RIG-I Works Double Duty

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The pathogen sensor RIG-I recognizes viral RNA and signals to induce an antiviral response. In this issue of *Cell Host & Microbe*, Weber et al. (2015), along with recent work by Sato et al. (2015), demonstrate that RIG-I directly inhibits viral replication independent of antiviral signaling.

Rapid and accurate recognition of viral invaders represents the first step to mounting an effective immune defense. Host factors known as pattern recognition receptors (PRRs) detect conserved viral signatures and subsequently trigger innate immune activation. One such PRR, RIG-I, surveys the cytosol for viral RNA and then signals via an adaptor protein, MAVS, to transcriptionally induce type I and III interferons (IFNs), leading to an antiviral state (reviewed in Chan and Gack, 2015). Extensive evidence has demonstrated that RIG-I is a key sensor of many RNA virus infections. In addition, there is increasing evidence that RIG-I detects DNA viruses, which also produce RNA species during their life cycles.

Influenza A virus (IAV) is a segmented negative-strand RNA virus of the family *Orthomyxoviridae*, which causes annual epidemics and occasional pandemic outbreaks and thus constitutes a major global health threat (Medina and García-Sastre, 2011). IAV can infect many different host species and can occasionally "jump" from one species to another, potentially causing severe disease. Wild birds are the main reservoir of IAV, and current research is intensely focused on the adaptation of avian IAV strains to mammals.

In regards to innate sensing of IAV infection, synthetic 5′triphosphate-containing short dsRNA, resembling the panhandle-like RNA configuration of IAV genomes, potently triggers RIG-I activation (Pichlmair et al., 2006). Physiologically, however, IAV genomic RNAs are not "naked," but rather packaged into eight rod-shaped ribonucleoproteins (RNPs), which together form the IAV nucleocapsid. Each RNP contains viral RNA, multiple nucleoproteins (NPs), and the viral polymerase complex (composed of PA, PB1, and PB2). Furthermore, IAV nucleocapsids are transported from the site of virus entry to the nucleus, where IAV replication takes place. Therefore, whether RIG-I can detect incoming IAV nucleocapsids during their short passage through the cytoplasm has been a longstanding question in the field.

The work of Weber et al. (2015), presented in this issue of *Cell Host & Microbe*, demonstrates that RIG-I recognizes the 5′-triphosphorylated, encapsidated genomic RNA of IAV in the cytoplasm shortly after infection (Figure 1). To explore whether RIG-I can detect incoming IAV nucleocapsids, the authors treated cells with chemical inhibitors that blocked the replication of viral genomes or their nuclear export, thereby allowing them to monitor RIG-I activation independent of viral RNA synthesis. Indeed, incoming IAV nucleocapsids triggered RIG-I activation within 1 hr of infection. Furthermore, RIG-I, but not the related sensor MDA5, physically interacted with the panhandle-RNA structure of incoming nucleocapsids. The authors next asked if naturally occurring mutations in components of the nucleocapsid affect sensing by RIG-I. Residue 627 in the polymerase subunit PB2 is an important determinant of IAV host adaptation and virulence (Hatta et al., 2001). Avian IAV strains generally carry a glutamate at this position (PB2-627E), while mammalian IAV strains usually harbor a lysine residue (PB2-627K). Weber et al. (2015) found that nucleocapsids containing avian-adapted PB2-627E are recognized by human RIG-I more efficiently than those containing mammalian-adapted PB2-627K, suggesting that the PB2-627K mutation in IAV has evolved to evade RIG-I-mediated immunity in mammalian hosts.

The authors further confirmed previous results that showed that PB2-627K has a higher binding affinity to IAV NP as compared to PB2-627E, suggesting that tighter binding of the viral polymerase to the nucleocapsid may prevent RIG-I from accessing viral RNA and thereby limit antiviral restriction. Intriguingly, gene targeting of the adaptor MAVS or the use of a signaling-inactive mutant of RIG-I did not affect RIG-I-mediated restriction of avian-adapted IAV. These results indicated that binding of RIG-I to nucleocapsids can directly restrict IAV infection in a signaling- and IFN-independent manner.

In a complementary paper recently published in *Immunity*, Sato et al. (2015) support the concept that RIG-I works double duty by functioning as both an innate receptor and antiviral effector protein (Figure 1). The authors found that RIG-I senses the 5′-ε stem-loop region of the pregenomic RNA (pgRNA) of hepatitis B virus (HBV), a DNA virus of the family *Hepadnaviridae* that is responsible for significant morbidity worldwide (Liaw and Chu, 2009). Sensing of pgRNA by RIG-I triggered robust production of type III IFNs but only minimal production of type I IFNs. The authors further observed that binding of RIG-I to pgRNA inhibited access of the HBV polymerase (P protein) to the 5′-ε stem-loop region, thereby directly suppressing HBV replication. In support of this direct antiviral activity of RIG-I, a signaling-inactive mutant of RIG-I was still able to block HBV replication, while a RIG-I mutant deficient in RNA binding failed to do so.

Since its discovery in 2004, a plethora of studies have cemented the importance of RIG-I in antiviral signaling and IFN induction. The studies by Weber et al. (2015) and Sato et al. (2015) now

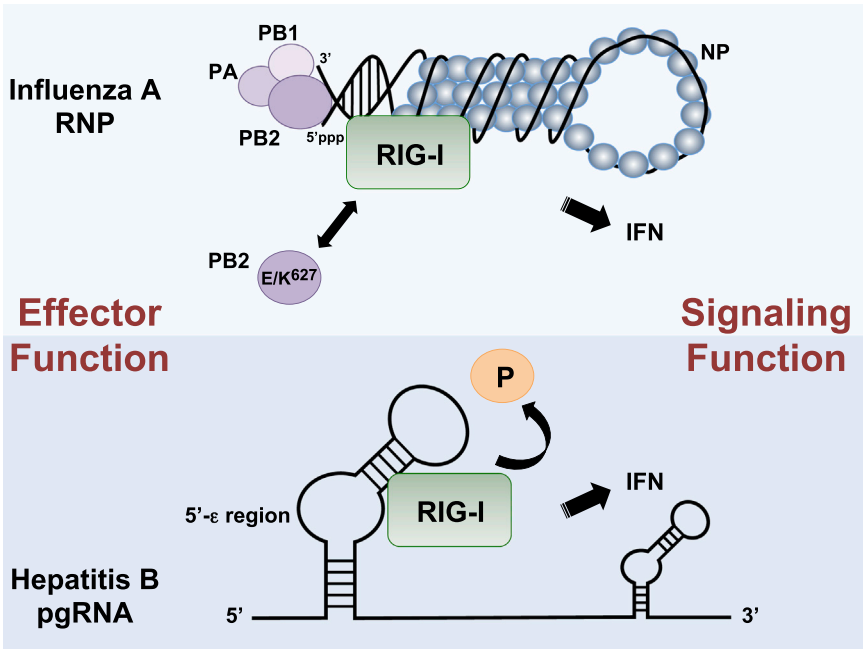

Figure 1. RIG-I Dually Functions as an Innate Immune Sensor Inducing IFN Expression and as a Direct Antiviral Restriction Factor

Upper panel: Ribonucleoproteins (RNPs) of the IAV nucleocapsid are composed of viral RNA, multiple nucleoproteins (NP), and the viral polymerase complex consisting of subunits PA, PB1, and PB2. RIG-I binds to the 5′-triphosphorylated panhandle-RNA structure of RNPs during their short passage through the cytoplasm. Binding of RIG-I to the viral RNA is modulated by residue 627 in PB2. The RNPs of mammalian-adapted IAV strains, harboring PB2-627K, are poorly recognized by RIG-I. The RNPs of avian-adapted IAV strains, containing PB2-627E, are efficiently bound by RIG-I, which directly inhibits viral replication. Furthermore, sensing of IAV RNAs by RIG-I leads to downstream signaling and induction of IFNs. Lower panel: RIG-I binds to the 5′-ε stem-loop region of the HBV pregenomic RNA (pgRNA). Binding of RIG-I to pgRNA counteracts the interaction of the HBV polymerase (P) with the 5′-ε region, thereby directly inhibiting viral replication. Furthermore, recognition of pgRNA by RIG-I leads to signaling and induction of predominantly type III IFN.

reveal that RIG-I not only acts as a sensor, but can also exert direct effector function to restrict viral replication. For HBV, RIG-I does so by binding the 5′-ε region of pgRNA to block binding of the P protein. For IAV, the mechanistic details of how viral RNA binding by RIG-I restricts virus replication are still unknown. It could be speculated that RIG-I disrupts binding of components of the IAV polymerase complex to the viral RNA. Furthermore, the binding of RIG-I to the IAV nucleocapsid is modulated by a well-known mammalian-adaptive mutation: an E627K substitution in PB2, which was previously described to allow efficient polymerase activity in mammalian cells.

While the two studies have considerably advanced our understanding of innate immune detection by RIG-I, they also raise several important questions. Does RIG-I displacement of viral polymerase protein(s) exclusively account for its direct effector function, or are there other activities of RIG-I that contribute to this antiviral effect? What are the relative contributions of RIG-I signaling and direct effector function toward host defense? In this regard, it is unclear whether these two antiviral modes of RIG-I happen simultaneously or in a temporally distinct fashion. Finally, as several upstream regulatory proteins are required for RIG-I-mediated antiviral signaling (reviewed in Chan and Gack, 2015), it can be speculated that there also exist host factors required for direct RIG-I effector function. Identification of such regulatory proteins would likely reveal further mechanistic details of how RIG-I directly restricts viral replication.

On the virus side, it remains to be elucidated whether RIG-I also restricts other viruses via direct effector function or if this function only applies to a small subset of viruses. Many viruses, however, have evolved means to block RIG-I-mediated antiviral signaling and IFN production. For example, the NS1 protein of IAV targets the ubiquitin E3 ligases TRIM25 and Riplet to inhibit RIG-I signal activation via K63-linked ubiquitination (Rajsbaum et al., 2012). The PB2-E627K substitution in mammalian-adapted IAV strains suggests that viruses may have also evolved means to evade RIG-I-mediated antiviral effector function. Furthermore, some virulent strains of IAV, such as the pandemic H1N1 virus of 2009 (pH1N1), do not contain PB2-E627K substitutions. Artificially introducing this substitution into pH1N1 did not increase its virulence (Herfst et al., 2010), suggesting that other adaptive mutations in IAV may exist to allow evasion of direct RIG-I antiviral function. In regards to the findings by Sato et al. (2015), it remains unclear why HBV infection preferentially triggers type III, but not type I, IFN induction upon RIG-I signaling. Recent work showing that peroxisomal-localized MAVS mediates type III IFN induction may provide a clue to the puzzle (Odendall et al., 2014). Alternatively, antagonistic proteins of HBV may specifically block the RIG-I-MAVS signaling axis that leads to type I IFN induction.

In conclusion, these two studies provide evidence that RIG-I exerts antiviral activity via two distinct mechanisms: the previously well-characterized innate sensing function of RIG-I, which leads to IFN gene expression, and the newly discovered antiviral effector function of RIG-I, which blocks binding of the viral polymerase to the RNA. A comprehensive view of how RIG-I controls viral replication will greatly enhance our understanding of innate immune restriction and may lead to novel antiviral therapies.

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Caps Off to Poxviruses

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In this issue of *Cell Host & Microbe*, Liu et al. (2015) and Burgess and Mohr (2015) describe how two poxvirus mRNA decapping enzymes hijack a host 5′-to-3′-exoribonuclease to evade antiviral innate immunity by limiting accumulation of double-stranded RNA.

A deadly chess game is in progress between viruses and their hosts that has extended over millions of years. Higher vertebrates have evolved elaborate and multilayered innate immune systems that, when successful, suppress or otherwise retard viral infections until the adaptive immune system can clear the infection. As a consequence, to propagate and spread, viruses must counteract host innate immunity.

Among viral pathogens that infect humans, the dsDNA genome poxvirus family includes one of the deadliest, variola virus, the causative agent of smallpox. Prior to development of an effective vaccine and its eradication in 1980, variola virus left a trail of destruction extending for many thousands of years. However, for virologists, poxviruses, in particular the smallpox vaccine strain, vaccinia virus (VACV), provide a treasure trove of information on this topic because of their relatively large genomes (including about 200 genes), the fact that they replicate entirely in the cytoplasm (where many innate immunity factors reside), and the many fascinating ways in which they counteract host immunity (McFadden, 2005).

To replicate and spread, poxviruses must antagonize two potent host antiviral pathways: the 2′,5′-oligoadenylate synthetase (OAS)/RNase L and double-stranded RNA (dsRNA)-dependent protein kinase R (PKR) systems (Sadler and Williams, 2008) (Figure 1). Interferons (IFNs) produced in response to viral infections induce through cell-surface receptors JAK-STAT signaling, resulting in transcription of a family of OAS genes and a single PKR gene. DsRNA produced by the virus activates both PKR and OAS proteins. PKR undergoes auto-phosphorylation and then it phosphorylates translation initiation factor eIF2α, inhibiting recycling of the GDP-bound form, causing cessation of protein synthesis. OAS proteins produce unusual 2′-to-5′ linked oligoadenylates known as "2-5A" from ATP. 2-5A binds with monomeric RNase L, causing it to dimerize into a catalytically active form that internally cleaves viral and cellular single-stranded RNA (Dong and Silverman, 1995). Therefore, both pathways block viral protein synthesis but by different mechanisms; one is direct (PKR), and the other is indirect (OAS/RNase L) by degrading mRNA and rRNA.

It has been known for 46 years that VACV produces dsRNA from annealing of complementary strands of viral RNA late in the poxvirus replication cycle (Duesberg and Colby, 1969). In perhaps the most important advance on this topic since then, back-to-back papers in this issue of *Cell Host & Microbe* describe how VACV blunts antiviral responses by limiting the accumulation of dsRNA (Burgess and Mohr, 2015; Liu et al., 2015) (Figure 1). Late (post DNA replication) in the poxvirus replication cycle, dsRNA is formed by base-pairing between converging viral transcripts produced from opposite strands of the viral genome. It turns out that VACV prevents high levels of dsRNA from accumulating through an RNA catabolic pathway that borrows from and mimics the host 5′-to-3′ pathway of mRNA decay (Figure 1). In eukaryotes, a decapping enzyme (Dcp2) containing a nudix/MutT hydrolase motif removes m^7-GDP caps allowing the 5′-to-3′-exoribonuclease, Xrn1, to progressively degrade the decapped 5′-monophosphorylated RNAs (Jones et al., 2012). VACV D9 and D10 proteins, nudix/MutT motif enzymes, are involved in suppressing translation of host mRNA, countering the host antiviral response while reducing competition for host translational machinery, and in maintaining divisions between different stages (early, intermediate, and late) of the virus-replication cycle (see Liu et al. [2015] for original references). D9 and D10 are early and late proteins, respectively, which have about 25% sequence identity and are widely conserved among chordopoxviruses, highlighting their importance. However, due to their compensating