REVIEW

# Regulation of virus-triggered type I interferon signaling by cellular and viral proteins

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**Abstract** Host pattern recognition receptors (PRRs) recognize invading viral pathogens and initiate a series of signaling cascades that lead to the expression of type I interferons (IFNs) and inflammatory cytokines. During the past decade, significant progresses have been made to characterize PRRs such as Toll-like receptors (TLRs) and RIG-I-like receptors (RLRs) and elucidate the molecular mechanisms of TLR- and RLR-mediated signaling. To avoid excessive and harmful immune effects caused by over-activation of these signaling pathways, host cells adopt a number of strategies to regulate them. In addition, invading viruses also employ a variety of mechanisms to inhibit the production of type I IFNs, thereby evading the supervision and clearance by the host. In this review, we briefly summarize the TLR- and RLR-mediated type I IFN signaling and then focus on the mechanisms by which host cellular and viral components regulate the expression of type I IFNs.

## 1 Introduction

Organisms, from unicellular bacteria to human, are exposed to invading pathogens all the time. To protect themselves from pathogenic effects caused by the invaders, hosts have evolved immune system to detect and prevent infection by pathogens. The immune system in mammals is traditionally divided into two branches: innate immunity and adaptive immunity. The adaptive immunity, which is able to generate specific immune responses mediated by antibodies and effector T cells, is highly specific. However, it is evolved only in higher organisms and there is usually a delay of 4–7 days before the initial adaptive immunity takes effects. In contrast, the non-specific innate immunity is evolutionarily conserved and begins to work minutes to hours after infection. Therefore, the innate immunity

constitutes the first line for defense against pathogens such as viruses.

There are wide spectrums of viral pathogens that are known to infect humans, which have been a great threat to human health. The early events of innate immunity against invading viruses include the recognition of viral components, initiation of signaling pathways and transcriptional induction of type I IFNs and other cytokines (Akira et al., 2006). The type I IFNs bind to IFN receptor (IFNR) in both autocrine and paracrine manners to initiate a series of signaling events leading to the expression of hundreds of downstream genes, collectively referred to as interferon stimulated genes (ISGs). Proteins encoded by the ISGs inhibit viral replication or cause apoptosis of infected cells, and therefore result in an antiviral effect (Sadler and Williams, 2008).

Because type I IFNs play a central role in antiviral immunity, great efforts have been made during the past decade to elucidate the mechanisms of virus-triggered type I IFN induction. However, over-produced type I IFNs cause excessive and harmful immune effects to the host (Theofilopoulos et al., 2005). As a result, the production of these cytokines should be tightly regulated. On the other hand, viruses have also evolved a variety of mechanisms to inhibit the production of type I IFNs, thereby evading the supervision and elimination by the innate immunity. In this review, we first briefly summarize the virus-triggered type I IFN signaling and then focus on the regulatory mechanisms exerted by cellular and viral proteins.

## 2 Viral infection-triggered type I IFN signaling: a brief introduction

As mentioned above, virus-triggered type I IFN signaling is initiated by the recognition of pathogen-associated molecular patterns (PAMPs) generated during viral infection and replication. So far there are at least five kinds of viral PAMPs that have been characterized, including

double-stranded RNA (dsRNA), 5'triphosphorylated single-stranded RNA (5'pppssRNA), viral envelope glycoprotein, unmethylated CpG DNA (CpG DNA), and AT-rich double-stranded DNA (the analog Poly dA:dT) (Kumar et al., 2009; Takeuchi and Akira, 2009). These PAMPs are recognized by host pathogen-recognition receptors (PRRs), which include Toll-like receptors (TLRs), RIG-I-like receptors (RLRs including RIG-I and MDA5), NOD-like receptors (NLRs), and the recently identified cytoplasmic DNA sensors DNA-dependent activator of interferon-regulatory factors (DAI), absent in melanoma 2 (AIM2) and RNA polymerase III (Pol-III) (Ablasser et al., 2009; Chiu et al., 2009; McCartney and Colonna, 2009). The PRRs, each specific for a distinct ligand set, and the PRRs-mediated signaling pathways have been extensively reviewed in several previous publications (Akira et al., 2006; Kawai and Akira, 2009; Kumar et al., 2009; Takeuchi and Akira, 2009; Yoneyama and Fujita, 2009) (Table 1).

Here, we choose TLR3, TLR7/8, TLR9, RLRs, DAI and Pol-III-mediated signaling for discussion because they all recognize the viral nuclear aids but induce the production of type I IFNs via three representative adaptor proteins. These pathways converge at the activation of several transcription factors such as interferon-regulated factor 3/7 (IRF3/7) and NF-κB which collaborate to regulate transcription of type I IFNs (Maniatis et al., 1998; Honda et al., 2006).

### 2.1    TRIF-dependent pathway

TLR3 is the first characterized mammalian PRR and TLR3-meidated signaling has been extensively studied. Upon stimulation of viral dsRNA or its synthetic analog poly(I:C), the intracellular domain of TLR3 recruits the adaptor protein Toll/interleukin receptor (TIR) domain-containing adaptor-inducing IFN-β (TRIF). TRIF has an N-terminal domain, a middle TIR domain and a C-terminal domain called receptor-interacting protein (RIP) homotypic interaction motif (RHIM) (Sato et al., 2003; Yamamoto et al., 2003). The adaptor protein TRIF undergoes oligomerization through its TIR and RHIM domains, and recruits the TRAF family-member-associated NF-κB activator (TANK) binding kinase 1 (TBK1) via its N-terminal domain to activate IRF3/7. It is also suggested that NF-κB activating kinase (NAK)-associated protein 1 (NAP1) and tumor necrosis factor (TNF) receptor-associated factor 3 (TRAF3) are involved in TRIF-mediated activation of IRF3/7 by facilitating TRIF and TBK1 interaction (Oganesyan et al., 2006; Saha et al., 2006; Ryzhakov and Randow, 2007). Collectively, dsRNA induces activation of IRF3/7 through TLR3-TRIF-NAP1/TRAF3-TBK1 pathway.

TRIF mediates NF-κB activation through two distinct pathways. TRIF contains a consensus TRAF6-binding motif in the N-terminal region and mutation of this motif

impairs TRIF-mediated NF-κB but not IRF3 activation (Han et al., 2004; Jiang et al., 2004). However, TLR3 signaling in TRAF6-deficient macrophages is not affected (Gohda et al., 2004), which indicates the existence of functional redundancy. It has been demonstrated that TRIF is also capable of activating NF-κB through its C-terminal RHIM, which is responsible for recruitment of RIP (Meylan et al., 2004). It has been shown that poly(I:C)-induced NF-κB activation is completely blocked in RIP-deficient MEFs (Cusson-Hermance et al., 2005). In addition, overexpression of TRIF induces apoptosis by interacting with RIP1 through a RIP1/Fas-associated death domain (FADD)/caspase 8-dependent and mitochondria-independent apoptotic pathway (Han et al., 2004). Collectively, the TLR3-triggered TRIF-dependent pathways activate IRF3/7 and NF-κB and induce apoptosis via TBK1, RIP1 and RIP1/FADD/caspase 8, respectively.

### 2.2    MyD88-dependent pathway

In contrast to the TRIF-dependent signaling triggered by TLR3, TLR7/8 and TLR9-mediated signaling depends exclusively on another adaptor protein MyD88 (myeloid differentiation primary response protein-88) (Akira and Takeda, 2004; Akira et al., 2006; Yoneyama and Fujita, 2009). The MyD88-dependent pathway includes a number of signaling molecules: the adaptor protein MyD88, IL-1Rassociated kinase 4/1 (IRAK4/1), transforming growth factor-β-activated kinase (TAK1), TRAF6 and TAK1 binding protein-1/2 (TAB1/2). Upon binding to their respective ligands, TLR7/8 and TLR9 recruit MyD88 and IRAK4 (Honda et al., 2004; Kawai et al., 2004). IRAK4 further recruits IRAK1 and TRAF6 and thereby phosphorylates and activates IRAK1 (Uematsu et al., 2005). The IRAK1-TRAF6 complex then disassociates from the receptor (Akira and Takeda, 2004). On one hand, the complex interacts with IKKα, TRAF3 and osteopontin, leading to phosphorylation and activation of IRF7 (Hoshino et al., 2006; Shinohara et al., 2006). On the other hand, it interacts with another complex consisting of TAK1, TAB1 and TAB2, followed by the phosphorylation and activation of TAK1. The activated TAK1 subsequently phosphorylates the IκB kinases (IKKs), leading to ubiquitination and degradation of IκB and activation of NF-κB (Akira and Takeda, 2004). Activation of TAK1 also results in the activation of MAPKs, including c-Jun N-terminal kinase (JNK), leading to activation of AP-1 (Akira and Takeda, 2004).

### 2.3    VISA-dependent pathway

RLR- and Pol-III-mediated signaling requires the mitochondrial adaptor protein virus-induced signaling adaptor (VISA, also called MAVS, IPS-1 and Cardif) (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Shinohara et al., 2005; Xu et al., 2005). Upon binding of dsRNA or

**Table 1**   Detection of pathogens by TLRs and RLRs

| PRRs | pathogens or RNAs | references |
|---|---|---|
| TLR3 | double-stranded RNA, poly(I:C) | Alexopoulou et al., 2001 |
| | West Nile virus | Wang et al., 2004 |
| | encephalomyocarditis virus | Wang et al., 2004 |
| | influenza A virus | Le Goffic et al., 2006 |
| | herpes simplex virus | Zhang et al., 2007 |
| | short interfering RNA (siRNA) | Kawai and Akira, 2008; Kleinman et al., 2008; |
| TLR7/8 | Imiquimod (R-837), Resiquimod (R-838), Loxoribine | Hemmi et al., 2002 |
| | guanosine and uridine-rich ssRNA | Diebold et al., 2004; Heil et al., 2004 |
| | human immunodeficiency virus | Diebold et al., 2004; Heil et al., 2004 |
| | influenza A virus | Diebold et al., 2004; Heil et al., 2004 |
| | short interfering RNA | Hornung et al., 2005 |
| TLR9 | bacterial and viral genomic DNA with CpG-DNA motif | Hemmi et al., 2000; Krieg, 2002 |
| | mouse cytomegalovirus | Krug et al., 2004a |
| | herpes simplex virus-1 | Krug et al., 2004b |
| | herpes simplex virus-2 | Lund et al., 2003 |
| RIG-I | Poly(I:C) | Yoneyama et al., 2004 |
| | short dsRNA, Reovirus (short fragment of genomic dsRNA), Poly(I:C)(< 1 kb) | Kato et al., 2008 |
| | 5'pppssRNA | Hornung et al., 2006; Pichlmair et al., 2006 |
| | 5'triphosphate RNA with a panhandle structure at 5' end | Schlee et al., 2009 |
| | *in vitro* transcribed RNA | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | influenza A virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | vesicular stomatitis virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | Newcastle disease virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | Sendai virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | Japanese encephalitis virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | hepatitis C virus | Saito et al.,2008; Yoneyama and Fujita, 2009 |
| | respiratory syncytial virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | Dengue virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | West Nile virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| MDA5 | Long dsRNA, poly(I:C) (about 2 kb) Reovirus (long fragment of genomic dsRNA) | Kato et al., 2008 |
| | encephalomyocarditis virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | Theiler's encephalomyelitis virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | Mengo virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | Dengue virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| | West Nile virus | Kato et al., 2006; Yoneyama and Fujita, 2009 |
| DAI | B-DNA, poly(dA:dT) | Takaoka et al., 2007 |
| RNA polymerase III | AT-rich dsDNA | Ablasser et al., 2009; Chiu et al., 2009 |
| | *Legionella pneumophila* | Chiu et al., 2009 |
| | herpes simplex virus, Epstein-Barr virus | Ablasser et al., 2009; Chiu et al., 2009 |

TLR: Toll-like receptor; RLR: RIG-I-like receptor; PRRs: pattern recognition receptors; DAI: DNA-dependent activator of interferon-regulatory factors.

5'pppssRNA, RLRs undergo conformational changes and are recruited to the adaptor protein VISA located on the mitochondrial outer-membrane. VISA interacts with TRAF6 and TRAF3 and further activates the canonical and noncanonical IKK protein kinase family members, respectively (Xu et al., 2005; Oganesyan et al., 2006; Saha et al., 2006). The canonical IKK complex IKKα/β/γ is essential for virus-triggered RLR-mediated NF-κB activation, and the noncanonical IKK family members TBK1 and IKKε are responsible for phosphorylation and

activation of IRF3 and IRF7. Other studies have also demonstrated the involvement of several other signaling components in virus-induced activation of NF-κB and/or IRF3, including TANK, TRADD, FADD and RIP (Kawai et al., 2005; Guo and Cheng, 2007; Michallet et al., 2008).

Recently, we and others identified a new adapter protein called mediator of IRF3 activation (MITA, also known as STING), which plays a critical role in virus-induced type I IFN expression (Ishikawa and Barber, 2008; Zhong et al., 2008). MITA has been found to localize to the outer-membrane of mitochondria or endoplasmic reticulum (ER). It has been demonstrated that MITA acts as an adapter to recruit TBK1 and IRF3 to the VISA-associated complex after viral infection. In this complex, TBK1 first phosphorylates MITA at Ser358, which is critical for subsequent phosphorylation of IRF3 (Zhong et al., 2008). There is also evidence that a number of molecules involved in protein transportation are also required for virus-triggered type I IFN production (Ishikawa and Barber, 2008; Ishikawa et al., 2009).

Viral DNA-triggered type I IFN production is mediated through at least three pathways. First, two recent publications reported that in transformed cells, AT-rich double-stranded DNA (poly(dA:dT)) and cytoplasmic viral or bacterial DNA is transcribed into RNA by Pol-III and the transcribed RNA, which probably contains 5'triphosphate structure and is then recognized by RIG-I and signals through VISA (Ablasser et al., 2009; Chiu et al., 2009). Second, in primary or low passage cells, transfection of dsDNA regardless of its sequences or infection by DNA viruses also triggers Pol-III-independent signaling, leading

to the expression of type I IFNs. The signaling pathway requires the signaling complex MITA-TBK1-IRF3 but not DAI or VISA (Chiu et al., 2009; Ishikawa et al., 2009). However, future studies are needed to characterize the sensors and adaptors that function upstream of MITA in the Pol-III-independent pathway. Third, DAI senses invading DNA to induce type I IFNs in L929 cells, which depends on TBK1 and IRF3 but not VISA (Takaoka et al., 2007). The adaptor protein for this process is currently unknown.

## 3  Regulation of PRR-triggered type I IFNs by cellular proteins

It is commonly observed that PRR-mediated signaling is rapidly activated after viral infection, leading to the production of type I IFNs and other cytokines. However, the overproduction of type I IFNs can cause unwanted or excessive immune responses that may lead to allergy, necrosis, autoimmune diseases and other harmful effects (Theofilopoulos et al., 2005). Therefore, PRR-mediated type I IFN signaling must be tightly regulated. For example, the C-terminus of RIG-I contains a repressor domain (RD) which masks the CARD domain and RNA helicase domain of RIG-I, thereby inhibiting the activation of RIG-I in uninfected cells (Cui et al., 2008; Takahasi et al., 2008; Yoneyama and Fujita, 2009). In addition to the autonomous inhibitory mechanism, the host has evolved several mechanisms to prevent unnecessary activation in steady-state cells or excessive signaling under viral infection conditions (Table 2).

**Table 2**  Cellular regulators of TLRs/RLRs signaling

| regulators | type of regulation | target(s) | proposed mechanisms | references |
|---|---|---|---|---|
| **sequestration of signaling molecules** | | | | |
| LGP2 | negative feedback | RIG-I | sequestration of RIG-I from RNA ligands inhibition of RIG-I oligomerization competitive interaction with VISA facilitationg recognition of | Yoneyama et al., 2005; Saito et al., 2007; Venkataraman et al., 2007 |
| | positive regulation | MDA5 | dsRNA by MDA5 | Pippig et al., 2009 |
| RIG-I-SV | negative feedback or dominant negative | RIG-I | competitive binding to dsRNA sequestration of RIG-I from VISA | Gack et al., 2008 |
| DAK | inhibition of steady-state cells | MDA5 | sequestration of MDA5 from activation of IRF3 | Diao et al., 2007 |
| Atg5-Atg12 | inhibition of steady- or activated state cells | RIG-I, MDA5, VISA | sequestration of RLRs from VISA | Jounai et al., 2007 |
| TBK1s | negative feedback | RIG-I | sequestration of RIG-I from VISA and TBK1 | Deng et al., 2008 |
| NLRX1 | inhibition of steady-state cells | VISA | sequestration of VISA from activation | Moore et al., 2008 |
| gC1qR | negative feedback | VISA | disruption of RLR-VISA interaction | Xu et al., 2009 |
| MyD88s | negative feedback | MyD88 | disruption of IRAK1/4-MyD88 association | Burns et al., 2003 |
| TRAF1 | negative feedback | TRIF | sequestration of TRIF by the cleaved N-terminus of TRAF1 by TRIF-activated caspases | Su et al., 2006 |

(*Continued*)

| regulators | type of regulation | target(s) | proposed mechanisms | references |
|---|---|---|---|---|
| ISG56 | negative feedback | MITA | sequestration of MITA from VISA and TBK1 | Li et al., 2009 |
| SIKE | inhibition of steady-state cells | TBK1, IKKε | sequestration of the targets from TRIF and IRF3 | Huang et al., 2005 |
| IRAK-M | negative feedback | IRAK1 | inhibition of IRAK1-MyD88 association | Kobayashi et al., 2002 |
| FLN29 | negative feedback | TRIF, VISA, TRAF3/6 | possible sequestration of its targets | Mashima et al., 2005; Sanada et al., 2008 |
| SHP-2 | negative feedback | TBK1 | possible dephosphorylation of TBK1 substrates | An et al., 2006 |
| **destabilization of key signaling molecules** | | | | |
| ISG15 | negative feedback | RIG-I | destabilization of RIG-I with UBE1L and UbcH8 | Zhao et al., 2005; Lu et al., 2006 |
| | positive regulation | IRF3 | protection of IRF3 from degradation | |
| RNF125 | negative feedback | RIG-I, MDA5, VISA | proteosomal degradation of targets | Arimoto et al., 2007 |
| RNF5 | negative feedback | MITA | proteosomal degradation of MITA | Zhong et al., 2009 |
| A20 | negative feedback | RIP1, TRAF6, TRIF | ubiquitination or deubiquitination of targets | Saitoh et al., 2005; Lin et al., 2006 |
| RBCK1 | negative feedback (TLRs/RLRs) | TAB2/3, IRF3 | proteosomal degradation of targets | Tian et al., 2007; Zhang et al., 2008 |
| Trim30α | negative feedback | TAB2, TAB3 | lysosomal degradation of TAB2 and TAB3 | Shi et al., 2008 |
| Triad3A | inhibition of steady-state conditions | TLR9 | proteosomal degradation of certain TLRs | Chuang and Ulevitch, 2004 |
| **cleavage of the polyubiquitin chains from signaling molecules** | | | | |
| DUBA | negative feedback | TRAF3 | deubiquitination of TRAF3 | Kayagaki et al., 2007 |
| CYLD | inhibition of steady- or activated state | RIG-I, NEMO | deubiquitination of targets | Friedman et al., 2008; Zhang et al., 2008 |
| **others** | | | | |
| Pin1 | negative feedback | IRF3 | conformational change-dependent degradation | Saitoh et al., 2006 |
| GSK3β | positive regulation negative feedback | p65 CREB | phosphorylation of p65 inhibition of phosphorylation of CREB | Martin et al., 2005; Hu et al., 2006 |
| Nrdp1 | inhibition of steady- or activated state positive regulation | MyD88 TBK1 | degradation of MyD88 by K48-linked ubiquitination activation of TBK1 by K63-linked ubiquitination | Wang et al., 2009 |
| SHP-1 | negative feedback positive regulation | IRAK1 | inhibition of proinflammatory cytokines expression enhancement of type I IFN production | An et al., 2008 |
| WDR34 | negative feedback | TAK1 | unclear | Gao et al., 2009 |
| Caspase 8 | negative feedback | TRIF, VISA | cleavage of the targets | Rebsamen et al., 2008 |
| TRIM21 | negative feedback positive regulation | IRF3 | proteosomal degradation of IRF3 inhibition of Pin1-induced degradation of IRF3 and sustaining activation of IRF3 | Higgs et al., 2008; Yang et al., 2009 |
| TANK | positive regulation inhibition of steady-state conditions | TRAF3, TBK1, IRF3 TRAF6 | scaffolding TRAF3-TBK1-IRF3 interaction deubiquitination of TRAF6 | Guo and Cheng, 2007; Gatot et al., 2007; Kawagoe et al., 2009 |
| SARM | negative feedback not negative feedback | TRIF unknown | sequestration of TRIF unclear/restriction of viral infection in brain region | Carty et al., 2006; Kim et al., 2007; Szretter et al., 2009 |
| IRF4 | negative feedback | IRF5 | competition with IRF5 for MyD88 | Tamura et al., 2008 |

## 3.1   Sequestering the interaction between signaling molecules

### 3.1.1   Sequestration of PRRs

**Laboratory of genetics and physiology (LGP2)** Compared to RIG-I and MDA5, LGP2 contains an RNA helicase domain and an RNA binding domain but lacks the CARD domain. Therefore, LGP2 binds to dsRNA competitively with RIG-I but does not initiate signaling. It has been shown that overexpression of LGP2 negatively regulates Sendai virus (SeV) and Newcastle disease virus (NDV)-triggered induction of type I IFNs, and RNAi-mediated knockdown of LGP2 can enhance expression of antiviral genes (Yoneyama et al., 2005). In addition, LGP2 interacts with RIG-I and inhibits oligomerization of RIG-I which is important for activation of the latter. LGP2 also disrupts VISA-TBK1/IKKε interaction to inhibit RLR-mediated type I IFN signaling (Saito et al., 2007). However, the *LGP2$^{-/-}$* mice are resistant to NDV infection which is sensed by RIG-I but sensitive to EMCV infection which is sensed by MDA5, suggesting that LGP2 differently regulates RIG-I- and MDA5-mediated signaling (Kato et al., 2006; Venkataraman et al., 2007). Recently, a study revealed the crystal structure of the repressor domain of LGP2, which suggests that LGP2 inhibits RIG-I-mediated signaling by competitively binding to dsRNA, while it positively regulates MDA5-mediated signaling by facilitating recognition of dsRNA by MDA5 (Pippig et al., 2009).

**RIG-I splice variant (RIG-I-SV)** It has been demonstrated that the full activation of RIG-I depends on its K63-linked ubiquitination at K172 by TRIM25 or RNF135 (Gack et al., 2008; Gao et al., 2009; Oshiumi et al., 2009). TRIM25 firstly interacts with RIG-I and then catalyzes the ubiquitination of RIG-I (Gack et al., 2007). The Thr55 of RIG-I is critical for its binding with TRIM25. Compared to the full-length RIG-I, RIG-I-SV lacks the aa36-80 region. Thus, RIG-I-SV does not interact with TRIM25 to initiate signaling (Gack et al., 2008). However, RIG-I-SV contains the intact RNA binding and helicase domain and interacts with RIG-I but not MDA5. Therefore, RIG-I-SV inhibits RIG-I- but not MDA5-mediated type I IFN signaling by competitively binding to dsRNA and VISA with RIG-I and inhibiting the oligomerization of RIG-I.

**Dihydroxyacetone kinase (DAK)** The protein kinase DAK was found to interact with MDA5 in yeast two-hybrid assays (Diao et al., 2007). DAK is a member of the evolutionarily conserved family of dihydroxyacetone kinases from bacteria to humans (Bachler et al., 2005; Cabezas et al., 2005). Mammalian DAK displays dual activities as flavin adenine dinucleotide (FAD)-adenosine monophosphate (AMP) lyase and ATP-dependent Pha kinase. However, the physiological functions of DAK in innate antiviral response were unknown. In co-immunoprecipitation experiments, DAK interacts with MDA5 but not RIG-I in untransfected cells or overexpressed conditions, and the CARD domain-containing fragment of MDA5 is sufficient for the association. Expression of DAK inhibits MDA5- but not RIG-I- or VISA-mediated induction of IFN-β, while RNAi knockdown of DAK has an opposite effect. Endogenous interaction between MDA5 and DAK is decreased when the cells are infected with the Sendai virus, suggesting that the association is disrupted upon virus infection. It is possible that upon binding to dsRNA, the conformational change of MDA5 results in its higher affinity to the downstream adaptor VISA (Diao et al., 2007). Therefore, DAK keeps MDA5 inactive under steady-state conditions.

**Atg5-Atg12** The Atg5-Atg12 conjugate plays a critical role in autophagic process. The autophagy has been implicated for defense against infections with intracellular bacteria and viruses (Gutierrez et al., 2004). However, autophagosomes have also been exploited by certain viruses as a place for viral replication. A recent study has demonstrated that VSV infection induces higher level of IRF3 phosphorylation and expression of IFN-β and IP10 in *Atg5$^{-/-}$* than in wild-type MEFs (Jounai et al., 2007). Further investigation suggests that the Atg5-Atg12 conjugate associates directly with the CARD domains of RIG-I, MDA5 and VISA and viral infection can enhance the interaction. Therefore, Atg5-Atg12 disrupts the CARD interactions between RIG-I or MDA5 and VISA, preventing the formation of the RLR-VISA complex. Atg7 is a critical scaffold protein that facilitates Atg12 to conjugate with Atg5 (Komatsu et al., 2006). Accordingly, Atg7 deficient MEFs produce higher amount of type I IFNs in response to cytoplasmic poly(I:C) stimulation (Jounai et al., 2007). Collectively, these data suggest that Atg5-Atg12 conjugate acts as a suppressor of RLR signaling by blocking the interaction between RLRs and VISA.

**TBK1s** Compared to the full-length TBK1, the splice variant TBK1s lacks exons three to six. It has been shown that TBK1s is expressed 3–6 hours after viral infection (Deng et al., 2008). TBK1s interacts with RIG-I and disrupts the interaction between RIG-I and downstream molecules, thereby inhibiting RIG-I-mediated activation of IRF3. Interestingly, TBK1s does not inhibit VISA- or TBK1-mediated activation of IFN-β, suggesting that TBK1 functions at RIG-I level as a negative feedback regulator.

It should be noted that although DAK and TBK1s disrupt interaction between MDA5 or RIG-I and VISA, neither of them inhibits RLR-mediated NF-κB activation. The mechanisms for this process need further investigation.

### 3.1.2   Sequestration of adaptor proteins

**NLRX1** NLRX1 belongs to a protein family containing nucleotide-binding domain (NBD) and leucine-rich repeat

(LRR) (NLR), which are structurally conserved and have been demonstrated to function in defense against bacterial infection (Akira et al., 2006). Expression of NLRX1 inhibits RLR-mediated expression of cytokines such as type I IFNs, IL-6 and TNFα, while knockdown of NLRX1 has an opposite effect. NLRX1 was found to reside at the outermembrane of mitochondria and associate with the CARD domain of VISA, thereby sequestering VISA from RLRs and inhibiting RLR-mediated signaling (Moore et al., 2008).

**gC1qR** Receptor for globular domain of complement component C1q (gC1qR) is located in mitochondria, nucleus, cytoplasm and on cell membrane (Xu et al., 2009). Overexpression of gC1qR inhibits RIG-I-mediated activation of IRF3 and NF-κB and production of IFN-β and other cytokines. Conversely, RNAi knockdown of gC1qR enhanced the production of type I IFNs. It is shown that mitochondrial gC1qR weakly interacts with VISA and SeV infection causes the translocation of gC1qR to mitochondria and enhances its association with VISA (Xu et al., 2009). Thus, it has been postulated that gC1qR plays a role as a negative feedback regulator in RLR-mediated signaling.

**MyD88s** MyD88s is an alternatively spliced form of MyD88, lacking the intermediate amino acids 110–157 of MyD88. Unlike MyD88, MyD88s does not induce IRAK1 phosphorylation, despite its interaction with IRAK1. The expression of MyD88s can be detected in spleen and brain tissues and is upregulated by LPS stimulation. MyD88s forms dimer or oligomer with MyD88 and interacts with IRAK1, sequestering IRAK1 from IRAK4 and inhibiting IRAK4-induced phosphorylation of IRAK1(Burns et al., 2003). Thus, MyD88s is a negative feedback regulator of TLR-induced MyD88-dependent signaling.

**TRAF1** TRAF1 belongs to the TRAF family. All members of this family except for TRAF1 contain a RING domain that bears an E3 ubiquitin ligase activity (Bradley and Pober, 2001). In 293 cells that stably express TLR3, overexpressed TRAF1 interacts with TRIF and effectively inhibits poly(I:C)-induced activation of NF-κB and the IFN-β promoter. Further studies suggest that C-terminal part of TRAF1 and the TIR domain of TRIF are responsible for their interaction. Interestingly, TRIF induces caspase-dependent cleavage of TRAF1, and the cleaved N-terminal but not C-terminal fragment of TRAF1 shows inhibitory effect. Inhibition of the cleavage of TRAF1 by mutating of the cleavage site or addition of caspase inhibitor impairs its ability to inhibit TRIF-dependent signaling (Su et al., 2006). Therefore, TRIF-induced cleavage of TRAF1 is required for its inhibition of TRIF signaling.

**ISG56** Interferon-stimulated gene 56 (ISG56) is one of the first identified proteins induced by virus and type I IFNs (Sadler and Williams, 2008). In immunoprecipitaion and mass-spectrometry assays, ISG56 was identified as a MITA-interacting protein (Li et al., 2009). ISG56 negatively regulates virus-triggered signaling at MITA level by disrupting TBK1-MITA and VISA-MITA inter-actions. Consistent with these observations, inhibition of ISG56 by RNAi enhances cellular antiviral responses as well as the expression of type I IFNs. It has been reported that ISG56 acts as a suppressor of viral replication and protein translation (Wang et al., 2003; Terenzi et al., 2006; Wacher et al., 2007). In this context, ISG56 might have multiple functions and is an important integrator of inhibition of viral replication and control of excessive antiviral responses. It is also possible that the functions of ISG56 are temporally and spatially regulated during viral infection. However, more studies are required for the full understanding of these processes (Li et al., 2009).

### 3.1.3 Sequestration of kinases

**SIKE** Suppressor of IKKε (SIKE) contains no other recognizable domain but two coiled-coil domains. In yeast two-hybrid screens, SIKE was identified as an IKKε-interacting protein (Huang et al., 2005). Because TBK1 functions in most types of cells and shows highly homology with IKKε which functions in limited types of cells and is viral infection inducible, studies are focused on the interaction between SIKE and TBK1 (Hemmi et al., 2004; Perry et al., 2004). SIKE interacts with TBK1 in uninfected cells, sequestering TBK1 from IRF3, while viral infection or poly(I:C) stimulation disrupts the interaction, thereby releasing TBK1 to interact with and phosphorylate IRF3. Consistently, overexpression of SIKE inhibits TLR3- and RLR-mediated signaling and knock-down of SIKE has an opposite effect (Huang et al., 2005). Therefore, SIKE might sequester TBK1/IKKε in inactive forms under steady-state conditions to avoid unnecessary activation of these kinases.

**IRAK-M** IRAK-M belongs to the IRAK family (Ring-wood and Li, 2008). However, it does not possess kinase activity like other IRAKs do. Unlike the ubiquitous expression profile of IRAK1/4, the expression of IRAK-M is limited to monocytes or macrophages. *IRAK-M*$^{-/-}$ macrophages show increased cytokine production when stimulated with bacteria (Kobayashi et al., 2002) and further studies suggest that IRAK-M prevents the associa-tion of IRAK-1 and IRAK-4 with the MyD88 complex, thereby negatively regulating TLR-mediated signaling in macrophages.

**FLN29** FLN29 is also an IFN-inducible protein. It contains a TRAF-type zinc finger domain at its N-terminus and a conserved TRAF6-binding motif which mediates its interaction with TRAF6. Overexpression of FLN29 inhibits TRIF-dependent NF-κB and MAPK activations (Mashima et al., 2005). *FLN29*$^{-/-}$ MEFs are highly resistant to VSV infection, and these cells produce more IFN-β than wild-type cells in response to poly(I:C). FLN29-deficient mice become more susceptible to poly(I:C)-induced septic shock compared with wild-type mice. Mechanistic studies show that FLN29 interacts with TRIF, VISA, TRAF3 and

TRAF6 and inhibits virus-triggered signaling at TRAF3/6 level (Sanada et al., 2008). However, the exact mechanism still needs further investigations.

**SHP-2** The Src homology 2 (SH2) domain-containing protein tyrosine phosphatase 2 (SHP-2) is an evolutionarily conserved tyrosine phosphatase (Qu, 2000; Neel et al., 2003), which has been demonstrated to positively regulate the signaling triggered by some cytokines such as IL-1, and negatively regulate the signaling triggered by IFN-α(You et al., 1999; You et al., 2001). Studies with SHP-2 deficient cells suggest that SHP-2 suppresses TLR3- but not TLR7- or TLR9-mediated production of type I IFNs and proinflammatory cytokines. The inhibitory function of SHP-2 depends on its phosphatase activity (An et al., 2006). Further investigations show that SHP-2 directly binds to TBK1, and the C-terminal domain of SHP-2 and the kinase domain of TBK1 are responsible for their interaction. It is possible that SHP-2 prevents TBK1-mediated phosphorylation of its substrates, thereby blocking TRIF-mediated signaling.

### 3.2 Modification of signaling molecules

#### 3.2.1 Inducing ubiquitination and degradation of key signaling molecules

**ISG15** ISG15 is one of the proteins extensively induced by viral infection (Theofilopoulos et al., 2005). The ubiquitin activating enzyme (E1) UBE1L and ubiquitin conjugating enzyme (E2) UbcH8 catalyze ISGylation of RIG-I by conjugating ISG15 to it, which is followed by ubiquitination and degradation of RIG-I (Zhao et al., 2005; Kim et al., 2008). Consistent with this observation, viral infection-induced expression of IFN-β is enhanced in $UBE1L^{-/-}$ than in wild-type MEFs. Also, the amount of RIG-I protein is more stable in $UBE1L^{-/-}$ than in wild-type MEFs (Kim et al., 2008). Collectively, these data suggest that the conjugation of ISG15 to RIG-I can inhibit RIG-I-mediated signaling by ubiquitination and degradation of RIG-I.

**RNF125** UbcH8 has been demonstrated to be associated with ubiquitination and degradation of RIG-I(Zhao et al., 2005). To identify the E3 ubiquitin ligase in this process, yeast two-hybrid assays were performed with UbcH8 as a bait. This effort led to the identification of RNF125 (Arimoto et al., 2007). RNF125 functions as an E3 ubiquitin ligase to catalyze ubiquitination of RIG-I. Thus, overexpression of RNF125 inhibits the virus-triggered and RIG-I-mediated type I IFN expression, while knockdown of RNF125 has an opposite effect. Furthermore, RNF125 is induced by IFN-α stimulation or viral infection, suggesting a negative feedback role played by RNF125 in innate antiviral signaling.

**RNF5** Ring-finger protein 5 is an E3 ubiquitin ligase that has been implicated in cell motility, protein quality control in the ER, cancer and degenerative myopathy (Kyushiki et al., 1997; Didier et al., 2003; Bromberg et al., 2007;

Delaunay et al., 2008). In a yeast two-hybrid assay, RNF5 was identified as a MITA-interacting protein (Zhong et al., 2009). Overexpression of RNF5 inhibits virus-triggered expression of type I IFNs in 293, HeLa as well as primary monocyte-derived macrophages and dendritic cells, while knockdown of RNF5 potentiates the expression of type I IFNs upon viral infection. A further study suggests that RNF5 interacts with MITA and catalyzes K48-linked ubiquitination of MITA at K150 after viral infection, thereby inhibiting excessive type I IFN response.

**A20** A20 was initially found to negatively regulate TNF-triggered NF-κB signaling (Opipari et al., 1990). A20 contains an ovarian tumor (OTU) domain in its N-terminus which has deubiquitination activity, and a C-terminus with ubiquitination activity (Wertz et al., 2004). Both domains are required for inhibition of TNF-induced activation of NF-κB. Similarly, A20 also inhibits RLR-mediated activation of IRF3 (Wang et al., 2004). Interestingly, the C-terminal domain alone of A20 is sufficient for its inhibitory function, suggesting that A20 negatively regulates the signaling through its ubiquitination activity (Saitoh et al., 2005; Lin et al., 2006). Moreover, macrophages derived from A20 deficient mice are incapable of terminating TLR-induced NF-κB activation (Boone et al., 2005). Further investigations show that A20 functions to cleave the polyubiquitin chains of TRAF6, which is critical for termination of TLR-mediated activation of NF-κB (Boone et al., 2004).

**RBCK1** RBCC protein interacting with PKC1 (RBCK1) belongs to the E3 ubiquitin ligase family (Marin and Ferrus, 2002). RBCK1 contains a ubiquitin-like (UBL) domain and a RING-IBR-RING (RBR) domain in its N- or C-terminus, respectively. RBCK1 interacts with the C-terminal ZNF domain of TAB2 and induces ubiquitination and degradation of TAB2, thereby inhibiting TNF-, IL-1- and RLR-induced activation of NF-κB (Tian et al., 2007). Further study also suggests that RBCK1 interacts with IRF3 and induces degradation of IRF3 after viral infection, indicating a negative feedback regulation of virus-triggered type I IFN signaling by RBCK1 (Zhang et al., 2008).

**TRIM30α** Tripartite motif (TRIM) family proteins are also known as "RBCC" proteins, as they contain an RBCC motif at their N-terminus consisting of a RING domain, one or two B-boxes and a coiled-coil region. TRIM family proteins have been demonstrated to function in the regulation of cell proliferation, differentiation, development, oncogenesis, apoptosis and antiviral responses (Nisole et al., 2005). In addition to the RBCC motif, TRIM30α also contains a SPRY domain at its C-terminus. The expression of TRIM30α is induced by the activation of NF-κB in various types of cells after stimulation with a variety of TLR ligands, such as poly(I:C) and CpG DNA. It has been shown that TRIM30α targets TAB2 and TAB3 for K63-linked ubiquitination and lysosome-dependent degradation and inhibits autoubiquitination of TRAF6,

contributing to the inhibition of TLR-mediated NF-κB activation. Further studies suggest that TRIM30α transgenic mice are more resistant to endotoxin shock, whereas *in vivo* knockdown of TRIM30α by siRNA reduces LPS-induced tolerance, which demonstrates that TRIM30α negatively regulated LPS-mediated signaling *in vivo* and functions as a negative modulator of the TLR signaling pathway (Shi et al., 2008).

### 3.2.2 Cleaving the polyubiquitin chains from signaling molecules

**DUBA** Like A20, DUBA is also a member of the deubiquitination (DUB) domain-containing protein family which contains an OTU domain. A screening of the RNAi library targeting DUB family proteins led to the identification of DUBA as a negative regulator of TLR3-mediated signaling (Kayagaki et al., 2007). Knockdown of DUBA potentiates poly(I:C)-induced expression of IFN-β. Conversely, overexpression of DUBA inhibits poly(I:C)-induced or RIG-I-mediated signaling. An unambiguous identification of DUBA-interacting proteins suggests that DUBA interacts with TRAF3, whose K63-linked polyubiquitination is required for TLR- and RLR-mediated signaling. DUBA cleaves the K63-linked polyubiquitin chains of TRAF3 and depletion of DUBA increases the level of ubiquinated TRAF3 both in steady-state cells and in ligands-stimulated cells. Furthermore, overexpression of DUBA also impairs interaction between TBK1 and IRF3, suggesting that ubiquitination of TRAF3 controls the activity of the TBK1-IRF3 complex.

**CYLD** CYLD is an OTU DUB family protein that has been identified to interact with IKKγ and deubiquitinate the K63-linked polyubiquitin chains of IKKγ, thereby negatively regulating TNF-induced activation of NF-κB (Ea et al., 2006). Interestingly, two groups independently reported that CYLD functions as a negative modulator of RIG-I-mediated signaling (Zhang et al., 2008; Friedman et al., 2008). Knockdown of CYLD results in an enhancement in SeV-triggered IFN-β secretion. Experiments using *CYLD⁻/⁻* MEFs and dendritic cells (DCs) show constitutive activation of TBK1/IKKε as well as hyper-induction of type I IFNs by VSV infection. Immunoprecipitation experiments show that CYLD coprecipitates not only with RIG-I but also with TBK1 and IKKε. Interestingly, TBK1 or IKKε specifically causes CYLD to shift to higher molecular bands, suggesting phosphorylation of CYLD by these kinases. The data indicate that CYLD probably regulates the activities of IKK-family kinases and leads to inactivation of the signaling (Zhang et al., 2008).

Experiments from another group show that CYLD associates with the CARD domain of RIG-I and removes K63-linked ubiquitin from RIG-I. Loss of CYLD in DCs causes accumulation of ubiquitinated RIG-I. Furthermore, the accumulation of ubiquitinated RIG-I in CYLD-deficient cells is associated with constitutive activation of TBK1. The expression of CYLD can be induced by TNF treatment or viral infection (Friedman et al., 2008). These data together suggest a working model of the mechanisms by which CYLD negatively regulates the signaling. In uninfected cells, RIG-I undergoes ubiquitination-deubiquitination kinetically, making RIG-I inactive. Upon viral infection, the E3 ubiquitin ligases TRIM25 and/or RNF135 mediate K63-linked ubiquitination of RIG-I, which subsequently activates downstream kinase TBK1 (Gack et al., 2007; Gao et al., 2009; Oshiumi et al., 2009). However, as viral infection goes on, CYLD accumulates and cleaves the K63-linked ubiquitin chains of RIG-I, inhibiting virus-triggered type I IFN signaling. Thus, CYLD is most likely a negative regulator that inhibits RIG-I in both steady-state and activated state to prevent unnecessary signaling events. However, the precise mechanism that controls the balance between TRIM25/RNF135-mediated ubiquitination and CYLD-mediated deubiquitination of RIG-I and the phosphorylation of CYLD by TBK1 in its inhibitory function remains to be elucidated.

### 3.3 Others

In addition to the mechanisms mentioned above, several proteins have been described as regulators in TLR- and RLR-mediated signaling. For example, GSK3β regulates TLR- and IFNγ-mediated production of a subset of inflammatory cytokines and facilitates production of anti-inflammatory cytokines (Martin et al., 2005; Woodgett and Ohashi, 2005; Hu et al., 2006). Nrdp1 mediates K63-linked ubiquitination and activation of TBK1 as well as K48-linked ubiquitination and degradation of MyD88, thereby enhancing production of type I IFNs and suppressing production of pro-inflammatory cytokines (Wang et al., 2009). SHP-1 interacts with the kinase domain of IRAK1 and inhibits IRAK1 activation, which may explain why SHP-1 inhibits the expression of pro-inflammatory cytokines (An et al., 2008). However, the exact mechanisms are unclear. Pin1 recognizes and binds to the Ser336 phosphorylated IRF3, leading to considerable conformational change of IRF3. The conformational change makes IRF3 accessible for binding with E3 ubiquitin ligases such as RBCK1, which catalyze ubiquitination and degradation of IRF3 (Saitoh et al., 2006; Zhang et al., 2008). WD40 domain repeat protein 34 (WDR34) interacts with TAK1 and negatively regulates TLR3-induced NF-κB activation, although the mechanism is unclear at this moment (Gao et al., 2009). There is evidence suggesting that caspase 8 cleaves VISA at D249 and TRIF at both D281 and D289, indicating a crosstalk between the apoptosis pathway and TLR-/RLR-mediated signaling (Rebsamen et al., 2008). It is possible that these proteins function together in a temporal and spatial manner to control harmful excessive immune responses and protect host against autoimmune diseases.

There are controversies about the functions of several proteins involved in virus-triggered signaling. For example, ISG15 covalently binds to and stabilizes IRF3, counteracting its negative regulation of RIG-I (Lu et al., 2006; Kim et al., 2008). Ro52 (also called TRIM21) has been demonstrated to catalyze the ubiquitination of IRF3 and induce its degradation, while another report suggests that Ro52/TRIM21 interacts with Pin1 and IRF3 and prevents Pin1-induced conformational change and degradation of IRF3, thereby sustaining IRF3 activation during viral infection (Higgs et al., 2008; Yang et al., 2009). TANK has been identified as a scaffold protein facilitating TRAF3-TBK1-IRF3 interaction and subsequent activation of IRF3 (Guo and Cheng, 2007; Gatot et al., 2007). However, experiments on TANK-deficient mice suggest that TANK is not involved in interferon responses but rather functions as a negative regulator of TLR-signaling and is critical for the prevention of autoimmune nephritis by regulating ubiquitination of TRAF6 (Kawagoe et al., 2009). SARM contains HEAT-armadillo motifs at its N-terminus, two sterile α motifs (SAM) in middle and a TIR domain at its C-terminus and is inducible by TLR ligands and acts as a negative regulator in TRIF- but not MyD88-dependent pathway (Carty et al., 2006). However, another study showed that SARM is not involved in TLR-mediated signaling, as evidenced by the equivalent production of TNF and MCP-1 by bone marrow-derived macrophages of SARM-deficient and wild-type mice in response to various TLR ligands. It is also reported that SARM functions to restrict viral infection and neuronal injury in a brain region-specific manner (Szretter et al., 2009). Further studies and more efforts are required to figure out these inconsistencies.

# 4  Regulation of PRR-triggered type I IFNs by viral proteins

Host and virus have mutually exerted powerful selective pressure to each other throughout their evolution. For example, the type I IFN system as a fast and primary defense against viral pathogenesis serves as a strong selective pressure for viral evolution (Akira and Takeda, 2004; Yoneyama and Fujita, 2009). On the other hand, various viral components, which play roles in viral replication, assembly and pathogenesis, also target the molecules involved in the system to evade host immunity against viruses (Bowie and Unterholzner, 2008). There is increasing evidence suggesting that viruses have evolved a number of strategies to evade the recognition by PRRs, to inhibit PRR-mediated signaling, and even to manipulate host signaling pathways for their own benefit (Table 3).

## 4.1   Evasion of recognition by PRRs

It has been demonstrated that RLR-mediated expression of type I IFNs in macrophages and cDCs represents the first line of defense against local viral infection (Kumagai et al., 2007). Experiments on RIG-I- or MDA5-deficient mice suggest that RIG-I and MDA5 recognize different RNA structures generated by distinct viruses (Kato et al., 2006). For example, RIG-I recognizes 5'pppssRNA, short dsRNA, Newcastle disease virus (NDV) and influenza A virus (IAV), while MDA5 recognizes long dsRNA and piconaviridae family such as encephalomyocarditis virus (EMCV). However, neither of them recognizes cellular mRNA which is protected by a methylguanosine cap (m7Gppp structure) or ribosomal RNA or tRNA which is

**Table 3**   Viral inhibitors of TLRs/RLRs signaling

| viruses | inhibitors | target(s) | proposed mechanisms | references |
|---|---|---|---|---|
| **evasion recognition by PRRs** | | | | |
| encephalomyocarditis virus poliovirus | VPg | viral RNA | sequestration of viral RNA from RIG-I | Flanegan et al., 1977; Lee et al., 1977 Hornung et al., 2004; Pichlmair et al., 2006 |
| severe acute respiratory syndrome coronavirus vesicular stomatitis virus | Nsp14, Nsp16 RDRP | viral RNA | addition of a m7Gppp structure to the 5'end of its RNA | von Grotthuss et al., 2003; Ogino and Banerjee, 2007; Chen et al., 2009 |
| Hantaan virus Crimean-Congo hemorrhagic fever virus Borna disease virus | phosphatase | viral RNA | monophosphorylation of 5'end of viral RNA | Schneider et al., 2005; Habjan et al., 2008 |
| human immunodeficiency virus adenovirus | unknown | viral RNA | using host RNA processing machinery to cap newly synthesized viral RNA | Furuichi and Shatkin, 2000 |
| influenza A virus | unknown | viral RNA | snatching 5'capped cellular mRNA to 5'end of viral RNA | Plotch et al., 1981 |
| Vaccinia virus | E3L | viral dsRNA | sequestration of viral dsRNA | Chang et al., 1992 |
| Ebola virus | VP35 | viral dsRNA, RIG-I | sequestration of viral dsRNA | Cardenas et al., 2006; Haasnoot et al., 2007 |
| human immunodeficiency virus 1 | Tat | viral dsRNA | sequestration of viral dsRNA | Weeks et al., 1990 |
| Reovirus | Sigma3 | viral RNA | sequestration of viral RNA | Olland et al., 2001 |

| viruses | inhibitors | target(s) | proposed mechanisms | references |
|---|---|---|---|---|
| **prevention of the interactions of signaling molecules** | | | | |
| influenza A virus | NS1 | RIG-I | sequestration of RIG-I from VISA | Mibayashi et al., 2007 |
| Picornavirus | V proteins | MDA5 | sequestration of MDA5 | Andrejeva et al., 2004 |
| human metapneumovirus | glycoprotein G | RIG-I | sequestration of RIG-I | Bao et al., 2008 |
| Vaccinia virus | A46R A52R N1L B14R K7R K1L | MyD88, TRIF IRAK2, TRAF6 TBK1 IKKβ DDX3 IκBα | sequestration of targets | Stack et al., 2005 Keating et al., 2007 DiPerna et al., 2004 Chen et al., 2008 Schroder et al., 2008 Rahman and McFadden, 2006; Roy and Mocarski, 2007 |
| Hepatitis C virus Borna disease virus Rabies virus | NS3 phosphoprotein phosphoprotein | TBK1 TBK1 TBK1 | sequestration of TBK1 from its upstream or downstream signaling proteins | Otsuka et al., 2005 Unterstab et al., 2005 Brzozka et al., 2005 20 20 |
| Hantavirus | G1 protein | TRAF3, TBK1 | disruption of TRAF3-TBK1 interaction | Alff et al., 2006 |
| West Nile virus | NS1 | NF-κB, IRF3 | inhibition of translocation of NF-κB and IRF3 to nucleus | Wilson et al., 2008 |
| herpes simplex virus | ICP0 | IRF3, CBP | inhibition of IRF3 binding to target promoters | Melroe et al., 2007 |
| mumps virus Parainfluenza virus 5 | V protein V protein | TBK1, IKKε | competition with IRF3 for phosphorylation by TBK1/IKK | Lu et al., 2008 |
| human herpesvirus 8 | vIRF1 | IRF3, CBP, p300 | disruption of IRF3/CBP/p300 association | Lin et al., 2001 |
| Kaposi's sarcoma-associated herpesvirus | vIRF3 K-bZIP | IRF7 IRF3 | inhibition of IRF7 DNA binding activity competition with host IRF3 for DNA binding | Joo et al., 2003 Lefort et al., 2004 |
| **cleavage or degradation of signaling proteins** | | | | |
| hepatitis C virus | NS3/4A NS5A | TRIF, VISA MyD88 | cleavage of targets | Li et al., 2005a; Li et al., 2005b; Meylan et al., 2005; Lin et al., 2006 Abe et al., 2007 |
| hepatitis A virus | 3ABC | VISA | cleavage of VISA | Yang et al., 2007 |
| Flavivirus GB Virus B | NS3/4A | VISA | cleavage of VISA | Chen et al., 2007 |
| poliovirus | NS5A | MDA5 | cleavage of MDA5 by caspases | Barral et al., 2007 |
| bovine herpesvirus | bICP0 | IRF3 | degradation of IRF3 | Saira et al., 2007 |
| classical swine fever virus | Protease N | IRF3 | degradation of IRF3 | Bauhofer et al., 2007 |
| human immunodeficiency virus 1 | Vpr and Vif | IRF3 | degradation of IRF3 | Okumura et al., 2008 |
| Rotavirus | NSP1 | IRF3, IRF5, IRF7 | destabilization of targets | Barro and Patton, 2007 |
| SARS-Coronavirus | PL[pro] | IRF3 | proteasomal degradation of IRF3 | Devaraj et al., 2007 |
| foot-and-mouth disease virus | L[pro] | NF-κB | degradation of p65 and/or RelA | Roy and Mocarski, 2007 |
| Kaposi's sarcoma-associated herpesvirus | RTA | IRF7 | proteasomal degradation of IRF7 | Mossman and Ashkar, 2005 |
| **others** | | | | |
| African swine fever virus | A238L A224L | NF-κB NF-κB | sequestration of NF-κB in cytoplasm activation of NF-κB and inhibition of caspases | Tait et al., 2000 Rodriguez et al., 2002 |
| Kaposi's sarcoma-associated herpesvirus | K13 | IKKα/β/γ complex | activation of NF-κB | Matta et al., 2007 |

monophosphated and/or modified with unusual bases (Hornung et al., 2006; Kato et al., 2006; Bowie and Unterholzner, 2008).

Viruses seem to be so smart that they are aware of the tricks by which the host distinguishes self and nonself RNA. A number of proteins encoded by viral genomes can process viral RNA and simulate the modifications of host RNA, thereby evading the supervision by PRRs (Furuichi and Furuichi, 2000). For example, picornaviruses such as EMCV protect the 5' end of its RNA with the covalently linked protein VPg (Flanegan et al., 1977). The non-structural protein 14 (nsp14) of severe acute respiratory syndrome (SARS) coronavirus functions as an N7 methyltransferase that catalyzes to form an m7Gppp structure at the 5'end of its RNA (von Grotthuss et al., 2003; Chen et al., 2009). Genomic RNAs from Hantaan virus (HTNV), Crimean-Congo hemorrhagic fever virus (CCHFV) and Borna disease virus (BDV) are 5'monophosphorylated (Schneider et al., 2005; Habjan et al., 2008). However, the exact mechanisms for these modifications are unknown. Human immunodeficiency virus (HIV) and adenovirus use host RNA processing machinery to cap newly synthesized viral RNA (Furuichi and Furuichi, 2000). Poxviruses replicate in the cytoplasm and encode their own RNA capping machinery. In addition, IAV 'snatches' capped 5' fragments from cellular mRNA to its own genomic RNA to mask its 5'ppp structure ( Plotch et al., 1981).

Many viruses also produce dsRNA at some stage during their life cycle, which is recognized by host PRRs. To avoid innate immune responses that are initiated by RLRs, some viral genomes encode dsRNA-binding proteins, including vaccinia virus (VACV) E3L(Chang et al., 1992), Ebola virus VP35 (Cardenas et al., 2006; Haasnoot et al., 2007) and HIV 1 Tat (Weeks et al., 1990). These proteins shield the dsRNA structures generated during infection and replication from recognition by RLRs. It is also possible for these proteins to inhibit TLR3-mediated signaling in virus- and viral dsRNA-containing endosomes. Taken together, these studies suggest that many viruses inhibit PRR-mediated type I IFN signaling at the very beginning of PRR-mediated recognition of viral nuclear acids.

### 4.2    Disruption of the interactions of signaling molecules

As mentioned above, virus-triggered type I IFN signaling depends on the interactions of various signaling molecules. To block the signaling process, many viral proteins interact with the key molecules involved in the process and thereby prevent signal transduction leading to the expression of type I IFNs. For example, some viral proteins bind to RLRs directly, thereby inhibiting RLR-mediated signaling effectively. Nonstructural protein 1 of IAV which binds to RIG-I and V proteins of picornavirus which bind to MDA5 are two such examples (Andrejeva et al., 2004; Mibayashi et al., 2007). The *Paramyxoviridae* family human

metapneumovirus (hMPV) G gene-encoded glycoprotein G specifically interacts with RIG-I and blocks RIG-I-mediated IFN-β induction (Bao et al., 2008). Recombinant hMPV lacking the G gene replicates efficiently *in vitro* but its virulence is highly attenuated *in vivo*. A46R of VACV is a multiple TIR domain-containing protein, which interacts with the adaptors TRIF and MyD88 and inhibits TLR-mediated production of type I IFNs. Consistent with the observation, the deletion of A46R attenuates but not abolishes viral virulence of VACV (Stack et al., 2005). N1L of VACV is associated with the kinase TBK1 (DiPerna et al., 2004). Other two VACV-encoded proteins, A52R which interacts with TRAF6 and IRAK2 and B14R which interacts with IKKβ inhibit production of type I IFNs and inflammatory cytokines (Chen et al., 2008; Schroder et al., 2008). Hepatitis C virus (HCV) NS3, rabies virus phosphoprotein and BDV phosphoprotein interact with TBK1, leading to sequestration of TBK1 from its upstream or downstream signaling proteins (Otsuka et al., 2005; Unterstab et al., 2005). The G1 protein of pathogenic Hantavirus can inhibit TBK1 function by disrupting the TRAF3-TBK1 interaction which is required for signaling (Alff P et al., 2006). Poxvirus protein N1L targets IKK complex and inhibits the activation of NF-κB and IRF3 by TLRs (DiPerna et al., 2004). Recent studies suggest that DEAD-box protein 3 (DDX3) is a critical component of the TBK1/IKKε complex in both RLRs and cytoplasmic DNA receptors-mediated signaling. K7R of VACV inhibits PRR-mediated induction of IFN-β by targeting DDX3, thereby preventing TBK1 or IKKε-mediated activation of IRFs (Schroder et al., 2008).

Several viral proteins inhibit signaling by targeting transcription factors. For example, NS1 of West Nile virus (WNV) inhibits TLR3-mediated induction of IFN-β by preventing the translocation of NF-κB and IRF3 to the nucleus (Wilson et al., 2008). Herpes simplex virus (HSV) infected cell protein 0 (ICP0) binds to IRF3 and sequesters IRF3 from binding host DNA (Melroe et al., 2007). There are also viral proteins homologous to host IRFs that inhibit IRF3 and IRF7 signaling. V proteins of mumps virus and parainfluenza virus 5 act as substrates of TBK1/IKKε and compete with IRF3 for phosphorylation by TBK1/IKKε (Lu et al., 2008). Human herpesvirus 8 encoded IRF homologue vIRF represses type I IFN induction by blocking the association of IRF3 with the coactivators CBP and p300 (Lin et al., 2001). Similarly, vIRF3, a Kaposi's sarcoma-associated herpesvirus (KSHV) viral IRF homologue, dimerizes with the host IRF7 and inhibits its DNA binding activity (Joo et al., 2007). Another KSHV protein, K-bZIP, competes with the host IRF3 for binding sites in the promoters of ISG genes, thereby modulating the expression of antiviral genes (Lefort et al., 2007).

### 4.3    Cleavage or degradation of signaling proteins

Many viral genomes are translated into polypeptide

precursors which need to be cleaved into mature and functional proteins during viral replication. The cleavage activity depends on several non-structural proteins encoded by the viruses. For example, HCV genome is translated to a polypeptide and NS3/4A is one of such proteases and responsible for the cleavage. On the other hand, such activity of these proteases provides the possibility that they may cleave the host signaling molecules as well. Not surprisingly, the adaptors TRIF and VISA are cleaved by NS3/4A into two polypeptides that impair their ability to mediate PRR-induced expression of type I IFNs (Li et al., 2005a; Li et al., 2005b; Lin et al., 2006). Further studies suggest that the protease precursor protein 3ABC of Picornavirus hepatitis A virus (HAV) and NS3/4A of Flavivirus GB Virus B cleave VISA on the mitochondrial membrane, which disrupts RLR-mediated signaling (Chen et al., 2007; Yang et al., 2007). NS5A of poliovirus induces the cleavage of MDA5 by caspases, although the exact mechanism is unclear (Abe et al., 2007).

In addition, a number of viral proteins destabilize IRFs and target them for degradation. For example, bICP0 of bovine herpesvirus targets IRF3 for degradation instead of temporarily sequestering it as ICP0 from HSV does (Barral et al., 2007). Flaviridae family classical swine fever virus protease N (Npro) and HIV proteins Vpr and Vif also induce proteasomal degradation of IRF3, suggesting a common mechanism used by viruses to evade antiviral responses (Bauhofer et al., 2007; Okumura et al., 2008). Rotavirus NSP1 antagonizes the function of IRF3, IRF5 and IRF7 by inducing their degradation, thereby inhibiting the expression of type I IFNs (Barro and Patton, 2007).

### 4.4 Others

In contrast to evasion of the host recognition and inhibition of the signaling, it is proposed that viruses can 'hijack' and even subvert aspects of PRR-mediated signaling. Viruses use living cells as hosts, which means that while viral infection and replication causes dysfunction of the hosts, viruses have to prevent the hosts from apoptosis at the same time. Not only is NF-κB involved in the production of IFN-β, it also inhibits apoptosis and promotes proliferation of the host cells. Therefore, NF-κB is a key molecule to be regulated and used by viruses. Taking the control of NF-κB activity by African swine fever virus (ASFV) as an example, the ASFV protein A238L is an early expressed homologue to the inhibitor of κBα (IκBα) that sequesters NF-κB in the cytoplasm, thereby inhibiting NF-κB activity (Tait et al., 2000). However, as infection and replication progress, A224L is expressed, which activates NF-κB and inhibits caspases, preventing cells from apoptosis (Rodriguez et al., 2002). Similarly, the K13 protein from KAHV interacts with the canonical IKKα/β/γ complex to selectively activate NF-κB (Matta et al., 2007). There are other examples of viruses that use and

manipulate PRR-signaling for their replication and survival. HIV employs DDX3 to export viral RNA from the nucleus to the cytoplasm (Yedavalli et al., 2004). WNV may use TLR3-mediated signaling to produce cytokines to create a microenvironment favored by the viruses, as evidenced by the fact that TLR3 deficiency mice are resistant to WNV infection (Wang et al., 2004). Collectively, viruses adopt different strategies to inhibit and manipulate PRR-mediated signaling. Although progresses have been made to elucidate the mechanisms in the past decade, we expect new insights into the strategies that are used by viruses to interfere with PRR-mediated signaling.

## 5   Perspectives

Just as host and virus exert selective pressure on each other, studies on hosts and investigations of viruses mutually facilitate our understandings about each other. Future studies will focus on systemic mechanisms by which hosts balance the expression of antiviral and inflammatory cytokines and the translation of viral evasion into benefit of human health. First, mice with deletion of some cellular negative regulators show resistance to viral infection and high viability, while mice defective in others are susceptible to viral infection-caused inflammatory responses. As a result, it is important to take a systemic view of the host regulators when using *in vivo* mouse models. Second, much more efforts are needed to direct *in vivo* studies relevant to humans. For example, TLR3 deficiency does not impair the host immune response to several viruses (Edelmann et al., 2004). Later, it was reported that TLR-deficient mice are more resistant to WNV infection (Wang et al., 2004). However, patients with mutations in TLR3 are related to HSV-associated encephalitis (Zhang et al., 2007). Finally, development of therapeutic drugs that targets cellular or viral inhibitors may provide new strategy to treat infection or immune dysfunction-caused diseases.

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