CellPress

A Sense of Self: RIG-I's Tolerance to Host RNA

John W. Schoggins[1,*]
[1]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, TX 75390, USA
*Correspondence: john.schoggins@utsouthwestern.edu
http://dx.doi.org/10.1016/j.immuni.2015.06.022

The innate immune sensor RIG-I recognizes viral RNA while avoiding unwanted activation by self RNA. In this issue of *Immunity*, Schuberth-Wagner et al. (2015) show that a histidine residue in the RNA binding pocket of RIG-I sterically excludes the cap1 structure of self RNA, thereby preventing downstream activation.

The innate immune sensors RIG-I and MDA5 sense RNA virus genomes, leading to activation of the transcription factor IRF3 and induction of interferon-mediated antiviral responses. Structural studies indicate that RIG-I recognizes the 5′ triphosphate terminus of double-stranded RNA (dsRNA) (reviewed in Kolakofsky et al., 2012), whereas MDA5 binds the internal duplex structure (Wu et al., 2013). Similar to viral RNAs, endogenous host RNAs also contain a 5′ triphosphate end. The mechanisms by which sensors like RIG-I and MDA5 can distinguish viral from self RNA have been under active investigation (Anchisi et al., 2015; Kato et al., 2011). Accumulating evidence suggests that enzymatic modification of host RNA confers molecular signatures that mask an otherwise stimulatory molecule (Hornung et al., 2006; Pichlmair et al., 2006).

Eukaryotic mRNA is characterized by a cap structure that consists of a 5′ triphosphate linked to a methylated guanosine at N_7 (^{m7}G). This cap0 structure is essential for translation initiation and mRNA stability. The mRNA of higher eukaryotes is also modified by 2′O-methylation at N_1 (cap1) and N_2 (cap2). Previous studies implicated the cap0 structure of RNA ligands as an inhibitory modification to RIG-I activation. However, additional work demonstrated that the in vitro transcription protocols typically used to generated these ligands can also generate aberrant 5′ triphosphate-containing RNAs that activate RIG-I (Schlee et al., 2009). A systematic characterization of the relative contributions of host modifications to the stimulatory or inhibitory properties of synthetic (not in vitro-transcribed) dsRNA has not been carried out.

In this study, Schuberth-Wagner et al. (2015) sought to characterize which of these 5′ mRNA modifications prevent RIG-I activation. In lieu of in vitro transcription, they synthesized a series of well-defined 24-mer RNA ligands containing a 5′-triphosphate (ppp-RNA) and one or more features of cap0, cap1, or cap2 RNAs. Single-stranded RNAs containing these modifications were hybridized to a complementary RNA to generate blunt-ended dsRNA molecules. The identity and purity of the capped RNAs were verified by mass spectrometry. The various RNA ligands were transfected into human peripheral blood mononuclear cells (PBMCs) or murine bone-marrow-derived dendritic cells, and RIG-I activation was measured by IFN-α production. The authors found that a single 2′O-methyl group at N_1 ($pppG_mA$, cap1 modification) completely abolished RIG-I activation (Figure 1), whereas the cap0 and cap2 modifications only modestly reduced IFN-α induction.

To determine structural features of RIG-I that confer protection from activation by self RNAs, the authors mutated selected amino acids in the RIG-I RNA binding pocket. The RIG-I mutants were assayed for activation by IP-10 induction in RIG-I-deficient 293 cells. An unmodified ppp-dsRNA unexpectedly showed higher activation in cells expressing RIG-I with a mutation (H830A) as compared to wild-type RIG-I. Because H830 is dispensable for activation and has been shown to contact the 2′OH of N_1 in activating ligands, the authors hypothesized that RNA ligands with a 2′O-methyl modification at N_1 might be subject to steric exclusion in the binding pocket, thereby precluding activation. To address this, the authors compared RIG-I(WT) and RIG-I(H830A) activation by their panel of 5′-modified 24-mer dsRNAs. Whereas RIG-I(WT) was not activated by ppp-dsRNA containing a 2′O-methyl group at N_1 ($pppG_mA$), RIG-I(H830A) was susceptible to activation by this ligand (Figure 1). A similar ligand with a ^{m7}G cap (^{m7}G-$pppG_mA$) also activated RIG-I(H830A).

In vitro binding studies using WT and H830A RIG-I largely confirmed the activation phenotypes. Ligands bearing cap1 modifications (^{m7}G-$pppG_mA$) bound RIG-I(H830A) but not RIG-I(WT). In the absence of the ^{m7}G modification, binding of the N_1-modified $pppG_mA$ dsRNA to RIG-I(H830A) was reduced but not abolished. The authors hypothesize that this unexpected result might be due to residual, non-competitive binding. Together, the results suggest that H830 sterically excludes ppp-dsRNA bearing 2′O-methylation at N_1.

Because H830 is strictly conserved across evolution and prevented RIG-I activation by N_1-2′O-methylated ppp-dsRNA, the authors proposed that self RNA bearing this protective 2′O-methylation might exist. They first showed that long-term expression of RIG-I(H830A) in cells lacking endogenous RIG-I(WT) could eventually trigger IP-10 production in the absence of an exogenous activating ligand, suggesting cellular accumulation of RNAs bearing 2′O-methylation at N_1. The authors then immunoprecipitated cellular RNAs that associated with a FLAG-tagged RIG-I truncation containing the ppp-dsRNA binding domain (RD domain). These purified RNAs activated RIG-I(H830A), but not RIG-I(WT), further implicating the existence of self RNAs that are modified to avoid RIG-I activation. Lastly, siRNA-mediated silencing of the endogenous cap1 methyltransferase (hMTr1) resulted in IFN induction in the presence, but not absence, of RIG-I. These results indicated that the host uses cap1 modification to prevent the formation of self RNAs with immunostimulatory properties.

To explore the relevance of 2′O-methylation at N_1 in the context of self versus non-self RNA, the authors turned to the flaviviruses, which express a 2′O-methyltransferase activity as part of the multifunctional non-structural protein 5 (NS5).

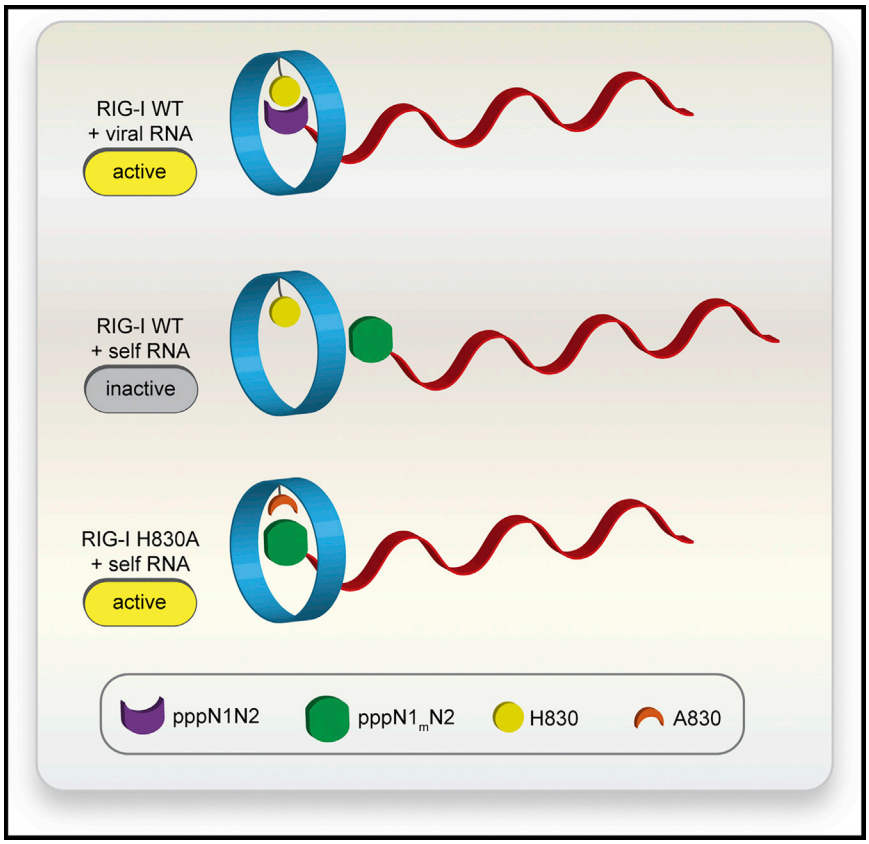

Figure 1. Schematic of RIG-I Exclusion of Self RNA

The RIG-I helicase and C-terminal regulatory domain (blue ring) recognize dsRNA (red helix) containing a 5′ triphosphate. In the absence of 2′O-methylation at N_1, a common feature of many viral RNAs, the dsRNA binds RIG-I to activate downstream responses. Self RNA (and some evasion-competent viral RNA, such as flavivirus) contains a 2′O-methyl group at N_1, which is excluded from the RIG-I binding pocket by a highly conserved H830 residue. With a H830A mutation, RIG-I is susceptible to activation by 2′O-methylated self RNAs. For simplicity, the ^{m7}G modification has been omitted from the illustration.

A yellow fever virus replicon (YFVR) bearing a NS5 E218A mutation, which abrogates 2′O-methylation activity, was used to determine whether the virus naturally uses 2′O-methylation to evade host recognition by RIG-I. In RIG-I-replete A549 cells, replication of the YFVR-E218A genome was attenuated when compared to YFVR-WT. By contrast, IFN-deficient Vero cells replicated both E218A and WT YFVR to similar levels. Similar results were obtained with fully infectious viruses. When RIG-I(WT) was expressed in RIG-I-deficient cells, YFVR-E218A stimulated higher amounts of IP-10 production when compared to YFVR-WT. By contrast, both YFVR-WT and YFVR-E218A activated cells expressing RIG-I(H830A) to similar levels. These data indicate that YFV NS5 methyltransferase marks the viral RNA with a 2′O-methylation that evades recognition and activation of RIG-I(WT) but not RIG-I(H830A).

The findings in this study potentially resolve the issue of how RIG-I can distinguish viral from self RNA. By capitalizing on a highly conserved H830 residue, RIG-I sterically avoids host RNAs containing a cap1 modification (Figure 1). This result highlights a new function for 2′O-methylation, a well-known but not necessarily well-understood RNA modification. Recent work has also indicated that 2′O-methylation of viral RNAs allows viruses to evade host restriction by MDA5 and IFIT family members (Daffis et al., 2010; Züst et al., 2011), suggesting an increasingly critical role for this RNA modification.

This study implicates the existence of a pool of self RNAs that can serve as endogenous ligands for RIG-I. The stimulatory activity of these RNAs could be revealed only by removing their 5′ modifications or by using cells expressing RIG-I(H830A) mutant. The identity and nature of these activating self RNAs was not characterized in this study. Thus, it is intriguing to speculate whether they are random and varied, or whether they fall into a specific class of RNAs that might have functional consequences to the immune system or the host cell in general. It will also be of great interest to determine whether a similar pool of self RNAs exists in vivo.

Another consideration stemming from this study is the potential for customizable RIG-I-based ligands. Whereas 2′O-methylation at N_1 abolished RIG-I activation, 2′O-methylation at N_2 was still partially activating, but not to the extent of non-methylated ppp-dsRNA. Thus, one can envision generating novel RIG-I ligands with titratable activation profiles depending on their modifications. Moreover, the finding that H830 in the dsRNA binding pocket of RIG-I is critical for excluding self RNAs suggests that the RIG-I binding pocket might also be customizable. Engineering custom RIG-I molecules with tailor-made ligands could provide useful tools for additional research into "self versus non-self" recognition, and might also serve as a platform for the development of novel therapeutics.

REFERENCES

Anchisi, S., Guerra, J., and Garcin, D. (2015). MBio *6*, e02349.

Daffis, S., Szretter, K.J., Schriewer, J., Li, J., Youn, S., Errett, J., Lin, T.Y., Schneller, S., Zust, R., Dong, H., et al. (2010). Nature *468*, 452–456.

Hornung, V., Ellegast, J., Kim, S., Brzózka, K., Jung, A., Kato, H., Poeck, H., Akira, S., Conzelmann, K.K., Schlee, M., et al. (2006). Science *314*, 994–997.

Kato, H., Takahasi, K., and Fujita, T. (2011). Immunol. Rev. *243*, 91–98.

Kolakofsky, D., Kowalinski, E., and Cusack, S. (2012). RNA *18*, 2118–2127.

Pichlmair, A., Schulz, O., Tan, C.P., Näslund, T.I., Liljeström, P., Weber, F., and Reis e Sousa, C. (2006). Science *314*, 997–1001.

Schlee, M., Roth, A., Hornung, V., Hagmann, C.A., Wimmenauer, V., Barchet, W., Coch, C., Janke, M., Mihailovic, A., Wardle, G., et al. (2009). Immunity *31*, 25–34.

Schuberth-Wagner, C., Ludwig, J., Bruder, A.K., Herzner, A.-M., Zillinger, T., Goldeck, M., Schmidt, T., Schmid-Burgk, J.L., Kerber, R., Wolter, S., Stümpel, J.-P., et al. (2015). Immunity *43*, this issue, 41–51.

Wu, B., Peisley, A., Richards, C., Yao, H., Zeng, X., Lin, C., Chu, F., Walz, T., and Hur, S. (2013). Cell *152*, 276–289.

Züst, R., Cervantes-Barragan, L., Habjan, M., Maier, R., Neuman, B.W., Ziebuhr, J., Szretter, K.J., Baker, S.C., Barchet, W., Diamond, M.S., et al. (2011). Nat. Immunol. *12*, 137–143.