Nucleoside modifications in RNA limit activation of 2'-5'-oligoadenylate synthetase and increase resistance to cleavage by RNase L

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ABSTRACT

The interferon-induced enzymes 2'-5'-oligoadenylate synthetase (OAS) and RNase L are key components of innate immunity involved in sensory and effector functions following viral infections. Upon binding target RNA, OAS is activated to produce 2'-5'-linked oligoadenylates (2-5A) that activate RNase L, which then cleaves single-stranded self and non-self RNA. Modified nucleosides that are present in cellular transcripts have been shown to suppress activation of several RNA sensors. Here, we demonstrate that in vitro transcribed, unmodified RNA activates OAS, induces RNase L-mediated ribosomal RNA (rRNA) cleavage and is rapidly cleaved by RNase L. In contrast, RNA containing modified nucleosides activates OAS less efficiently and induces limited rRNA cleavage. Nucleoside modifications also make RNA resistant to cleavage by RNase L. In contrast, RNA containing modified nucleosides activates OAS less efficiently and induces limited rRNA cleavage. Nucleoside modifications also make RNA resistant to cleavage by RNase L. Examining translation in RNase L/C0/C0 cells and mice confirmed that RNase L activity reduces translation of unmodified mRNA, which is not observed with modified mRNA. Additionally, mRNA containing the nucleoside modification pseudouridine (Ψ) is translated longer than wild-type mRNA. The observation that modified nucleosides in RNA reduce 2-5A pathway activation joins OAS and RNase L to the list of RNA sensors and effectors whose functions are limited when RNA is modified, confirming the role of nucleoside modifications in suppressing immune recognition of RNA.

INTRODUCTION

The antiviral 2-5A system is initiated when double-stranded (ds)RNA is bound by 2'-5'-oligoadenylate synthetases (OAS). There are four OAS genes in humans, OAS1, OAS2, OAS3 and OASL, encoding 8–10 isoforms due to alternative splicing. Activated OAS (other than enzymatically inactive OASL) uses ATP as substrate to produce unique, short 2'-5'-linked oligomers called 2-5A [p,5′A(2p5′A), x = 1–3; n ≥ 2] that activate the latent endoribonuclease RNase L. Binding of 2-5A to the N-terminal region of RNase L monomers causes RNase L dimerization and activates the C-terminal nuclease domain. Activated RNase L cleaves single-stranded (ss)RNA preferentially after UU and UA dinucleotide motifs. [For a recent review of the 2-5A system, see ref. (1).]

Although nucleoside modifications are common in RNA, how this influences 2-5A system activity is unknown. RNA contains more than 100 different modified nucleosides. Nucleoside modifications are produced naturally during RNA maturation and are introduced post-transcriptionally in a site-specific manner. Pseudouridine (Ψ) is the most prevalent modified nucleoside found in RNA (2,3). One function of Ψ at specific locations in tRNA and ribosomal RNA (rRNA) is to stabilize crucial secondary structure (4). However, no physiologic role has been identified for the majority of RNA modification sites, and the effect of nucleoside modifications on most RNA-binding proteins has not been established.

In vitro transcribed RNA containing modified nucleosides has been shown to be less stimulatory to several host defense RNA sensors, including protein kinase R (PKR), toll-like receptor (TLR)3, TLR7, TLR8 and retinoic acid-
inducible gene I (RIG-I) (5–8). We previously reported the production of in vitro transcribed mRNA in which every uridine is replaced by pseudouridine (ψ-mRNA) and found that protein expression from ψ-mRNA is higher than from unmodified in vitro transcribed mRNA (9), and this enhanced translation is due in part to reduced activation of PKR by ψ-mRNA (6).

Here, we report that the presence of modified nucleosides in RNA has two effects on the 2-5A pathway. Certain unmodified in vitro transcribed mRNAs activate OAS, resulting in rRNA cleavage and reduced translation. Additionally, unmodified RNA is more rapidly cleaved by activated RNase L. In contrast, RNAs containing certain modified nucleosides fail to activate OAS and are resistant to cleavage by RNase L. Modified RNA is therefore identified as a distinguishing pattern for 2-5A system activity.

MATERIALS AND METHODS

Cells and reagents

Human embryonic kidney (HEK) 293T cells were obtained from the American Type Culture Collection and were used to generate recombinant human RNase L and p32[2′5′-A]2A (trimer 2-5A) as described (11,12). Dual-labeled fluorescent probe 6-FAM-UUA CAA AUC UCUC UUG CCC CAU UUU UUU GGU UUA-BHQ-1 was custom synthesized by Integrated DNA Technologies. In vitro OAS activation and measurement of functional 2-5A

Recombinant hexahistidine-tagged human OAS1 p42 was a gift of Rune Hartmann (University of Aarhus, Denmark) (10). Recombinant human RNase L and p32[2′5′-A]2A (trimer 2-5A) were prepared as described (11,12). Dual-labeled fluorescent probe 6-FAM-UUA CAA AUC UCUC UUG CCC CAU UUU UUU GGU UUA-BHQ-1 was custom synthesized by Integrated DNA Technologies. In vitro OAS1 activation was performed as described (13). Briefly, 20 μg/ml of OAS1 was activated with 2.0 μg/ml RNA for the indicated time in buffer consisting of 20 mM HEPES pH 7.5, 20 mM Mg(OAc)2, 20 mM KCl, 1 mM EDTA and 10 mM ATP. Reactions were stopped by heating to 95°C for 3 min. Rate of functionally active 2-5A produced was measured using a fluorescence resonance energy transfer (FRET) based assay as described previously (14,15). Synthesized p32[2′5′-A]2A (trimer 2-5A) (12) was purified using HPLC and used for generating standard curves (13).

rRNA cleavage

One day prior to transfection, WT or RNase L−/− MEF cells were seeded into 96-well plates at a density of 5.0 × 10⁴ cells/well and treated with 1000 U/ml interferon-α2A/D (Sigma). Poly(I:C) or mRNAs were complexed with lipofectin as described (7). Cells were exposed to 50 μl DMEM containing lipofectin-complexed RNA (2.5 μg) for 1 h, which was then replaced with complete medium and further cultured. At 3 h post-transfection, total RNA was recovered from cells using Trizol (Invitrogen). RNA was separated by agarose gel electrophoresis, stained with SybrGold reagent (Invitrogen) and detected using UV fluorescence and a GelDoc 2000 imager (Bio-Rad Laboratories).

In vitro RNA cleavage by RNase L

Recombinant human RNase L was prepared as described (14). For oligo RNAs, 12.5 nM RNase L was activated on ice with 100 nM trimer 2-5A for 30 min in RNase L cleavage buffer (25 mM Tris–HCl pH 7.4, 100 mM KCl, 10 mM MgCl₂, 50 mM ATP and 7 mM β-mercaptoethanol). Then 100 nM 5′-end-labeled oligo RNA [32P]pC11U2C7 or [32P]pC11Ψ2C7 was added and reactions were incubated at 30°C. At the indicated times, reactions were stopped by the addition of urea-TBE loading buffer (Bio-Rad) and heating to 95°C for 3 min. Aliquots were separated by 15% polyacrylamide gel electrophoresis, gels were dried, and samples were imaged using a phosphor storage screen (Molecular Dynamics) and detected using a Typhoon PhosphorImager (GE Healthcare). Cleavage of mRNA was performed similarly, using 10 nM RNase L, 10 nM trimer 2-5A and 100 nM of metabolically labeled firefly luciferase mRNA. Reactions were stopped by heating to 95°C for 5 min. The mRNA was...
recovered by phenol:chloroform extraction and detected by northern blotting.

**RNA stability in rabbit reticulocyte lysate**

Equal mass (25 ng/μl) or equal molar (40 μM) mRNAs-encoding firefly and Renilla luciferases were incubated in 15 μl rabbit reticulocyte lysate (RLL) (Promega) at 30°C. At the indicated times, a 2 μl aliquot was removed and the RNA was recovered using Trizol for subsequent detection by northern blotting.

**RNA stability in cell culture**

HEK293T, WT MEF or RNase L−/− MEF cells were nucleofected with 5 μg mRNA using nucleofector program T-020 and nucleofector V kit (Lonza). After 15 min recovery in RPMI, cells were plated in complete media and incubated at 37°C. At the indicated time, RNA was recovered from cells using Trizol for subsequent detection by northern blotting.

**Northern blotting**

RNA was isolated from RRL or cells using Trizol. Samples were processed and analyzed on northern blots as previously described (16). Probes were derived from plasmids and were specific for the coding region of firefly luciferase or Renilla luciferase.

**Half-life calculation**

After performing northern blots, images were scanned from film and ImageJ (version 1.44 p) was used to measure the density of the band corresponding to the full-length mRNA. For each data point, the log10 was taken and the values were plotted as a function of time. The slope of best fit line (k) was used to calculate the mRNA half-life using the equation \( t_{1/2} = 0.693/k \) (17).

**Detection of reporter proteins in RNA-transfected cells**

Cells were seeded into 96-well plates at a density of 2–5 × 10^4 cells/well one day prior to transfection. Cells were exposed to 50 μl DMEM containing lipofectin-complexed RNA (0.25 μg) for 1 h, which was then replaced with complete medium and further cultured. Cells were incubated in methionine/cysteine-free medium (Invitrogen) for 1 h, then pulsed with complete medium supplemented with 35S-methionine/cysteine (140 mCi/ml) (PerkinElmer) for 3 h prior to lysis in 50 μl RIPA buffer supplemented with protease inhibitor cocktail (Sigma). Renilla luciferase was immunoprecipitated from lysates using an anti-Renilla luciferase antibody (PM047, Medical & Biological Laboratories) and protein G-coated Dynabeads (Invitrogen) and separated by 15% polyacrylamide gel electrophoresis. Gels containing the labeled samples were treated with 1 M sodium salicylate, dried and a fluorogram was generated by exposure to BioMax MS film (Kodak).

**Statistical analysis**

All data are reported as mean ± standard error of the mean (SEM). Statistical differences between treatment groups were calculated by the Student’s t-test using Microsoft Excel. For all statistical testing, a \( P < 0.05 \) was considered significant.

**RESULTS**

RNA containing nucleoside modifications activates OAS less than unmodified RNA

We first compared the activation of purified human OAS1 by unmodified RNA or RNA with identical sequence containing the modified nucleosides \( \Psi, N^\bullet\text{-methyladenosine} \) (m6A) or 2-thiouridine (s2U). The amount of functionally active 2-5A (trimer or higher) produced was quantified using a FRET-based RNase L activation assay (12,13). The unmodified RNA efficiently activated OAS1. In contrast, RNA containing \( \Psi, \text{m}^\bullet\text{A or s}^2\text{U} \) were poor activators of OAS and did not induce production of functionally active 2-5A (Figure 1). Because \( \Psi \) is the most prevalent modified nucleoside (2,3) and has also been shown to reduce the RNA activation of other RNA sensors (5–8), subsequent experiments focused on the comparison of unmodified RNA to \( \Psi \)-containing RNA with identical sequence.

**Pseudouridine-containing RNA induces less rRNA cleavage than unmodified RNA**

OAS activation by unmodified RNA leads to activation of RNase L, which mediates the effector function of the 2-5A system by cleaving ssRNA. RNase L-mediated cleavage at exposed loops of rRNAs in intact ribosomes results in well-defined cleavage patterns in rRNA (18). Therefore, the integrity of rRNA following RNA transfection was examined. Lipofectin-complexed RNA was transfected...
to WT and RNase L−/− MEF cells, and total RNA was subsequently recovered and examined by agarose gel electrophoresis and UV imaging. Cells mock transfected with no RNA were included as a negative control. In WT cells, delivery of unmodified in vitro transcribed RNA induced cleavage of rRNA, but significantly less rRNA was cleaved when the transfected RNA contained Ψ. Transfection of the same set of RNAs into RNase L−/− MEF cells did not generate the specific rRNA degradation profile (Figure 2).

RNase L cleaves uridine-containing RNA more readily than Ψ-containing RNA

Activated RNase L cleaves preferentially after UpNp in ssRNA. Therefore, to compare the ability of RNase L to cleave Ψ-containing RNA, purified recombinant human RNase L was activated with trimer 2-5A and mixed with 5′-[32P] end-labeled oligo RNA containing a single RNase L cleavage site (C11U2C7 or C11Ψ2C7). The oligo RNA containing unmodified uridine was rapidly cleaved, while there was no significant cleavage of the oligo RNA containing Ψ (Figure 3A and B). Full-length firefly luciferase mRNA metabolically labeled with 32P was then analyzed for cleavage by RNase L. Both unmodified and Ψ-RNA could be cleaved by RNase L. However, consistent with the results obtained with oligo RNAs, Ψ-containing RNA was cleaved less efficiently by RNase L than unmodified RNA (Figure 3C).

Nucleoside-modified RNA has an increased half-life

We next examined the stability of unmodified and Ψ-containing RNA by northern blot analysis. Both unmodified and Ψ-RNA were equally stable at room temperature through experimental time courses and indefinitely in storage at −20°C. Unmodified and Ψ-RNAs were added to RRL or transfected to HEK293T cells. When transfecting cells using cationic lipids, a portion of RNA complexed with transfection reagents persist as an extracellular, nuclease-protected fraction. Therefore, for these experiments, we used nucleofection to deliver naked RNA and confirmed the rapid degradation of extracellular RNA by serum nucleases in the culture media. Total RNA was subsequently re-isolated and aliquots were examined by northern blot to compare degradation rates of the reporter RNAs. Two reporter RNAs, firefly and Renilla luciferase, were studied simultaneously to ensure that stability differences were not a result of delivery conditions. Ψ-modified RNAs had longer half-lives than unmodified RNAs in RRL (Figure 4A). Similarly, in HEK293T cells the half-life of Ψ-modified firefly luciferase RNA increased ~2-fold to 6.1 h compared to 3.2 h for unmodified RNA (Figure 4B).

Subsequently, the influence of RNase L on the stability of unmodified and Ψ-containing RNA was also compared using RNase L−/− MEF cells. As in HEK293T cells, the
RNA was delivered by nucleofection. Total RNA was recovered from cell culture and firefly and Renilla luciferase RNA were assessed by northern blot. In both WT and RNase L/C0/MEF cells, 3'-modified RNA half-life was increased by 50% to 3.8 h compared to 2.5 h for unmodified RNA (Figure 4C).

**Translational advantage of 3'-RNA is reduced in the absence of RNase L.**

Considering that 3'-modification of RNA reduced activation of OAS1 and RNA induced rRNA degradation, and
that RNA containing $\Psi$ was cleaved by RNase L less efficiently, we asked how the absence of RNase L influences translation of unmodified and $\Psi$-containing mRNA.

Thus, mRNAs encoding luciferase were transfected into WT and RNase L$^{-/-}$ MEF cell lines and translation was assessed by measuring luciferase activity. In WT cells, more protein was translated from the $\Psi$-containing mRNA than from the unmodified mRNA. In the RNase L$^{-/-}$ cell line, there was lower level translation of both mRNAs and the translational advantage of $\Psi$-mRNA over unmodified mRNA was dramatically reduced (Figure 5A).

A similar pattern of translation occurred in the spleens of mice following injection of mRNA. Either WT C57Bl/6 or RNase L$^{-/-}$ mice were given lipofectin-complexed luciferase mRNA by tail vein injection. Luciferase activity was assessed in spleen lysate 4 h later. In WT mice, $\Psi$-containing mRNA was translated at higher levels than unmodified mRNA. In RNase L$^{-/-}$ mice, translation of $\Psi$-containing mRNA reached the same level as observed in WT mice, but translation of unmodified mRNA was increased relative to WT ($P < 0.05$), (Figure 5B).

**DISCUSSION**

We investigated how the enzymes of the 2-5A system interact with RNA and the role that modified nucleosides play in altering activation and effector function. Our data show that in vitro transcribed unmodified RNA activates OAS1, but this activation is reduced when the RNA contains modified nucleosides. OAS activation by unmodified RNA leads to RNase L-mediated rRNA cleavage, which is reduced by $\Psi$-RNA. Furthermore, RNase L cleaves unmodified RNA more efficiently than RNase L$^{-/-}$.

**Translation of pseudouridine-containing mRNA continues for a longer time than unmodified mRNA**

Having seen that the presence of modified nucleosides in mRNA increases its half-life and translation efficiency, we compared the translation over time, to determine how modified nucleosides influence the duration of translation.

RNA was complexed to lipofectin and delivered to cells that were subsequently pulsed with $^{35}$S-methionine/cysteine for 3 h at 1, 21 and 45 h post-transfection. Translation of the mRNA was assessed by immunoprecipitating the encoded Renilla luciferase protein and measuring $^{35}$S incorporation. There was a higher level of total translation of $\Psi$-containing mRNA at each time point (Figure 6A), and ongoing translation of $\Psi$-containing mRNA continued after at least 48 h when detectable translation of unmodified mRNA ceased (Figure 6B).
characterized as requiring OAS family of proteins. Activation of OAS was originally shown with 20 bp long dsRNA (19), but subsequently other structures with significant single-strandedness have been proven to be potent OAS activators, including aptamers (20), viral RNAs (21–24) and some cellular RNAs (13,25). However, dsRNA generated from homopolymers contained 2'-O-methylated nucleosides (26,27) or 5-methyl-uridines (28) did not activate OAS. Here, we report that unmodified in vitro transcribed RNA activated OAS1 to generate 2-5A, but this was substantially reduced when RNA contained \( \Psi \), \( m^\Psi \)A or \( s^\Psi \)U. Recently, the consensus sequence nnWWnnnnnnnWGn (W = U or A) was demonstrated to be important for OAS1 activation by dsRNA, and this interaction was dependent on the minor groove and free OH groups on the critical base pairs (29). The requirement that three out of the four critical base pairs in this sequence must be U:A highlights the importance of uridine for OAS1 activation. However, pseudouridine forms hydrogen bonds with adenosine in the same manner that uridine does (Figure 7), and the imino group of \( \Psi \) is oriented toward the major groove (30), so how \( \Psi \) disrupts OAS1 activation remains unclear.

The presence of \( \Psi \) stabilizes secondary structure and adds rigidity to both ss and dsRNA [reviewed in (4)]. In this capacity, \( \Psi \) could affect OAS activation by altering the equilibrium structure of the RNA, rather than directly affecting OAS binding.

Activation of OAS leads to production of 2-5A, which binds to the latent endoribonuclease RNase L, the effector enzyme of the 2-5A pathway. Activated RNase L cleaves various ssRNA including specific sites of rRNAs accessible in the intact ribosome, resulting in RNase L-specific cleavage products visible by gel electrophoresis (18). In WT MEF, unmodified RNA induced rRNA cleavage, which was reduced if RNA contained \( \Psi \). However, none of the RNAs caused rRNA cleavage in RNase L-/- cells, confirming that the 2-5A system is required for RNA-induced rRNA cleavage. High levels of 2-5A result in global rRNA cleavage by RNase L (31), and when sustained ultimately lead to apoptosis (32,33). In comparison, the level of rRNA cleavage induced here by transfection of in vitro transcribed RNA is relatively small, and may not be expected to induce high levels of apoptosis. On the other hand, this level of rRNA cleavage is sufficient to have a profound impact on translation of the reporter mRNA. We propose that unmodified RNA induces local OAS and RNase L activation, as demonstrated with viral RNAs and ssRNA covalently linked to dsRNA (34,35). Accordingly, locally activated RNase L cleavage likely reduces translation of unmodified mRNA through local cleavage of rRNA without inducing global rRNA cleavage and apoptosis.

The presence of \( \Psi \) has been shown to enhance the stability of RNA secondary structures, but has not previously been demonstrated to cause resistance to nucleases. RNA containing \( \Psi \) was cleaved efficiently by RNase A, RNase H (36), RNase T1, RNase T2, nuclease P1 and snake venom phosphodiesterase, although there is some indication that pancreatic diesterase and snake venom phosphodiesterase may cleave \( \Psi \)-RNA with reduced efficiency (37). A previous report based on cleavage of a C11N2C7 oligo RNA showed that RNA containing 2'-deoxy-2'-\( \alpha \)-fluorouridine was bound by RNase L but cleaved slowly, whereas RNA containing 2'-O-methyluridine was not bound by RNase L (38). Here, we used a similar approach and demonstrated that purified RNase L readily cleaved the oligo ssRNA C11U2C7 but not when the cleavage site contained \( \Psi \). We also extended those findings to the examination of long in vitro transcribed RNA and showed that unmodified RNA was cleaved by purified RNase L, but cleavage of \( \Psi \)-RNA occurred more slowly. The cleavage of \( \Psi \)-RNA despite inactivity toward C11U2C7 is not surprising considering the substrate specificity of RNase L. RNase L cleaves preferentially UpNp, with highest activity following UU, UA and AU, but it is also capable of cleaving RNA following dinucleotide motives that avoid U (e.g. AA, AC and CA) (39–41).

We also examined the effect of \( \Psi \)-modification on the stability of in vitro transcribed RNA. In RRL and in cell
culture, \(\Psi\)-RNA was degraded more slowly than unmodified RNA. Previous experiments also suggested that \(\Psi\)-RNA is retained longer following injection in mice (9). Despite the rapid cleavage of unmodified RNA by RNase L \textit{in vitro}, the half-life of unmodified RNA did not increase to the level of \(\Psi\)-RNA in RNase L \(\gamma^{-/}\) cells. This suggests that in addition to RNase L, other intracellular nucleases also cleave unmodified RNA more efficiently than \(\Psi\)-containing RNA.

As seen in previous reports (6,9), in WT cells, there was significantly higher translation of \(\Psi\)-mRNA than unmodified mRNA. In contrast, in RNase L \(\gamma^{-/}\) MEF cells the translational advantage of \(\Psi\)-mRNA over the unmodified mRNA was limited. Similarly, the translational advantage of \(\Psi\)-mRNA was reduced in RNase L \(\gamma^{-/}\) mice relative to WT mice. Notably, however, the absolute translation level of \(\Psi\)-mRNA remained equal in WT and RNase L \(\gamma^{-/}\) mice, while the translation of unmodified mRNA increased in RNase L \(\gamma^{-/}\) mice. This indicates that neither the presence of RNase L nor \(\Psi\)-mRNA alone significantly affects translation of \(\Psi\)-mRNA, but rather that unmodified RNA causes translational inhibition through RNase L activation. Moreover, these results are consistent with the \textit{in vitro} activation of OAS1 by unmodified RNA that we observed. Furthermore, \(\Psi\)-mRNA continued to be actively translated for a longer duration than unmodified mRNA. In RNase L \(\gamma^{-/}\) cells and mice, the translational advantage of \(\Psi\)-mRNA is reduced, despite the observation that the absence of RNase L in cells does not significantly alter the stability of either U-RNA or \(\Psi\)-mRNA. Therefore, we propose that in cells the mechanism by which the 2-5A system enhances translation of \(\Psi\)-mRNA is not primarily through reduced degradation of the \(\Psi\)-mRNA itself, but instead through decreased rRNA cleavage resulting from diminished OAS activation. Thus, OAS activation by unmodified mRNA results in RNase L activation, which reduces translation due to rRNA cleavage rather than through cleavage of the activating transfected mRNA.

In addition to viral RNA, select cellular mRNAs from prostate cancer cells have been shown to activate OAS (13). Additionally, cleavage of cellular and viral RNAs by RNase L produces short RNAs, which can activate the cytoplasmic RNA sensor RIG-I, leading to interferon production (42–44). The presence and effects of modified nucleosides in these RNase L-generated short RNAs has not been investigated. Because RNA containing modified nucleosides activates OAS less and is less efficiently cleaved by RNase L, if viral infection or cancer development were to alter the level of nucleoside modification, it could lead to modified RIG-I activation and ultimately change immune responsiveness and disease progression. Consistent with this possibility, some viral mRNAs are hyper-methylated compared to mammalian mRNA (45,46). Viral-encoded 2'-O-methyltransferases extensively modify the 5'-ends of their capped mRNA. This modification is critical for the virus to avoid interferon induction and evade detection by the immune system. (47,48). Additionally, a recent study of non-small-cell lung cancer identified the upregulation of small nucleolar RNAs (snoRNAs), which function in directing pseudouridylation and 2'-O-methylation of RNA (49).

Nucleases play a central role in host defense through destruction of pathogenic nucleic acids. The 2-5A system functions to detect and degrade danger-associated intracellular RNAs. Activation of RNase L also leads to reduced translation due to rRNA cleavage and when sustained, results in apoptosis, further limiting replication of the pathogens. Here, we identify that some unmodified RNAs serve as a molecular pattern recognized by OAS and RNase L. The 2-5A system activity is decreased when RNA contains nucleoside modifications, which reduce both OAS activation and cleavage by RNase L. Other RNA sensors, including PKR, TLR3, TLR7, TLR8 and RIG-I (5–8) also exhibit reduced activation by RNA containing modified nucleosides. Therefore, this work supports the proposal made by us and others that RNA sensors recognize certain RNAs that contain unmodified nucleosides as a danger-associated molecular pattern, as part of the extensive system of innate host defenses against pathogenic RNA, but that nucleoside modification suppresses RNA immunogenicity.

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