LSm14A is a processing body-associated sensor of viral nucleic acids that initiates cellular antiviral response in the early phase of viral infection

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Recognition of viral nucleic acids by pattern recognition receptors initiates type I IFN induction and innate antiviral immune response. Here we show that LSm14A, a member of the LSm family involved in RNA processing in the processing bodies, binds to synthetic or viral RNA and DNA and mediates IRF3 activation and IFN-β induction. Knockdown of LSm14A inhibits cytosolic RNA- and DNA-trigger type I IFN production and cellular antiviral response. Moreover, LSm14A is essential for early-phase induction of IFN-β after either RNA or DNA virus infection. We further found that LSm14A-mediated IFN-β induction requires RIG-I–VISA or MITA after RNA or DNA virus infection, respectively, and viral infection causes translocation of LSm14A to peroxisomes, where RIG-I, VISA, and MITA are located. These findings suggest that LSm14A is a sensor for both viral RNA and DNA and plays an important role in initiating IFN-β induction in the early phase of viral infection.

The innate immune response is the first line of host defense against viral infection, which is mediated by pathogen recognition receptors (PRRs) after recognition of viral nucleic acids, replicative intermediates, and transcription products (1–3). It has been well established that viral RNA is sensed by endosomal Toll-like receptors (TLRs) and cytosolic RIG-I–like receptors (RLRs). Recognition of viral RNAs by these receptors links them to downstream adapter proteins, including TRIF, VISA/MAVS/IPS-1/Cardif (4–7), and MITA/STING (8, 9), leading to activation of the kinases TBK1 and IKKβ. These kinases phosphorylate and activate the transcription factors IRF3 and NF-κB, respectively, which cooperatively induce transcription of a set of antiviral genes including type I IFNs (10).

In contrast to recognition of viral RNA, the mechanisms by which cytosolic viral or microbial DNA induces type I IFN and proinflammatory cytokines are not well understood. Particularly, the sensors that detect cytosolic viral DNA and the signaling mechanisms of the subsequent IFN induction pathways are still unclear or controversial. It has been demonstrated that TLR9 recognizes CpG DNA derived from viruses and bacteria in the endolysosomes, leading to IFN-α induction via MyD88 and IKKα (11, 12). However, exogenous dsDNA introduced into the cytoplasm, as would happen during infection by a DNA virus, triggers IFN-β induction through MITA-TBK1–dependent pathways (13). To date, several cytoplasmic DNA sensors have been reported, including DAI, RNA polymerase III, IFI16, and DDX41 (14–17). However, it seems that none of the identified sensors plays a universal role in detecting viral and microbial DNA in distinct cell types. In addition, it is unknown whether the sensing of viral or microbial nucleic acids is temporally regulated by distinct receptors.

In the present study, we identified a component of the processing bodies (P-bodies), LSm14A (also called RAP55), as an activator of IRF3 in expression screen experiments (18). Our results indicated that LSm14A bound to synthetic or viral RNA and DNA and was essential for initiating the induction of IFN-β in the early phase of virus infection. We further found that viral infection caused LSm14A translocation to peroxisomes, where LSm14A initiated IFN-β induction via RIG-I–VISA or MITA after RNA and DNA virus infection, respectively. These findings suggest that LSm14A is a sensor for both viral RNA and DNA and provide a mechanism for temporal regulation of type I IFN induction and cellular antiviral innate immunity.

Results

Identification of LSm14A as a Mediator of IFN-β Induction. ISRE (IFN-stimulated response element) is an enhancer motif bound by activated IRF3/7, which is essential for transcriptional induction of type I IFN genes (19, 20). To identify candidate molecules involved in virus-triggered innate immune response, we screened ∼10,000 independent human cDNA expression plasmids for their ability to regulate ISRE activity by reporter assays. These efforts led to the identification of LSm14A, a member of the LSm family of proteins that are involved in RNA metabolism (18). As shown in Fig. 1*A*, overexpression of LSm14A activated ISRE and potentiated SeV-triggered ISRE activation in a dose-dependent manner. The role of LSm14A in mediating ISRE activation is specific to the LSm family proteins because 11 other examined members of the LSm family proteins had no marked effects on ISRE activation either in the absence or presence of SeV infection (Fig. S1*A*). Overexpression of LSm14A also activated NF-κB and potentiated SeV-induced NF-κB activation (Fig. 1*B*). Consistently, LSm14A activated the IFN-β promoter and potentiated SeV-induced activation of the IFN-β promoter (Fig. 1*B*), which requires coordinative and cooperative activation of IRF3 and NF-κB. Furthermore, overexpression of LSm14A markedly potentiated SeV-induced transcription of endogenous *IFNB1* gene (Fig. 1*C*), as well as secretion of IFN-β cytokine (Fig. 1*D*). Interestingly, LSm14A had no marked effects on transcriptional activation of promoters of the IFN-α family genes, including IFN-α1, IFN-α4, IFN-α7, and IFN-α14 (Fig. S1*B*). These results suggest that LSm14A differentially regulates type I IFN expression, consistent with previous observations that expression of IFN-β and IFN-α family members are differentially regulated after viral infection (19).

Because LSm14A mediates virus-triggered induction of IFN-β, we next determined whether LSm14A plays a role in cellular antiviral response. In plaque assays, overexpression of LSm14A inhibited vesicular stomatitis virus (VSV) replication and further enhanced the inhibition of VSV replication triggered by cytoplasmic poly(I:C) (Fig. 1*E*). Similar results were obtained with GFP-tagged Newcastle disease virus (NDV). As shown in Fig. 1*F*, overexpression of LSm14A inhibited NDV replication, as suggested by diminished GFP expression. Collectively, these data suggest that LSm14A is involved in cellular antiviral responses.

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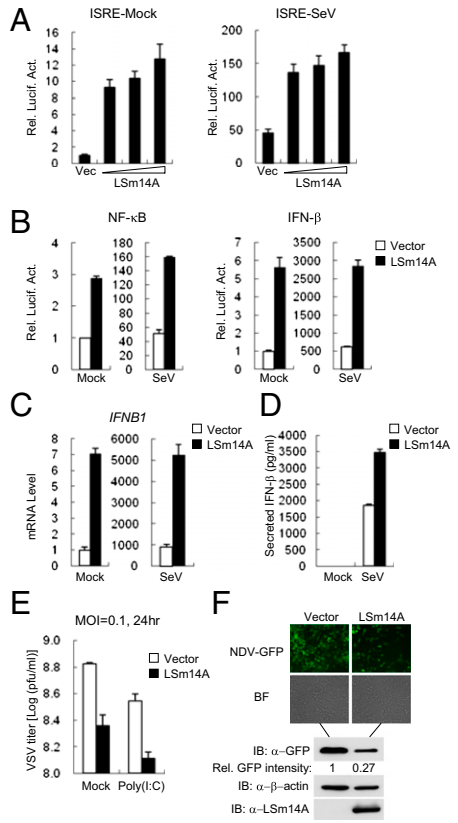

Fig. 1. Identification of LSm14A. (*A*) LSm14A activated ISRE in a dose-dependent manner. The 293 cells were transfected with ISRE reporter and increased amount of LSm14A plasmid for 18 h, and then left uninfected or infected with SeV for 10 h before luciferase assays. (*B*) LSm14A activated NF-κB and the IFN-β promoter. Reporter assays were performed similarly as in *A*. (*C*) LSm14A increased *IFNB1* gene expression. The 293 cells were transfected with LSm14A plasmid for 18 h and then infected with SeV for 10 h before RT-PCR analysis for *IFNB1* mRNA was performed. (*D*) LSm14A increased the production of IFN-β. The 293 cells were transfected and infected with SeV as in *C*. Secretion of IFN-β was measured by ELISA at 18 h after SeV infection. (*E*) Overexpression of LSm14A inhibited VSV replication. The 293 cells were transfected with LSm14A plasmid for 18 h and then further transfected with poly(I:C) for an additional 24 h. The transfected cells were infected with VSV [multiplicity of infection (MOI) = 0.1] for 24 h before culture medium was harvested for measurement of VSV production by plaque assays. (*F*) Overexpression of LSm14A inhibited NDV replication. The 293 cells were transfected with LSm14A plasmid for 18 h. The cells were then infected with NDV-GFP (MOI = 0.01) for another 36 h. The replications of NDV were analyzed by microscopy (*Upper*) and analyzed by immunoblots with anti-GFP (*Lower*). The intensities of GFP bands were quantitated using the Bio-Rad Quantity One Program and normalized to that of β-actin levels. BF, bright field. For *A*–*E*, graphs show mean ± SD, $n = 3$.

LSm14A Recognizes Synthetic or Viral RNA and DNA. LSm14A is a member of the LSm family of proteins, which have been shown to be involved in RNA processing events (18, 21). The N terminus of LSm14A, amino acids 1–76, is a conserved LSm domain, whereas the C terminus contains two domains called "DFDF box" (amino acids 291–316) and "FDF_TFG box" (amino acids 361–397), respectively (Fig. 2*A*). Considering its predictive ability of binding to RNA, we examined whether LSm14A binds to poly(I:C), a synthetic dsRNA. Poly(I:C) pull-down experiments showed that both LSm14A and RIG-I could bind to poly(I:C) individually or simultaneously (Fig. 2*B*). As shown in Fig. 2*C*, LSm14A also bound to both biotinylated 5′-ppp-RNA (ssRNA) and poly(dA:dT) (dsDNA). Competitive poly(dA:dT) pull-down experiments indicated that the association of LSm14A with poly (dA:dT) could be inhibited by unlabeled poly(dA:dT) but not poly(dG:dC) or plasmid DNA (Fig. 2*D*), suggesting that LSm14A has higher affinity to poly(dA:dT) than poly(dG:dC) or plasmid DNA.

Because ISD (a 45-bp dsDNA), HSV 60mer (a 60-bp dsDNA oligonucleotide derived from the HSV-1 genome), and VACV 70mer (a 70-bp dsDNA conserved in various poxviral genomes, such as VACV) could activate IFN-β response (16), we detected the interactions of GST-tagged LSm14A with the above dsDNAs by GST pull-down experiments. As shown in Fig. 2*E*, LSm14A bound to ISD, HSV 60mer, and VACV 70mer, as well as poly(dA:dT). Taken together, these findings suggest that LSm14A binds to synthetic ssRNA, dsRNA, dsDNA, and viral nucleic acids.

To map domains of LSm14A that are responsible for nucleic acid binding, we made various truncation mutants of LSm14A (Fig. 2*A*). Pull-down assays indicated that the FDF_TFG box was required and sufficient for the ability of LSm14A to bind to 5′ ppp-RNA and poly(dA:dT) (Fig. 2*C*). Consistent with its ability to bind to poly(I:C), 5′ppp-RNA, and poly(dA:dT), LSm14A dramatically potentiated activation of the IFN-β promoter triggered by transfection of these nucleic acids but not poly(dG:dC) or plasmid DNA (Fig. 2*F*), whereas the truncation mutant containing the FDF_TFG box acted as a dominant negative mutant and markedly inhibited activation of the IFN-β promoter triggered by poly(I:C), poly(dA:dT), and SeV infection (Fig. 2*G*).

LSm14A Is Required for Induction of IFN-β Triggered by RNA and DNA Virus. To investigate the physiological functions of LSm14A in innate antiviral response, we constructed five RNAi plasmids targeting different sites of human LSm14A mRNA. Reporter assays indicated that knockdown of LSm14A inhibited SeV-induced activation of the IFN-β promoter (Fig. 3*A*). The inhibitory efficiencies of LSm14A-RNAi plasmids on SeV-induced IFN-β activation were correlated with their abilities to down-regulate endogenous LSm14A expression (Fig. 3*A*). An RNAi off-target LSm14A mutant with three nucleotides nonsense mutations in the target sequence of the #3 LSm14A-RNAi plasmid, rescued the #3 LSm14A-RNAi–mediated inhibition of SeV-induced activation of the IFN-β promoter (Fig. 3*B*), further confirming that LSm14A plays an important role in SeV-induced IFN-β induction. Knockdown of LSm14A by RNAi also markedly inhibited SeV-induced ISRE and NF-κB activation (Fig. 3*C*), suggesting that LSm14A is involved in both SeV-induced IRF3 and NF-κB activation pathways.

Because LSm14A also binds to DNA, we examined whether it is involved in DNA virus-triggered IFN-β induction. As shown in Fig. 3*D*, knockdown of LSm14A dramatically inhibited activation of the IFN-β promoter triggered by HSV-1 in hepatic Huh7 or colon HCT116 cells, suggesting that LSm14A plays an important role in DNA virus-triggered IFN-β induction. Consistent with its involvement in both RNA and DNA virus-triggered IFN-β induction, knockdown of LSm14A also inhibited IFN-β promoter activation triggered by transfected cytoplasmic poly(I:C), 5′ppp-RNA, and poly(dA:dT) (Fig. 3*E*). In contrast, knockdown of LSm14A had no marked inhibitory effects on TLR3-mediated activation of the IFN-β promoter triggered by poly(I:C) (Fig. 3*F*), as well as TNFα- or IL-1β–induced NF-κB activation (Fig. S2). These data suggest that LSm14A is specifically involved in both RNA and DNA virus-triggered and cytoplasmic PRR-mediated induction of IFN-β.

In plaque assays, knockdown of LSm14A enhanced VSV replication and markedly reversed cytoplasmic poly(I:C)-mediated inhibition of VSV replication (Fig. 3*G*). Replications of NDV were also enhanced in LSm14A knock-down cells (Fig. 3*H*). Plaque assays also indicated that knockdown of LSm14A enhanced HSV-1 replication and reversed cytoplasmic poly (dA:dT)-mediated inhibition of HSV-1 replication (Fig. 3*I*). These data suggest that LSm14A is required for efficient cellular antiviral responses.

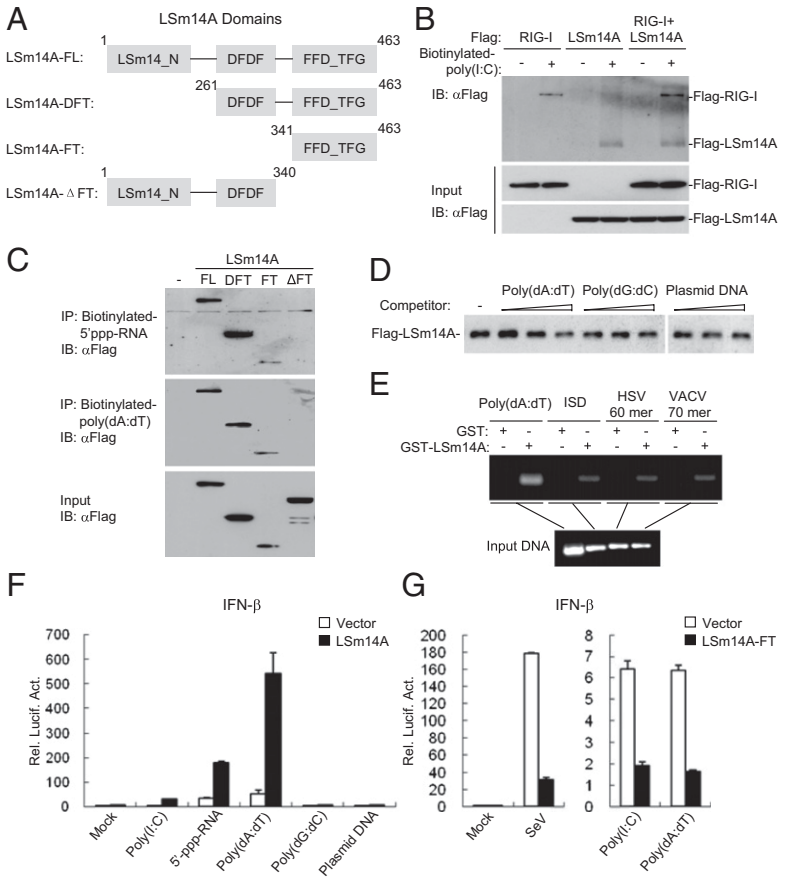

Fig. 2. LSm14A binds to synthetic or viral nucleic acids. (*A*) Schematic presentation of human LSm14A and its truncation mutants. (*B*) LSm14A bound to poly(I:C). The 293 cells were transfected with the indicated plasmids. Cell lysates were incubated with biotinylated-poly(I:C) and streptavidin-Sepharose. Bound proteins were analyzed by immunoblots with anti-Flag. (*C*) LSm14A bound to 5′ppp-RNA and poly(dA:dT) through its C terminus. The experiments were similarly performed as in *B*. (*D*) Competitive poly(dA:dT) pull-down. The 293 cells were transfected with Flag-LSm14A. Cell lysates were incubated with an increased amount of the indicated dsDNA (2, 10, 50 μg/mL) and then incubated with biotinylated-poly (dA:dT) and streptavidin-Sepharose. Bound proteins were analyzed by immunoblot with anti-Flag. (*E*) LSm14A bound to viral dsDNA. Recombinant GST-tagged LSm14A was incubated with the indicated dsDNA and GST beads. Bound dsDNA was analyzed by electrophoresis. (*F*) LSm14A potentiated cytoplasmic dsRNA- or dsDNA-triggered activation of the IFN-β promoter. The 293 cells were transfected with IFN-β promoter reporter and LSm14A plasmid for 18 h and then further transfected with the indicated nucleic acids for 20 h before luciferase assays. (*G*) Function of LSm14A C terminus in virus-induced signaling. Experiments were performed as in *E*, except LSm14A-FT was used instead of full-length LSm14A. For *F* and *G*, graphs show mean ± SD, $n = 3$.

It has been demonstrated that LSm14A is localized at the P-bodies (18). To further exclude the possibility that the defects in the innate response in LSm14A knockdown cells are due to disrupting the function of the P-bodies, we designed two RNAi vectors for DDX6 (also named p54), which is a critical component of the P-bodies, and knockdown of DDX6 leads to disassembly of the P-bodies (22, 23). As shown in Fig. S3, knockdown of DDX6 had minor effects on SeV-induced activation of IFN-β, whereas knockdown of LSm14A significantly inhibited SeV-induced IFN-β activation. These data suggest that knockdown of LSm14A directly leads to the defects in innate response.

LSm14A Mediates Induction of IFN-β in the Early Phase of Viral Infection. To explore a possible temporal role of LSm14A in virus-triggered IFN-β induction, we dynamically analyzed the effects of LSm14A deficiency on IFN-β induction in different phases of virus infection. As shown in Fig. 4*A*, knockdown of LSm14A markedly decreased transcription of the *IFNB1* gene, as well as other downstream genes, including *RANTES*, *ISG56*, and *RIG-I*, at 6- and 9-h time points after SeV infection. However, the expression levels of these genes in LSm14A-knockdown cells were mostly recovered to levels of control cells at 12 or 24 h after SeV infection. Consistently, ELISAs showed that the secretion of IFN-β was markedly decreased in LSm14A-knockdown cells in comparison with control cells at 8 and 12 h after SeV infection, but recovered at later time points (Fig. 4*B*). These results suggest that LSm14A mediates induction of IFN-β in the early phase of SeV infection. Furthermore, we examined the nuclear translocations of IRF3 and NF-κB during the early time points of SeV infection. As shown in Fig. 4*C*, nuclear IRF3 was decreased in LSm14A-knockdown cells at 4 and 6 h after SeV infection but recovered to the level of control cells at later time points. Consistently, cytoplasmic IRF3 was reversely changed. In these experiments, nuclear p65 was also decreased in LSm14A-knockdown cells as early as 4 h after SeV infection. Similarly, RT-PCR experiments indicated that IFN-β mRNA levels induced by HSV-1 infection in THP-1 cells were markedly decreased by LSm14A knockdown from 4 to 12 h after HSV-1 infection and restored to levels comparable to control cells at 24 h after infection (Fig. 4*D*). These results suggest that LSm14A is also important for DNA virus-triggered IFN-β induction at early phase of infection.

LSm14A-Mediated IFN-β Induction Requires RIG-I–VISA or MITA. Previously, various studies have demonstrated that the cytoplasmic receptor RIG-I and adapter protein VISA are required for IFN-β induction triggered by a majority of examined RNA viruses (24, 25). Reporter assays showed that activation of ISRE by overexpression of LSm14A was abolished in *Rig-i*$^{-/-}$ and *Visa*$^{-/-}$ MEF cells, whereas reconstitution of the deficient cells with RIG-I or VISA restored the ability of LSm14A to potentiate ISRE activation (Fig. S4*A*). In real-time PCR experiments, SeV-induced expression of endogenous IFN-β mRNA was abolished in RIG-I- or VISA-deficient mouse embryonic fibroblasts (MEFs), whereas reconstitution of RIG-I or VISA but not LSm14A restored *Ifnb1* gene transcription induced by SeV infection (Fig. S4 *B* and *C*). In addition, LSm14A failed to activate ISRE and the IFN-β promoter in *Rig-i*$^{-/-}$ MEFs but restored its ability to enhance SeV-triggered activation of ISRE and the IFN-β promoter in *Rig-i*$^{-/-}$ cells reconstituted with RIG-I (Fig. S4*D*). Consistently, knockdown of LSm14A did not inhibit ISRE activation by overexpression of RIG-I (Fig. S4*E*). These results suggest that LSm14A signals through RIG-I and VISA in SeV-triggered IFN-β induction pathways.

A recent study suggested that a family of cytosolic dsRNA sensor (such as DDX21) signals through TRIF and VISA (26). To explore the role of TRIF in LSm14A-mediated signaling, we used two TRIF-RNAi plasmids (27). As shown in Fig. S4*F*,

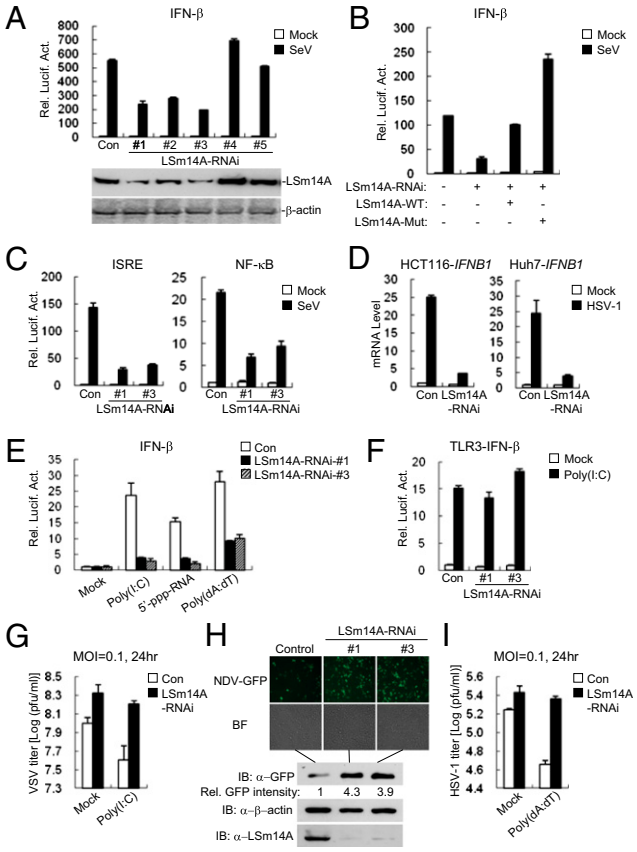

Fig. 3. LSm14A mediates RNA and DNA virus-triggered IFN-β production. (*A*) Effect of LSm14A-RNAi on SeV-induced activation of the IFN-β promoter. *Upper*: The 293 cells were transfected with IFN-β reporter and the indicated RNAi plasmids for 24 h and then infected with SeV for 12 h before reporter assays. *Lower*: Effects of LSm14A-RNAi plasmids on the expression of endogenous LSm14A were analyzed by immunoblots. (*B*) Rescue of LSm14A-RNAi effect by an RNAi-resistant mutant. The 293 cells were transfected with LSm14A-RNAi plasmid, together with wild-type or RNAi-resistant mutant LSm14A plasmid for 24 h. Cells were then left uninfected or infected with SeV for 12 h before luciferase assays. (*C*) Effects of LSm14A-RNAi on SeV-induced ISRE and NF-κB activation. Experiments were similarly performed as in *B*. (*D*) Effect of LSm14A-RNAi on HSV-1–induced IFN-β production. HCT116 or Huh7 cells stably transduced with LSm14A-RNAi plasmid were infected with HSV-1 for 24 h before RT-PCR analysis for *IFNB1* mRNA was performed. (*E*) Effect of LSm14A-RNAi on IFN-β promoter activation triggered by cytoplasmic nucleic acids. The 293 cells were transfected with LSm14A-RNAi plasmid and IFN-β promoter reporter for 24 h and then further transfected with the indicated nucleic acids for 20 h before luciferase assays. (*F*) Effect of LSm14A-RNAi on TLR3-dependent signaling. The 293 cells were transfected with IFN-β reporter, TLR3 plasmid, and LSm14A-RNAi plasmid for 24 h. Cells were then treated with poly(I:C) (20 μg/mL) for 12 h before luciferase assays. (*G*) Knockdown of LSm14A increased VSV replication. The 293 cells were transfected with LSm14A-RNAi plasmid and then infected with VSV (MOI = 0.1) for 24 h before culture medium was harvested for measurement of VSV production by plaque assays. (*H*) Knockdown of LSm14A increased NDV replication. The 293 cells were transfected with LSm14A-RNAi plasmid, then infected with NDV-GFP (MOI = 0.01) for another 36 h. The replications of NDV were analyzed by microscopy (*Upper*) or immunoblots with anti-GFP (*Lower*). The intensities of GFP bands were quantitated using Bio-Rad Quantity One Program and normalized to that of β-actin levels. BF, bright field. (*I*) Knockdown of LSm14A increased HSV-1 replication. HCT116 cells stably transduced with LSm14A-RNAi plasmid were transfected with poly(dA:dT) (1 μg) for 20 h and then infected with HSV-1 (MOI = 0.1) for 24 h before the culture medium was analyzed for HSV-1 production by plaque assays.

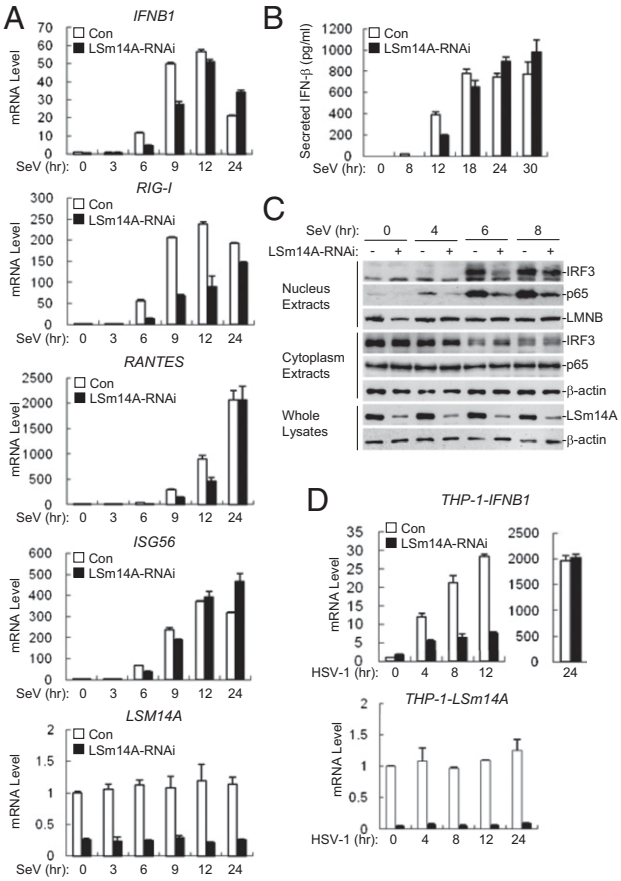

Fig. 4. LSm14A mediates IFN-β induction in the early phase of viral infection. (*A*) Effects of LSm14A knockdown on induction of antiviral genes in different phases of SeV infection. The 293 cells were transfected with LSm14A-RNAi plasmid for 24 h, then infected with SeV for the indicated times before RT-PCR was performed. (*B*) Effects of LSm14A knockdown on SeV-induced IFN-β production. Experiments were performed as in *A*, except that secretion of IFN-β in the culture medium was measured by ELISA. (*C*) Effects of LSm14A knockdown on nuclear translocation of IRF3 and p65. The 293 cells were transfected with LSm14A-RNAi plasmid for 24 h, then infected with SeV for the indicated times before cellular fractionation and immunoblots analyses were performed. (*D*) Effects of LSm14A knockdown on HSV-1–induced *IFNB1* mRNA expression. THP-1 cells were stably transduced with LSm14A-RNAi plasmid, and infected with HSV-1 for the indicated times before RT-PCR was performed for *IFNB1* (*Upper*) and *LSm14A* (*Lower*) mRNA.

knockdown of TRIF inhibited LSm14A-mediated activation of the IFN-β promoter in 293 cells. These data suggest that TRIF plays a role in LSm14A-mediated signaling.

Consistent with previous reports that DNA virus-triggered IFN-β induction does not require RIG-I and VISA (25, 28, 29), HSV-1 infection could fully induce *Ifnb1* gene transcription in RIG-I- or VISA-deficient MEFs (Fig. S4 *G* and *H*). In RIG-I–deficient MEFs, LSm14A could still potentiate HSV-1-induced *Ifnb1* gene transcription (Fig. S4*G*), suggesting that LSm14A-mediated IFN-β induction after DNA virus infection is independent of RIG-I pathways. Previous studies have suggested a role for the adapter protein MITA in DNA virus-induced IFN induction (13). Interestingly, in MITA-deficient MEFs, HSV-1 failed to induce *Ifnb1* gene transcription, and overexpression of LSm14A also failed to potentiate HSV-1–induced *Ifnb1* gene transcription (Fig. S4*I*). These results suggest that LSm14A signals through MITA in HSV-1–triggered IFN-β induction pathways.

LSm14A Is Translocated to Peroxisomes After Viral Infection. To further determine how LSm14A is spatially related to RIG-I, VISA, and MITA in virus-triggered signaling, we analyzed the localizations of LSm14A by cell fractionation and confocal immunofluorescent microscopy. Cell fractionation experiments indicated that LSm14A was localized in the cytosol and membrane but not mitochondrial and nuclear fractions, and the localization of LSm14A overlapped with DCP1a, a marker for P-bodies (Fig. S5*A*). In these experiments, RIG-I was induced by SeV infection and also existed in the cytosol and membrane fractions, which was similar to LSm14A.

To facilitate analysis of endogenous LSm14A, we produced Flag epitope tag knock-in HCT116 cell lines by a recently reported genetic approach (30) (Fig. S5*B*). In these cells, which are designated as HCT116-LSm14A-3xFlag, approximately half of the endogenous LSm14A protein could be detected by anti-Flag antibody (Fig. S5*B*). The cellular localization of LSm14A in these cells was examined by immunofluorescent microscopy with anti-Flag antibody. The results indicated that LSm14A was mostly colocalized with GFP-DCP1a in both uninfected and infected cells (Fig. 5*A*). In similar experiments, LSm14A did not colocalize with the mitochondria, endoplasmic reticulum, Golgi complex, endosomes, or lysosomes (Fig. S5*C*).

We next examined whether RIG-I, VISA, and MITA localize at P-bodies. Immunofluorescent microscopy showed that RIG-I, VISA, and MITA did not colocalize with P-bodies (Fig. S5*D*). Recently, it has been demonstrated that VISA is localized at peroxisomes that are platforms for early-phase innate antiviral response (31). We found that in addition to VISA, a fraction of RIG-I and MITA was also localized at the peroxisomes in both uninfected and viral infected cells (Fig. 5*B*). Interestingly, LSm14A was rarely detected in the peroxisomes in uninfected cells, but ~50% of LSm14A protein was translocated to the peroxisomes upon infection by either SeV or HSV-1 (Fig. 5*C*). These results suggest that LSm14A is recruited to the peroxisomes after viral infection, which might be platforms for RIG-I-, VISA-, and MITA-mediated innate antiviral signaling.

We next asked whether the translocation of LSm14A to the peroxisomes requires RIG-I or VISA. Immunofluenscent microcopy showed that LSm14A was translocated to peroxisomes in both wild-type and *Rig-i*$^{-/-}$ or *Visa*$^{-/-}$ MEFs (Fig. S6). These data suggest that the translocation of LSm14A to peroxisomes is RIG-I- or VISA-independent.

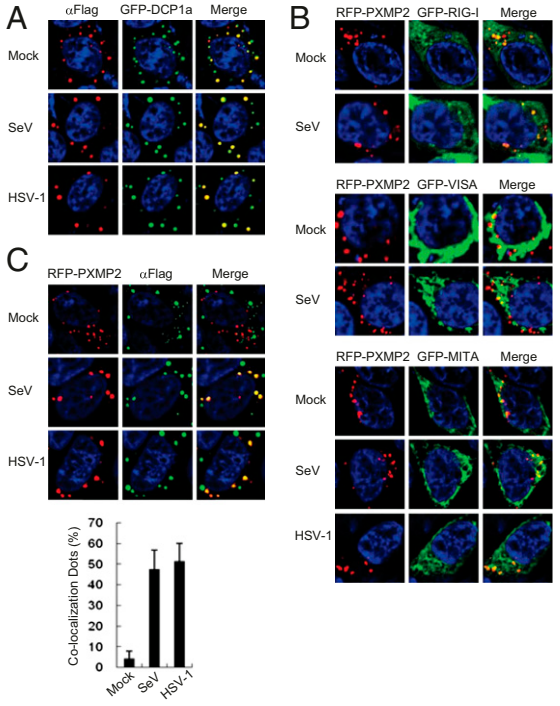

Fig. 5. LSm14A is localized at P-bodies and translocated to the peroxisomes after virus infection. (*A*) Endogenous LSm14A was localized at the P-bodies. HCT116-LSm14A-3xFlag knock-in cells were transfected with GFP-DCP1a (a P-body marker) and then left uninfected or infected with SeV or HSV-1 for 4 h before immunofluorescent staining with anti-Flag (red). (*B*) Colocalization of RIG-I, VISA, and MITA with peroxisomes. HCT116 cells were transfected with RFP-PXMP2 (red, a peroxisome marker) and the indicated GFP-tagged expression plasmids (green) and then infected with SeV or HSV-1 for 4 h before confocal microscopy. (*C*) Endogenous LSm14A was translocated to peroxisomes after viral infection. Experiments were performed as in *A*. Histograph shows the percentage of colocalization dots obtained from five cells.

Discussion

LSm14A was originally discovered to be a component of the P-bodies (18). Our study demonstrate that LSm14A is a sensor for both viral RNA and DNA and acts as a switch point on viral RNA- and DNA-induced IFN pathways, respectively. We found that knockdown of LSm14A inhibited SeV-induced expression of *IFNB1* gene and its downstream genes *RANTES*, *ISG56*, and *RIG-I* only at the early but not late phase of virus infection. LSm14A is constitutively expressed in various cells, whereas the expression level of RIG-I is quite low in resting cells and can be strongly induced by IFN-β. These observations suggest that LSm14A mediates the initial induction of type I IFNs, which promote RIG-I expression to amplify cellular antiviral response at the late phase of viral infection. Interestingly, LSm14A could not induce IFN-β or potentiate SeV-triggered induction of IFN-β in RIG-I- and VISA-deficient cells, and reconstitution of these cells with RIG-I or VISA restored the ability of LSm14A to mediate IFN-β induction, suggesting that LSm14A signals through RIG-I and VISA after infection with RNA viruses. In light of the fact that RIG-I is expressed at low level in resting cells, and LSm14A is only required for the early-phase induction of IFN-β after SeV infection, it is possible that LSm14A acts as an essential cofactor for RIG-I–mediated IFN-β induction when the concentration of RIG-I is low, and LSm14A is not required for RIG-I–mediated signaling when the concentration of RIG-I is high. Alternatively, detection of viral RNA may be controlled in a temporal and spatial manner. In the early phase of virus infection, the original incoming viral RNA is transported to the P-bodies and sensed by LSm14A, where it initiates early IFN-β and RIG-I induction. In the late phase, the replicated viral RNA in the cytoplasm is directly detected by cytoplasmic RIG-I. This may provide an additional mechanism for temporal and spatial regulation of type I IFN induction and cellular antiviral response.

Genetic studies demonstrate that MITA but not RIG-I and VISA is essential in type I IFN induction triggered by cytosolic synthetic DNA and DNA virus (13, 25). Interestingly, our results indicated that LSm14A was required for IFN-β induction and cellular antiviral response triggered by cytoplasmic poly(dA:dT) and the DNA virus HSV-1. Moreover, DNA virus-triggered and LSm14A-mediated IFN-β induction was normal in RIG-I- and VISA-deficient MEFs but abolished in MITA-deficient MEFs. These results suggest that MITA is required for LSm14A-mediated IFN-β induction after DNA virus infection. Our findings demonstrate that LSm14A can mediate both viral RNA- and DNA-triggered IFN-β induction through distinct downstream signaling pathways. So far, we have not been able to identify a physical association between LSm14A and RIG-I/VISA or MITA with or without viral infection. It is possible that other components exist between LSm14A and RIG-I/VISA or MITA. In addition, how LSm14A selectively activates distinct downstream pathways after infection with different types of viruses needs further investigation. One of the possibilities is that viral RNA leads to the formation of LSm14A–RIG-I complex, which initiates VISA-mediated signaling pathways, whereas viral DNA

is directly recognized by LSm14A alone, which signals through MITA-mediated pathways.

A previous study suggested that peroxisome-targeting VISA mediated viperin but not IFN-β expression upon viral infection (31). We reconstituted *Visa*$^{-/-}$ MEFs with wild-type, mitochondria-, or peroxisome-targeting VISA and found that mitochondria-targeting VISA (VISA-Mito) could mediate a strong IFN-β and viperin induction upon SeV infection, whereas peroxisome-targeting VISA (VISA-Pex) could only weakly mediate IFN-β and viperin induction upon SeV infection or overexpression of LSm14A (Fig. S7). In these experiments, however, the degree of VISA-Pex–mediated IFN-β induction was comparable to that of VISA-Pex–mediated viperin induction (Fig. S7). The published study (31) indicated that (*i*) VISA-Pex could still mediate IFN-β expression upon reovirus infection even though at a low level; and (*ii*) different viruses had different levels of response on VISA-Pex–mediated induction of viperin. It seems that influenza was much weaker than reovirus to induce VISA-Pex–mediated viperin expression (31). Taken together, it is possible that different viruses may have a different dependency on peroxisome-located VISA for induction of downstream antiviral proteins.

LSm14A was previously reported to be a component of the P-bodies (18), which was confirmed in our experiments. Interestingly, we found that viral infection induced translocation of a fraction of LSm14A to peroxisomes, where RIG-I, VISA, and MITA were also found. The simplest explanation for these observations is that LSm14A recognizes viral nucleic acids at the P-bodies, then translocates to the peroxisomes, and signals through RIG-I–VISA or MITA for induction of IFN-β and other antiviral genes (a working model is shown in Fig. S8).

Recently, some new sensors for viral RNA or DNA have been reported, such as HMGBs, IFI16, IFIT1, and DDX41 (16, 17, 32, 33). These molecules recognize different types of nucleic acids or function in different cell types. Despite the progress in recent years, how viral nucleic acids are recognized by host cells is still enigmatic. LSm14A is a newly identified sensor that mediates both RNA and DNA virus-triggered induction of antiviral genes in the early phase of viral infection. Importantly, our findings suggest that P-bodies are probably new cellular structures for detection of viral nucleic acids, and the P-body peroxisome may act as a new route of innate antiviral signaling. Collectively, these observations will certainly help to eventually decipher the complicated networks of IFN induction and innate antiviral immunity.

Materials and Methods

Cell Lines and Retroviral Gene Transfer. Transduction of LSm14A-RNAi plasmid to THP-1, HCT116, and Huh7 cells and reconstitution of RIG-I, VISA, or LSm14A into MEFs and bone marrow-derived dendritic cells (BM-DCs) were performed by retroviral-mediated gene transfer, as described in *SI Materials and Methods*.

Generation and Transfection of *Mita*$^{-/-}$ BM-DCs. Single-cell suspensions of bone marrow cells were cultured in RPMI 1640 medium containing 10% FBS, supplemented with murine GM-CSF (50 ng/mL) and IL-4 (10 ng/mL) (R&D Systems). Fresh GM-CSF was added on days 3 and 5. Transfection of primary BM-DCs was performed with retroviral infection as described above on day 6, and transduced cells were further assayed 1 d after infection.

Other Materials and Methods. Other materials and methods used in this study were previously reported (8) or are described in *SI Materials and Methods*.

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