# The Role of Ubiquitination in Regulation of Innate Immune Signaling

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**Abbreviations:** See Table 1.

**Abstract**
Ubiquitination, also denoted ubiquitylation, is a posttranslational modification that has been implicated in the regulation of both innate and adaptive immune responses. Ubiquitination plays crucial roles in innate immune signaling by ensuring the proper orchestration of several signaling mediators that constitute a functional immune response. Herein, we briefly summarize the latest discoveries concerning the molecular ubiquitination-related machinery that senses, assembles, and disassembles innate immune signaling mediators.

## Introduction

The mammalian immune system, which involves a complex yet tightly regulated network of interactions among different types of cells, cell receptors, and signaling pathways, constantly battles invading pathogens. In addition to the aforementioned immune-system participants, its specificity and complexity also depend on posttranslational modifications of proteins involved in the initiation, maintenance, and termination of immune responses. These posttranslational modifications involve the addition of a chemical group or another protein(s) at one or more site of substrate. To date, more than 200 types of posttranslational modifications have been reported (Kho et al., 2004); with phosphorylation, ubiquitination, and sumoylation being the most extensively studied and well characterized.

Ubiquitination is a key posttranslational modification regulating numerous biological processes at various cellular levels, e.g., protein trafficking, the cell cycle, and immune responses. The addition of ubiquitin to a substrate protein usually involves three main steps: activation by a ubiquitin-activating enzyme (E1), conjugation by a ubiquitin-conjugating enzyme (E2), and ligation by a ubiquitin ligase (E3) (Pickart, 2001). The addition of ubiquitin can affect the substrate protein in one of several ways: it can alter its sub-cellular location, mediate its potential for proteasomal degradation, and/or modify its interaction with another protein. During ubiquitination, a single ubiquitin (mono-ubiquitination) or multiple ubiquitin adducts (polyubiquitination) are being added to lysine residue of a substrate protein (Hershko and Ciechanover, 1998). Each ubiquitin molecule contains seven lysine residues and a free N terminus (K6, K11, K27, K29, K33, K48, and K63) thus allowing the formation of diversified ubiquitin linkages via polyubiquitination. In addition, a donor ubiquitin molecule can also attach to a receptor ubiquitin via the amino terminal methionine (M1) resulting in M1 or linear-linkages (Kirisako et al., 2006). These complex and varied structures enable ubiquitination to transmit diverse functional signals that determine the fate of a substrate protein. The well-studied K63-linked chains mediate the functions of various cellular proteins involved in inflammatory signaling complexes, whereas K48-linked chains predominantly facilitate the proteasomal-mediated degradation of substrates (Hershko and Ciechanover, 1998; Chen and Sun, 2009). To date, other types of ubiquitin linkages have not been well studied and are usually referred to as atypical ubiquitinations. The functions so far attributed to atypical linkages are very diverse but also suggest roles in inflammation, the immune system, and/or the cell cycle (Morris and Solomon, 2004; Boname et al., 2010; Gerlach et al., 2011; Wickliffe et al., 2011).

E3s generally determine substrate specificity and have been divided into three subfamilies based on their domain structure: HECT, RING domain or RBR ligases. E3s containing the RING domain directly transfer ubiquitin to a target protein, whereas E3s containing the HECT domain facilitate the transfer of E2-loaded ubiquitin to the substrate (Pickart, 2001; Rotin and Kumar, 2009). Recently, it has been shown that RBR ligases possess features of both RING and HECT E3s (Wenzel et al., 2011; Smit et al., 2012). DUBs can reverse ubiquitination. Several DUBs have been identified that fine tune the specific ubiquitination process and regulate diverse signaling pathways. Generally, DUBs are classified as one of five types depending upon their protease domain, i.e., ubiquitin-specific protease (USP), ovarian tumor protease (OTU), Machado–Joseph-disease protease (Josephins), ubiquitin C-terminal hydrolase, or JAB1/MPN/Mov34 metalloenzyme (Nijman et al., 2005; Komander et al., 2009a; Reyes-Turcu et al., 2009). DUBs are also categorized into three subclasses defined by their functional attribute, i.e., DUBs that cleave ubiquitin precursors, DUBs that rescue proteins from ubiquitin-mediated degradation and reverse the related biological process(es) by removing ubiquitin chains from the substrate, and DUBs that edit ubiquitin chains to

**Table 1.** Glossary

| | |
|---|---|
| ABIN1 | A20 binding inhibitor of NF-κB 1 |
| cIAP | Cellular inhibitor of apoptosis protein |
| DUBs | Deubiquitinating enzymes |
| FADD | Fas-associated protein with death domain |
| HECT | Homology to E6AP C-terminus |
| HOIL-1 | Heme-oxidized iron-responsive element-binding protein 2 ubiquitin ligase-1 |
| HOIP | HOIL-1-interacting protein |
| IKK | Inhibitor of κB kinase |
| IRAK | IL1-R-associated kinase |
| LGP2 | Laboratory of genetics and physiology gene 2 |
| LUBAC | Linear ubiquitin chain assembly complex |
| MAPK | Mitogen-activated protein kinase |
| MAVS | Mitochondrial antiviral signaling protein |
| MDA5 | Melanoma differentiation-associated gene 5 |
| MyD88 | Myeloid differentiation primary response gene 88 |
| MEF | Mouse embryonic fibroblast |
| NEMO | NF-κB kinase B essential modulator |
| OTULIN | OTU DUB with linear linkage specificity |
| RING | Really Interesting New Gene |
| RBR | RING-between-RING |
| RNF | RING-finger protein |
| RIP | Receptor interacting protein |
| SHARPIN | SH3 and multiple ankyrin repeat domain protein (SHANK)-associated RBCK1 homology (RH)-domain interacting Protein |
| TAB | TAK1-binding protein |
| TAK | Transforming growth factor-β activated kinase 1 |
| TNF | Tumor necrosis factor |
| TNFR1 | TNF receptor 1 |
| TRADD | TNFR1-associated death-domain (DD) |
| TRAF | TNF receptor-associated factor |
| TRIF | TIR-domain-containing adaptor protein inducing interferon-β -mediated transcription-factor |
| TRIM25 | Tripartite motif containing protein 25 |
| TAX1BP1 | Human T-cell leukemia virus type I (Tax1) binding protein 1 |
| USP | Ubiquitin-specific protease |
| XIAP | X-linked inhibitor of apoptosis protein |

modify signaling pathways (Komander et al., 2009a; Reyes-Turcu et al., 2009). Although some evidence suggests important roles for DUBs in immune response regulation, including the negative regulation of NF-κB signaling by CYLD, a USP DUB, and A20, an OTU DUB (Trompouki et al., 2003; Wertz et al., 2004), the target substrates and physiological functions of most DUBs have yet to be identified.

In short, ubiquitination is mediated by hundreds of E3, several E2s, and DUBs that dictate specificity of deubiquitinating molecule. In the following sections we summarize what is known about the integral roles of ubiquitination in host innate-immune defense mechanisms.

**Ubiquitination mediates innate immune responses**

Germ-line pattern-recognition receptors (PRRs) recognize pathogen-associated molecular patterns and initiate the host immune response against invading pathogens. This family of receptors includes membrane-bound toll-like receptors (TLRs) and cytosolic receptors, e.g., nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) and retinoic acid-inducible gene I (RIG-I)-like

helicases **or** RIG-I-like receptors (RLRs). PRR activation results in the activation of the NF-κB and MAPK signaling pathways leading to the production of proinflammatory cytokines, chemokines, and interferons (IFNs). These inflammatory mediators initiate an immune response and begin the recruitment of immune cells to the site of infection.

Over the last decade, many studies have partially characterized the diversified roles of ubiquitination in immune system regulation. Although ubiquitination is an integral part of inflammatory signaling, its role(s) in cell death still needs to be more fully explored. Below, we summarize our current understanding of how ubiquitination and deubiquitination control diverse innate immune responses.

## TLR signaling

TLRs are membrane-bound PRRs that sense a broad array of pathogens and have been divided into two main groups: expressed in the plasma membrane (TLR1, 2, 4, 5, 6, and 10) or located in the endosome (TLR3, 7, 8, and 9). TLR activation leads to the recruitment of several downstream signaling mediators and ultimately results in the production of pro-inflammatory cytokines and/or type I IFNs (Takeda et al., 2003). TLR signaling can also induce cell death (Feoktistova et al., 2011; Kaiser et al., 2013). MyD88-dependent signaling, engaged by TLR4 recruits IRAK1 and IRAK4 that subsequently interact with TRAF3, TRAF6 (a molecule shared by several signaling pathways), and cIAPs (Figure 1) (Tseng et al., 2010). In this cascade, TRAF6 interacts with a multi-molecular E2 complex denoted Ubc13/Uev1A, resulting in the ubiquitinosome (Deng et al., 2000). Subsequently, within this TLR4 signaling complex (TLR4-SC) TRAF6 is self-conjugated to K63-linkages and also ubiquitinates cIAP1/2, which then recruits TAB-TAK and IKK-NEMO, resulting in NF-κB and MAPK signaling (Kanayama et al., 2004; Wu et al., 2006; Vallabhapurapu and Karin, 2009). Interestingly, detachment of TLR4-SC from the plasma membrane and translocation to the cytosol, which is required for the activation of the

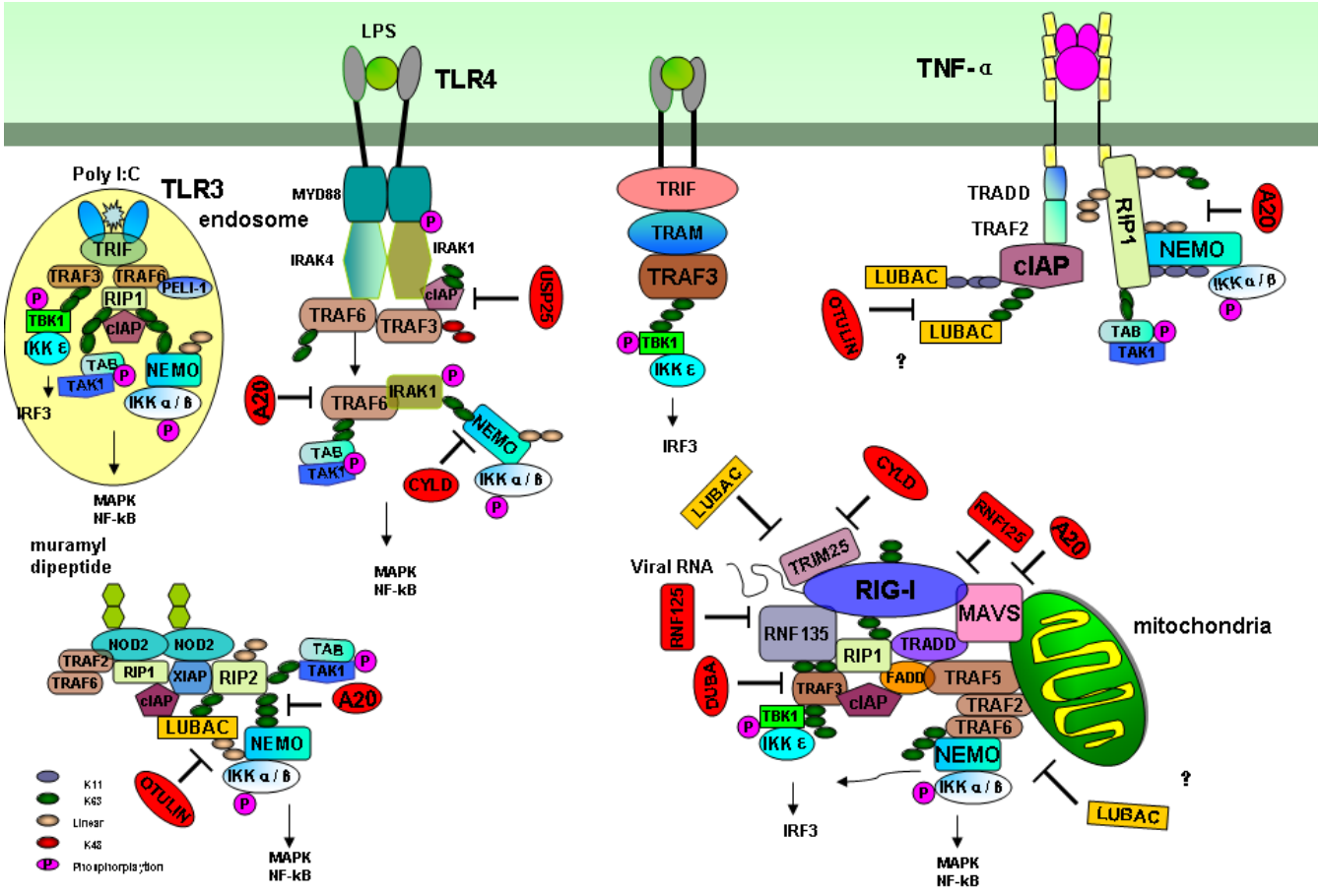


**Figure 1. Ubiquitination is involved in PRR signaling**. Several E3s are required for TLR3, TLR4, NOD2, RIG-1, and TNFR signaling. In TLR3-mediated signaling, TRIF, TRAF3, and TRAF6 are recruited. TRAF3 recruitment results in IRF3 activation and subsequent production of IFN. TRAF6 is required for NF-κB kinase B activation. TRAF3 and TRAF6 also regulate the TLR4 signaling pathway. RIG-I signaling is regulated by TRIM25 and TRAF6, which promote the recruitment of NEMO and the IKK complex. In the NOD2 signaling complex, XIAP and LUBAC promote ubiquitination of RIP2, thus allowing the activation of the NF-κB kinase B signaling pathway. In TNFR-mediated signaling, cIAPs, RIP1, and LUBAC play important roles in activating subsequent inflammatory pathways.

TAB-TAK complex, is facilitated by cIAP1/2 K48-mediated ubiquitination of TRAF3 (Matsuzawa et al., 2008; Tseng et al., 2010). Upon TLR3 and TLR4 activation, TRIF is also recruited, which then binds to RIP1 and TRAF6. Both TRAF6 and RIP1 are subsequently polyubiquitinated, and activates NF-κB and/or type-I IFNs, depending upon the E3s recruited to the TLR-SC (Chang et al., 2009; Vallabhapurapu and Karin, 2009) (Figure 1). Despite the key role of K63-linked ubiquitin chains in TLR signaling, the requirements for and recruitment patterns of the major E2s and E3s to facilitate K63-linked ubiquitination remain elusive. In response to IL-1β or LPS, TRAF6 is thought to interact with the E2 Ubc13/Uev1A to facilitate K63-linked polyubiquitination of IRAK1 and TAK1 (Wang et al., 2001; Conze et al., 2008; Windheim et al., 2008). Genetic analysis has provided conflicting results on the role of Ubc13 in TLR4 signaling. Deletion of Ubc13 in bone marrow-derived macrophages, MEFs, and B cells does not impair TRAF6-mediated NF-κB activation in the IL-1R/TLR4 signaling complex (Yamamoto et al., 2006). Conversely, macrophages and splenocytes derived from Ubc13+/− mice show reduced NF-κB activation following LPS stimulation (Fukushima et al., 2007). Furthermore, the exact role of the RING domain in TRAF6 during IL-1R/TLR4 signaling is not clear, although TRAF6-deficient cells have significantly reduced NF-κB activation following LPS or IL-1β stimulation (Lomaga et al., 1999). Introduction of a TRAF6 mutant lacking the RING domain into TRAF6-deficient cells rescues the effect of TRAF6 deficiency on NF-κB activation in the IL-1R/TLR4 signaling complex (Kobayashi et al., 2001), whereas a RING-domain point mutant of TRAF6 fails to restore NF-κB activation in TRAF6-deficient cells (Lamothe et al., 2007). Therefore, more genetic and biochemical studies are needed to probe the precise mechanism of Ubc13–TRAF6-mediated regulation of NF-κB activation in IL-1R/TLR4 signaling.

Several other common E3s play important roles in TLR signaling. PELI-1 regulates TRIF-dependent TLR signaling (Chang et al., 2009), and cIAPs regulates cell death and inflammation (Feoktistova et al., 2011; Estornes et al., 2012). Recently, the LUBAC complex (consisting of the E3 ligases HOIL-1L and HOIP, and the accessory protein SHARPIN) has been suggested to regulate TLR signaling (Ikeda et al., 2011; Sasaki et al., 2013). LUBAC catalyzes the linear polyubiquitination of NEMO upon activation by IL-1R/TLR agonists, leading to IKK activation (Tokunaga et al., 2009). Although it is not clear whether there is functional redundancy between K63-linked ubiquitination and linear ubiquitination, a recent study by Emmerich et al. (2013) suggests that linear and K63-linked ubiquitin chains work together to modify the functions of signaling molecules such as MyD88 and IRAK4 in IL-1R/TLR4 signaling (Emmerich et al., 2013). There is some uncertainty regarding how IKK activation is positively regulated by linear ubiquitination as mediated by LUBAC, because SHARPIN-deficient macrophages exhibit no reduction in IKK activation following TLR stimulation (Zak et al., 2011). Recent biochemical studies also revealed another type of ubiquitin chain-mediated regulation of IKK activation. Unanchored K63-linked polyubiquitin chains (which are not conjugated to a protein) are polymerized by the TRAF6–Ubc13 complex, which subsequently activates TAK1 through the binding of TAB2 (Xia et al., 2009). Further studies are needed to determine the functional *in vivo* significance of unanchored ubiquitin chains in IL-1R/TLR signaling pathways.

To tightly regulate immune-response activation, DUBs act as important ubiquitination-regulating agents (Figure 1). Recently, USP25 was described as an off switch for TLR4-mediated MAPK signaling. Interestingly, USP25 only limits the production of pro-inflammatory cytokines by interacting with MyD88 and stabilizing TRAF3, resulting in a balance between TLR-triggered cytokines and type-I IFNs (Zhong et al., 2013). Another important DUB, USP7, stabilizes NF-κB by preventing its proteasomal degradation in response to diverse TLR signaling cascades (Colleran et al., 2013). In addition, another study suggests that USP7 has DUB activity for TRAF6 in the TLR pathway (Daubeuf et al., 2009). A20, a member of a multi-protein complex that includes Itch, RNF11, TAX1BP1, and ABIN1/2/3, can neutralize the TRAF6 ubiquitinosome by targeting polyubiquitinated TRAF6 (Lin et al., 2008). Macrophages deficient in A20 show prolonged TRAF6 ubiquitination following LPS stimulation (Boone et al., 2004). A recent study has revealed that A20 inhibits IL-1β-induced interactions of TRAF6 with Ubc13 or UbcH5, which triggers their proteasomal degradation (Shembade et al., 2010a). Recent evidence suggests that CYLD is also a TLR-modulating DUB, as it negatively regulates NF-κB activation of TLR4 signaling (Sun, 2010).

**RLR and NLR signaling**

RLRs and NLRs are cytosolic PPRs. Three types of RLRs exist, namely RIG-I, MDA5, and LGP2. Upon sensing viral particles, RIG-I becomes K63 ubiquitinated by RNF135 and TRIM25 and by free K63-linked chains, which results in binding of RIG-I to the adaptor protein MAVS (Takeuchi and Akira, 2010; Jiang et al., 2012). Upon activation, MAVS-recruited TRADD further recruits multiple downstream mediators to trigger antiviral responses (Liu et al., 2013). The recruitment of the TRAF3 results in the activation of IRF3, which leads to production of IFN (Michallet et al., 2008). Conversely, TRAF6 recruitment, in conjunction with the adaptor molecules TRAF2 and TRAF5, results in activation of NF-κB and IRF3 (Liu et al., 2013). In addition, TRADD recruits RIP1, caspase-8, and FADD, which are required for IRF3 and NF-κB activation (Takahashi et al., 2006; Michallet et al., 2008). Interestingly, RIP1 ubiquitination is involved in maximal activation of the RIG-I mediated antiviral response and its subsequent termination. Although RIP1 ubiquitination acts as a positive regulator of RIG-I signaling, it is also required for its cleavage by caspase-8 in the RIG-I signaling complex, resulting in down regulation of RIG-I-mediated IFN production (Rajput et al., 2011). The types of ubiquitination linkages involved in this dual function of RIP1 still need to be determined.

Other E3 ligases, e.g., LUBAC, RNF125, and cIAP1/2, also mediate RIG-I signaling cascades. cIAP1/2 facilitates activation of IRF3 and NF-κB by mediating K63 polyubiquitination of TRAF3 and TRAF6 (Mao et al., 2010). In contrast, RNF125 and LUBAC negatively regulate RIG-I-mediated immune responses (Figure 1). RNF125 attaches K48-linked chains to RIG-I and MAVS, targeting them for

proteasomal degradation (Arimoto et al., 2007), whereas LUBAC disrupts the TRIM25–RIG-I interaction directly or by ubiquitin-mediated proteasomal degradation of TRIM25 (Inn et al., 2011). In line with these observations, infection with Sendai virus induces K-48 and linear polyubiquitination of TRIM25, whereas HOIP-knockdown cells show increased IFN production following infection (Inn et al., 2011). Further, LUBAC can also linearly ubiquitinate NEMO and then negatively regulate the RIG-I-mediated immune responses (Belgnaoui et al., 2012). Accordingly, vesicular stomatitis virus replication was decreased due to increased IFN production in SHARPIN-deficient MEFs (Belgnaoui et al., 2012). It has, therefore, been proposed that linear ubiquitination has an opposing role in the context of the RIG-I-mediated antiviral response compared with other signaling pathways, e.g., those triggered by TLR (Sasaki et al., 2013), IL-1 (Emmerich et al., 2013), TNF (Haas et al., 2009; Gerlach et al., 2011), and NOD2 (Damgaard et al., 2012; Fiil et al., 2013). The detailed mechanisms of linear ubiquitination-mediated RIG-I signaling require further investigation to be fully understood.

RIG-I-mediated immune responses are also regulated by several DUBs, including CYLD, which deubiquitinates RIG-I (Zhang et al., 2008) thereby inhibiting its signaling. In response to Sendei virus infection, enhanced IFN production has been observed following CYLD knockdown, whereas CYLD ectopic expression inhibits this enhancement (Friedman et al., 2008). Moreover, bone marrow-derived cells and fibroblasts derived from CYLD-deficient mice show constitutive activation of TBK1 (Zhang et al., 2008). Another DUB, DUBA, also modulates RIG-I-mediated immune signaling by deubiquitinating TRAF3. Overexpression of DUBA has been shown to disrupt the TRAF3-TBK1 interaction (Kayagaki et al., 2007). Recently, A20 was reported to counteract RIG-I-mediated IRF3 and NF-κB responses, but if A20 directly interacts with RIG-I and/or MAVS still needs to be determined (Lin et al., 2006; Harhaj and Dixit, 2012). Furthermore, Itch, a member of the A20 complex has been reported to attach K-48-linked polyubiquitination chains to MAVS (You et al., 2009), but, similarly, its direct interaction with MAVS has not been reported.

NLR signaling also relies on ubiquitination-regulated mechanisms. Two of the most well studied NLRs are NOD1 and NOD2. Stimulation of NLRs results in a robust immune response via production of cytokines, chemokines, and IFNs. The NOD complex includes RIP2, which is ubiquitinated by cIAPs (Bertrand et al., 2009). Specifically, cIAP1 and 2 induce K63-linked ubiquitination of RIP2 (Bertrand et al., 2009). Moreover, E3 ligases, e.g., TRAF2, 5, and 6, are also recruited to the NOD signaling complex, where they function as adaptor molecules and also cross-talk with components of other signaling pathways, e.g., those involving TLRs and RLRs (Figure 1). XIAP is also recruited to the NOD signaling complex and directly binds to RIP2. Furthermore, XIAP mediates recruitment of LUBAC, which adds linear ubiquitin chains to RIP2 (Damgaard et al., 2012; Fiil et al., 2013). This results in the activation of TAB-TAK and IKK-NEMO complexes and subsequent activation of the NF-κB and MAPK signaling pathways. The importance of XIAP in the innate immune response has been shown using several intracellular bacterial infection models (Lopez and Meier, 2010). XIAP-deficient mice suffer from increased pulmonary infectivity and are unable to clear bacteria following *Chlamydophila pneumoniae* infection (Prakash et al., 2010). Similarly, XIAP-deficient mice exhibit decreased survival following *Listeria monocytogenes* intraperitoneal injection (Bauler et al., 2008).

A20 negatively regulates NLR signaling by deubiquitinating RIP2 (Hitotsumatsu et al., 2008). In the absence of A20, increased RIP2 ubiquitination and prolonged NF-κB activation are observed both *in vitro* and *in vivo* (Hitotsumatsu et al., 2008). Recent studies have also identified the DUB OTULIN as negatively regulating NOD2 signaling by limiting linear ubiquitination of LUBAC (Figure 1; (Fiil et al., 2013). Overexpression of OTULIN results in inhibition of LUBAC and to a lesser extent XIAP-mediated NF-κB activation, whereas OTULIN knockdown results in over-activation of NF-κB following stimulation by the NOD2 ligand MDP (Fiil et al., 2013).

The crucial role of ubiquitination in NLRP3-mediated immune responses has also been demonstrated (Dempsey, 2013). Although cIAPs are implicated in the regulation of inflammasome activation, two opposing views exist regarding the role of cIAPs in the NLRP3 signaling complex. One report suggests that cIAPs directly mediate K63-polyubiquitination of caspases-1-containing complexes, thus positively regulating their activation (Labbé et al., 2011). In contrast, cIAPs and XIAP have been reported to negatively regulate the NLRP3-mediated inflammasome (Vince et al., 2012). Recently, the positively regulating DUB BRCC3 was identified which directly deubiquitinates NLRP3 and stabilizes the NLRP3-inflammasome. Knockdown of BRCC3 results in increased NLRP3 ubiquitination. The DUB activity of BRCC3 is also required for caspase-1 activation and IL-1 processing (Py et al., 2013).

Taken together, these studies provide new insights into the importance of ubiquitination in NLR signaling. To comprehensively understand NLR signaling and the inflammasome, further studies are needed to determine the precise role of other E3s and DUBs.

**TNF signaling**

Activation of the TNF signaling pathway can result in two opposing outcomes. On the one hand, this signaling cascade results in inflammation and cellular protection, and, on the other hand, it results in the activation of the cellular apoptosis-signaling cascade (Micheau and Tschopp, 2003). TNF-α, a major immune effector, binds to TNFR1 to trigger activation of the NF-κB and MAPK pathways in an ubiquitin-dependent manner (Zinngrebe et al., 2014). Initial recruitment of TRADD and TRAF2, or alternately TRAF5, allows the subsequent recruitment of cIAPs and RIP1 (Figure 1). These cIAPs along with TRAF2/5 facilitate K63-linked ubiquitination of RIP1, resulting in the assembly of downstream mediators and activation of the NF-κB and MAPK pathways (Figure 1; (Wu et al., 2006; Ea et al., 2006; Bertrand et al., 2008; Varfolomeev et al., 2008). TNF-α-induced ubiquitination of RIP1 is not observed in TRAF2-deficient MEFs, but it remains unclear whether TRAF2 directly catalyzes the

polyubiquitination of RIP1 (Lee et al., 2004). cIAP1 and cIAP2 are also RING domain-containing E3s reported to catalyze RIP1 polyubiquitination (Mahoney et al., 2008), but a recent study also suggests that cIAP1, along with UbcH5, generates K11-linked polyubiquitin chains on RIP1 in the TNFR1 signaling complex to activate NF-κB in a nondegradative manner (Dynek et al., 2010). Although several studies clearly demonstrate the pivotal roles of cIAP1/2 in RIP1 polyubiquitination (Mahoney et al., 2008; Varfolomeev et al., 2008), the detailed mechanisms underlying cIAP1/2 and TRAF2/5 interplay remain elusive.

The interaction between polyubiquitinated RIP1 and TAB2/3 facilitates the recruitment of TAK1 to TNFR1, which aids in the activation of IKK (Kanayama et al., 2004). Although it is clear that TAK1 activates IKK by phosphorylation (Blonska et al., 2005), it is still unclear whether TAK1 phosphorylates IKK directly or through a mediator. Mutation of K377 in RIP1, a key residue for its ubiquitination, prevents the downstream recruitment of the IKK complex to TNFR1 and results in inactivation of IKK (Ea et al., 2006).

Ubc13/Uev1A is an E2 that specifically catalyzes addition of K63-linked ubiquitin chains. Although the crucial role of Ubc13/Uev1A in NF-κB activation by TNF-α has been described (Deng et al., 2000), several other studies have reported a very limited role for Ubc13 in TNF-α-mediated NF-κB activation. For example, MEFs deficient in Ubc13 show no effect on MAPK activation by TNF stimulation (Yamamoto et al., 2006). Furthermore, human cells expressing an endogenous K63 mutant exhibit no effect on IKK activation by TNF-α (Xu et al., 2009). Moreover, they showed that Ubc5, but not Ubc13, is required for IKK activation by TNF-α (Xu et al., 2009). These results suggest that either polyubiquitin chains or other E2s and E3s are involved in the TNF-α-mediated NF-κB signaling pathway.

Recently, the central role of LUBAC in TNFR1-mediated NF-κB activation was determined (Tokunaga et al., 2009; Haas et al., 2009; Iwai, 2012). LUBAC regulates IKK activation in TNF signaling complex. Deletion and overexpression studies revealed that the LUBAC catalyzes the addition of linear polyubiquitination chains to NEMO, resulting in NF-κB activation (Gerlach et al., 2011; Haas et al., 2009). This linear ubiquitination facilitates the recruitment of the IKK complex, which appears to be similar to RIP1-mediated IKK activation by K63-linked polyubiquitin chains (Gerlach et al., 2011; Tokunaga et al., 2011). Although K63 and linear ubiquitination exhibit equivalent open conformations, these two chains are recognized by distinct components of the NF-κB signaling pathway (Komander et al., 2009b). Further studies are needed to determine the precise roles of the LUBAC in the TNF signaling pathway.

DUBs play important roles in NF-κB signaling pathways by regulating the effects of ubiquitination. The two well-studied DUBs A20 and CYLD negatively regulate TNF-mediated NF-κB and MAPK activation (Kovalenko et al., 2003; Wertz et al., 2004; Jono et al., 2004). A20 acts as a dual-function ubiquitin-editing enzyme (Wertz et al., 2004). K63-linked polyubiquitin chains are first removed by the OTU DUB domain of A20, and then A20 conjugates K48-linked polyubiquitination to RIPI through its C-terminal E3 domain to facilitate its proteasome-mediated degradation (Wertz et al., 2004). A20-deficient mice exhibit prolonged NF-κB responses and develop severe multi-organ inflammation (Lee et al., 2000). A20, in conjunction with TAXBP1, Itch, and RNF11, assembles a complex that attenuates TNFR1 signaling (Shembade et al., 2007, 2008, 2009, 2010b). Recent studies also suggest that A20 can inhibit LUBAC-mediated NF-κB responses in a DUB-independent manner (Tokunaga et al., 2012; Verhelst et al., 2012). The DUB CYLD is also known to inhibit NF-κB signaling. CYLD possesses a USP-type DUB domain that acts on linear and K63-linkages (Brummelkamp et al., 2003; Kovalenko et al., 2003; Trompouki et al., 2003). The DUB OTULIN (also named gumby) is the most recently characterized member of the OTU family of DUBs. OTULIN exclusively recognizes linear ubiquitin chains and inhibits TNF-α-induced NF-κB responses by preventing NEMO-RIP1 association (Fiil et al., 2013; Keusekotten et al., 2013). Several other DUBs, e.g., USP31 (Tzimas et al., 2006), USP21 (Xu et al., 2010), Cezanne (Enesa et al., 2008), USP11 (Sun et al., 2010; Yamaguchi et al., 2007), and USP4 (Zhou et al., 2012), have also been shown to regulate TNF-α signaling by terminating NF-κB activation. Further genetic and biochemical studies are required to determine the exact roles of these DUBs in the TNF-α-induced NF-κB signaling pathway.

**Summary**

The mammalian immune system possesses remarkable abilities to precisely modulate defense mechanisms against invading pathogens, sensing when, where, and for how long defenses are needed. The immune system works in a highly sophisticated manner, although we do not fully understand its molecular interactions and functionalities. Herein, by summarizing some of the available evidences**,** we propose that ubiquitination and deubiquitination play pivotal roles in regulating immune responses. Ubiquitination and deubiquitination of key proteins within signaling cascades tightly regulate the complex mechanisms of immune signaling. Defects in the ubiquitin pathway can greatly alter the immune response, resulting in increased pathogenicity of invading pathogens. Although recent findings have improved our understanding of the various ubiquitination pathways, we still lack a complete understanding of the exact roles of this modification. There is a great need to elucidate the precise roles of individual ubiquitinating/deubiquitinating molecules in regulating host immune responses and also to determine how invading pathogens exploit the host ubiquitin system for their own good. Detailed investigations of these modifications may also reveal novel therapeutic options for several diseases.

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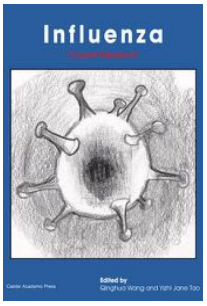


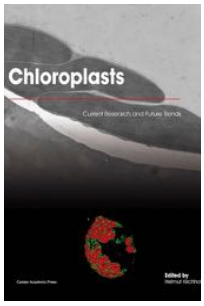


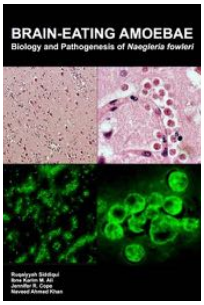


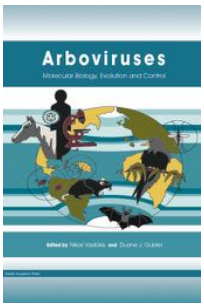



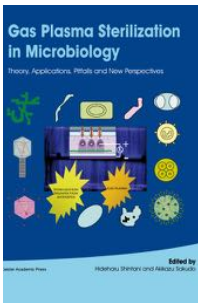


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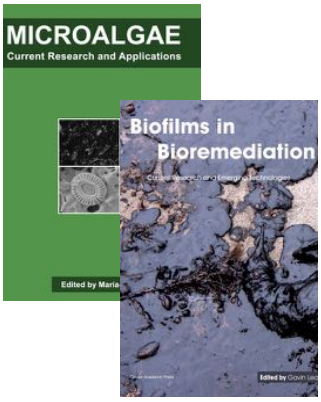


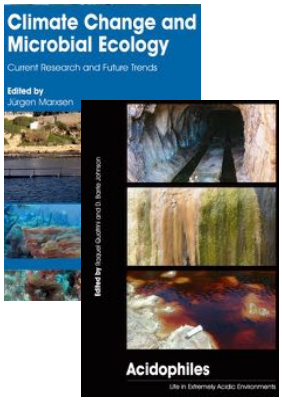


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