THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 38, pp. 32216–32221, September 14, 2012


# TBK1-associated Protein in Endolysosomes (TAPE)/CC2D1A Is a Key Regulator Linking RIG-I-like Receptors to Antiviral Immunity*[S]

Received for publication, June 26, 2012 Published, JBC Papers in Press, July 25, 2012, DOI 10.1074/jbc.C112.394346

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**Background:** RIG-I-like receptor (RLR) signaling to antiviral immunity is important but remains elusive.
**Results:** TAPE/CC2D1A functionally and physically regulates the RLR-IPS-1 pathways.
**Conclusion:** TAPE/CC2D1A is essential for linking RLRs to antiviral immunity.
**Significance:** This current work uncovers TAPE as a novel regulator of RLR signaling and provides novel insights into the early events of RLR-mediated antiviral responses.

**Retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) are key RNA viral sensors for triggering antiviral immunity. The underlying mechanisms for RLRs to trigger antiviral immunity have yet to be explored. Here we report the identification of TAPE (TBK1-associated protein in endolysosomes) as a novel regulator of the RLR pathways. TAPE functionally and physically interacts with RIG-I, MDA5, and IPS-1 to activate the IFN-$\beta$ promoter. TAPE knockdown impairs IFN-$\beta$ activation induced by RLRs but not IPS-1. TAPE-deficient cells are defective in cytokine production upon RLR ligand stimulation. During RNA virus infection, TAPE knockdown or deficiency diminishes cytokine production and antiviral responses. Our data demonstrate a critical role for TAPE in linking RLRs to antiviral immunity.**

Recognition of virus invasion in mammalian cells is mediated by pattern recognition receptors (PRRs)[2] to detect viral nucleic acids, thereby triggering antiviral responses (1). Among these PRRs, Toll-like receptor (TLR) family members, including TLR3, TLR7/8, and TLR9, are responsible for recognizing viral nucleic acids in endolysosomes of macrophages and dendritic cells. RIG-I-like receptors (RLRs) act as cytosolic sensors to detect viral RNA in a wide range of cells. Notably, cytosolic RNA polymerase III can transcribe B-form DNA into double-stranded RNA for RIG-I sensing (2, 3), suggesting that RNA polymerase III and RIG-I cooperate to detect viral DNA. The RLR family includes three members, RIG-I, MDA5, and LGP2. RIG-I and MDA5 are shown to detect distinct molecular signatures of viral RNA during RNA virus infection (4, 5). RIG-I preferentially recognizes viral RNA containing a 5′-triphosphate moiety from RNA viruses such as influenza viruses and vesicular stomatitis virus (VSV). MDA5 selectively detects long viral dsRNA from picornaviridae such as encephalomyocarditis virus (EMCV). A recent study further indicated that MDA5 is responsible for sensing viral RNA lacking a ribose 2′-*O*-methylation modification (6). Several other regulators involved in the RLR pathways have recently been identified (1, 4). A mitochondrial adaptor IPS-1 (also called MAVS, Cardif, or VISA) is essential for linking RIG-I and MDA5 to downstream pathways (1, 4). Stimulator of, also known as MITA or ERIS, is a transmembrane protein on the endoplasmic reticulum organelles to involve in the RIG-I and DNA pathways (7–9). Zinc-finger CCCH-, is a regulator facilitating RIG-I oligomerization and signaling during antiviral innate immune responses (10).

TBK1 is a protein kinase coupling several PRRs to IFN-$\beta$ production by phosphorylation and activation of transcription factors IRF3 and IRF7. These PRRs include endosomal TLRs (TLR3 and TLR4), cytosolic RLRs (RIG-I and MDA5), and DNA sensors (DAI, IFI16, and DDX41). TBK1-associated protein in endolysosomes (TAPE), also called CC2D1A/Freud-1/Aki-1, has recently been identified as a TBK1-interacting protein that regulates the TLR3 and TLR4 pathways (11) and also as an NF-$\kappa$B activator through the canonical pathway (12). TAPE and its *Drosophila* ortholog Lethal (2) giant disc (Lgd) are endolysosomal adaptors implicated in endosomal trafficking

* This work was supported by grants from the National Science Council in Taiwan (Grants NSC 97-2320-B-006-014-MY3, NSC 100-2320-B-006-013-MY3, and NSC 100-2321-B-002-013).
[S] This article contains supplemental Figs. S1 and S2 and Table 1.
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[2] The abbreviations used are: PRR, pattern recognition receptor; TLR, Toll-like receptor; RLR, RIG-I-like receptor; RIG-I, retinoic acid-inducible gene I; MDA5, melanoma differentiation-associated gene 5; IPS-1, interferon-$\beta$ promoter stimulator 1; TAPE, TBK1-associated protein in endolysosomes; h, human; m, mouse; VSV, vesicular stomatitis virus; EMCV, encephalomyocarditis virus; 5′-ppp dsRNA, 5′-triphosphate double strand RNA; MEF, mouse embryonic fibroblast; IAV, influenza A virus; MOI, multiplicity of infection; TRIF, TIR-domain-containing adapter-inducing interferon-$\beta$; TIR, Toll/interleukin-1 receptor.

(11, 13, 14). We are interested in investigating whether TAPE, like TBK1, plays a critical role in regulating multiple PRR pathways mentioned above. Through this current work, we have uncovered a novel functional role for TAPE in regulating RLR signaling.

## EXPERIMENTAL PROCEDURES

*Cell, Viruses, and Reagents*—Human embryonic kidney 293 (HEK293), HEK293T, and Vero cells were described previously (11). A549 cells were grown in DMEM with 10% cosmic calf serum (HyClone). VSV was described previously (11). EMCV and influenza A/WSN/33 viruses (H1N1) were originally obtained from ATCC. Eight plasmids for generation of influenza A/PR/8 viruses were obtained from Robert G. Webster (St. Jude Children's Research Hospital, Memphis, TN) and were generated by co-transfection of eight plasmids into Madin-Darby canine kidney/293T cells. Influenza A viruses was amplified using Madin-Darby canine kidney cells, and the viral titer was determined by plaque assays. Poly(dA:dT), poly(I-C), and 5′-triphosphate double strand RNA (5′-ppp dsRNA) were purchased from InvivoGen. Anti-actin and anti-FLAG antibodies were described previously (11). Anti-GFP (G1544, Sigma), anti-$His_6$ tag (R940-25, Invitrogen), anti-MDA5 (IMG-3202, Imgenex), and anti-CC2D1A/TAPE (16816-1-AP, Proteintech) were used for Western blot.

*Plasmids and Primers*—HA-hTAPE, HA-hTAPE-N, HA-hTAPE-C, HA-hTAPE-△C2, hTAPE-EGFP, mTAPE, IKKϵ-K38A-Myc, and IFN-β-Luc were described previously (11). pBABE-puro SV40 LT (Addgene plasmid 13970) was developed by Thomas Roberts (Harvard Medical School). Mouse IPS-1 (mIPS-1, accession number BC037391) and human MDA5 (hMDA5, accession number BC111750) were purchased from Open Biosystems. Mouse IPS-1 and human MDA5 were amplified by PCR and cloned into a pcDNA4/HisMax-TOPO vector (Invitrogen). FLAG-RIG-I was kindly provided by T. Fujita (Kyoto University, Japan). ΔRIG-I (amino acids 1–284) was amplified by PCR and cloned into a pcDNA6/Myc-His vector. Primers used for cloning, genotyping, and quantitative PCR were listed in supplemental Table 1.

*Generation of Tape Knock-out Mice, MEF, and Fetal Liver Macrophages—Tape/Cc2d1a* gene-targeted ES clone (B6J, design ID: 49663 (72_A06)) was obtained from the Knockout Mouse Project (KOMP) organization (University of California, Davis, CA) and then used to generate chimeric mice. Germline transmitted animals were bred at the National Cheng Kung University and Taiwan National Health Research Institutes. All animal care and handling were approved by the Institutional Animal Care and User Committees. Wild type and TAPE knock-out ($TAPE^{-/-}$) mouse embryonic fibroblasts (MEFs) were prepared from day 13.5 embryos and cultured in DMEM with 10% FBS, nonessential amino acids, and L-glutamine. MEFs were immortalized by SV40 large T antigen and selected with puromycin. Fetal liver macrophages were obtained from $TAPE^{+/+}$ or $TAPE^{-/-}$ neonatal liver within 24 h of birth. Cells were cultured with RPMI including the 30% macrophage CSF supernatant from L929 for a week, and this medium was replaced with fresh medium at days 4 and 7.

*RNA Interference*—Control (GFP), sihTAPE-1, sihTAPE-2, siTBK1, siTRIF, and sihIPS-1 were described previously (11). The sequence of influenza A virus (IAV) NS1 (PR8) small interfering RNA (siRNA) is 5′-GCUGGAGACUCUAAUAUUA-3′. HEK293 cells seeded onto a 24-well plate were transfected with the indicated siRNAs using RNAiMAX (Invitrogen). A549 cells seeded onto a 24-well plate were transfected with indicated siRNAs using Lipofectamine 2000 (Invitrogen). 48 h after transfection, cells were used for luciferase reporter, ELISA, or plaque assays.

*ELISA, Plaque, and Reporter Assay*—Human IFN-β, human IL-6, mouse IFN-β mouse RANTES (regulated on activation normal T cell expressed and secreted), and mouse IL-6 in the supernatants were measured by ELISA. ELISA kits were as follows: human IFN-β (Antigenix America), mouse IFN-β (PBL Interferon Source), and human IL-6, mouse IL-6, and mouse RANTES (R&D Systems). Virus titer was measured by plaque assays. HEK293 cells were silenced with the indicated siRNAs for 48 h and then transfected with the indicated receptors. After VSV infection, VSV titers in culture supernatants were collected and measured as described previously (11). Reporter assays were performed to measure the IFN-β promoter activity in HEK293 cells by the Dual-Luciferase assay kit as described previously (11). Values represent the means ± S.E. of duplicated samples. Data are representative of two or three experiments. For reporter assays in MEFs, Microporator (Invitrogen) was used to transfect reporter plasmids.

*RNA Analysis and Preparation of Influenza Virus-infected RNA*—RNA was extracted by RNAzol (Molecular Research Center) according to the manufacturer instruction, and cDNA was synthesized using the high capacity cDNA reverse transcription kit (Applied Biosystems). Quantitative RT-PCR analysis was performed with the Applied Biosystems StepOne and SYBR Green system (Kapa Biosystems). Data presented were normalized to β-actin. Murine actin and IFN-β primers are listed in supplemental Table 1. For preparing influenza virus-infected RNA, HEK293 cells were uninfected (control) or infected by influenza A virus. 20 h after infection, total RNA was extracted using TRIzol reagent (Invitrogen) and then treated with DNase.

## RESULTS AND DISCUSSION

*TAPE Is Involved in the RIG-I and MDA5 Pathways*—To investigate whether TAPE is involved in the RLR pathways, we first employed the siRNA approach to examine the effect of TAPE knockdown on RIG-I-mediated IFN-β promoter induction. Results from luciferase reporter assays showed that knockdown of TAPE, like the RLR downstream regulator IPS-1, impaired RIG-I-mediated IFN-β promoter induction in HEK293 cells upon poly(I-C) transfection (Fig. 1*A*). In contrast, either control or TRIF siRNA failed to block RIG-I-mediated IFN-β promoter induction. Similar blocking effects by TAPE knockdown were also observed by stimulation with RIG-I-specific ligands including 5′-ppp dsRNA and IAV-infected RNA from HEK293 cells, both of which contain a 5′-triphosphate moiety specific for RIG-I recognition (supplemental Fig. S1*A*). RIG-I has recently been shown to cooperate with RNA polymerase III to detect viral and B-form DNA. Thus, we also

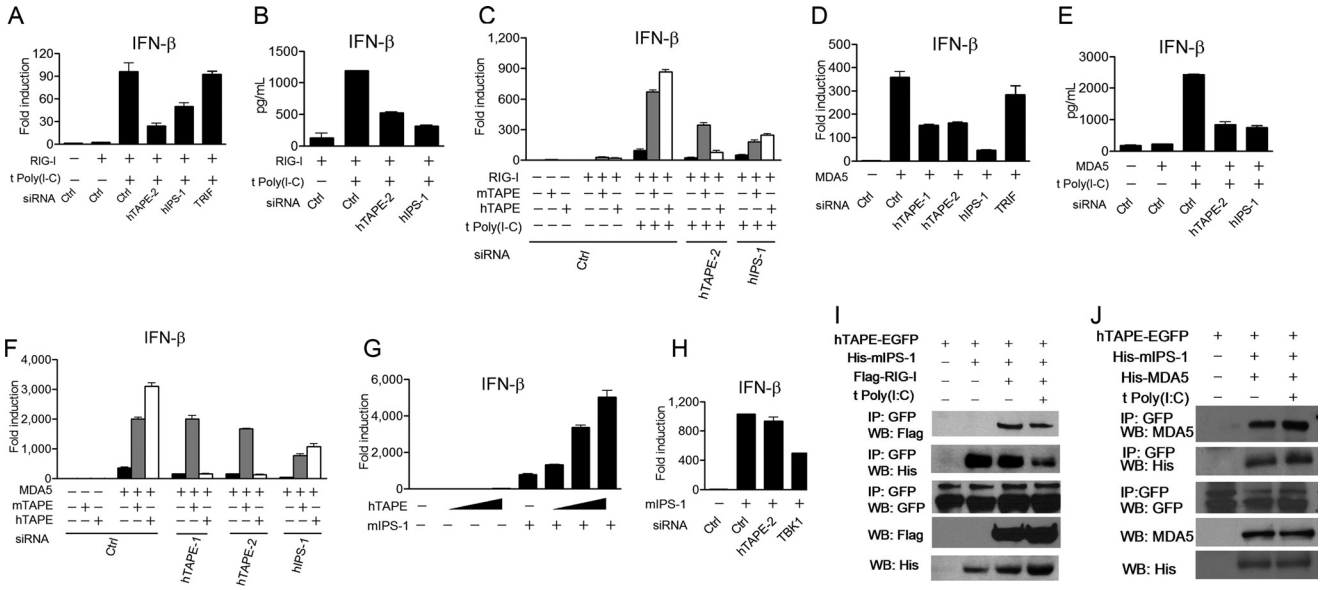


FIGURE 1. **TAPE is involved in the RIG-I and MDA5 pathways.** *A* and *C*, 293 cells treated with the indicated siRNAs were transfected with IFN-β-Luc together with RIG-I, mTAPE, or hTAPE as indicated and then treated with poly(I-C) transfection (*tPoly(I-C)*) (0.2 μg/ml) to analyze the IFN-β promoter activity. Results were separately displayed as *panels A* and *C*. *Ctrl*, control. *B* and *E*, 293 cells treated with the indicated siRNAs were transfected with RIG-I (*B*) or MDA5 (*E*) for poly(I-C) transfection. Supernatants were measured by ELISA for human IFN-β production. *D* and *F*, IFN-β reporter assays as in *panels A* and *C* were performed using MDA5. Results were separately displayed as *panels D* and *F*. *G*, 293 cells were transfected with IFN-β-Luc together with mIPS-1 and increasing amounts of hTAPE to analyze the synergistic effect. *H*, 293 cells treated with the indicated siRNAs were transfected with IFN-β-Luc together with mIPS-1 to analyze the IFN-β promoter activity. *I* and *J*, 293T cells were transfected with FLAG-RIG-I (*I*) or His-MDA5 (*J*), together with hTAPE-EGFP and His-mIPS-1 as indicated, and then treated with poly(I-C) transfection. Cell lysates were subjected to immunoprecipitation (*IP*) and Western blot (*WB*) analysis.

assessed whether TAPE is involved in RIG-I-mediated DNA detection. Our results showed that TAPE knockdown abolished the RNA polymerase III-RIG-I pathway to the activation of the IFN-β promoter upon B-DNA stimulation (supplemental Fig. S1*B*).

To rule out the off-target silencing effect by siRNA, we used mouse TAPE (mTAPE) and human TAPE (hTAPE) expression constructs for rescuing experiments. In the presence of control siRNA, co-expression of either mTAPE or hTAPE with RIG-I led to synergistic IFN-β promoter activation upon poly(I-C) transfection (Fig. 1*C*). However, only expression of mTAPE was able to rescue the impairment of RIG-I-mediated IFN-β activation caused by hTAPE siRNA and further synergized with RIG-I for IFN-β activation (Fig. 1*C*). Interestingly, knockdown of IPS-1 blocked synergistic IFN-β activation induced by cooperation of RIG-I with mTAPE or hTAPE (Fig. 1*C*). In addition to the siRNA approach, we also used deletion mutants of TAPE and RIG-I to characterize their interplay. These experiments demonstrated that the wild type TAPE cooperated with an active mutant ΔRIG-I containing the CARD domains to induce synergistic IFN-β promoter activation, whereas a TAPE C-terminal mutant (TAPE-C) blocked ΔRIG-I-induced IFN-β promoter activation (supplemental Fig. S1*C*). Next we attempted to extend the TAPE knockdown effect to the protein level of IFN-β production measured by ELISA. Knockdown of TAPE, like IPS-1, impaired RIG-I-mediated IFN-β cytokine production in HEK293 cells in response to poly(I-C) transfection (Fig. 1*B*). Together, these data suggest that TAPE is a novel regulator linking RIG-I signaling to type I IFN production.

Having shown the importance of TAPE in the RIG-I pathway, we next explored the potential role of TAPE in the MDA5 pathway. Results from luciferase reporter assays revealed that knockdown of endogenous TAPE by two different siRNAs impaired MDA5-induced IFN-β promoter induction in HEK293 cells (Fig. 1*D*). Likewise, our results from ELISA further confirmed the TAPE knockdown effect on MDA5-mediated IFN-β production in HEK293 cells upon poly(I-C) transfection (Fig. 1*E*). We further confirmed the specificity of TAPE knockdown by rescuing experiments. In the presence of control siRNA, expression of mTAPE or hTAPE with MDA5 induced synergistic IFN-β promoter activation (Fig. 1*F*). In the presence of hTAPE siRNAs, mTAPE not only rescued the impairment of MDA5-induced IFN-β promoter activation caused by hTAPE siRNAs but also synergized with MDA5 to activate the IFN-β promoter (Fig. 1*F*). Knockdown of IPS-1 blocked synergistic IFN-β activation induced by co-expression of MDA5 with hTAPE or mTAPE (Fig. 1*F*). In addition to the siRNA approach, TAPE deletion mutants were also used to study its functional interaction with MDA5. Our results demonstrated that the TAPE deletion mutants impaired MDA5-induced IFN-β promoter activation (supplemental Fig. S1*D*). These data support a critical role for TAPE in coupling MDA5 to type I IFN induction.

Because IPS-1 is a mitochondrial adaptor essential for relaying RLR signaling, we were thus interested in assessing the relationship between IPS-1 and TAPE. By luciferase reporter assays, our results showed that expression of TAPE potently facilitated IPS-1 to activate the IFN-β promoter in a dose-dependent manner (Fig. 1*G*). Interestingly, knockdown of TAPE in HEK293 cells failed to block IPS-1-induced IFN-β promoter activation, whereas knockdown of TBK1 impaired this activation (Fig. 1*H*). Furthermore, we assessed the physical interactions of TAPE, IPS-1, and RLRs. Results from co-immunoprecipitation experiments showed that expression of TAPE, RIG-I,

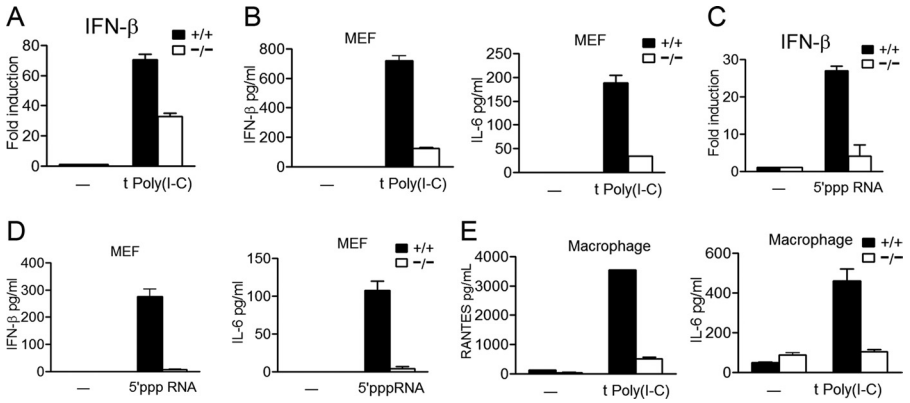


FIGURE 2. **Tape deficiency impairs IFN-β production upon RLR ligand stimulation.** *A* and *C*, *Tape* $^{+/+}$ and *Tape*$^{-/-}$ MEFs transfected with IFN-β-Luc were treated with poly(I-C) (0.2 μg/ml) (*A*) or 5′-ppp dsRNA (0.5 μg/ml) (*C*) transfection to analyze the IFN-β promoter activity. *t Poly(I-C)*, poly(I-C) transfection. *B* and *D*, likewise, supernatants from treated MEFs were measured by ELISA for IFN-β (*left*) or IL-6 (*right*) production. *E*, supernatants from *Tape* $^{+/+}$ and *Tape*$^{-/-}$ fetal liver macrophages with poly(I-C) transfection were measured by ELISA for RANTES (*left*) or IL-6 (*right*) production.

and IPS-1 led to a trimolecule complex formation constitutively in HEK293T cells (Fig. 1*I*). Poly(I-C) stimulation did not further enhance this complex formation, probably because overexpressed proteins overcame the need of ligand stimulation. Likewise, TAPE was shown to associate with IPS-1 and MDA5 in HEK293T cells before or after poly(I-C) transfection (Fig. 1*J*). Together, these results suggest that TAPE functions upstream of IPS-1 to promote the complex formation between RLRs and IPS-1.

*Generation of TAPE-deficient Mice*—To explore *in vivo* functions of TAPE in the host defense, we generated *Tape/Cc2d1a* knock-out mice by the gene trap targeting strategy. The gene trap allele was designed to insert a FRT-flanked LacZ reporter and neomycin cassette in intron 11 (supplemental Fig. S2*A*, *top panel*). In addition, loxP sites were inserted flanking exons 12–14. For the conditional knock-out purpose, the gene trap allele could be converted into the conditional allele following Flip-induced recombination first to delete the FRT-flanked cassette and then Cre-induced recombination to delete the loxP-flanked exons 12–14. The gene trap allele leads to the generation of a truncated TAPE/CC2D1A polypeptide containing amino acids 1–400 (supplemental Fig. S2*A*, *bottom panel*). The deletion of *Tape/Cc2d1a* in MEFs was verified by PCR and Western blotting (supplemental Fig. S2, *B* and *C*). Similar to a recent study (15), TAPE/CC2D1A-deficient mice died within 1 day of birth, whereas heterozygous mice were viable and fertile.

*TAPE-deficient MEFs and Macrophages Are Impaired in Type I IFN Induction upon RLR Ligand Stimulation*—Because of the neonatal lethality of TAPE-deficient mice, TAPE-deficient MEFs and fetal liver macrophages were used to further assess the importance of TAPE in innate immune responses. Our results demonstrated that poly(I-C)-induced IFN-β promoter induction was evidently diminished in TAPE-deficient MEFs as compared with wild type MEFs (Fig. 2*A*). Further, TAPE deficiency in MEFs led to the decreased cytokine production of IFN-β and IL-6 in response to poly(I-C) transfection (Fig. 2*B*). Another RIG-I-specific ligand, 5′-ppp dsRNA, was also used for analyses. We found that TAPE deficiency in MEFs resulted in the impairment of 5′-ppp dsRNA-induced IFN-β and IL-6 induction at the promoter and/or protein level (Fig. 2, *C* and *D*). In addition to MEFs, TAPE deficiency in fetal liver macrophages also decreased the cytokine production of RANTES and IL-6 upon poly(I-C) transfection (Fig. 2*E*). Data from this gene knock-out approach further support the essential role for TAPE in regulating RLR signaling in mouse primary cells.

*TAPE Is Required for Triggering Antiviral Responses during RNA Virus Infection*—Next we assessed whether TAPE plays a key role in antiviral responses during RNA virus infection. It is established that RIG-I is a major RNA sensor for detecting IAV infection. Results from luciferase reporter assays indicated that like IPS-1, hTAPE knockdown impaired RIG-I-mediated IFN-β promoter induction in HEK293 cells upon IAV infection (Fig. 3*A*). Rescuing experiments further demonstrated that expression of mTAPE but not hTAPE was able to rescue the impairment caused by hTAPE siRNA (Fig. 3*A*). We also investigated the functional role of TAPE in a human lung epithelial cell line A549, which may provide a physiologically relevant condition for IAV infection. Silencing of TAPE, like IPS-1, diminished the cytokine production of IFN-β and IL-6 in A549 cells after IAV infection (Fig. 3*B*). Similarly, we noticed that TAPE-deficient MEFs, as compared with wild type MEFs, were reduced in chemokine RANTES production and IFN-β mRNA expression upon IAV infection (Fig. 3*C*). In addition to IAV, we also assessed the role of TAPE in response to VSV, which is another RNA virus recognized by RIG-I. Results from plaque assays showed that expression of RIG-I lowered the VSV titer in HEK293 cells (Fig. 3*D*). However, silencing of hTAPE, like IPS-1, resulted in the increased VSV titer in HEK293 cells expressing RIG-I or a control vector (Fig. 3*D*). We also investigated the effect of TAPE on EMCV infection, which is detected by MDA5. Results from reporter assays indicated that hTAPE knockdown impaired MDA5-mediated IFN-β promoter induction in HEK293 cells upon EMCV infection (Fig. 3*E*). Collectively, our data demonstrate a central role for TAPE in linking RLRs to antiviral immunity.

*Concluding Remarks*—Our previous work has established the involvement of TAPE in the TLR3 and endocytic TLR4-TRIF pathways (11). Here the current work further extends the biological role of TAPE to the regulation of RLR signaling. To our knowledge, TAPE is the first innate immune regulator implicated in both TLR and RLR signaling at a very early step. TAPE may link to either TRIF-mediated or IPS-1-mediated down-

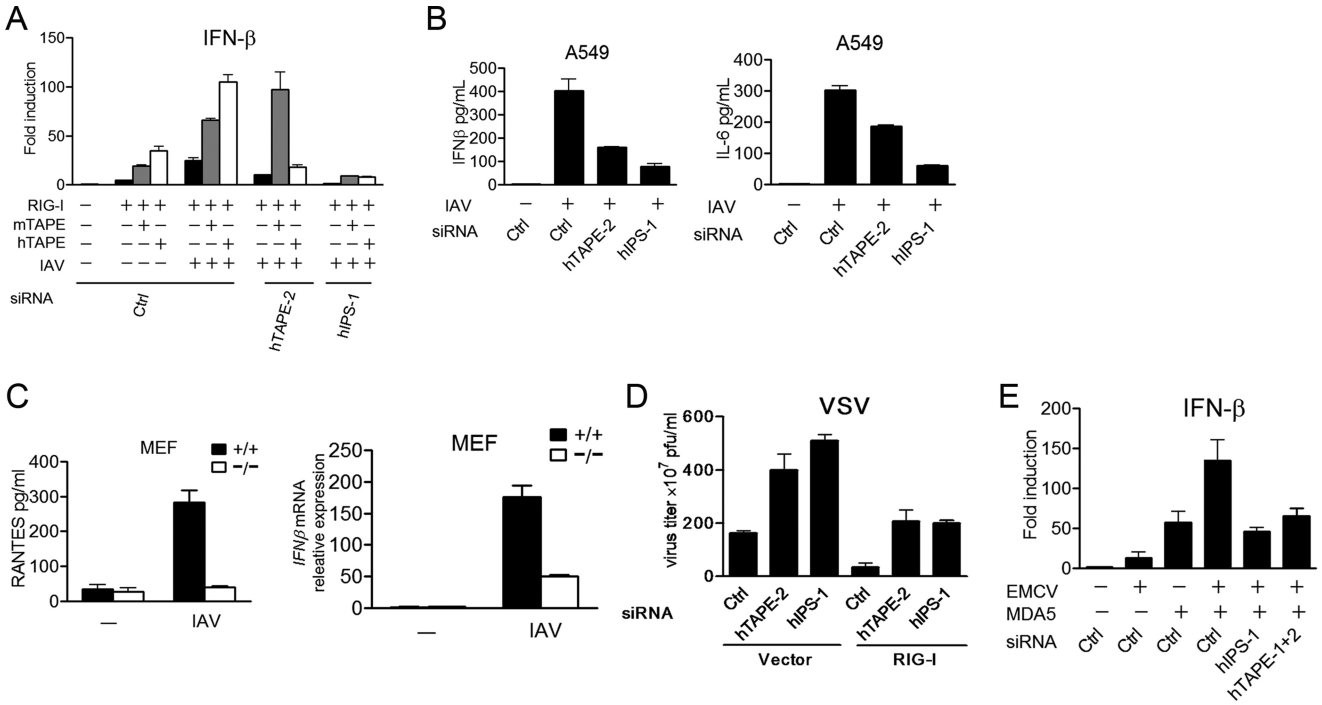


FIGURE 3. **Roles of TAPE in antiviral immune responses.** *A*, 293 cells treated with the indicated siRNAs were transfected with IFN-β-Luc, together with RIG-I, mTAPE, or hTAPE as indicated, and then infected with IAV (A/WSN/33) (4 MOI) to analyze the IFN-β promoter activity. *Ctrl*, control. *B*, A549 cells treated with the indicated siRNAs together with NS1 siRNA were infected with IAV (3 MOI) for 24 h. NS1 siRNA was used to knockdown IAV NS1 protein, which is known to antagonize IFN-β induction. Supernatants were measured by ELISA for IFN-β (*left*) or IL-6 (*right*) production. *C*, supernatants from *Tape* $^{+/+}$ and *Tape* $^{-/-}$ MEFs infected with IAV (A/WSN/33) (3 MOI) were measured for RANTES production by ELISA (*left*). *Ifnb* mRNA expression levels in MEFs infected with IAV (PR8 strain) (3 MOI for 10 h) were monitored by quantitative RT-PCR. *D*, 293 cells treated with the indicated siRNAs were transfected with RIG-I or a control vector and then infected with VSV (0.001 MOI). Viral titers in supernatants were measured by plaque assays. *E*, 293 cells treated with the indicated siRNAs were transfected with IFN-β-Luc, together with MDA5, and then infected with EMCV (2 MOI) to analyze the IFN-β promoter activity.

stream pathways in a PRR-dependent manner. TAPE acts upstream of TRIF to regulate the endosomal TLR3 and TLR4 pathways, whereas during RLR signaling, TAPE couples RLRs to IPS-1-mediated downstream pathways. It is evident that endolysosomes are key cellular compartments for endosomal TLRs to trigger downstream pathways (16). Interestingly, a lysosome-related organelle-related adaptor protein-3 (AP-3) has recently been shown to play a critical role in escorting endosomal TLRs to lysosome-related organelles for type I IFN production (17). TAPE/CC2D1A is located in different subcellular compartments, including the nucleus, centrosome, and endolysosomes, to play diverse biological roles (11, 18, 19). Thus, it is reasonable to speculate that while located in the endolysosomes, TAPE may function as an adaptor or a scaffold to bridge TLR3 or TLR4 to TRIF. It will be interesting to assess whether TAPE is involved in regulating the endosomal TLR7/8 and TLR9 pathways. Our current findings raise an intriguing possibility that endolysosomes are implicated in RLR signaling. Several lines of evidence seem to support this idea. A previous study indicated that high mobility group box protein 1 (HMGB1) acts as a universal nucleic acid-binding protein for multiple nucleic acid sensing pathways and is co-localized with RIG-I and Rab5 in mammalian cells, suggesting a role for endosomes in RIG-I signaling (20). Second, our previous work indicated that the TAPE deletion mutants are shown to impair the distribution of endolysosomes (11). These mutants were also shown to block the RIG-I pathway to IFN-β activation (supplemental Fig. S1), suggesting a link between the role of TAPE in endosomal trafficking and RLR signaling. Interestingly, recent studies indicated that TAPE/CC2D1A binds to and negatively regulates CHMP4B, a component of the endosomal sorting complexes required for transport (ESCRT) pathway, leading to the inhibition of HIV-1 budding (21, 22). These findings further support the importance of TAPE in regulating the endolysosomal compartments. Given these facts, we propose a model to illustrate a role for TAPE in endolysosomes for relaying RLR signaling to downstream pathways (supplemental Fig. S2*D*). However, currently we cannot rule out the possibility that during RLR signaling, TAPE may couple RLRs onto mitochondria to engage with IPS-1. It would be intriguing to further investigate whether and how TAPE regulates endolysosomes for cytosolic RLR signaling. Future studies of TAPE using conditional knock-out mice would shed light on *in vivo* functions of TAPE in the host defense.

*Acknowledgments—We thank other scientists for providing plasmids described under "Experimental Procedures." We thank Hsueh-Chi Lu and Miao-Ting Lin for technical assistance. We thank the Transgenic Mouse Model Core Facility of the National Core Facility Program for Biotechnology (NCFPB), National Science Council, Taiwan, for the provision of technical services.*

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