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Review

# Pathogen-Mediated Posttranslational Modifications: A Re-emerging Field

David Ribet[1,2,3] and Pascale Cossart[1,2,3,*]
[1]Institut Pasteur, Unité des Interactions Bactéries-Cellules, Département de Biologie Cellulaire et Infection, F-75015 Paris, France
[2]INSERM, U604, F-75015 Paris, France
[3]INRA, USC2020, F-75015 Paris, France
*Correspondence: pascale.cossart@pasteur.fr
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**Posttranslational modifications are increasingly recognized as key strategies used by bacterial and viral pathogens to modulate host factors critical for infection. A number of recent studies illustrate how pathogens use these posttranslational modifications to target central signaling pathways in the host cell, such as the NF-kB and MAP kinase pathways, which are essential for pathogens' replication, propagation, and evasion from host immune responses. These discoveries open new avenues for investigating the fundamental mechanisms of pathogen infection and the development of new therapeutics.**

Posttranslational modifications (PTMs) of proteins provide highly versatile tools and tricks used by both prokaryotic and eukaryotic cells to regulate the activity of key proteins. PTMs include the addition of simple chemical groups, such as a phosphate, acetyl, methyl, or hydroxyl groups; more complex groups, such as AMP, ADP-ribose, sugars, or lipids; and small polypeptides, such as ubiquitin or ubiquitin-like proteins. They also include modifications of specific amino acid side chains (e.g., deamidation of glutamine residues) and the cleavage of a peptide bond (i.e., proteolysis).

PTMs represent efficient strategies to modify activities, half-lives, or the intracellular localization of host proteins that are critical for infection. The first report that a pathogen could mediate a PTM occurred 40 years ago with the discovery that diphtheria toxin, produced by *Corynebacterium diphtheriae*, ADP-ribosylates and thus inhibits the host Elongation Factor-2 (EF-2) (Collier and Cole, 1969). This modification blocks translation in the intoxicated cells and thereby leads to cell death.

Since then, a considerable number of host PTMs mediated, induced, or counteracted by different pathogen-encoded virulence factors have been reported (for reviews, see Ribet and Cossart, 2010; Randow and Lehner, 2009). In this Review, we discuss new discoveries in the modulation of PTMs by pathogens. In the first part, we focus on ubiquitin and ubiquitin-like proteins, which have emerged as central regulating modules targeted by both viral and bacterial pathogens. We then discuss two recently identified PTMs catalyzed by bacterial pathogens, AMPylation and eliminylation. In the third part, we describe how pathogens hijack certain PTMs to preferentially target specific host pathways to promote their replication, propagation, and escape from the immune system.

## Ubiquitin and Ubiquitin-like Modifications Targeted by Pathogens

### *Ubiquitination*

Ubiquitination is the covalent attachment of ubiquitin, a small polypeptide of 76 amino acids, to a target protein. Ubiquitin is generally linked to the lysine residue of the target protein; however, a cysteine, serine, threonine, or N-terminal amino group of a protein can also be modified. This conjugation requires the successive activities of an E1-activating enzyme, an E2-conjugating enzyme, and then an E3 ligase. Ubiquitination is a fundamental PTM involved in many different cellular functions, including the trafficking of membrane proteins, endocytosis, signal transduction, DNA repair, and transcription regulation. Ubiquitin itself contains seven lysines, K6, K11, K27, K29, K33, K48, and K63. Therefore, chains of ubiquitin can be formed by attaching additional ubiquitin molecules to a lysine residue of the previously attached ubiquitin.

K48-linked polyubiquitin chains play a fundamental role in protein degradation by targeting proteins to the proteasome. In contrast, K63-linked polyubiquitin chains are involved in nonproteolytic processes, such as DNA repair and vesicular trafficking. In addition to these "homotypic" K48- or K63-linked chains, in which only one type of ubiquitin linkage is involved, mixed K11/K63-linked chains have also recently been described (Boname et al., 2010). The discovery of these "mixed" chains highlights that ubiquitin chains are probably more diverse and complex than appreciated until now.

Ubiquitination is reversible because eukaryotic cells encode proteases that are specific for ubiquitin. These proteases, called deubiquitinases (DUBs), remove ubiquitin from their targets or cleave the bond between two linked ubiquitins.

Ubiquitination constitutes an attractive target for a wide range of pathogens because it regulates many pathways in eukaryotic cells. Indeed, viruses and pathogenic bacteria can modulate the ubiquitination level of host proteins by inducing their monoubiquitination, their polyubiquitination with K48-linked chains (which then triggers their degradation), their polyubiquitination with other types of ubiquitin chains, or their deubiquitination (reviewed in Ribet and Cossart, 2010; Randow and Lehner, 2009).

Some pathogen-encoded effectors display E3 ubiquitin ligase activities. An important fraction of these viral or bacterial E3

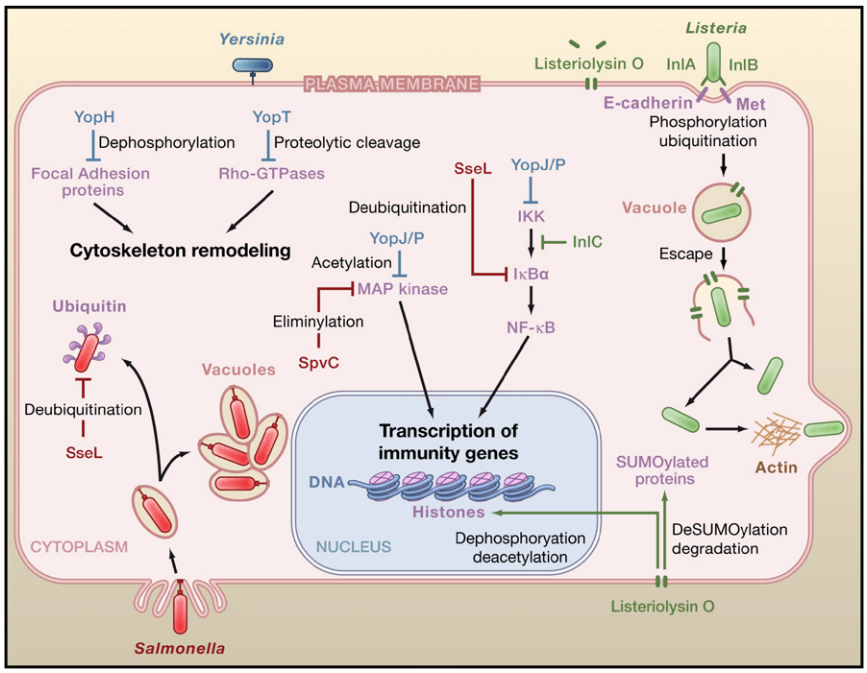


**Figure 1. Posttranslational Modification of Host Proteins during Infection**

*Yersinia* (blue) is an extracellular pathogen that injects effectors into the host cell's cytoplasm using a specialized type III secretion system (T3SS). *Salmonella* (red) triggers its own entry into host cells and replicates in a remodeled vacuole. It also secretes T3SS-dependent effectors. After cell invasion, *Listeria* (green) escapes from vacuoles and resides free in the cytoplasm, where it replicates and starts moving using the host cell's actin. Interactions with host factors are mediated by bacterial surface or secreted proteins. Effectors from all three of these bacteria (blue for *Yersinia* effectors, red for *Salmonella* effectors, and green for *Listeria* effectors) alter posttranslational modifications of host proteins (purple) to facilitate pathogens' replication, propagation, and evasion from host immune responses .

ligases shares structural homologies with eukaryotic E3 ligases, which are classically divided into HECT and RING E3s depending on their structures and mechanistic properties (reviewed in Kerscher et al., 2006). HECT E3 ligases transiently bind ubiquitin before transferring it to the target protein. In contrast, RING E3 ligases do not link ubiquitin directly but rather facilitate ubiquitination by binding simultaneously to the charged E2 enzyme and the protein target.

Recent studies have identified a new family of bacterial E3 ligases with a structural domain completely distinct from the eukaryotic RING and HECT domains (Hicks and Galán, 2010). Studies have also identified viral E3 ligases structurally distinct from eukaryotic ones (Randow and Lehner, 2009). Whether these new E3 ligases also exist in eukaryotes is still unknown. Whereas pathogens may have acquired eukaryotic-like E3 ligases by horizontal transfer from diverse eukaryotic sources, the noneukaryotic E3 ligases may represent novel structures evolved by pathogens to mimic the function of these essential enzymes of the host cell.

In addition to encoding their own E3 ligases, some pathogens may encode adaptor proteins that bind host E3 enzymes and redirect them to specific targets. For example, two decades ago, a study found that this strategy is used by some human papillomaviruses (HPVs), which are associated with the development of uterine cervix cancer. The E6 oncoproteins of HPV serotype 16 and 18 recruit a host E3 ligase to induce the degradation of the p53 tumor suppressor, thereby facilitating transformation of the infected cells (Scheffner et al., 1990).

In addition to E3 ubiquitin ligases, pathogens also encode DUB-like proteins. A few viral DUBs have been identified, but their roles in vivo, as well as their host targets, are unknown. In contrast, several DUB-like proteins have been characterized in pathogenic bacteria. *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) is an invasive pathogen of the small intestine that, in mice, causes a disease similar to human typhoid fever. SseL, an effector secreted by this bacterium, displays deubiquitinating activity in vitro. It suppresses ubiquitination and degradation of IκBα, a central regulator of the NF-κB pathway (see below) (Figure 1) (Le Negrate et al., 2008). Infection with a strain of *S.* Typhimurium lacking *sseL* leads to the accumulation of ubiquitinated proteins at the site of replicating intracellular bacteria (Rytkönen et al., 2007). Strikingly, the decoration of intracytosolic bacteria with polyubiquitinated proteins has recently been proposed as a signal used by host cells to sense intracellular invaders (Figure 1). This signal triggers cytosolic defense pathways, such as autophagy, although the nature of ubiquitinated proteins is unknown (Perrin et al., 2004; Thurston et al., 2009). Bacterial DUBs may decrease this accumulation of polyubiquitinated proteins and thus might represent a strategy developed by intracellular bacteria to escape these specific host defense systems.

Interestingly, pathogen-encoded proteins can also be directly ubiquitinated by the host cell machinery. A striking example in which PTMs by the host cell strongly alter the behavior of bacterial effectors is the *Salmonella* SopE and SptP proteins. These two effectors contribute to the transient remodeling of the host cell's cytoskeleton during bacterial entry into the cell. SopE acts as a GEF (guanine nucleotide exchange factor) and activates host Rho-GTPases, resulting in actin cytoskeleton rearrangement, membrane ruffling, and subsequent bacterial uptake. In contrast, SptP acts as a GAP (GTPase-activating protein) to deactivate Rho-GTPases and allow the recovery of the actin cytoskeleton's normal architecture a few hours after infection. Although SopE and SptP are codelivered by *Salmonella*, they exhibit different half-lives. SopE is rapidly polyubiquitinated and degraded by the host proteasome, whereas SptP exhibits much slower degradation kinetics (Kubori and Galán, 2003). Recent studies found that *Salmonella* also hijacks the ubiquitination machinery to control one of its effectors, SopB, which displays two different activities depending on whether the protein is ubiquitinated or not (Patel et al., 2009; Knodler

et al., 2009). Thus, by actively co-opting the ubiquitination machinery of the host cell, *Salmonella* regulates the half-lives and activities of some of its key virulence factors.

### SUMOylation

In addition to ubiquitin, other polypeptides can be covalently linked to cellular proteins to modify their fate and functions. These polypeptides, which belong to the ubiquitin-like protein family, share high structural homology with ubiquitin, ranging from ~15% to 50% sequence similarity with it. SUMO (small ubiquitin-like modifier) belongs to the ubiquitin-like protein family and is ubiquitous in the eukaryotic kingdom. The human genome encodes three functional SUMO isoforms that can be linked to hundreds of different targets. Similar to the ubiquitin system, the conjugation of SUMO onto the lysine of a target protein requires an E1, an E2, and an E3 SUMO enzyme. In parallel, deSUMOylases regulate the SUMOylation level of cellular proteins by removing SUMO from its targets.

SUMOylation is a fundamental PTM involved in transcription regulation, intracellular transport, stress responses, the maintenance of genome integrity, and many other biological processes. Although SUMOylation was first thought not to play a role in protein degradation, recent findings show that SUMO can trigger the recruitment of ubiquitin E3 ligases, such as RNF4 (RING finger protein 4), leading to the ubiquitination and proteasomal degradation of some SUMOylated proteins (Lallemand-Breitenbach et al., 2008; Tatham et al., 2008).

As with the ubiquitin system, several bacterial and viral factors target or mimic components of the SUMOylation machinery, thereby increasing or decreasing the SUMOylation level of host proteins (reviewed in Boggio and Chiocca, 2006; Ribet and Cossart, 2010). For example, KSHV (Kaposi's sarcoma-associated herpes virus), a herpes virus responsible for Kaposi's sarcoma development, encodes an enzyme, K-bZip, which displays E3 SUMO ligase activity. This protein directly participates in catalyzing SUMO conjugation to host targets, such as p53 and Retinoblastoma (Rb) protein (Chang et al., 2010). These modifications are proposed to play a role in modulating host genes expression in the early stage of viral infection (Chang et al., 2010).

VP35, a protein encoded by Ebola virus, does not display E3-like activity, but it binds to the host E3 SUMO enzyme PIAS1 (protein inhibitor of activated STAT 1) and increases the SUMOylation level of IRF7 (interferon regulatory factor 7) (Chang et al., 2009). This SUMOylation of IRF7 downregulates interferon transcription and may contribute to the dampening of the antiviral response induced upon infection of Ebola virus (Chang et al., 2009).

Gam1, a protein encoded by an avian adenovirus, has an opposite effect on SUMOylation; it targets the host E1 SUMO enzyme to proteasomal degradation, thereby inhibiting the SUMOylation machinery and altering host transcription (Boggio et al., 2004). Degradation of the SUMOylation machinery is a strategy also used by *Listeria monocytogenes*, a food-borne bacterial pathogen responsible for listeriosis. Indeed, infection by *L. monocytogenes* leads to the degradation of Ubc9, the human E2 SUMO enzyme (Ribet et al., 2010). Listeriolysin O is a pore-forming toxin secreted by this bacterium, which plays a fundamental role in bacterial virulence (Figure 1). Listeriolysin O triggers the degradation of Ubc9, as well as the degradation of some SUMOylated host proteins (Ribet et al., 2010). In contrast to the ubiquitin system, which includes dozens of E2 enzymes in humans, the SUMO system has only one E2 enzyme. Therefore, this degradation of Ubc9 leads to a blockade of the SUMOylation machinery and to a global decrease in the level of SUMO-conjugated host proteins in infected cells. Thus, by decreasing SUMOylation in infected cells, *Listeria* may alter the activities of host factors critical for infection (Ribet et al., 2010).

Pathogen-encoded deSUMOylases can also cause a decrease in the SUMOylation level of host proteins. Indeed, this is the case for XopD, a protein injected by the plant pathogen *Xanthomonas campestris* into the cytoplasm of plant cells. This protein is a SUMO-specific protease, which induces deSUMOylation of several host factors when it is expressed in plant cells (Hotson et al., 2003). XopD is known to alter host transcription, to promote pathogen multiplication, and to delay the onset of leaf chlorosis and necrosis. However, the exact roles of deSUMOylation in XopD's effects are unknown (Kim et al., 2008).

In addition to the induction or inhibition of SUMOylation of host proteins, viral proteins can be SUMOylated themselves. However, the role that these modifications play in virulence is unknown in most cases (Boggio and Chiocca, 2006). Surprisingly, examples of bacterial factors directly SUMOylated by host enzymes have not been identified. It is, however, likely that future studies will unveil the existence of such modifications, as well as their role in bacterial infection or in antibacterial defenses.

### Neddylation

Neddylation is another PTM that pathogens target during infection. Nedd8, which is a member of the ubiquitin-like protein family, can be linked to cellular proteins in a fashion similar to ubiquitin (reviewed in Rabut and Peter, 2008). The major class of currently known Nedd8 substrates is Cullins. Cullins act as scaffolding proteins in the assembly of multisubunit RING E3 ubiquitin enzymes, called Cullin RING ligases (CRLs). Neddylation of Cullins controls the activity of CRLs and thereby the ubiquitination and degradation kinetics of CRLs substrates. As with ubiquitin, Nedd8 can be deconjugated from its targets by deneddylases.

Bacterial and viral pathogens can interfere with the neddylation of host proteins. For example, the Epstein-Barr virus encodes a protein BPLF1, which displays deneddylase activity (Gastaldello et al., 2010). During infection, BPLF1 deneddylates Cullins, thereby inhibiting the activity of CRLs and stabilizing several CRL substrates. In particular, this leads to the deregulation of the cell cycle and the establishment of an S-phase-like cellular environment, which is required for efficient replication of virus DNA (Gastaldello et al., 2010).

A recent study also reported that Cif (cycle-inhibiting factor), a cyclomodulin translocated into cells by enteropathogenic and enterohemorrhagic *Escherichia coli*, binds to Nedd8-conjugated CRLs of the host. This interaction inhibits the activity of the CRLs, leading to a deregulation of the host cell cycle (Jubelin et al., 2010). Proteins with in vitro deneddylase activity have also been described in *Chlamydia trachomatis*, an obligate intracellular bacterial pathogen. However, the role these deneddylases play in infection remains unknown (Misaghi et al., 2006).

### *ISGylation*

ISG15 (interferon stimulated gene 15) is an ubiquitin-like protein with two ubiquitin domains. The expression of ISG15 is induced in response to type I interferons (IFN), a family of cytokines involved in the antiviral response. Consistent with this induction in response to IFN, a growing number of studies are now highlighting the roles ISG15 plays in antiviral defense against several types of viruses (reviewed in Skaug and Chen, 2010; Jeon et al., 2010). Conjugation of ISG15 to target proteins requires the activity of E1, E2, and E3 enzymes, which are also induced by IFN. In contrast to the ubiquitin system, which includes hundreds of E3 enzymes, one unique E3 ISG15 enzyme, namely HERC5, modifies the vast majority of ISG15 substrates in human cells. Like with other ubiquitin-like modifications, ISGylation is reversible; specific proteases, called deISGylases, remove ISG15 from its targets.

The antiviral activity of ISG15 can be due to either the ISGylation of host proteins critical for infection or the direct ISGylation of viral proteins (Skaug and Chen, 2010; Jeon et al., 2010). This latter case has been described for the NS1 protein of influenza A virus (NS1A), which is ISGylated during infection. This modification of NS1A was linked to an impairment of influenza replication, although the precise effect of the ISG15 addition on NS1A remains to be determined (Zhao et al., 2010; Tang et al., 2010).

Interestingly, recent studies also proposed that the ISG15 conjugation system may modify broadly, and somehow nonspecifically, newly synthesized proteins in a cotranslational manner (Durfee et al., 2010). This implies that, in the context of an interferon response, viral proteins, rather than cellular proteins, may be the principal targets of ISGylation (Durfee et al., 2010). Although only a small fraction of viral proteins might be ISGylated, it was proposed that ISGylation of viruses' structural proteins, which precisely assemble into high-order structures, might impair the production of infectious viral particles. Indeed, this was demonstrated for the human papillomavirus HPV16. ISGylation of a small proportion of its structural protein L1 was sufficient to have a dominant-negative effect on virus infectivity (Durfee et al., 2010). The authors postulated that the ISGylation of host proteins could thus only be a side effect of the cell's effort to target viral proteins.

Consistent with the role of ISG15 in antiviral defense, several viruses have evolved strategies to impair ISGylation (Skaug and Chen, 2010; Jeon et al., 2010). In particular, studies have identified several viral proteins that can either mimic deISGylases or interfere with the ISGylation machinery of the infected cell. Indeed, the papain-like protease of SARS coronavirus and the ovarian tumor domain-containing proteases of nairo- and arteriviruses all display ISG15-deconjugating activities (Lindner et al., 2005; Frias-Staheli et al., 2007). On the other hand, NS1 protein of influenza B virus binds to ISG15 and inhibits its conjugation to target proteins (Yuan and Krug, 2001). By inhibiting ISG15 conjugation or increasing ISG15 deconjugation, all these effector proteins were proposed to decrease the potential antiviral effect of ISGylation.

The role of ISG15 in bacterial infections remains completely unknown. According to the study by Durfee et al. (2010), the participation of ISG15 in antibacterial defenses, if any, will probably rely on the ISGylation of cellular proteins rather than bacterial proteins because the latter are not translated by the host cell machinery. Nevertheless, investigating the role of ISG15 in infections by bacterial pathogens will undoubtedly provide exciting insights into the field of host-pathogens interactions.

## AMPylation and Eliminylation, New PTMs Mediated by Bacteria

### *AMPylation*

AMPylation is the addition of an adenosine monophosphate (AMP) group onto a threonine, tyrosine, or, possibly, serine residue of a protein. The AMPylation of host proteins by bacterial pathogens was recently detected in cells during an infection with *Vibrio parahaemolyticus*, a human pathogen causing acute gastroenteritis, and *Histophilus somni*, a pathogen responsible for respiratory diseases and septicemia in cattle. Two virulence factors produced by these extracellular bacteria, namely VopS and IbpA, are able to reach the cytoplasm of host cells during infection, where they use ATP to transfer an AMP moiety to host Rho-GTPases (Figure 2) (Yarbrough et al., 2009; Worby et al., 2009). This AMPylation alters the activity of Rho-GTPases, which regulate the dynamics of the cell cytoskeleton.

The catalytic domain responsible for AMPylation was mapped to the Fic domain (filamention induced by cAMP) of VopS and IbpA. Fic domains are defined by a core sequence of nine amino acids containing an invariant histidine residue that is essential for the AMPylation (Yarbrough et al., 2009). Interestingly, proteins containing Fic domains are found not only in prokaryotes but also in eukaryotes, and the existence of eukaryotic proteins able to catalyze AMPylation has been proposed (Worby et al., 2009; Kinch et al., 2009). Thus, AMPylation might represent a new and important posttranslational modification in eukaryotic cells.

*Legionella pneumophila* is a human pathogen of the respiratory tract responsible for a severe form of pneumonia, called Legionnaire's disease. *L. pneumophila* encodes a factor, DrrA, which AMPylates the host protein Rab1b, a small GTPase involved in intracellular vesicular transport (Muller et al., 2010). AMPylation of Rab1b leads to its constitutive activation, which not only alters vesicular transport in infected cells but also contributes to the formation of *Legionella* intracellular vacuoles and aids bacterial replication.

Interestingly, the catalytic domain of DrrA is distinct from the Fic domains observed in VopS and IbpA (Muller et al., 2010). Thus, a wide diversity of both prokaryotic and eukaryotic enzymes may catalyze AMPylation, a posttranslational modification that might represent an unsuspected way of regulating various signaling pathways in the cell.

### *Eliminylation*

Phosphorylation was the first covalent protein modification described. Since its discovery in the late 1950s, phosphorylation has emerged as a common and fundamental PTM. Phosphorylation involves the reversible attachment of a phosphate group to target proteins by forming a phosphoester bond. This addition generally occurs on hydroxyl groups of serine, threonine, or tyrosine residues. Phosphorylation is reversible; phosphatases can hydrolyze the phosphoester bond to release the phosphate group and restore the amino acid in its unphosphorylated form.

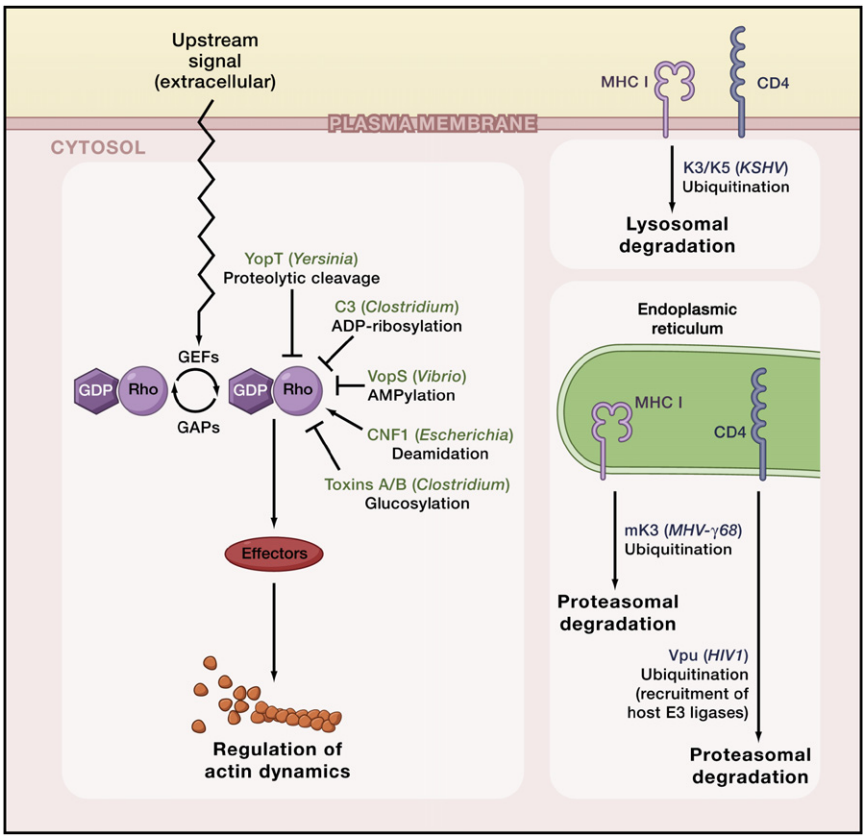


**Figure 2. Pathogen-Mediated PTMs Target the Cytoskeleton and Immunoreceptors**

Bacteria effector proteins (green) control the dynamics of the host cell's actin cytoskeleton by posttranslationally modifying Rho-GTPases (left). Viral effector proteins (blue) regulate posttranslational modification of immunoreceptors, such as the major histocompatibility complex class I (MHC I) and the CD4 (cluster of differentiation 4) molecules (right), thereby decreasing their expression at the cell surface and dampening immune responses.

Interestingly, a previously unknown enzymatic activity, called phosphothreonine lyase, was recently identified in three different bacterial factors (Li et al., 2007; Mazurkiewicz et al., 2008; Zhang et al., 2007). These enzymes remove the phosphate group from a threonine residue but, in contrast to classical phosphatases, do not regenerate the hydroxyl group. Instead, this reaction, nicknamed eliminylation, modifies threonine into dehydrobutyrine, a residue that can no longer be phosphorylated (Brennan and Barford, 2009).

The first factor identified with such activity is OspF, a protein produced by *Shigella flexneri*, the causative agent of bacillary dysentery in humans (Li et al., 2007). During infection, bacteria directly secrete OspF into the host cell cytoplasm, where OspF helps to dampen the host immune responses by irreversibly dephosphorylating host MAP (mitogen-activated protein) kinases (Figure 3) (Li et al., 2007; Arbibe et al., 2007). Phosphothreonine lyases have been described only in *S. flexneri*, *S.* Typhimurium, and the plant pathogen *Pseudomonas syringae*, and MAP kinases are the only known targets of this PTM. However, we can expect that, as with AMPylation, some eukaryotic enzymes may also display this activity and that eliminylation might regulate numerous signaling pathways in eukaryotic cells.

## Signaling Pathways Preferentially Targeted by Pathogens by Alteration of Host PTMs

Some pathogens produce several effectors that modulate the activity of host cell proteins by stimulating or counteracting their PTMs. In this section, we will focus on several key cellular pathways that are preferentially targeted by pathogens through these PTMs.

### *Regulation of the Cytoskeleton Dynamics by PTMs*

The niches occupied by pathogens within their hosts are quite diverse. Whereas some bacterial pathogens remain strictly extracellular, other bacteria, as well as viruses, invade host cells and replicate therein. For viruses, entry into host cell is strictly required for the synthesis of viral proteins and the production of new infectious viral particles. Bacteria take refuge inside host cells to escape humoral immune response and to replicate in a well-protected environment. To enter the cell and create such niches requires extensive remodeling of the host cell cytoskeleton, a multiprotein assembly of structural and regulatory elements. Indeed, many pathogen-induced PTMs target structural or regulatory components of the host cell's cytoskeleton.

*Listeria monocytogenes* is a bacterium that can induce its own entry into a wide range of cells that are normally nonphagocytic. This internalization requires interactions between surface proteins of *Listeria* and host receptors. After successive PTMs, these interactions trigger the recruitment of host factors and the remodeling of host cell cytoskeleton required for internalization of the bacteria (Figure 1). For example, the interaction between the *Listeria* surface protein InlA and its cellular receptor E-cadherin promotes *Listeria's* invasion into epithelial cells of the intestine. Activation of E-cadherin by InlA leads to phosphorylation and ubiquitination of E-cadherin by the Src kinase and the Hakai E3 ligase, respectively. These PTMs trigger the recruitment of the host's clathrin-mediated endocytic machinery followed by rearrangements of the actin cytoskeleton and internalization of the bacteria (Bonazzi et al., 2008).

In contrast, entry of *Listeria* into cells that do not express E-cadherin is mediated by another surface protein, InlB, which interacts with and activates Met, the hepatocyte growth factor (HGF) receptor (Figure 1). Similar to HGF activation, Met activation by InlB induces its autophosphorylation and subsequent monoubiquitination by the host E3 ligase Cbl. This leads to the recruitment of the host's clathrin-dependent endocytic

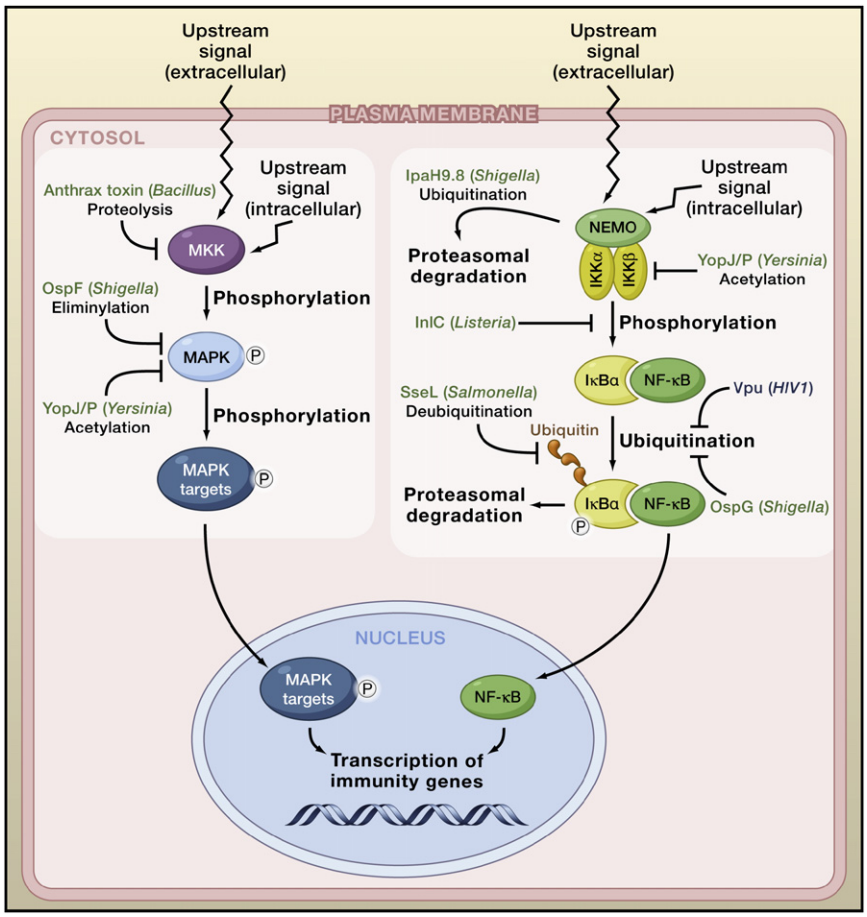


**Figure 3. Pathogen-Mediated PTMs Target the MAP Kinase and NF-κB Signaling Pathways**

The MAP kinase (left) and NF-κB (right) signaling cascades trigger immune responses in the host cell during infections. Both bacterial (green) and viral (blue) effectors weaken these immune responses by inducing or counteracting post-translational modifications of key components in these critical pathways.

machinery, actin rearrangements, and ultimately, the internalization of the bacteria (Veiga and Cossart, 2005; Veiga et al., 2007).

To avoid being killed, pathogens can also actively inhibit their engulfment by professional phagocytes. The mechanisms involved in this process may also require various pathogen effectors to regulate the PTMs of host proteins (Figure 1). Pathogenic *Yersinia* species are involved in human diseases, ranging from enteric disorders to the plague. One virulence factor secreted by *Yersinia,* YopH, displays potent phosphatase activity. It decreases phosphorylation levels of host proteins involved in focal adhesion complexes and impairs the cytoskeleton rearrangements required for bacterial uptake. Another factor of *Yersinia*, YopT, is a protease that cleaves the membrane-anchoring domain of host Rho-GTPases, leading to their irreversible detachment from the plasma membrane and their inactivation (Figure 2 and Figure 1) (Shao et al., 2002). Thus, YopT contributes to the inhibition of bacterial phagocytosis by preventing rearrangements of the actin cytoskeleton.

Finally, some bacterial pathogens, such as *Clostridium difficile*, secrete several toxins that posttranslationally modify host Rho-GTPases, leading to their constitutive activation, inactivation, or degradation (Figure 2). This alteration of Rho-GTPases is widespread and allows bacteria to regulate the host cell's cytoskeleton in numerous ways, as well as gene transcription and cytokine expression (reviewed in Aktories and Barbieri, 2005).

### *Inhibition of the NF-kB Pathway*

The NF-κB pathway is an example of a pathway tightly regulated by ubiquitination (Figure 3). The NF-κB pathway plays a central role in inflammation and in the establishment of both innate and immune responses. Specific signals, such as cytokines or microbial signatures, activate this pathway by switching on the IkB kinase (IKK) complex. This leads to the phosphorylation of IkBα, an inhibitor protein that sequesters transcription factors of the NF-κB family in the cytoplasm. Phosphorylated IkBα is then recognized by specific ubiquitin E3 ligases, polyubiquitinated with K48-linked chains, and targeted to the proteasome for degradation. Destroying IkBα leads to the release of NF-κB transcription factors, allowing them to translocate into the nucleus and initiate transcription of various genes involved in host immune responses. Because the NF-κB pathway plays a central role in immune responses, there is a strong evolutionary pressure on pathogens to prevent activation of this pathway during infection.

One possibility for dampening this pathway is to block the ubiquitination of IkBα, thereby inhibiting its proteasomal degradation and the translocation of NF-κB factors into the nucleus (Figure 3). In numerous cases, factors achieve this goal by interfering with the host ubiquitination machinery. For example, *S. flexneri* secretes the effector OspG into the host cell's cytoplasm, where it binds to and inhibits UbcH5, a host E2 ubiquitin enzyme involved in IkBα ubiquitination (Kim et al., 2005). The accessory protein Vpu (viral protein U) of HIV1 also interferes with IkBα ubiquitination by inhibiting the E3 ubiquitin ligase involved in IkBα's modification (Bour et al., 2001). The DUB-like SseL factor produced by *S.* Typhimurium inhibits IkBα ubiquitination in response to the TNF-α cytokine, suggesting that SseL acts directly by removing the K48-linked chains of IkBα (Le Negrate et al., 2008).

Numerous factors also target the IKK complex directly (Figure 3). For example, in addition to producing OspG, *S. flexneri* also secretes IpaH9.8, an effector with E3 ubiquitin ligase activity. IpaH9.8 polyubiquitinates the NEMO/IKKγ protein of the IKK complex and targets it to the proteasome, thereby

impairing the phosphorylation and subsequent degradation of IkBα (Rohde et al., 2007; Ashida et al., 2010). *L. monocytogenes* intracellularly secretes InlC, which directly interacts with the IKKα protein to block the phosphorylation of IkBα (Gouin et al., 2010). Similarly, YopJ/P, an effector produced by pathogenic *Yersinia* species, mediates the acetylation of the IKKα and β proteins, which prevents their activation and subsequent IkBα phosphorylation (Mittal et al., 2006).

Interestingly, commensal bacteria of the human intestine can also act on the NF-κB pathway. Indeed, some bacterial fermentation products, such as butyrate or other short-chained fatty acids, can stimulate the local production of reactive oxygen species in intestinal epithelial cells. This leads to the inactivation of some redox-sensitive enzymes, such as E2 Nedd8 enzyme, and therefore a decrease in the neddylation level of host proteins. In this context, reduced neddylation levels, in particular the decrease in Cullin-1 neddylation, have been associated with a downregulation of the NF-κB pathway and hypothesized to contribute to the inflammatory tolerance of the intestinal epithelium toward commensal bacteria (Kumar et al., 2009).

### *Targeting of MAP Kinase Pathway*

Similar to the NF-κB pathway, the MAP kinase pathway is another central signaling cascade that is essential for the activation of host innate immune responses. Therefore, not surprisingly, pathogens often target the MAP kinase pathway in order to facilitate their infection (Figure 3). One effector protein secreted intracellularly by *Shigella* is OspF, which possesses phosphothreonine lyase activity. OspF irreversibly dephosphorylates host MAP kinases and, therefore, was proposed to participate in the dampening of host immune responses (Li et al., 2007; Arbibe et al., 2007). Interestingly, other bacterial virulence factors, such as SpvC from *S.* Typhimurium or HopAI1 from the plant pathogen *P. syringae*, possess the same phosphothreonine lyase activity as OspF and also target MAP kinases of their hosts (Mazurkiewicz et al., 2008; Zhang et al., 2007). In addition to these factors, the *Yersinia* YopJ/P effector can inactivate host MAP kinases by catalyzing their acetylation (Mittal et al., 2006; Mukherjee et al., 2006). Finally, the anthrax lethal factor, a subunit of the Anthrax toxin encoded by *Bacillus anthracis,* cleaves host MAP kinases, leading to their irreversible inactivation (reviewed in Turk, 2007).

### *Regulation of Cellular Immunoreceptors*

To avoid detection by the immune system, some pathogens restrict the surface expression of fundamental molecules of the immune system by subverting host ubiquitination (Figure 2). For example, KSHV encodes two E3 ubiquitin ligases, K3 and K5, which both target the host protein's major histocompatibility complex class I (MHC I). An essential player of the immune response, MHC I alerts the immune system to intracellular pathogens by sampling the protein repertoire of host cells and then presenting peptides to cytotoxic T lymphocytes. K3 rapidly mediates the polyubiquitination of MHC I molecules at the surface of the cell with K63-linked chains, leading to their endocytosis and degradation. Interestingly, K5 also mediates polyubiquitination of MHC I but with mixed K63 and K11 chains, instead of homotypic chains. Indeed, these mixed chains are required for the internalization of MHC I by K5, thus highlighting, for the first time, the putative importance of such mixed polyubiquitin chains in the control of immune responses (Boname et al., 2010). Some herpesvirus E3 ubiquitin ligases downregulate MHC I molecules by triggering their degradation by the ERAD (endoplasmic reticulum-associated protein degradation) pathway (reviewed in Randow and Lehner, 2009). Some viral proteins, such as HIV Vpu accessory protein, can act as adaptors of host E3 ubiquitin ligases to induce the proteasomal degradation of other types of host immunoreceptors, such as CD4 (cluster of differentiation 4) receptor on T cells (Schubert et al., 1998). Finally, bacterial pathogens, such as *Salmonella*, can decrease the expression of MHC class II molecules at the cell surface by modulating their ubiquitination, which also leads to the dampening of host immune responses (Lapaque et al., 2009).

## Conclusion

Researchers have known for decades that pathogens interfere with the host's PTMs. However, the current ''re-emergence'' of this field of research reflects the importance of controlling PTMs during infection and the complexity of these processes in host-pathogen interactions. In this Review, we focused on how pathogens manipulate host PTMs and how they use these PTMs to solve their own biological needs.

It should be stressed that pathogens may also actively co-opt or be the passive targets of the host cell's PTM machinery. As mentioned above, pathogen-encoded proteins can indeed be ubiquitinated, SUMOylated, or ISGylated, and like with host proteins, PTMs of pathogen-encoded proteins regulate these factors' half-lives, activities, intracellular localization, or binding to other host- or pathogen-encoded factors. Therefore, it is tempting to speculate that the diversity of known PTMs affecting pathogen-encoded proteins will greatly increase in the near future.

As the number of studies reporting crosstalk between different PTMs increases, an emerging idea is that PTMs are more complex than originally anticipated. For example, in the NF-kB signaling pathway alone, phosphorylation, SUMOylation, K63-polyubiquitination, and K48-polyubiquitination act in synergy to regulate the activation or the inhibition of transcriptional responses. Targeting of these pathways by pathogens, therefore, often requires a tightly controlled orchestration of multiple levels of PTMs.

Studies on pathogen interference with host protein PTMs has provided numerous insights into cell biology over the years. In particular, some pathogen effectors serve as invaluable tools to study particular aspects of cell biology. For example, the C3 exoenzyme from *Clostridium* ADP-ribosylates and inhibits multiple Rho-GTPases. Therefore, the C3 protein has been used successfully to highlight the specific role of the Rho-GTPase in stress fiber formation and to study the regulation of the actin cytoskeleton dynamics in eukaryotic cells (Ridley and Hall, 1992; Ridley et al., 1992).

Finally, the development of new technologies, such as improvements in mass spectrometry (especially the SILAC [stable isotope labeling of amino acids in cell culture] technique; Mann, 2006), will undoubtedly increase the list of currently known PTMs and facilitate the understanding of their roles in host-pathogen interactions. Identifying pathogen-encoded

enzymes that catalyze specific PTMs critical for infection will provide valuable new targets for drug development. Indeed, the selective inhibition of these enzymes may constitute a promising strategy to counter these insidious invaders.

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## Note Added in Proof

It came recently to our attention that AMPylation is also called adenylylation and was first discovered in the late sixties as regulating the glutamine synthetase of *E. coli* by modifying a tyrosine residue (Stadtman, E.R., 2001, The story of glutamine synthetase regulation. J. Biol. Chem. *276*, 44357–44364).