
223 been identified in bacterial pathogens including *Salmonella*, *Shigella*, *Chlamydia*, and
224 *Legionella*, some of them being specific for one UBL while others display cross-reactivity
225 between different UBLs [43,44]. Interestingly, several bacterial effectors possessing a
226 deubiquitinase activity display a strong preference for K63-linked chains over K48 or K11
227 chains [44]. This may reveal a significant selection pressure for bacteria to interfere with this
228 specific Ubiquitin-modification in order to promote infection.

229

230 *Direct targeting of Ubiquitin and UBL polypeptides*

231 Ubiquitin itself, as well as other UBLs, can be directly targeted and inactivated by bacterial
232 effectors (Fig. 1). Phosphoribosylation of Ubiquitin for example, catalyzed by the *Legionella*
233 SdeA effector, was reported to interfere with multiple steps of the ubiquitination cascade [38].
234 The presence of phosphoribosylated Ubiquitin in chains further confers resistance to various
235 deubiquitinases [45]. SdeA, by both triggering E1 and E2-independent ubiquitination of
236 specific host targets and by inhibiting ubiquitination of others, thus efficiently controls the host
237 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

263 signaling cascade, reflects the diversity of danger signals sensed by the host and triggering this
264 pathway.

265 One common strategy used by bacterial pathogens to dampen the NF- κ B signaling cascade
266 consists in conjugating K48-Ubiquitin chains to essential components of this pathway thereby
267 triggering their proteasome-dependent degradation. *Shigella flexneri*, for example, uses at least
268 five different effectors to inhibit essential branches of the NF- κ B pathway: IpaH1.4 and IpaH2.5
269 ubiquitinate LUBAC, a complex involved in the activation of the NF- κ B pathway that
270 conjugates linear Ubiquitin chains to the NF- κ B modulator NEMO [50]; IpaH0722
271 ubiquitinates TRAF2, a factor involved in the NF- κ B pathway activation following the
272 detection of intracytosolic bacteria [51]; IpaH9.8 ubiquitinates NEMO and thereby perturbs the
273 NF- κ B activation triggered by bacterial peptidoglycan detection [52].

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

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

288

289 *Host cytoskeleton*

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

302

303 *Transcription factors*

304 In order to exploit host functions, bacterial pathogens remodel the proteome of infected cells.
305 This remodeling may result from deregulation of gene transcription by injection of bacterial
306 proteins such as nucleomodulins that act directly on host nucleus [61], or by interference with
307 host transcription factors, some of them being regulated by Ubiquitin or UBLs. *Listeria*
308 *monocytogenes*, for example, dampens the SUMOylation of numerous transcription factors
309 during infection [28]. As SUMO conjugation either increases or decreases transcription factors
310 activity, this decrease in SUMOylation may modulate the expression of specific subset of genes
311 and lead to a reprogramming of host gene expression. As mentioned above, decreasing the
312 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 Leukemia 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

338 and may change their intracellular localization, their stability or their interaction with other
339 bacterial or host factors. Post-translational modification of bacterial proteins couples their
340 activity to their arrival into the host cell cytoplasm. Interestingly, post-translational
341 modification of bacterial proteins can also be used by the host to tag exogenous proteins and
342 target them for degradation.

343 Ubiquitination of *Salmonella* proteins constitutes a nice example illustrating the versatility of
344 consequences of this post-translational modification on bacterial proteins activity. SopE and
345 SptP are two *Salmonella* effectors that contribute to the transient remodeling of the host cell's
346 cytoskeleton. These two effectors, which are delivered simultaneously by *Salmonella*, exhibit
347 different half-lives. SopE, which is involved in actin cytoskeleton rearrangement, membrane
348 ruffling and bacteria uptake, is rapidly polyubiquitinated and degraded by the host proteasome
349 [63]. SptP, which displays an opposite activity to SopE, exhibits a much slower degradation
350 kinetics, allowing recovery of the actin cytoskeleton's normal architecture a few hours after
351 infection [63]. Conjugation of Ubiquitin to SopB, a phosphoinositide phosphatase secreted by
352 *Salmonella* via T3SS, modifies its cellular localization [64]. Upon delivery, SopB associates
353 with the host plasma membrane where it participates to actin-mediated bacterial entry. Later
354 on, Ubiquitination of SopB by TRAF6 leads to its translocation to the *Salmonella*-containing
355 vacuoles, where it modulates vesicle trafficking and interferes with the delivery of these
356 vacuoles to lysosomes [64,65]. Mass spectrometry-based large-scale analysis of the
357 Ubiquitinome of cells infected by *Salmonella* recently provided additional examples of
358 bacterial proteins modified by Ubiquitin [58]. In addition to the previously reported SopE and
359 SopB, several effectors were identified as being ubiquitinated during infection. Interestingly,
360 integral outer membrane proteins were reported to be conjugated to Ubiquitin and may
361 represent the targets forming the Ubiquitin coat surrounding cytosolic bacteria and involved in
362 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.

387 Thanks to the continuous improvement in proteomic analyses, the list of proteins known to be
388 modified by Ubiquitin or UBLs has greatly expanded during these last years. It is in particular
389 now feasible to compare the variations of the ubiquitinome (or other “UBL-ome”) of cells
390 during infection by a pathogen or after exposure to a bacterial toxin [28,58]. Some of these
391 techniques are furthermore compatible with *in vivo* analysis and the comparison of the content
392 of proteins modified by Ubi/UBL in organs from infected or control animals is now possible
393 [71,72]. Interestingly, current proteomic-based approaches not only reveal the identity of the
394 proteins modified by Ubi/UBL but also the modifications sites. These data are critical for
395 further analysis of the role of these PTM in the function of the identified protein and hence, to
396 decipher the consequences of bacterial alteration of these PTMs. Several recent studies on
397 ubiquitin conjugation revealed that ubiquitination establishes a much more complex code than
398 originally thought. Indeed, in addition to “mixed” ubiquitin chains involving different types of
399 linkages between Ubiquitin monomers, chains mixing ubiquitin and other UBLs such as SUMO
400 have also been reported [1,2,73]. In addition, Ubiquitin has recently been found to be itself
401 post-translationally modified by acetylation or phosphorylation, which further expands the
402 repertoire of ubiquitination [1,2,73]. We are only beginning to understand the tremendous
403 diversity of Ubiquitin modifications and their roles in cell biology but it is very likely that
404 bacterial pathogens have long learned how to break this so-called “Ubiquitin code” and
405 efficiently use it for their own profit (see Outstanding Questions).

406 Finally, while this review focused on pathogenic bacteria, some non pathogenic bacteria such
407 as commensals of the intestinal microbiota were also reported to interfere with host Ubi/UBL
408 systems [74]. For example, production of butyrate by commensal bacteria leads to the
409 inactivation of the E2 NEDD8 enzyme in intestinal epithelial cells and was proposed to
410 participate to the inflammatory tolerance of gut bacteria [75,76]. Some intestinal bacteria may
411 even usurp host ubiquitin for their own purpose. Indeed, even though most bacteria lack

412 Ubiquitin or UBL genes, a Ubiquitin gene has been identified in the genome of some
413 *Bacteroides fragilis* strains [77,78]. Interestingly, this eukaryotic-like Ubiquitin, which was
414 probably acquired via horizontal gene transfer, does not seem to be involved in bacterial protein
415 modification since it lacks the critical terminal glycine residue. This protein is instead secreted
416 and acts as a bacterial toxin targeting and killing other intestinal bacteria [78]. Many other
417 surprises like this one are probably still awaiting to be discovered and, even though the first
418 report of a bacterium post-translationally modifying a host protein occurred almost 50 years
419 ago [79], the field of pathogen and host post-translational modifications is, without a doubt, still
420 very promising.

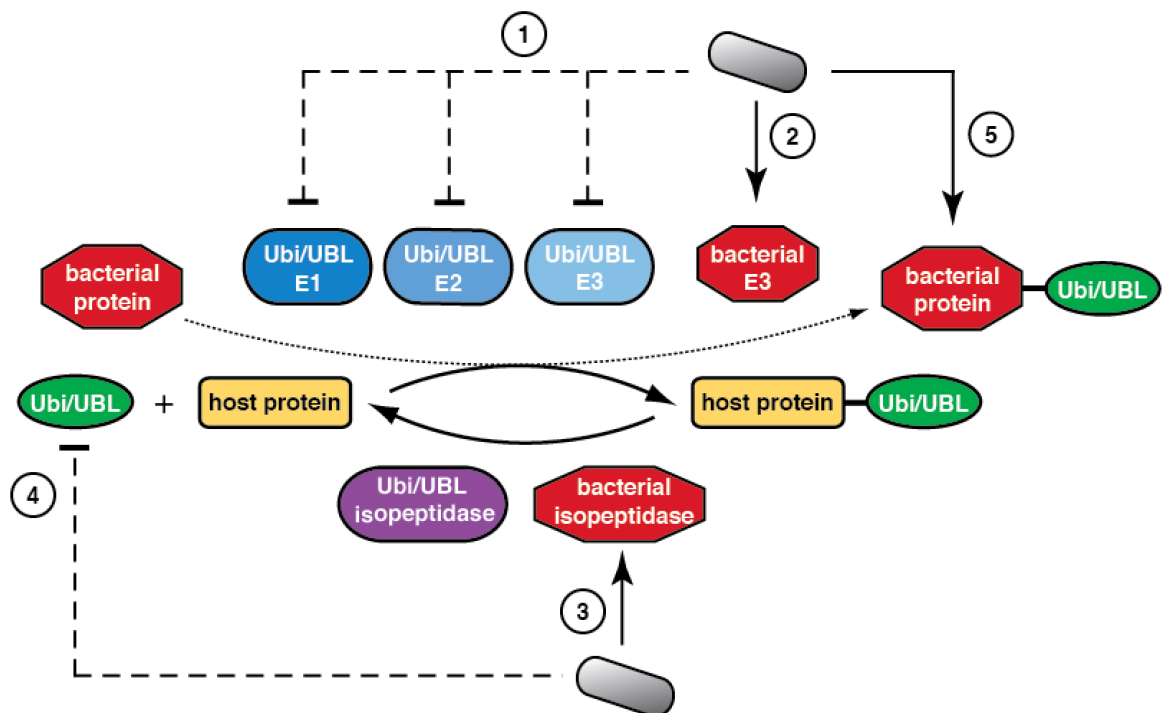


Figure 1 : Main strategies used by bacterial pathogens to interfere with host Ubiquitin or Ubiquitin-like protein modifications.

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 1 : Examples of bacterial proteins interfering with Ubi/UBL conjugation to host proteins

Ubi/UBL target	Bacteria	Extra/intracellular bacteria	Effector	Enzymatic activity	Effect	Refs
Ubiquitin	<i>Salmonella Typhimurium</i>	intracellular	SopA	E3 Ubi ligase (HECT)	Regulation of host inflammation	80
Ubiquitin	EPEC, EHEC	extracellular	NleL	E3 Ubi ligase (HECT)	Regulation of actin pedestal formation	81
Ubiquitin	EPEC, EHEC	extracellular	NleG	E3 Ubi ligase (RING)	?	82
Ubiquitin	<i>Pseudomonas syringae</i>	extracellular	AvrPtoB	E3 Ubi ligase (U-box)	Inhibition of plant pattern-triggered immunity	83,84
Ubiquitin	<i>Shigella flexneri</i>	Intracellular	OspI	Gln deamidase	Inactivation of UBE2N/UBC13 (E2 Ubi enzyme NF- κ B pathway)	25
Ubiquitin	<i>Shigella flexneri</i>	intracellular	IpaH1.4	E3 Ubi ligase (NEL)	Ubiquitination of LUBAC (NF- κ B pathway)	50
Ubiquitin	<i>Shigella flexneri</i>	intracellular	IpaH2.5	E3 Ubi ligase (NEL)	Ubiquitination of LUBAC (NF- κ B pathway)	50
Ubiquitin	<i>Shigella flexneri</i>	intracellular	IpaH0722	E3 Ubi ligase (NEL)	Ubiquitination of TRAF2 (NF- κ B pathway)	51
Ubiquitin	<i>Shigella flexneri</i>	intracellular	IpaH9.8	E3 Ubi ligase (NEL)	Ubiquitination of NEMO (NF- κ B pathway)	52
Ubiquitin	<i>Legionella pneumophila</i>	intracellular	SdeA	non eukaryotic Ubi ligase	E1/E2-independent ubiquitination of Rab GTPases and RTN4	37-39
Ubiquitin	<i>Shigella flexneri</i>	intracellular	OspG	kinase	Inhibition of UBC5 (E2 Ubi enzyme; NF- κ B pathway)	53
Ubiquitin	EPEC, EHEC	extracellular	NleB	Glycosyltransferase	Inhibition of TRAF2 ubiquitination (NF- κ B pathway)	54
Ubiquitin	EPEC	extracellular	?	?	Downregulation of UBE1 and UBA6 (E1 Ubi enzymes)	26
Ubiquitin	EPEC	extracellular	NleE	Cys methyltransferase	Inactivation of TAB2 and TAB3 (NF- κ B pathway)	55, 85
Ubiquitin	<i>Legionella pneumophila</i>	intracellular	SidJ	deubiquitylase	?	40

Ubiquitin	<i>Shigella flexneri</i>	intracellular	ShICE	deubiquitylase	?	44
Ubiquitin	<i>Chlamydia trachomatis</i>	intracellular	ChlaDUB1	deubiquitylase	Inhibition of NF-κB pathway activation	86, 87
Ubiquitin	<i>Burkholderia pseudomallei</i>	extracellular	CHBP	Gln deamidase	Deamidation of Ubiquitin	47
SUMO	<i>Listeria monocytogenes</i>	intracellular	LLO	Pore-forming toxin	Downregulation of UBE2I/UBC9 (E2 SUMO enzyme)	27
SUMO	<i>Clostridium perfringens</i>	extracellular	PFO	Pore-forming toxin	Downregulation of UBE2I/UBC9 (E2 SUMO enzyme)	27
SUMO	<i>Streptococcus pneumoniae</i>	extracellular	PLY	Pore-forming toxin	Downregulation of UBE2I/UBC9 (E2 SUMO enzyme)	27
SUMO	<i>Shigella flexneri</i>	intracellular	? / Ca ²⁺ influx	?	Proteolytic cleavage of UBA2/SAE2 (E1 SUMO enzyme)	31
SUMO	<i>Salmonella Typhimurium</i>	intracellular	? / miRNAs	?	Downregulation of UBE2I/UBC9 (E2 SUMO enzyme)	30
SUMO	<i>Xanthomonas euvesicatoria</i>	extracellular	XopD	deSUMOylase	DeSUMOylation of SIERF4 (plant immune response)	41,42
NEDD8	EPEC	extracellular	CIF	Gln deamidase	Deamidation of NEDD8	47,48
NEDD8	<i>Chlamydia trachomatis</i>	intracellular	ChlaDUB1	deNeddylase	Inhibition of NF-κB pathway activation	86, 87

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