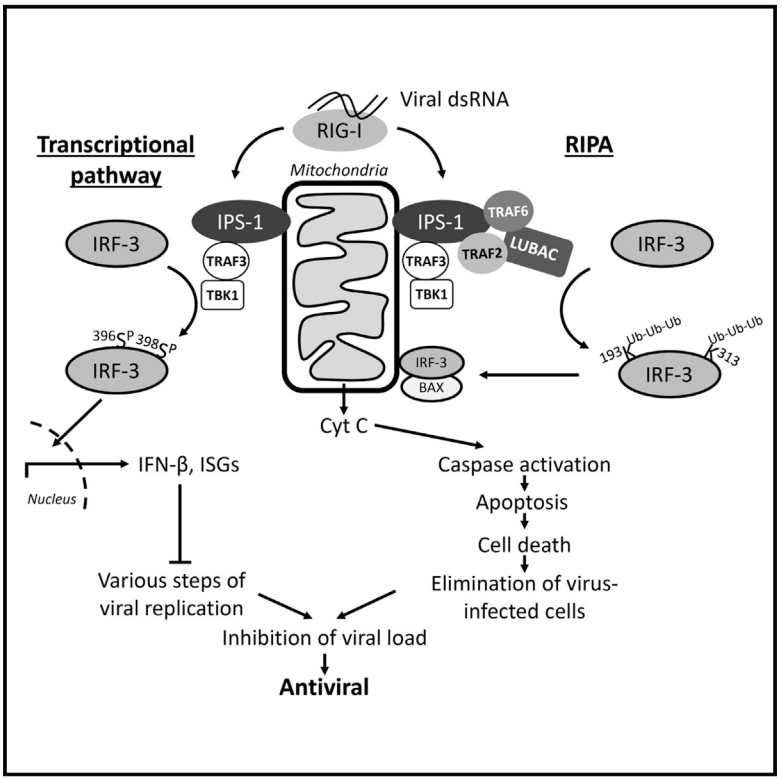
Article

Immunity

Ubiquitination of the Transcription Factor IRF-3 Activates RIPA, the Apoptotic Pathway that Protects Mice from Viral Pathogenesis

Graphical Abstract



Highlights

- RLR-signaling activates IRF-3 to trigger RIPA, the pro-apoptotic antiviral pathway
- RIPA can protect the host in the absence of antiviral gene induction
- RIPA requires ubiquitination of IRF-3 on specific lysines

Authors

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In Brief

The antiviral effects of IRF-3 are known to involve transcription of interferon stimulated genes as well as inducing apoptosis in infected cells. Sen and colleagues report that these two antiviral pathways mediated by IRF-3 are independent and that the apoptotic pathway is sufficient to inhibit viral pathogenesis

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Ubiquitination of the Transcription Factor IRF-3 Activates RIPA, the Apoptotic Pathway that Protects Mice from Viral Pathogenesis

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SUMMARY

The transcription factor IRF-3 mediates cellular antiviral response by inducing the expression of interferon and other antiviral proteins. In RNA-virus infected cells, IRF-3's transcriptional activation is triggered primarily by RIG-I-like receptors (RLR), which can also activate the RLR-induced IRF-3-mediated pathway of apoptosis (RIPA). Here, we have reported that the pathway of IRF-3 activation in RIPA was independent of and distinct from the known pathway of transcriptional activation of IRF-3. It required linear polyubiquitination of two specific lysine residues of IRF-3 by LUBAC, the linear polyubiquitinating enzyme complex, which bound IRF-3 in signal-dependent fashion. To evaluate the role of RIPA in viral pathogenesis, we engineered a genetically targeted mouse, which expressed a mutant IRF-3 that was RIPA-competent but transcriptionally inert; this single-action IRF-3 could protect mice from lethal viral infection. Our observations indicated that IRF-3-mediated apoptosis of virus-infected cells could be an effective antiviral mechanism, without expression of the interferon-stimulated genes.

INTRODUCTION

Virus infection triggers complex host responses, which determine the final outcome of the infection. Innate immune responses of the infected cell is the first line of host defense mechanism, which helps initiate and shape the subsequent adaptive immune responses, to eliminate the virus from the host. Various pattern-recognition receptors (PRRs) recognize viral components in the infected cell and trigger signaling pathways that lead to transcriptional induction of antiviral genes. Many RNA viruses, including Paramyxoviruses, and some DNA viruses activate the cytoplasmic RIG-I-like receptors (RLR) (Ramos and Gale, 2011); signaling by these receptors causes activation of transcription factors, such as IRF-3, NF-κB, and AP1, which, in turn, induce the expression of many genes, including the interferon (IFN) and the IFN-stimulated genes (ISG).

The biochemistry of RLR-signaling has been extensively investigated; a large number of adaptors, enzymes, and scaffolding proteins regulate this process both positively and negatively. Several components of this cascade are activated by phosphorylation or dephosphorylation and ubiquitination or deubiquitination. RLR activation requires its signal-dependent dephosphorylation, followed by K63-linked polyubiquitination of specific lysine residues. The two RLRs, RIG-I and MDA-5, exist as phosphorylated proteins in unstimulated cells; virus infection causes their dephosphorylation by the protein phosphatase PP1 to activate these receptors (Wies et al., 2013). RIG-I is further activated by ubiquitination of a specific lysine residue (K172); TRIM25, an E3 ligase, catalyzes the conjugation of K63-linked polyubiquitin chains on this lysine residue of RIG-I (Gack et al., 2007). Another E3 ligase, Riplet, catalyzes K63-linked polyubiquitin conjugation near the C terminus of RIG-I (Oshiumi et al., 2010). Deubiquitination by USP15 counteracts TRIM25 activity, thereby limiting signaling by RIG-I (Pauli et al., 2014). RIG-I can also be activated by non-covalent interaction with unanchored K63-linked polyubiquitin chains (Zeng et al., 2010). The activated RIG-I associates with mitochondrial outer membrane-bound protein, IPS1, which recruits the TRAF family of E3 ligases. TRAF3, specifically required for IRF-3 activation, undergoes K63 polyubiquitination upon recruitment to the RIG-I and IPS-1 containing complex (Mao et al., 2010). The polyubiquitinated TRAF3 interacts with the Ser/Thr kinase TBK1, which is activated by autophosphorylation and K63-linked polyubiquitination to function as a kinase for IRF-3 (Tu et al., 2013). DUBA deubiquitinates TRAF3 to downregulate its interaction with TBK1 and consequent activation of IRF-3 (Kayagaki et al., 2007).

RLR-signaling activates IRF-3 as a transcription factor by TBK1-mediated phosphorylation of its specific Ser residues, thereby changing its conformation. Phosphorylated IRF-3 dimerizes, translocates from the cytoplasm to the nucleus, and binds to the IFN-stimulated response elements (ISRE) in the promoters of the target genes to induce their transcription (Hiscott, 2007; Ikushima et al., 2013). To function as a transcription factor, IRF-3 requires the co-activator, β-catenin, which needs