Ubiquitin in the activation and attenuation of innate antiviral immunity

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Viral infection activates danger signals that are transmitted via the retinoic acid–inducible gene 1–like receptor (RLR), nucleotide-binding oligomerization domain-like receptor (NLR), and Toll-like receptor (TLR) protein signaling cascades. This places host cells in an antiviral posture by up-regulating antiviral cytokines including type-I interferon (IFN-I). Ubiquitin modifications and cross-talk between proteins within these signaling cascades potentiate IFN-I expression, and inversely, a growing number of viruses are found to weaponize the ubiquitin modification system to suppress IFN-I. Here we review how host- and virus-directed ubiquitin modification of proteins in the RLR, NLR, and TLR antiviral signaling cascades modulate IFN-I expression.

The frontline in the cellular response to viral infection is comprised of the specific and general effectors of the innate immune system. Effector molecule production is initiated by immune sentinels known as pattern recognition receptors (PRRs), which screen the intra- and extracellular environment for molecular motifs uniquely associated with pathogens. PRR engagement transduces pro-immune signals into the nucleus via protein signaling cascades that self-limit to mitigate autoimmunity as the infection clears (Crampton et al., 2012). Protein posttranslational modifications (PTMs) form part of this exquisite system of regulation, with ubiquitin and ubiquitin-like modifications key among them.

The retinoic acid–inducible gene 1 (RIG-I)–like receptors (RLRs) and nucleotide-binding oligomerization domain (NOD)–like receptors (NLRs) are intracellular PRRs. The RLRs sense invasive RNA produced during infection by both RNA and DNA viruses (Schlee, 2013). RLR engagement up-regulates type-I IFN (IFN-I) expression, which in turn stimulates transcription of hundreds of IFN-stimulated genes (ISGs) that commit host and nearby cells to an antiviral posture. Recognized for their role in antibacterial immunity, the NLRs are emerging as antiviral mediators that regulate both IFN-I and NF-κB activation. These are also activated by TLRs, a cell-specific class of extracellular and endosomal transmembrane PRRs that sense a broad spectrum of pathogenic motifs. RLR, NLR, and TLR signaling proteins must be spatially and temporally coordinated for efficient immune signal transduction.

Ubiquitination is a PTM involving the covalent attachment of the 8.6-kD protein ubiquitin to target proteins. Ubiquitination is catalyzed by the ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2), and ubiquitin protein ligase (E3). The E3 largely dictates substrate specificity, with at least 617 genes encoding putative ubiquitin and ubiquitin-like E3s annotated in the human genome (Li et al., 2008). Ubiquitin can undergo ubiquitination itself at its seven lysine residues (K6/K11/K27/K29/K33/K48/K63), building lysine-linked polyubiquitin chains, or its N-terminal methionine (M1), forming linear polyubiquitin chains. Alternatively, ubiquitin chains may be noncovalently associated with target proteins. Furthermore, ubiquitin chains may be remodeled by deubiquitinating enzymes (DUbs; Fig. 1). The function, abundance, or subcellular distribution of proteins involved in almost every cellular process is regulated in this way, with an increasingly clear role in regulating innate immunity.

Viruses are obligate intracellular parasites that facilitate their own replication by manipulating the host cell environment. Thus, the ubiquitin modification system presents a key manipulation target for viruses to circumvent antiviral signaling pathways. Methods for this include substrate molecular mimicry, binding and blocking E3-substrate pairs, expressing virally encoded E3s/DUbs, and hijacking host E3s/DUbs. Additionally, a novel mechanism involving ubiquitin chain packaging into nascent virions for subsequent redeployment

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Abbreviations used: CARD, caspase activation and recruitment domain; CCD, coiled-coil domain; DUb, deubiquitinating enzyme; HOIP, HOIL-1–interacting protein; IAV, influenza A virus; IKK, inhibitor of NF-κB kinase; IRF, IFN regulatory factor; ISG, IFN-stimulated gene; KSHV, Kaposi's sarcoma–associated herpesvirus; LUBAC, linear ubiquitin assembly complex; MAVS, mitochondrial antiviral signaling protein; NEMO, NF-κB essential modulator; NLR, NOD-like receptor; PRR, pattern recognition receptor; PTM, posttranslational modification; RAUL, RTA-associated ubiquitin ligase; RD, repressor domain; RING, really interesting new gene; RLR, RIG-I–like receptor; RTA, replication and transcription factor; SUMO, small ubiquitin-like modifier; TRAF, TNF receptor–associated factor; TRIF, TIR domain–containing adaptor-inducing IFN-β.

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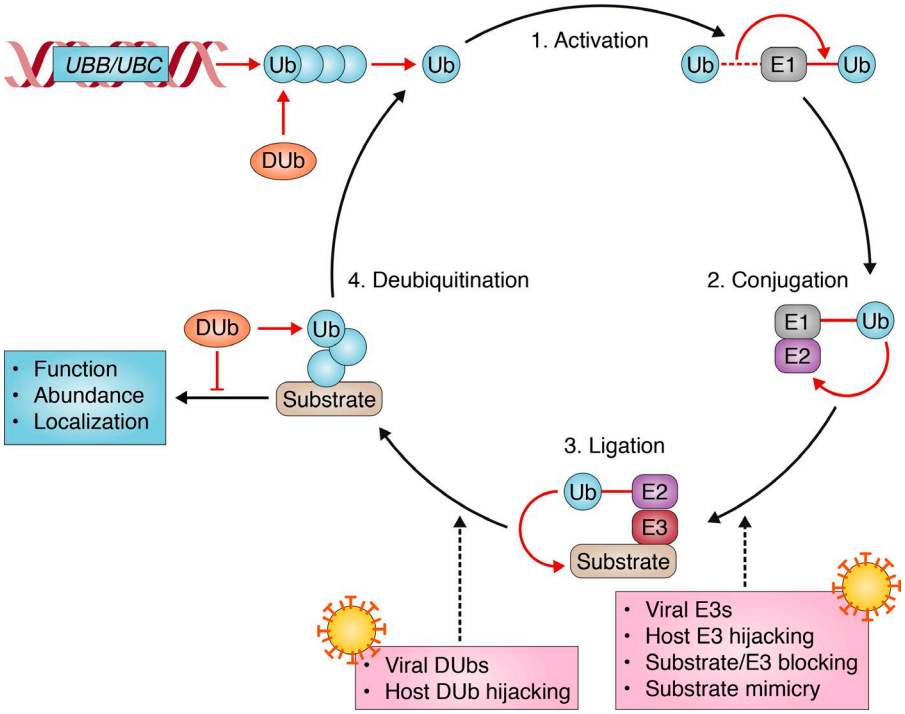

Figure 1. **The ubiquitin modification system and mechanisms of viral manipulation.** (1) Ubiquitin (Ub) expresses as an inactive polyprotein, encoded by the *UBB* and *UBC* genes. DUbs cleave this polyprotein into monomers that are activated by the E1-activating enzyme, involving the energy-dependent adenylation of the ubiquitin C-terminal glycine. The ubiquitin-adenylate intermediate (dashed line) converts into a covalent thioester bond (solid line). (2) Ubiquitin transfers to the active site cysteine residue of an E2-conjugating enzyme. (3) The E3 directly or indirectly transfers the E2-bound ubiquitin to a substrate acceptor residue, forming an isopeptide bond. (4) DUbs remodel ubiquitin modifications and antagonize ubiquitin-driven functional outcomes.

against the host was recently described (Banerjee et al., 2014). Although characterizing the molecular mechanisms of ubiquitin-dependent immune signaling remains challenging, this information is essential in understanding innate immune regulation and mechanisms of viral immune evasion.

RLR signaling

Pattern recognition and cascade initiation. RIG-I and melanoma differentiation-associated gene 5 receptor (MDA5), together with the regulatory homologue laboratory of genetics and physiology 2 (LGP2), form the apex of the RLR signaling cascade. All three are expressed ubiquitously, but only RIG-I and MDA5 possess N-terminal caspase activation and recruitment domains (CARDs) that are capable of downstream immune signal transduction.

Based on their distinctive C-terminal domains that sense different types of invasive RNA, the RLRs are activated by different viruses. RNA lacking a 5′-7-methylguanosine cap, a feature of mature eukaryotic RNA, potently activates MDA5-mediated signaling (Züst et al., 2011). Except during viral infection, the mammalian cytosol is normally vacant of immature 5′-triphosphorylated RNA, certain kinds of which activate RIG-I (Schlee et al., 2009).

RLR activation up-regulates expression of two IFN-I isotypes, IFN-α and IFN-β, which regulate transcription of hundreds of ISGs during infection (Fig. 2, middle). IFN regulatory factors (IRFs), including IRF3 and IRF7, dimerize and translocate into the nucleus to drive transcription of various IFN-α/β subtypes upon phosphorylation by TNF receptor–associated factor (TRAF) family member–associated NF-κB activator (TANK)–binding kinase 1 (TBK1). TBK1 localization changes from the cytosol to distinct subcellular compartments depending on upstream signaling events (Goncalves et al., 2011). In promoting IFN-I signaling, TBK1 associates with RIG-I as well as key adaptor proteins, including TANK and NF-κB essential modulator (NEMO; Guo and Cheng, 2007; Zhao et al., 2007; Wang et al., 2012a). This facilitates interactions between TBK1, inhibitor of NF-κB kinase subunit ε (IKKε) and TRAFs, particularly TRAF3. Upon RLR activation, these proteins are colocalized at the cytosolic surface of the mitochondrial outer membrane (Parvatiyar et al., 2010; van Zuylen et al., 2012), coordinated by the mitochondrial antiviral-signaling protein (MAVS; also termed VISA/Cardif/IPS-1).

At rest, the RLR cascade is maintained in an inactive but tensioned state through an intricate negative feedback system involving protein expression levels, conformational changes, compartmentalization, and PTMs. Part of this system operates at the receptor through conformational auto-inhibition of the RIG-I CARDs. The RIG-I C-terminal repressor domain (RD) audits the cytosol for viral RNA, binding of which induces a major structural rearrangement in the RD and CARD (Saito et al., 2007). Conversely, MDA5 oligomerizes along the length of RNA ligands, forming immunogenic filaments that are potentiated by ATP hydrolysis and interaction with LGP2 (Peisley et al., 2012; Bruns et al., 2014).

RIG-I activation depends on ubiquitin. The unfurled RIG-I CARDs undergo tetramerization upon K63-linked polyubiquitination or unanchored polyubiquitin chain association (Peisley et al., 2014). These modifications drive mitochondrial accumulation of RIG-I, promoting CARD–CARD interac-

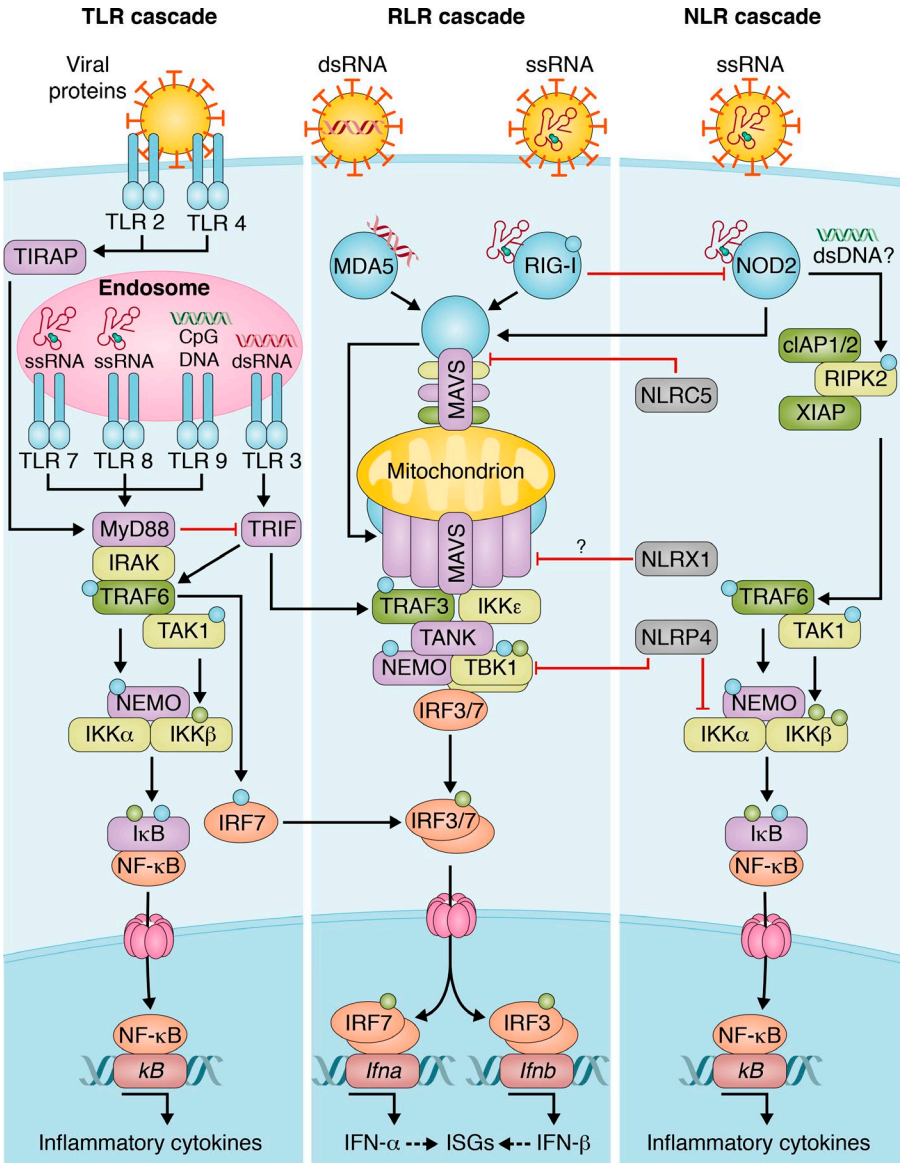

Figure 2. **Schematic of the TLR, RLR, and NLR antiviral protein signaling cascades and modes of cross-talk.** PRRs (blue) screen the intracellular and extracellular environment for pathogenic motifs. Ligand-activated PRRs bind adaptor proteins (purple) and recruit protein kinases (yellow) and ubiquitin-protein ligases (green). These regulate immune signal transduction to transcription factors (orange) through PTM of signaling cascade proteins. Other regulatory proteins (gray) support or sequester these signaling proteins. Immune signaling scaffolds such as mitochondria typically coordinate these actions. Activated transcription factors translocate into the nucleus and bind to promoter response elements, stimulating appropriate antiviral gene transcription. Blue and green circles represent ubiquitination and phosphorylation, respectively. Black arrows, activation; red lines, deactivation.

tions with MAVS and inducing its oligomerization and filamentation. The RIG-I CARDs contain a high proportion of hydrophobic residues and are prone to aggregation, thus oligomerization and polyubiquitination may stabilize the activated CARDs or elicit a separate mitochondrial targeting signal. Conversely, ubiquitination has no known role in MDA5 or LGP2 activation.

The first virus-triggered RIG-I ubiquitination site described, K172, depends on the E3 activity of tripartite motif protein 25 (TRIM25; Gack et al., 2007). Plausibly as a means of restricting escape mutant selection, this activation mechanism now appears to have evolved with partial redundancy using alternate E3s. TRIM4 was recently described to modify this same site in addition to two other CARD residues: K154 and K164 (Yan et al., 2014). Furthermore, these same three residues are reportedly ubiquitinated by really interesting new gene (RING) finger protein-135 (RNF135; also termed Riplet/REUL; Gao et al., 2009), although this is controversial (Fig. 3; Oshiumi et al., 2010). Underscoring the importance of these modifications, ubiquitin-specific protease 3 (USP3) and ubiquitin C-terminal hydrolase are DUbs that inhibit IFN-I production by removing such chains from RIG-I (Friedman et al., 2008; Cui et al., 2014).

Both TRIM25 and RNF135 are targets of the influenza A virus (IAV) nonstructural protein 1 (NS1), which blocks their E3 activity and ubiquitin-dependent RIG-I activation (Fig. 3; Gack et al., 2009; Rajsbaum et al., 2012). IAV-NS1 binds the central coiled-coil domain (CCD) of TRIM25 and is postulated to prevent CCD-mediated homo-oligomerization. Although the NS1-binding site on RNF135 is unknown, RNF135 and TRIM25 share a similar RING-CCD-B30.2/SPRY (sp1A and ryanodine receptors) domain

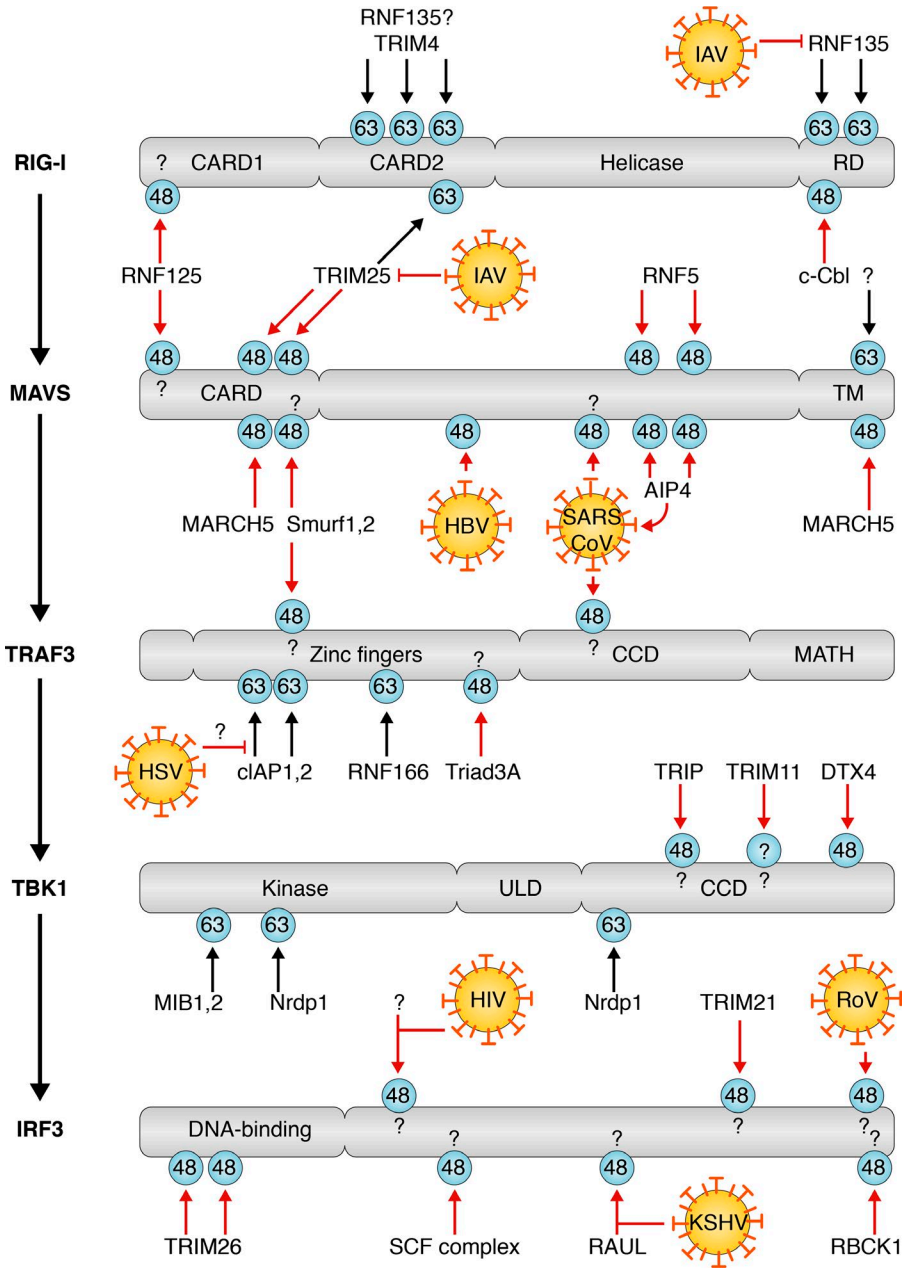

Figure 3. **Effect on IFN-I expression of ubiquitin modifications to key RLR cascade proteins and mechanisms of manipulation by human-tropic viruses.** Ubiquitin modification site and ubiquitin chain linkage type are shown in blue circles. Ubiquitin modifications that up-regulate or down-regulate IFN-I expression are shown with black or red arrows, respectively. Question marks indicate where the modification site, ubiquitin chain linkage, or modifying E3 are unknown. MATH, meprin and TRAF homology domain (also termed TRAF-C); RoV, rotavirus; SARS-CoV, severe acute respiratory syndrome–coronavirus; TM, transmembrane domain; ULD, ubiquitin-like domain.

organization. However, the CCDs differ in size and sequence markedly, suggesting that IAV-NS1 may bind multiple sites on TRIM25 and RNF135. Alternatively, given that CCDs often mediate protein–protein interactions, IAV-NS1 may sense and subvert CCD-interacting domains more broadly. Notably, the IAV-NS1:RNF135 interactions observed by Rajsbaum et al. (2012) were strain dependent.

RNF135 enables RIG-I CARD activation by TRIM25 upon ubiquitinating RD residues K849 and K851. RNF135 knockdown inhibits interaction between RIG-I :TRIM25 and eliminates TBK1 recruitment (Oshiumi et al., 2009), revealing an ordered functional interplay between ubiquitination and phosphorylation in coordinating RIG-I activation. Hepatitis C virus (HCV) NS3-4A protease exploits this concept by targeting RNF135 for proteolytic cleavage (Oshiumi et al., 2013). Furthermore, numerous herpesviruses encode their own DUbs that inhibit IFN-I expression by stripping ubiquitin modifications from activated RIG-I (Inn et al., 2011b). Accordingly, HCV and herpesvirus infections are treatable with IFN (Oberman and Panet, 1988; Nguyen et al., 2014), although this can carry significant side effects. Endogenous IFN-I expression and self-regulation may be restored by defeating such mechanisms of viral antagonism.

Ubiquitin in the return to homeostasis. RLR signaling is also counterbalanced and diminished through ubiquitin modifica-

tion as the antiviral posture becomes unnecessary. RNF125 forms part of this process, ligating K48-linked polyubiquitin chains to the activated CARD of RIG-I and MDA5, leading to proteasome-mediated degradation of both receptors and diminished IFN-I signaling. USP4 is a DUb that sustains RLR signaling by specifically removing such chains (Wang et al., 2013a). In the same way, RNF125 ubiquitinates and degrades activated MAVS (Arimoto et al., 2007), suggesting that RNF125 is an E3 that destabilizes proteins containing activated CARDs. Given how commonly CARD-containing proteins and their homotypic interactions feature in immune signaling pathways (Bouchier-Hayes and Martin, 2002), RNF125 may represent a general immune signaling antagonist. Conversely, the 52-kD repressor of the inhibitor of the protein kinase (p52rIPK) binds and enhances the stability of RIG-I by blocking its ubiquitin-mediated degradation (Now and Yoo, 2011). Accordingly, the properties of p52rIPK or RNF125 may be exploitable in the treatment of viral infections or autoimmune disorders.

The linear ubiquitin assembly complex (LUBAC), containing SHANK-associated RH domain–interacting protein (SHARPIN), heme-oxidized IRP2 ubiquitin ligase 1L (HOIL-1L), and HOIL-1–interacting protein (HOIP), was proposed to negatively regulate RLR-mediated IFN-I expression via two independent mechanisms (Inn et al., 2011a). First, HOIL-1L competes with TRIM25 for RIG-I CARD binding, abrogating the RIG-I:MAVS interaction. Second, HOIP promotes M1- and K48-linked polyubiquitination of TRIM25 and induces its proteasomal degradation, thereby decreasing TRIM25-mediated activation of RIG-I. If LUBAC were capable of ligating K48-linked polyubiquitin chains to substrates, TRIM25 would be the first example to our knowledge.

Another route of RLR inhibition involves tetraspanin-6 ubiquitination by an unknown E3. During RLR activation, polyubiquitinated tetraspanin-6 is recruited to MAVS and blocks the RLR:MAVS interaction, thereby impeding recruitment of the downstream signaling apparatus (Wang et al., 2012b).

Convergence at MAVS

Mitochondria, peroxisomes, and endoplasmic reticulum function as immune signaling platforms linking viral pattern recognition with effector molecule production (Fig. 2, middle). Although this process remains poorly characterized, the nature and context of viral ligands detected by PRRs drives accumulation of the downstream signaling apparatus to these platforms. Adaptor proteins mediate this accumulation: mitochondria and peroxisomes by MAVS (Dixit et al., 2010) and endoplasmic reticulum by stimulator of IFN genes (STING; Ishikawa and Barber, 2008). Cyclic GMP-AMP synthase (cGAS) and AIM2-like receptors (ALRs), which include AIM2 and human IFN-inducible protein 16 (IFI16), have also emerged as important DNA virus PRRs. cGAS signals via STING and AIM2 generates inflammasome oligomers, whereas IFI16 can stimulate both signaling mechanisms (Diner et al., 2015).

RNA-activated RIG-I and MDA5 colocalize with MAVS, inducing its filamentation. It remains unclear why these filaments are potent inducers of downstream signaling; however, RLR cascade proteins including NEMO, IKKε, and various TRAFs possess MAVS-targeting signals (Paz et al., 2011). Furthermore, TBK1 and other key RLR cascade proteins interact with these proteins but are activated only upon oligomerization. Thus, steady state isolation of MAVS may represent a spatiotemporal barrier that restrains innate immune signaling, overcome through coordinating these proteins into signaling complexes upon MAVS multimerization. In this way, it is conceivable how immunomodulating E3s/DUbs may be compartmentalized together with their substrates.

Ubiquitin stringently regulates the MAVS signalosome. The central position that MAVS occupies within the RLR cascade is commensurate with the many PTMs that modulate its role. To our knowledge, MAVS ubiquitination has not been observed in resting cells using a variety of proteomic and biochemical approaches, indicating that MAVS ubiquitination occurs specifically during viral infection. At least seven E3s ubiquitinate MAVS, leading to MAVS degradation in almost every case, as described later in this section (Fig. 3). At least five of these modify other substrates within the same cascade, highlighting MAVS as a crucial locus of RLR regulation. Accordingly, MAVS is targeted by numerous viruses in a variety of ways; however, with the exception of HBV and severe acute respiratory syndrome–coronavirus (SARS-CoV; Fig. 3; Wei et al., 2010; Shi et al., 2014), this is usually achieved by means other than manipulating MAVS ubiquitination, likely given the extensive ubiquitin-mediated negative regulatory systems already in place.

MAVS aggregation is a key feature of RLR cascade activation, but how these aggregates are resolved during deactivation is only beginning to be clarified. In addition to ubiquitinating RIG-I and enhancing its association with MAVS, TRIM25 ubiquitinates MAVS at Lys7 and Lys10 and induces its partial proteolysis (Castanier et al., 2012). This was proposed as a means of discharging the activated RLR signalosome from the mitochondrial recruitment platform and would begin to address how IRF3 and other RLR signalosome components traffic correctly after activation. More recently, Lys7 and Lys500 were shown to be polyubiquitinated by membrane-associated RING finger protein 5 (MARCH5), a mitochondrial membrane-bound E3 that effectively dissolves MAVS aggregates by specifically targeting them for degradation. MARCH5 is an important regulator of mitochondrial fission and fusion whose expression is up-regulated during infection (Yoo et al., 2015). These mechanisms of MAVS aggregate resolution may be nonredundant, with the TRIM25 mechanism occurring throughout the immune response and the MARCH5 mechanism amplifying gradually in an IFN-I negative feedback loop.

Complete MAVS degradation is independently promoted by the E3s RNF5, RNF125, atrophin-1–interacting protein 4 (AIP4; also termed ITCH), SMAD ubiquitination regulatory factor 1 (Smurf1), and Smurf2 (Fig. 3). RNF5 polyubiquitinates MAVS at Lys362 and Lys461, whereas the adjacent residues Lys371 and Lys420 are polyubiquitinated by AIP4 upon recruitment by poly(rC)-binding protein 1 (PCBP1) or PCBP2 (You et al., 2009; Zhong et al., 2010; Zhou et al., 2012). AIP4 additionally inhibits IFN-I as well as NF-κB activation by ubiquitinating the inhibitor of apoptosis protein 1 (cIAP1), targeting it for lysosomal degradation (Tigno-Aranjuez et al., 2013). cIAP1 is an E3 that activates TRAF3/6 during viral infection (Mao et al., 2010), revealing that AIP4 broadly and multiply inhibits NLR-, RLR-, and TLR-mediated immune signaling. The acceptor site or sites for RNF125-induced MAVS ubiquitination are unknown; however, given that RNF125 also ubiquitinates the activated CARDs of RIG-I and MDA5 (Arimoto et al., 2007), the MAVS CARD appears a likely candidate. NEDD4 family–interacting protein 1 (Ndfip1) binds MAVS and recruits Smurf1 and possibly Smurf2, facilitating ubiquitination of unknown sites within MAVS (Wang et al., 2012c; Pan et al., 2014). Moreover, numerous TRAFs, including TRAF3 and TRAF6, interact with MAVS, and Smurf1 also targets these for degradation (Li et al., 2010a). Finally, Lys500 was reported as a single site of IFN-I–activating polyubiquitination by an unknown E3, inhibiting NF-κB activation by sequestering IKKε (Paz et al., 2009).

TRAF ubiquitination orients immune signal transmission

The TRAFs are six multifunctional adaptor proteins that regulate both NF-κB activation and IFN-I expression via the RLR, NLR, and TLR protein signaling cascades (Fig. 2). TRAF-mediated signaling outcomes are augmented by ubiquitin, and, excepting TRAF1, all TRAFs possess a RING finger domain and multiple zinc coordination sites, features typical of ubiquitin E3s. K63-linked autoubiquitination at Lys124 is a key activation mechanism of TRAF6 (Lamothe et al., 2007; Jiao et al., 2015) and possibly TRAF2 (Habelhah et al., 2004), TRAF4 (Marinis et al., 2012), and TRAF5 (Zhong et al., 2012). In vitro TRAF3 ubiquitination assays and analysis of recombinant TRAF3ΔRING isolated from mammalian cell lysates are also consistent with an autoubiquitination activation mechanism for TRAF3 (Kayagaki et al., 2007; Zeng et al., 2009).

TRAF3 and TRAF6 are among the first molecules activated by MAVS in the RLR pathway (Fig. 2, middle). Furthermore, there is increasing evidence of ubiquitin-mediated cross-talk between TRAFs. TRAF3 promotes IFN-I expression by activating TBK1/IRF3 (Parvatiyar et al., 2010), whereas TRAF6 activates mitogen-activated protein kinase kinase kinase 1 (MEKK1) to activate NF-κB, which also enhances IFN-I expression (Yoshida et al., 2008). Simultaneously, TRAF3 suppresses NF-κB by inhibiting IKK activation upon binding TRAF2 (Zarnegar et al., 2008), likely as a mechanism to skew innate immune effector molecule expression as required. Inversely, the E3 cIAP2, after itself being ubiquitinated by TRAF6, promotes TRAF3 degradation by ligating K48-linked polyubiquitin chains to TRAF3 at residues K107 and K156, thereby restoring NF-κB activation (Tseng et al., 2010). However, as well as degrading TRAF3, cIAP1/2 can also activate TRAF3 by catalyzing its K63-linked polyubiquitination (Fig. 3; Mao et al., 2010). This suggests that the context-dependent ubiquitination state of cIAP1/2 determines its effect on TRAF3. The E3 RNF166 was recently reported to ubiquitinate and activate both TRAF3 and TRAF6 (Chen et al., 2015). Finally, the RIG-I–activating E3 TRIM25 was reported to enhance MDA5-mediated NF-κB activation at the level of TRAF6 (Lee et al., 2015), although mechanistic details remain unclear.

TRAF-mediated signaling is also terminated by ubiquitin in numerous ways. HSV encodes the DUb UL36USP, which strips K63-linked polyubiquitin chains from TRAF3 to prevent downstream protein recruitment (Fig. 3; Wang et al., 2013b), possibly antagonizing cIAP1/2-mediated ubiquitination. TRAF3 and TRAF6 are both deactivated by the DUbs otubain 1 (OTUB1) and OTUB2, which remove K63-linked polyubiquitin chains (Li et al., 2010b). TRAF3 is further deactivated by the deubiquitinase DUBA, which removes K63-linked polyubiquitin chains from TRAF3 (Kayagaki et al., 2007). Furthermore, the E3 Triad3A redirects TRAF3 to the proteasome by ligating K48-linked polyubiquitin chains (Nakhaei et al., 2009). Altogether, this constitutes a ubiquitin-dependent feedback mechanism that enables TRAFs to dictate the direction of immune signal transmission in a context-dependent manner.

The NLRs: An emerging force in antiviral immunity

In contrast to the three RLR receptors, the 22 NLRs have diverse expression patterns and largely under-characterized functions. The NLRs are well recognized for their roles in regulating NF-κB activation and antibacterial immunity; however, at least five members have emerging roles in antiviral immune signaling: NOD1, NOD2, NLRC5 (NLR family CARD domain–containing protein 5), NLRP4 (NACHT, LRR, and PYD domain–containing protein 4), and NLRX1 (NLR family member X1; Fig. 2, right). Although NLRs recruit E3s and modulate the ubiquitination of other proteins, including several in the RLR cascade, the role of PTMs in NLR regulation remains under-defined.

NLR regulation and innate immune signaling cross-talk. The PRRs NOD1 and NOD2 are the best characterized NLRs. NOD1 is expressed ubiquitously, whereas NOD2 is expressed mainly in cells of myeloid and lymphoid origin and is up-regulated during bacterial and viral infection. The classic NOD2-activating ligand is bacterial muramyl dipeptide (MDP), which promotes NF-κB activation. However, NOD2 also promotes IFN-I expression during infection by numerous RNA viruses, in part through recognizing single-stranded

RNA (ssRNA) and interacting with MAVS. NOD2 may also promote IFN-I expression during infection by particular DNA viruses by an undefined mechanism (Sabbah et al., 2009; Kapoor et al., 2014). Accordingly, NOD2 dysfunction leads to inefficient innate and adaptive immune responses to viral infection (Lupfer et al., 2014).

NOD2 features regularly in the immune signaling landscape, yet mechanisms of NOD2 regulation and cross-talk are only beginning to be revealed. Upon activation by MDP, NOD2 is ubiquitinated by TRIM27, leading to NOD2 degradation and NF-κB inhibition (Zurek et al., 2012). NOD2 signaling is further suppressed by AIP4, which ubiquitinates Lys209 of receptor-interacting serine/threonine protein kinase 2 (RIPK2), the immediate downstream interacting partner of NOD2 (Fig. 2, right; Tao et al., 2009). Conversely, NOD2-driven NF-κB activation is enhanced by LUBAC, a negative regulator of RLR signaling, as well as X-linked IAP (XIAP), which respectively ligate M1- and K63-linked polyubiquitin chains to NOD2 and RIPK2 (Damgaard et al., 2012). These activating ubiquitin chains may be antagonized by the ubiquitin-editing enzyme A20 (Hitotsumatsu et al., 2008), which also disrupts ubiquitin-mediated TBK1 activation in the RLR signaling cascade as well as ubiquitin-mediated TRAF6 activation in the TLR cascade (Turer et al., 2008; Parvatiyar et al., 2010).

Another mitochondrial link between the NLR and RLR signaling pathways is the ubiquitously expressed NLRX1 (Fig. 2, right), whose role in IFN-I regulation is controversial. NLRX1 is localized to the mitochondrial outer membrane and was reported to inhibit MAVS-dependent IFN-I signaling by blocking the interaction between activated RIG-I/MDA5 and MAVS, although viral replication experiments using gene knockout cells have produced conflicting results (Soares et al., 2013). NLRX1 also potentiates NF-κB signaling by promoting reactive oxygen species production during bacterial infection, linking the mitochondrial immune signaling platform with proinflammatory cytokine generation (Tattoli et al., 2008).

NLRC5 was initially described to enhance IFN-γ and IFN-α expression and inhibit NF-κB and IFN-β, in the latter case through sequestering the activated effector domains of RIG-I and MDA5 (Cui et al., 2010; Kuenzel et al., 2010). NLRC5 has also been shown to bind and inhibit TBK1-mediated IFN-β induction in HEK-293T cells, although *NLRC5*$^{-/-}$ mice show relatively normal cytokine responses upon exposure to RLR-, TLR-, and NLR-activating stimuli (Kumar et al., 2011). Still other findings indicate that the RIG-I:NLRC5 interaction also positively regulates IFN-β expression, and this interaction is targeted by the IAV-NS1 protein (Fig. 2, right; Neerincx et al., 2010; Ranjan et al., 2015). These disparate conclusions may reflect cell-specific differences given that NLRC5 is predominantly expressed in hematopoietic cells or differences between mouse and human signaling pathways, suggesting that the NLRC5 regulatory framework is complex. Adding to this, ubiquitination plays an uncharacterized role in regulating NLRC5 upon LPS stimulation and may be induced by NLRC5 overexpression (Cui et al., 2010; Kuenzel et al., 2010). Given the diversity of interactions that NLRC5 takes part in, it is likely that further PTMs will be shown to regulate NLRC5 during viral infection.

NLRP4 has gained prominence as another negative regulator of multiple immune signaling pathways that is more widely expressed than NLRC5. NLRP4 was initially described to inhibit IKKα-mediated NF-κB activation (Fiorentino et al., 2002). Upon RLR cascade activation, NLRP4 also inhibits IRF3 activation by recruiting the E3 deltex-4 (DTX4) to ubiquitinate and degrade TBK1 (Cui et al., 2012), revealing yet another route for RLR/NLR cross-talk (Fig. 2, right).

TLR signaling

TLRs are differentially expressed in a wide range of cell populations. TLR3, TLR7, TLR8, and TLR9 are expressed in endosomal vesicles, whereas TLR2 and TLR4 are expressed on the cell surface. TLR3 recognizes double-stranded RNA, activating NF-κB–mediated proinflammatory cytokine production and strongly up-regulating TBK1/IRF3-dependent IFN-I expression. TLR7 and TLR8 recognize ssRNA, up-regulating IFN-α and proinflammatory cytokine production. TLR9 recognizes unmethylated cytosine-phosphate-guanine (CpG) DNA, a common feature of nonmammalian genomes, and stimulates IFN-α production. TLR2 and TLR4 are activated by a variety of microbial ligands, including specific viral proteins, resulting in proinflammatory cytokine expression (Fig. 2, left).

NF-κB activation and IFN-I up-regulation. TLR2, TLR4, TLR7, TLR8, and TLR9 signaling is mediated through the adaptor protein myeloid differentiation primary response gene 88 (MyD88; Fig. 2, left). MyD88 recruits NF-κB and IFN-I signaling components, including interleukin-1 receptor–associated kinase 1 (IRAK1), IRAK4, TRAF6, and IRF7. Activated TRAF6 ubiquitinates IRF7, leading to IFN-α expression (Kawai et al., 2004). TRAF6 also promotes K63-linked polyubiquitination of NEMO, enabling recruitment of the TGF-β–activated kinase (TAB)–TAK1 kinase complex (Tseng et al., 2010). Subsequent association between NEMO and M1-polyubiquitin chains induces TAK1-mediated phosphorylation of IKKα and IKKβ, priming them for full transactivation through autophosphorylation (Zhang et al., 2014). Activated IKKα phosphorylates the IκBα subunit, leading to its ubiquitination and proteasomal degradation and releasing NF-κB for nuclear translocation. Furthermore, MyD88, IRAK1/4, and TAB2/3 are also modified and activated by K63-linked polyubiquitin chains. Such chains were recently described as substrates for M1-polyubiquitination by HOIP, resulting in hybrid chains that may connect the MyD88/IRAK and TAK1/IKK signaling apparatus (Emmerich et al., 2013). TLR3 signaling is mediated by TIR domain–containing adaptor-inducing IFN-β (TRIF). TRIF activates TRAF3, which promotes IRF3/IRF7 activation (Tseng et al., 2010),

and also TRAF6, which promotes IKK activation (Fig. 2, left; Jiang et al., 2004).

Ubiquitin regulates the MyD88- and TRIF-dependent pathways. Although TLRs undergo extensive PTM, ubiquitination performs no known role in regulating TLRs directly. Instead, ubiquitination modulates their downstream signaling targets, particularly MyD88, TRIF, and TRAF6, and it is often here that viruses terminate TLR-mediated immunity.

Nrdp1 is an E3 that promotes IFN-I expression at the expense of proinflammatory cytokines. TBK1 polyubiquitination by Nrdp1 activates TBK1 in TRIF-mediated IFN-I expression, which simultaneously K48-polyubiquitinates and down-regulates MyD88-mediated NF-κB activation (Wang et al., 2009). Conversely, the ubiquitin-editing enzyme A20 inhibits ubiquitin-mediated activation of TRAF6, inhibiting NF-κB activation via the TLR and NLR cascades, as well as TBK1, inhibiting IFN-I expression (Hitotsumatsu et al., 2008; Turer et al., 2008; Parvatiyar et al., 2010). Additional avenues of signaling cross-talk include Smurf1 and Smurf2, which degrade MAVS and inhibit IFN-I activation. Smurf1 and Smurf2 also degrade MyD88, inhibiting TLR-mediated NF-κB activation (Lee et al., 2011).

cIAP2 is an E3 that, after itself being ubiquitinated by TRAF6, targets TRAF3 for degradation (Tseng et al., 2010). This is important in promoting TLR4-mediated signaling and cytokine production at the expense of type I IFN production (Zhong et al., 2013). Furthermore, TRAF6 itself promotes proinflammatory cytokine production at the expense of IFN-I. TRAF6 is activated by trans-autoubiquitination at K124, abolition of which eliminates NEMO ubiquitination and TAK1 activation (Lamothe et al., 2007). This mechanism is exploited by HSV, which uses the virally encoded E3 infected cell polypeptide 0 (ICP0) to recruit USP7 to deubiquitinate NEMO and TRAF6 (Daubeuf et al., 2009). In addition, ICP0 directly catalyzes ubiquitination and degradation of MyD88 and TIRAP (Toll/interleukin-1 receptor domain–containing adaptor protein; van Lint et al., 2010). Kaposi's sarcoma–associated herpesvirus (KSHV) encodes replication and transcription factor (RTA), an E3 that activates latent virus. This activation process involves suppression of antiviral cytokines, partly involving RTA-catalyzed ubiquitination and degradation of MyD88 and TRIF (Ahmad et al., 2011; Zhao et al., 2015), thereby impairing all TLR-mediated immune signaling pathways.

The final relay: IRFs transmit danger signals into the nucleus

TLR, NLR, and RLR IFN-I signaling converges at IRF activation, the penultimate step toward IFN-I transcription. IRF3 is constitutively expressed in most cell types, residing inactive in the cytosol until phosphorylation by TBK1/IKKε within two activation clusters (Ser385/Ser386 and Ser396/Ser398/Ser402/Thr404/Ser405), resulting in homodimerization, nuclear accumulation, DNA binding, and participation in *IFNB* gene transcription (Lin et al., 1998). IFN-β acts in an autocrine and paracrine manner upon its cognate receptor, IFN-α/β receptor (IFNAR), thereby activating JAK/STAT signaling and ISG expression. IRF7 expression is up-regulated in this way, which in turn activates *IFNA* transcription and additional ISG expression by a similar mechanism.

Ubiquitin is an IRF master toggle. The IRFs are among the most tightly controlled IFN-I signaling proteins through an interplay of PTMs, including phosphorylation, ubiquitination, and ubiquitin-like modifications. Phosphorylation of IRF7 at Ser477 and Ser479 by TBK1/IKKε is required for its activation (tenOever et al., 2004). However, ubiquitination by TRAF6 at nearby residues Lys444, Lys446, and Lys452 appears to be a prerequisite to this and serves as a link between the NF-κB and IFN-I activation pathways (Ning et al., 2008). Furthermore, TRIM28 binds active IRF7 and ligates small ubiquitin-like modifier (SUMO) at two of these ubiquitination sites, Lys444 and Lys446, negatively regulating virus-triggered IFN-α production (Liang et al., 2011), indicating that as yet unidentified DUbs or deSUMOylating enzymes participate in regulating IRF7.

Reminiscent of IRF7, IRF3 residues Lys70 and Lys87 accept both polyubiquitin chains and SUMO, and competition between these modifications can determine the fate of IFN-I signal transduction. At steady state, the SUMO-conjugating enzyme ubiquitin carrier protein 9 (Ubc9) protects IRF3 from ubiquitin-mediated degradation by occupying these sites with SUMO. Alternatively, the deSUMOylating enzyme sentrin-specific protease 2 (SENP2) removes SUMO from IRF3, enabling its K48-linked polyubiquitination (Ran et al., 2011). Subsequent work identified TRIM26 as an E3 that conjugates K48-linked polyubiquitin chains to these same sites (Fig. 3; Wang et al., 2015), triggering degradation of the active, nuclear-localized form of IRF3. Furthermore, activated IRF3 undergoes phosphorylation at Ser339. This promotes interaction with peptidyl-prolyl cis/trans isomerase NIMA-interacting 1 (Pin1), a nuclear-localized protein that promotes IRF3 degradation (Saitoh et al., 2006). The E3 recruited by Pin1 for this purpose is unknown; however, TRIM26 is also localized to the nucleus and seems a strong candidate.

IRF3 degradation is undesirable at early stages of the innate immune response and is limited in several ways. The IRF3-Pin1 interaction is inhibited by the HECT (homologous to the E6-AP C terminus) domain and RCC1-like domain–containing protein 5 (HERC5), which ligates another ubiquitin-like protein, ISG15, onto IRF3 at Lys193, Lys360, and Lys366, thereby sustaining IRF3 activation (Shi et al., 2010). TRIM21 is a ubiquitin E3 described to both inhibit the IRF3-Pin1 interaction and target IRF3 for proteasomal degradation (Higgs et al., 2008; Yang et al., 2009). TRIM21 reportedly also targets IRF7 for degradation upon TLR7 or TLR9 activation (Higgs et al., 2010), although the TRIM21-dependent IRF3/IFR7 ubiquitin acceptor sites remain undefined (Fig. 3).

Several additional E3s regulate IRF abundance. The Skp-Cullin–F-box (SCF)–containing complex, of which cullin1 (Cul1) is a core component, catalyzes IRF3 degradation as well as IκB degradation, promoting NF-κB activation (Fig. 3; Bibeau-Poirier et al., 2006). RanBP-type and C3HC4-type zinc finger–containing protein 1 (RBCK1) catalyzes K48-linked polyubiquitination and degradation of IRF3 during viral infection (Fig. 3; Zhang et al., 2008). Finally, the forkhead box protein O1 (FoxO1), a regulator of insulin signaling, binds IRF3 and promotes its degradation by recruiting an unknown E3. FoxO1 also negatively regulates IRF7 transcription (Lei et al., 2013), altogether implying a link between metabolism and innate immune induction. Expression of Cul1 and the E3s RBCK1, TRIM21, TRIM26, and HERC5 is IFN-I inducible (Henig et al., 2013), constituting a multiply redundant negative feedback web in which IFN-I expression is self-restraining.

Not so fast: Seizing the penultimate step toward antiviral gene transcription. IRFs are a significant target of viral disruption, usually resulting in their proteasome-mediated degradation. Rotavirus (RoV) nonstructural protein 1 blocks NF-κB signaling and usurps the ubiquitin modification system to redirect IRF3/5/7/9 to the proteasome in a strain-specific manner (Fig. 3; Morelli et al., 2015). Cells produce trace quantities of IFN-I at rest through basal activation of endogenous IRF3/IRF7, the intracellular concentration of which are regulated by the E3 RTA-associated ubiquitin ligase (RAUL). KSHV exploits this mechanism to diminish immune signaling, recruiting USP7 to deubiquitinate RAUL and thereby maintain RAUL-mediated IRF3/IRF7 degradation (Yu and Hayward, 2010). The RAUL-dependent ubiquitin acceptor sites on IRF3/IRF7 remain unknown (Fig. 3), but better characterization of the RAUL-IRF interaction may have implications for antiviral and autoimmunity treatments. Furthermore, the KSHV RTA protein catalyzes polyubiquitination and degradation of IRF7 and MyD88 (Yu et al., 2005). Thus, KSHV effectively terminates several signaling pathways at multiple stages.

Similar to KSHV, HIV infection fails to stimulate activation of IRF3, endogenous levels of which are quickly reduced upon infection. Underscoring the importance for HIV to disrupt early IFN-I–mediated immunity, IRF3 degradation is independently promoted by two viral accessory proteins, viral infectivity factor (Vif) and viral protein R (Vpr). The E3s hijacked for this purpose are unknown (Fig. 3), although Vif and Vpr recruit SCF-related components to degrade other antiviral proteins (Okumura et al., 2008).

Concluding remarks

The innate immune signaling architecture is complex and has coevolved with the pathogens it guards against, meanwhile restraining autoimmunity through an elaborate negative feedback scheme. A cornerstone of this dynamic regulatory framework is the ubiquitin modification system, which is manipulated by viruses relevant to human disease. Going forward in understanding mechanisms of infection and autoimmunity, we must address significant gaps in knowledge regarding the specificity and context-dependent regulation of E3s and DUbs and the consequences of ubiquitin modification. This will expose further cross-talk between the immune signaling cascades, revealing a functional and self-regulating whole. In the search for a new generation of antiviral and autoimmune treatments, we continue to learn from the pathogens that have long adapted to exploit this ready-made system of functional regulation; humans possess hundreds of specific- and general-effect E3s and DUbs, many of which could be harnessed for therapeutic use.

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REFERENCES

Ahmad, H., R. Gubbels, E. Ehlers, F. Meyer, T. Waterbury, R. Lin, and L. Zhang. 2011. Kaposi sarcoma-associated herpesvirus degrades cellular Toll-interleukin-1 receptor domain-containing adaptor-inducing β-interferon (TRIF). *J. Biol. Chem.* 286:7865–7872. http://dx.doi.org/10.1074/jbc.M110.191452

Arimoto, K., H. Takahashi, T. Hishiki, H. Konishi, T. Fujita, and K. Shimotohno. 2007. Negative regulation of the RIG-I signaling by the ubiquitin ligase RNF125. *Proc. Natl. Acad. Sci. USA.* 104:7500–7505. http://dx.doi.org/10.1073/pnas.0611551104

Banerjee, I., Y. Miyake, S.P. Nobs, C. Schneider, P. Horvath, M. Kopf, P. Matthias, A. Helenius, and Y. Yamauchi. 2014. Influenza A virus uses the aggresome processing machinery for host cell entry. *Science.* 346:473–477. http://dx.doi.org/10.1126/science.1257037

Bibeau-Poirier, A., S.P. Gravel, J.F. Clément, S. Rolland, G. Rodier, P. Coulombe, J. Hiscott, N. Grandvaux, S. Meloche, and M.J. Servant. 2006. Involvement of the IκB kinase (IKK)-related kinases tank-binding kinase 1/IKKi and cullin-based ubiquitin ligases in IFN regulatory factor-3 degradation. *J. Immunol.* 177:5059–5067. http://dx.doi.org/10.4049/jimmunol.177.8.5059

Bouchier-Hayes, L., and S.J. Martin. 2002. CARD games in apoptosis and immunity. *EMBO Rep.* 3:616–621. http://dx.doi.org/10.1093/embo-reports/kvf139

Bruns, A.M., G.P. Leser, R.A. Lamb, and C.M. Horvath. 2014. The innate immune sensor LGP2 activates antiviral signaling by regulating MDA5-RNA interaction and filament assembly. *Mol. Cell.* 55:771–781. http://dx.doi.org/10.1016/j.molcel.2014.07.003

Castanier, C., N. Zemirli, A. Portier, D. Garcin, N. Bidère, A. Vazquez, and D. Arnoult. 2012. MAVS ubiquitination by the E3 ligase TRIM25 and degradation by the proteasome is involved in type I interferon production after activation of the antiviral RIG-I-like receptors. *BMC Biol.* 10:44. http://dx.doi.org/10.1186/1741-7007-10-44

Chen, H.W., Y.K. Yang, H. Xu, W.W. Yang, Z.H. Zhai, and D.Y. Chen. 2015. Ring finger protein 166 potentiates RNA virus-induced interferon-β production via enhancing the ubiquitination of TRAF3 and TRAF6. *Sci. Rep.* 5:14770. http://dx.doi.org/10.1038/srep14770

Crampton, S.P., J.A. Deane, L. Feigenbaum, and S. Bolland. 2012. Ifih1 gene dose effect reveals MDA5-mediated chronic type I IFN gene signature,

viral resistance, and accelerated autoimmunity. *J. Immunol.* 188:1451–1459. http://dx.doi.org/10.4049/jimmunol.1102705

Cui, J., L. Zhu, X. Xia, H.Y. Wang, X. Legras, J. Hong, J. Ji, P. Shen, S. Zheng, Z.J. Chen, and R.F. Wang. 2010. NLRC5 negatively regulates the NF-κB and type I interferon signaling pathways. *Cell.* 141:483–496. http://dx.doi.org/10.1016/j.cell.2010.03.040

Cui, J., Y. Li, L. Zhu, D. Liu, Z. Songyang, H.Y. Wang, and R.F. Wang. 2012. NLRP4 negatively regulates type I interferon signaling by targeting the kinase TBK1 for degradation via the ubiquitin ligase DTX4. *Nat. Immunol.* 13:387–395. http://dx.doi.org/10.1038/ni.2239

Cui, J., Y. Song, Y. Li, Q. Zhu, P. Tan, Y. Qin, H.Y. Wang, and R.F. Wang. 2014. USP3 inhibits type I interferon signaling by deubiquitinating RIG-I-like receptors. *Cell Res.* 24:400–416. http://dx.doi.org/10.1038/cr.2013.170

Damgaard, R.B., U. Nachbur, M. Yabal, W.W. Wong, B.K. Fiil, M. Kastirr, E. Rieser, J.A. Rickard, A. Bankovacki, C. Peschel, et al. 2012. The ubiquitin ligase XIAP recruits LUBAC for NOD2 signaling in inflammation and innate immunity. *Mol. Cell.* 46:746–758. http://dx.doi.org/10.1016/j.molcel.2012.04.014

Daubeuf, S., D. Singh, Y. Tan, H. Liu, H.J. Federoff, W.J. Bowers, and K. Tolba. 2009. HSV ICP0 recruits USP7 to modulate TLR-mediated innate response. *Blood.* 113:3264–3275. http://dx.doi.org/10.1182/blood-2008-07-168203

Diner, B.A., K.K. Lum, and I.M. Cristea. 2015. The emerging role of nuclear viral DNA sensors. *J. Biol. Chem.* 290:26412–26421. http://dx.doi.org/10.1074/jbc.R115.652289

Dixit, E., S. Boulant, Y. Zhang, A.S. Lee, C. Odendall, B. Shum, N. Hacohen, Z.J. Chen, S.P. Whelan, M. Fransen, et al. 2010. Peroxisomes are signaling platforms for antiviral innate immunity. *Cell.* 141:668–681. http://dx.doi.org/10.1016/j.cell.2010.04.018

Emmerich, C.H., A. Ordureau, S. Strickson, J.S. Arthur, P.G. Pedrioli, D. Komander, and P. Cohen. 2013. Activation of the canonical IKK complex by K63/M1-linked hybrid ubiquitin chains. *Proc. Natl. Acad. Sci. USA.* 110:15247–15252. http://dx.doi.org/10.1073/pnas.1314715110

Fiorentino, L., C. Stehlik, V. Oliveira, M.E. Ariza, A. Godzik, and J.C. Reed. 2002. A novel PAAD-containing protein that modulates NF-κB induction by cytokines tumor necrosis factor-α and interleukin-1β. *J. Biol. Chem.* 277:35333–35340. http://dx.doi.org/10.1074/jbc.M200446200

Friedman, C.S., M.A. O'Donnell, D. Legarda-Addison, A. Ng, W.B. Cárdenas, J.S. Yount, T.M. Moran, C.F. Basler, A. Komuro, C.M. Horvath, et al. 2008. The tumour suppressor CYLD is a negative regulator of RIG-I-mediated antiviral response. *EMBO Rep.* 9:930–936. http://dx.doi.org/10.1038/embor.2008.136

Gack, M.U., Y.C. Shin, C.H. Joo, T. Urano, C. Liang, L. Sun, O. Takeuchi, S. Akira, Z. Chen, S. Inoue, and J.U. Jung. 2007. TRIM25 RING-finger E3 ubiquitin ligase is essential for RIG-I-mediated antiviral activity. *Nature.* 446:916–920. http://dx.doi.org/10.1038/nature05732

Gack, M.U., R.A. Albrecht, T. Urano, K.S. Inn, I.C. Huang, E. Carnero, M. Farzan, S. Inoue, J.U. Jung, and A. García-Sastre. 2009. Influenza A virus NS1 targets the ubiquitin ligase TRIM25 to evade recognition by the host viral RNA sensor RIG-I. *Cell Host Microbe.* 5:439–449. http://dx.doi.org/10.1016/j.chom.2009.04.006

Gao, D., Y.K. Yang, R.P. Wang, X. Zhou, F.C. Diao, M.D. Li, Z.H. Zhai, Z.F. Jiang, and D.Y. Chen. 2009. REUL is a novel E3 ubiquitin ligase and stimulator of retinoic-acid-inducible gene-I. *PLoS One.* 4:e5760. http://dx.doi.org/10.1371/journal.pone.0005760

Goncalves, A., T. Bürckstümmer, E. Dixit, R. Scheicher, M.W. Górna, E. Karayel, C. Sugar, A. Stukalov, T. Berg, R. Kralovics, et al. 2011. Functional dissection of the TBK1 molecular network. *PLoS One.* 6:e23971. http://dx.doi.org/10.1371/journal.pone.0023971

Guo, B., and G. Cheng. 2007. Modulation of the interferon antiviral response by the TBK1/IKKi adaptor protein TANK. *J. Biol. Chem.* 282:11817–11826. http://dx.doi.org/10.1074/jbc.M700017200

Habelhah, H., S. Takahashi, S.G. Cho, T. Kadoya, T. Watanabe, and Z. Ronai. 2004. Ubiquitination and translocation of TRAF2 is required for activation of JNK but not of p38 or NF-κB. *EMBO J.* 23:322–332. http://dx.doi.org/10.1038/sj.emboj.7600044

Henig, N., N. Avidan, I. Mandel, E. Staun-Ram, E. Ginzburg, T. Paperna, R.Y. Pinter, and A. Miller. 2013. Interferon-beta induces distinct gene expression response patterns in human monocytes versus T cells. *PLoS One.* 8:e62366. http://dx.doi.org/10.1371/journal.pone.0062366

Higgs, R., J. Ní Gabhann, N. Ben Larbi, E.P. Breen, K.A. Fitzgerald, and C.A. Jefferies. 2008. The E3 ubiquitin ligase Ro52 negatively regulates IFN-β production post-pathogen recognition by polyubiquitin-mediated degradation of IRF3. *J. Immunol.* 181:1780–1786. http://dx.doi.org/10.4049/jimmunol.181.3.1780

Higgs, R., E. Lazzari, C. Wynne, J. Ní Gabhann, A. Espinosa, M. Wahren-Herlenius, and C.A. Jefferies. 2010. Self protection from anti-viral responses—Ro52 promotes degradation of the transcription factor IRF7 downstream of the viral Toll-Like receptors. *PLoS One.* 5:e11776. http://dx.doi.org/10.1371/journal.pone.0011776

Hitotsumatsu, O., R.C. Ahmad, R. Tavares, M. Wang, D. Philpott, E.E. Turer, B.L. Lee, N. Shiffin, R. Advincula, B.A. Malynn, et al. 2008. The ubiquitin-editing enzyme A20 restricts nucleotide-binding oligomerization domain containing 2-triggered signals. *Immunity.* 28:381–390. http://dx.doi.org/10.1016/j.immuni.2008.02.002

Inn, K.S., M.U. Gack, F. Tokunaga, M. Shi, L.Y. Wong, K. Iwai, and J.U. Jung. 2011a. Linear ubiquitin assembly complex negatively regulates RIG-I- and TRIM25-mediated type I interferon induction. *Mol. Cell.* 41:354–365. http://dx.doi.org/10.1016/j.molcel.2010.12.029

Inn, K.S., S.H. Lee, J.Y. Rathbun, L.Y. Wong, Z. Toth, K. Machida, J.H. Ou, and J.U. Jung. 2011b. Inhibition of RIG-I-mediated signaling by Kaposi's sarcoma-associated herpesvirus-encoded deubiquitinase ORF64. *J. Virol.* 85:10899–10904. http://dx.doi.org/10.1128/JVI.00690-11

Ishikawa, H., and G.N. Barber. 2008. STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. *Nature.* 455:674–678. http://dx.doi.org/10.1038/nature07317

Jiang, Z., T.W. Mak, G. Sen, and X. Li. 2004. Toll-like receptor 3-mediated activation of NF-κB and IRF3 diverges at Toll-IL-1 receptor domain-containing adapter inducing IFN-β. *Proc. Natl. Acad. Sci. USA.* 101:3533–3538. http://dx.doi.org/10.1073/pnas.0308496101

Jiao, S., Z. Zhang, C. Li, M. Huang, Z. Shi, Y. Wang, X. Song, H. Liu, C. Li, M. Chen, et al. 2015. The kinase MST4 limits inflammatory responses through direct phosphorylation of the adaptor TRAF6. *Nat. Immunol.* 16:246–257. http://dx.doi.org/10.1038/ni.3097

Kapoor, A., M. Forman, and R. Arav-Boger. 2014. Activation of nucleotide oligomerization domain 2 (NOD2) by human cytomegalovirus initiates innate immune responses and restricts virus replication. *PLoS One.* 9:e92704. http://dx.doi.org/10.1371/journal.pone.0092704

Kawai, T., S. Sato, K.J. Ishii, C. Coban, H. Hemmi, M. Yamamoto, K. Terai, M. Matsuda, J. Inoue, S. Uematsu, et al. 2004. Interferon-α induction through Toll-like receptors involves a direct interaction of IRF7 with MyD88 and TRAF6. *Nat. Immunol.* 5:1061–1068. http://dx.doi.org/10.1038/ni1118

Kayagaki, N., Q. Phung, S. Chan, R. Chaudhari, C. Quan, K.M. O'Rourke, M. Eby, E. Pietras, G. Cheng, J.F. Bazan, et al. 2007. DUBA: a deubiquitinase that regulates type I interferon production. *Science.* 318:1628–1632. http://dx.doi.org/10.1126/science.1145918

Kuenzel, S., A. Till, M. Winkler, R. Häsler, S. Lipinski, S. Jung, J. Grötzinger, H. Fickenscher, S. Schreiber, and P. Rosenstiel. 2010. The nucleotide-binding oligomerization domain-like receptor NLRC5 is involved in IFN-dependent antiviral immune responses. *J. Immunol.* 184:1990–2000. http://dx.doi.org/10.4049/jimmunol.0900557

Kumar, H., S. Pandey, J. Zou, Y. Kumagai, K. Takahashi, S. Akira, and T. Kawai. 2011. NLRC5 deficiency does not influence cytokine induction by virus and bacteria infections. *J. Immunol.* 186:994–1000. http://dx.doi.org/10.4049/jimmunol.1002094

Lamothe, B., A. Besse, A.D. Campos, W.K. Webster, H. Wu, and B.G. Darnay. 2007. Site-specific Lys-63-linked tumor necrosis factor receptor-associated factor 6 auto-ubiquitination is a critical determinant of IκB kinase activation. *J. Biol. Chem.* 282:4102–4112. http://dx.doi.org/10.1074/jbc.M609503200

Lee, N.R., H.I. Kim, M.S. Choi, C.M. Yi, and K.S. Inn. 2015. Regulation of MDA5-MAVS antiviral signaling axis by TRIM25 through TRAF6-mediated NF-κB activation. *Mol. Cells.* 38:759–764. http://dx.doi.org/10.14348/molcells.2015.0047

Lee, Y.S., J.S. Park, J.H. Kim, S.M. Jung, J.Y. Lee, S.J. Kim, and S.H. Park. 2011. Smad6-specific recruitment of Smurf E3 ligases mediates TGF-β1-induced degradation of MyD88 in TLR4 signalling. *Nat. Commun.* 2:460. http://dx.doi.org/10.1038/ncomms1469

Lei, C.Q., Y. Zhang, T. Xia, L.Q. Jiang, B. Zhong, and H.B. Shu. 2013. FoxO1 negatively regulates cellular antiviral response by promoting degradation of IRF3. *J. Biol. Chem.* 288:12596–12604. http://dx.doi.org/10.1074/jbc.M112.444794

Li, S., K. Lu, J. Wang, L. An, G. Yang, H. Chen, Y. Cui, X. Yin, P. Xie, G. Xing, et al. 2010a. Ubiquitin ligase Smurf1 targets TRAF family proteins for ubiquitination and degradation. *Mol. Cell. Biochem.* 338:11–17. http://dx.doi.org/10.1007/s11010-009-0315-y

Li, S., H. Zheng, A.P. Mao, B. Zhong, Y. Li, Y. Liu, Y. Gao, Y. Ran, P. Tien, and H.B. Shu. 2010b. Regulation of virus-triggered signaling by OTUB1- and OTUB2-mediated deubiquitination of TRAF3 and TRAF6. *J. Biol. Chem.* 285:4291–4297. http://dx.doi.org/10.1074/jbc.M109.074971

Li, W., M.H. Bengtson, A. Ulbrich, A. Matsuda, V.A. Reddy, A. Orth, S.K. Chanda, S. Batalov, and C.A.P. Joazeiro. 2008. Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. *PLoS One.* 3:e1487. http://dx.doi.org/10.1371/journal.pone.0001487

Liang, Q., H. Deng, X. Li, X. Wu, Q. Tang, T.H. Chang, H. Peng, F.J. Rauscher III, K. Ozato, and F. Zhu. 2011. Tripartite motif-containing protein 28 is a small ubiquitin-related modifier E3 ligase and negative regulator of IFN regulatory factor 7. *J. Immunol.* 187:4754–4763. http://dx.doi.org/10.4049/jimmunol.1101704

Lin, R., C. Heylbroeck, P.M. Pitha, and J. Hiscott. 1998. Virus-dependent phosphorylation of the IRF-3 transcription factor regulates nuclear translocation, transactivation potential, and proteasome-mediated degradation. *Mol. Cell. Biol.* 18:2986–2996. http://dx.doi.org/10.1128/MCB.18.5.2986

Lupfer, C., P.G. Thomas, and T.D. Kanneganti. 2014. Nucleotide oligomerization and binding domain 2-dependent dendritic cell activation is necessary for innate immunity and optimal $CD8^+$ T Cell responses to influenza A virus infection. *J. Virol.* 88:8946–8955. http://dx.doi.org/10.1128/JVI.01110-14

Mao, A.P., S. Li, B. Zhong, Y. Li, J. Yan, Q. Li, C. Teng, and H.B. Shu. 2010. Virus-triggered ubiquitination of TRAF3/6 by cIAP1/2 is essential for induction of interferon-β (IFN-β) and cellular antiviral response. *J. Biol. Chem.* 285:9470–9476. http://dx.doi.org/10.1074/jbc.M109.071043

Marinis, J.M., J.E. Hutti, C.R. Homer, B.A. Cobb, L.C. Cantley, C. McDonald, and D.W. Abbott. 2012. IκB kinase α phosphorylation of TRAF4 downregulates innate immune signaling. *Mol. Cell. Biol.* 32:2479–2489. http://dx.doi.org/10.1128/MCB.00106-12

Morelli, M., A.F. Dennis, and J.T. Patton. 2015. Putative E3 ubiquitin ligase of human rotavirus inhibits NF-κB activation by using molecular mimicry to target β-TrCP. *MBio.* 6:e02490-14. http://dx.doi.org/10.1128/mBio.02490-14

Nakhaei, P., T. Mesplede, M. Solis, Q. Sun, T. Zhao, L. Yang, T.H. Chuang, C.F. Ware, R. Lin, and J. Hiscott. 2009. The E3 ubiquitin ligase Triad3A negatively regulates the RIG-I/MAVS signaling pathway by targeting TRAF3 for degradation. *PLoS Pathog.* 5:e1000650. http://dx.doi.org/10.1371/journal.ppat.1000650

Neerincx, A., K. Lautz, M. Menning, E. Kremmer, P. Zigrino, M. Hösel, H. Büning, R. Schwarzenbacher, and T.A. Kufer. 2010. A role for the human nucleotide-binding domain, leucine-rich repeat-containing family member NLRC5 in antiviral responses. *J. Biol. Chem.* 285:26223–26232. http://dx.doi.org/10.1074/jbc.M110.109736

Nguyen, N.H., S.A. McCormack, B.E. Yee, P. Devaki, D. Jencks, D.T. Chao, and M.H. Nguyen. 2014. Meta-analysis of patients with hepatitis C virus genotype 6: 48 weeks with pegylated interferon and ribavirin is superior to 24 weeks. *Hepatol. Int.* 8:540–549. http://dx.doi.org/10.1007/s12072-014-9570-4

Ning, S., A.D. Campos, B.G. Darnay, G.L. Bentz, and J.S. Pagano. 2008. TRAF6 and the three C-terminal lysine sites on IRF7 are required for its ubiquitination-mediated activation by the tumor necrosis factor receptor family member latent membrane protein 1. *Mol. Cell. Biol.* 28:6536–6546. http://dx.doi.org/10.1128/MCB.00785-08

Now, H., and J.Y. Yoo. 2011. A protein-kinase, IFN-inducible double-stranded RNA dependent inhibitor and repressor of p58 (PRKRIR) enhances type I IFN-mediated antiviral response through the stability control of RIG-I protein. *Biochem. Biophys. Res. Commun.* 413:487–493. http://dx.doi.org/10.1016/j.bbrc.2011.08.127

Oberman, F., and A. Panet. 1988. Inhibition of transcription of herpes simplex virus immediate early genes in interferon-treated human cells. *J. Gen. Virol.* 69:1167–1177. http://dx.doi.org/10.1099/0022-1317-69-6-1167

Okumura, A., T. Alce, B. Lubyova, H. Ezelle, K. Strebel, and P.M. Pitha. 2008. HIV-1 accessory proteins VPR and Vif modulate antiviral response by targeting IRF-3 for degradation. *Virology.* 373:85–97. http://dx.doi.org/10.1016/j.virol.2007.10.042

Oshiumi, H., M. Matsumoto, S. Hatakeyama, and T. Seya. 2009. Riplet/RNF135, a RING finger protein, ubiquitinates RIG-I to promote interferon-β induction during the early phase of viral infection. *J. Biol. Chem.* 284:807–817. http://dx.doi.org/10.1074/jbc.M804259200

Oshiumi, H., M. Miyashita, N. Inoue, M. Okabe, M. Matsumoto, and T. Seya. 2010. The ubiquitin ligase Riplet is essential for RIG-I-dependent innate immune responses to RNA virus infection. *Cell Host Microbe.* 8:496–509. http://dx.doi.org/10.1016/j.chom.2010.11.008

Oshiumi, H., M. Miyashita, M. Matsumoto, and T. Seya. 2013. A distinct role of Riplet-mediated K63-Linked polyubiquitination of the RIG-I repressor domain in human antiviral innate immune responses. *PLoS Pathog.* 9:e1003533. http://dx.doi.org/10.1371/journal.ppat.1003533

Pan, Y., R. Li, J.L. Meng, H.T. Mao, Y. Zhang, and J. Zhang. 2014. Smurf2 negatively modulates RIG-I-dependent antiviral response by targeting VISA/MAVS for ubiquitination and degradation. *J. Immunol.* 192:4758–4764. http://dx.doi.org/10.4049/jimmunol.1302632

Parvatiyar, K., G.N. Barber, and E.W. Harhaj. 2010. TAX1BP1 and A20 inhibit antiviral signaling by targeting TBK1-IKKi kinases. *J. Biol. Chem.* 285:14999–15009. http://dx.doi.org/10.1074/jbc.M110.109819

Paz, S., M. Vilasco, M. Arguello, Q. Sun, J. Lacoste, T.L. Nguyen, T. Zhao, E.A. Shestakova, S. Zaari, A. Bibeau-Poirier, et al. 2009. Ubiquitin-regulated recruitment of IκB kinase ε to the MAVS interferon signaling adapter. *Mol. Cell. Biol.* 29:3401–3412. http://dx.doi.org/10.1128/MCB.00880-08

Paz, S., M. Vilasco, S.J. Werden, M. Arguello, D. Joseph-Pillai, T. Zhao, T.L. Nguyen, Q. Sun, E.F. Meurs, R. Lin, and J. Hiscott. 2011. A functional C-terminal TRAF3-binding site in MAVS participates in positive and negative regulation of the IFN antiviral response. *Cell Res.* 21:895–910. http://dx.doi.org/10.1038/cr.2011.2

Peisley, A., M.H. Jo, C. Lin, B. Wu, M. Orme-Johnson, T. Walz, S. Hohng, and S. Hur. 2012. Kinetic mechanism for viral dsRNA length discrimination

by MDA5 filaments. *Proc. Natl. Acad. Sci. USA.* 109:E3340–E3349. http://dx.doi.org/10.1073/pnas.1208618109

Peisley, A., B. Wu, H. Xu, Z.J. Chen, and S. Hur. 2014. Structural basis for ubiquitin-mediated antiviral signal activation by RIG-I. *Nature.* 509:110–114. http://dx.doi.org/10.1038/nature13140

Rajsbaum, R., R.A. Albrecht, M.K. Wang, N.P. Maharaj, G.A. Versteeg, E. Nistal-Villán, A. García-Sastre, and M.U. Gack. 2012. Species-specific inhibition of RIG-I ubiquitination and IFN induction by the influenza A virus NS1 protein. *PLoS Pathog.* 8:e1003059. http://dx.doi.org/10.1371/journal.ppat.1003059

Ran, Y., T.T. Liu, Q. Zhou, S. Li, A.P. Mao, Y. Li, L.J. Liu, J.K. Cheng, and H.B. Shu. 2011. SENP2 negatively regulates cellular antiviral response by deSUMOylating IRF3 and conditioning it for ubiquitination and degradation. *J. Mol. Cell Biol.* 3:283–292. http://dx.doi.org/10.1093/jmcb/mjr020

Ranjan, P., N. Singh, A. Kumar, A. Neerincx, E. Kremmer, W. Cao, W.G. Davis, J.M. Katz, S. Gangappa, R. Lin, et al. 2015. NLRC5 interacts with RIG-I to induce a robust antiviral response against influenza virus infection. *Eur. J. Immunol.* 45:758–772. http://dx.doi.org/10.1002/eji.201344412

Sabbah, A., T.H. Chang, R. Harnack, V. Frohlich, K. Tominaga, P.H. Dube, Y. Xiang, and S. Bose. 2009. Activation of innate immune antiviral responses by Nod2. *Nat. Immunol.* 10:1073–1080. http://dx.doi.org/10.1038/ni.1782

Saito, T., R. Hirai, Y.M. Loo, D. Owen, C.L. Johnson, S.C. Sinha, S. Akira, T. Fujita, and M. Gale Jr. 2007. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. *Proc. Natl. Acad. Sci. USA.* 104:582–587. http://dx.doi.org/10.1073/pnas.0606699104

Saitoh, T., A. Tun-Kyi, A. Ryo, M. Yamamoto, G. Finn, T. Fujita, S. Akira, N. Yamamoto, K.P. Lu, and S. Yamaoka. 2006. Negative regulation of interferon-regulatory factor 3-dependent innate antiviral response by the prolyl isomerase Pin1. *Nat. Immunol.* 7:598–605. http://dx.doi.org/10.1038/ni1347

Schlee, M. 2013. Master sensors of pathogenic RNA - RIG-I like receptors. *Immunobiology.* 218:1322–1335. http://dx.doi.org/10.1016/j.imbio.2013.06.007

Schlee, M., A. Roth, V. Hornung, C.A. Hagmann, V. Wimmenauer, W. Barchet, C. Coch, M. Janke, A. Mihailovic, G. Wardle, et al. 2009. Recognition of 5′ triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. *Immunity.* 31:25–34. http://dx.doi.org/10.1016/j.immuni.2009.05.008

Shi, C.S., H.Y. Qi, C. Boularan, N.N. Huang, M. Abu-Asab, J.H. Shelhamer, and J.H. Kehrl. 2014. SARS-coronavirus open reading frame-9b suppresses innate immunity by targeting mitochondria and the MAVS/TRAF3/TRAF6 signalosome. *J. Immunol.* 193:3080–3089. http://dx.doi.org/10.4049/jimmunol.1303196

Shi, H.X., K. Yang, X. Liu, X.Y. Liu, B. Wei, Y.F. Shan, L.H. Zhu, and C. Wang. 2010. Positive regulation of interferon regulatory factor 3 activation by Herc5 via ISG15 modification. *Mol. Cell. Biol.* 30:2424–2436. http://dx.doi.org/10.1128/MCB.01466-09

Soares, F., I. Tattoli, M.E. Wortzman, D. Arnoult, D.J. Philpott, and S.E. Girardin. 2013. NLRX1 does not inhibit MAVS-dependent antiviral signalling. *Innate Immun.* 19:438–448. http://dx.doi.org/10.1177/1753425912467383

Tao, M., P.C. Scacheri, J.M. Marinis, E.W. Harhaj, L.E. Matesic, and D.W. Abbott. 2009. ITCH K63-ubiquitinates the NOD2 binding protein, RIP2, to influence inflammatory signaling pathways. *Curr. Biol.* 19:1255–1263. http://dx.doi.org/10.1016/j.cub.2009.06.038

Tattoli, I., L.A. Carneiro, M. Jéhanno, J.G. Magalhaes, Y. Shu, D.J. Philpott, D. Arnoult, and S.E. Girardin. 2008. NLRX1 is a mitochondrial NOD-like receptor that amplifies NF-κB and JNK pathways by inducing reactive oxygen species production. *EMBO Rep.* 9:293–300. http://dx.doi.org/10.1038/sj.embor.7401161

tenOever, B.R., S. Sharma, W. Zou, Q. Sun, N. Grandvaux, I. Julkunen, H. Hemmi, M. Yamamoto, S. Akira, W.C. Yeh, et al. 2004. Activation of TBK1 and IKKε kinases by vesicular stomatitis virus infection and the role of viral ribonucleoprotein in the development of interferon antiviral immunity. *J. Virol.* 78:10636–10649. http://dx.doi.org/10.1128/JVI.78.19.10636-10649.2004

Tigno-Aranjuez, J.T., X. Bai, and D.W. Abbott. 2013. A discrete ubiquitin-mediated network regulates the strength of NOD2 signaling. *Mol. Cell. Biol.* 33:146–158. http://dx.doi.org/10.1128/MCB.01049-12

Tseng, P.H., A. Matsuzawa, W. Zhang, T. Mino, D.A. Vignali, and M. Karin. 2010. Different modes of ubiquitination of the adaptor TRAF3 selectively activate the expression of type I interferons and proinflammatory cytokines. *Nat. Immunol.* 11:70–75. http://dx.doi.org/10.1038/ni.1819

Turer, E.E., R.M. Tavares, E. Mortier, O. Hitotsumatsu, R. Advincula, B. Lee, N. Shifrin, B.A. Malynn, and A. Ma. 2008. Homeostatic MyD88-dependent signals cause lethal inflammation in the absence of A20. *J. Exp. Med.* 205:451–464. http://dx.doi.org/10.1084/jem.20071108

van Lint, A.L., M.R. Murawski, R.E. Goodbody, M. Severa, K.A. Fitzgerald, R.W. Finberg, D.M. Knipe, and E.A. Kurt-Jones. 2010. Herpes simplex virus immediate-early ICP0 protein inhibits Toll-like receptor 2-dependent inflammatory responses and NF-κB signaling. *J. Virol.* 84:10802–10811. http://dx.doi.org/10.1128/JVI.00063-10

van Zuylen, W.J., P. Doyon, J.F. Clément, K.A. Khan, L.M. D'Ambrosio, F. Dô, M. St-Amant-Verret, T. Wissanji, G. Emery, A.C. Gingras, et al. 2012. Proteomic profiling of the TRAF3 interactome network reveals a new role for the ER-to-Golgi transport compartments in innate immunity. *PLoS Pathog.* 8:e1002747. http://dx.doi.org/10.1371/journal.ppat.1002747

Wang, C., T. Chen, J. Zhang, M. Yang, N. Li, X. Xu, and X. Cao. 2009. The E3 ubiquitin ligase Nrdp1 'preferentially' promotes TLR-mediated production of type I interferon. *Nat. Immunol.* 10:744–752. http://dx.doi.org/10.1038/ni.1742

Wang, L., S. Li, and M.E. Dorf. 2012a. NEMO binds ubiquitinated TANK-binding kinase 1 (TBK1) to regulate innate immune responses to RNA viruses. *PLoS One.* 7:e43756. http://dx.doi.org/10.1371/journal.pone.0043756

Wang, L., W. Zhao, M. Zhang, P. Wang, K. Zhao, X. Zhao, S. Yang, and C. Gao. 2013a. USP4 positively regulates RIG-I-mediated antiviral response through deubiquitination and stabilization of RIG-I. *J. Virol.* 87:4507–4515. http://dx.doi.org/10.1128/JVI.00031-13

Wang, P., W. Zhao, K. Zhao, L. Zhang, and C. Gao. 2015. TRIM26 negatively regulates interferon-β production and antiviral response through polyubiquitination and degradation of nuclear IRF3. *PLoS Pathog.* 11:e1004726. http://dx.doi.org/10.1371/journal.ppat.1004726

Wang, S., K. Wang, J. Li, and C. Zheng. 2013b. Herpes simplex virus 1 ubiquitin-specific protease UL36 inhibits beta interferon production by deubiquitinating TRAF3. *J. Virol.* 87:11851–11860. http://dx.doi.org/10.1128/JVI.01211-13

Wang, Y., X. Tong, E.S. Omoregie, W. Liu, S. Meng, and X. Ye. 2012b. Tetraspanin 6 (TSPAN6) negatively regulates retinoic acid-inducible gene I-like receptor-mediated immune signaling in a ubiquitination-dependent manner. *J. Biol. Chem.* 287:34626–34634. http://dx.doi.org/10.1074/jbc.M112.390401

Wang, Y., X. Tong, and X. Ye. 2012c. Ndfip1 negatively regulates RIG-I-dependent immune signaling by enhancing E3 ligase Smurf1-mediated MAVS degradation. *J. Immunol.* 189:5304–5313. http://dx.doi.org/10.4049/jimmunol.1201445

Wei, C., C. Ni, T. Song, Y. Liu, X. Yang, Z. Zheng, Y. Jia, Y. Yuan, K. Guan, Y. Xu, et al. 2010. The hepatitis B virus X protein disrupts innate immunity by downregulating mitochondrial antiviral signaling protein. *J. Immunol.* 185:1158–1168. http://dx.doi.org/10.4049/jimmunol.0903874

Yan, J., Q. Li, A.P. Mao, M.M. Hu, and H.B. Shu. 2014. TRIM4 modulates type I interferon induction and cellular antiviral response by targeting

RIG-I for K63-linked ubiquitination. *J. Mol. Cell Biol.* 6:154–163. http://dx.doi.org/10.1093/jmcb/mju005

Yang, K., H.X. Shi, X.Y. Liu, Y.F. Shan, B. Wei, S. Chen, and C. Wang. 2009. TRIM21 is essential to sustain IFN regulatory factor 3 activation during antiviral response. *J. Immunol.* 182:3782–3792. http://dx.doi.org/10.4049/jimmunol.0803126

Yoo, Y.S., Y.Y. Park, J.H. Kim, H. Cho, S.H. Kim, H.S. Lee, T.H. Kim, Y. Sun Kim, Y. Lee, C.J. Kim, et al. 2015. The mitochondrial ubiquitin ligase MARCH5 resolves MAVS aggregates during antiviral signalling. *Nat. Commun.* 6:7910. http://dx.doi.org/10.1038/ncomms8910

Yoshida, R., G. Takaesu, H. Yoshida, F. Okamoto, T. Yoshioka, Y. Choi, S. Akira, T. Kawai, A. Yoshimura, and T. Kobayashi. 2008. TRAF6 and MEKK1 play a pivotal role in the RIG-I-like helicase antiviral pathway. *J. Biol. Chem.* 283:36211–36220. http://dx.doi.org/10.1074/jbc.M806576200

You, F., H. Sun, X. Zhou, W. Sun, S. Liang, Z. Zhai, and Z. Jiang. 2009. PCBP2 mediates degradation of the adaptor MAVS via the HECT ubiquitin ligase AIP4. *Nat. Immunol.* 10:1300–1308. http://dx.doi.org/10.1038/ni.1815

Yu, Y., and G.S. Hayward. 2010. The ubiquitin E3 ligase RAUL negatively regulates type I interferon through ubiquitination of the transcription factors IRF7 and IRF3. *Immunity.* 33:863–877. http://dx.doi.org/10.1016/j.immuni.2010.11.027

Yu, Y., S.E. Wang, and G.S. Hayward. 2005. The KSHV immediate-early transcription factor RTA encodes ubiquitin E3 ligase activity that targets IRF7 for proteosome-mediated degradation. *Immunity.* 22:59–70. http://dx.doi.org/10.1016/j.immuni.2004.11.011

Zarnegar, B., S. Yamazaki, J.Q. He, and G. Cheng. 2008. Control of canonical NF-κB activation through the NIK-IKK complex pathway. *Proc. Natl. Acad. Sci. USA.* 105:3503–3508. http://dx.doi.org/10.1073/pnas.0707959105

Zeng, W., M. Xu, S. Liu, L. Sun, and Z.J. Chen. 2009. Key role of Ubc5 and lysine-63 polyubiquitination in viral activation of IRF3. *Mol. Cell.* 36:315–325. http://dx.doi.org/10.1016/j.molcel.2009.09.037

Zhang, J., K. Clark, T. Lawrence, M.W. Peggie, and P. Cohen. 2014. An unexpected twist to the activation of IKKβ: TAK1 primes IKKβ for activation by autophosphorylation. *Biochem. J.* 461:531–537. http://dx.doi.org/10.1042/BJ20140444

Zhang, M., Y. Tian, R.P. Wang, D. Gao, Y. Zhang, F.C. Diao, D.Y. Chen, Z.H. Zhai, and H.B. Shu. 2008. Negative feedback regulation of cellular antiviral signaling by RBCK1-mediated degradation of IRF3. *Cell Res.* 18:1096–1104. http://dx.doi.org/10.1038/cr.2008.277

Zhao, Q., D. Liang, R. Sun, B. Jia, T. Xia, H. Xiao, and K. Lan. 2015. Kaposi's sarcoma-associated herpesvirus-encoded replication and transcription activator impairs innate immunity via ubiquitin-mediated degradation of myeloid differentiation factor 88. *J. Virol.* 89:415–427. http://dx.doi.org/10.1128/JVI.02591-14

Zhao, T., L. Yang, Q. Sun, M. Arguello, D.W. Ballard, J. Hiscott, and R. Lin. 2007. The NEMO adaptor bridges the nuclear factor-κB and interferon regulatory factor signaling pathways. *Nat. Immunol.* 8:592–600. http://dx.doi.org/10.1038/ni1465

Zhong, B., Y. Zhang, B. Tan, T.T. Liu, Y.Y. Wang, and H.B. Shu. 2010. The E3 ubiquitin ligase RNF5 targets virus-induced signaling adaptor for ubiquitination and degradation. *J. Immunol.* 184:6249–6255. http://dx.doi.org/10.4049/jimmunol.0903748

Zhong, B., X. Liu, X. Wang, S.H. Chang, X. Liu, A. Wang, J.M. Reynolds, and C. Dong. 2012. Negative regulation of IL-17-mediated signaling and inflammation by the ubiquitin-specific protease USP25. *Nat. Immunol.* 13:1110–1117. http://dx.doi.org/10.1038/ni.2427

Zhong, B., X. Liu, X. Wang, X. Liu, H. Li, B.G. Darnay, X. Lin, S.C. Sun, and C. Dong. 2013. Ubiquitin-specific protease 25 regulates TLR4-dependent innate immune responses through deubiquitination of the adaptor protein TRAF3. *Sci. Signal.* 6:ra35. http://dx.doi.org/10.1126/scisignal.2003708

Zhou, X., F. You, H. Chen, and Z. Jiang. 2012. Poly(C)-binding protein 1 (PCBP1) mediates housekeeping degradation of mitochondrial antiviral signaling (MAVS). *Cell Res.* 22:717–727. http://dx.doi.org/10.1038/cr.2011.184

Zurek, B., I. Schoultz, A. Neerincx, L.M. Napolitano, K. Birkner, E. Bennek, G. Sellge, M. Lerm, G. Meroni, J.D. Söderholm, and T.A. Kufer. 2012. TRIM27 negatively regulates NOD2 by ubiquitination and proteasomal degradation. *PLoS One.* 7:e41255. http://dx.doi.org/10.1371/journal.pone.0041255

Züst, R., L. Cervantes-Barragan, M. Habjan, R. Maier, B.W. Neuman, J. Ziebuhr, K.J. Szretter, S.C. Baker, W. Barchet, M.S. Diamond, et al. 2011. Ribose 2′-*O*-methylation provides a molecular signature for the distinction of self and non-self mRNA dependent on the RNA sensor Mda5. *Nat. Immunol.* 12:137–143. http://dx.doi.org/10.1038/ni.1979