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# DNA Topoisomerase 1 Facilitates the Transcription and Replication of the Ebola Virus Genome

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**Ebola virus (EBOV) protein L (EBOL) acts as a viral RNA-dependent RNA polymerase. To better understand the mechanisms underlying the transcription and replication of the EBOV genome, we sought to identify cellular factors involved in these processes via their coimmunoprecipitation with EBOL and by mass spectrometry. Of 65 candidate proteins identified, we focused on DNA topoisomerase 1 (TOP1), which localizes to the nucleus and unwinds helical DNA. We found that in the presence of EBOL, TOP1 colocalizes and interacts with EBOL in the cytoplasm, where transcription and replication of the EBOV genome occur. Knockdown of TOP1 markedly reduced virus replication and viral polymerase activity. We also found that the phosphodiester bridge-cleaving and recombination activities of TOP1 are required for the polymerase activity of EBOL. These results demonstrate that TOP1 is an important cellular factor for the transcription and replication of the EBOV genome and, as such, plays a key role in the EBOV life cycle.**

Ebola virus (EBOV), a member of the family *Filoviridae*, in the order *Mononegavirales*, causes a severe hemorrhagic fever in humans and nonhuman primates, with high fatality rates (1). Its nonsegmented, negative-strand RNA genome encodes seven structural proteins. Three of these proteins, glycoprotein (GP), VP24, and VP40, are membrane-associated proteins that form the filamentous virions that are characteristic of EBOV (2–4). The four others, nucleoprotein (NP), VP30, VP35, and L, constitute a helical nucleocapsid that is required for the transcription and replication of the viral genomic RNA (5, 6). The nucleocapsid-associated L protein functions as an RNA-dependent RNA polymerase, with the assistance of VP35.

During the infection of host cells, EBOV proteins must interact with various host proteins for virus replication. Recently, Niemann-Pick C1, T-cell immunoglobulin, and mucin domain 1, which bind to EBOV GP, were identified as important cellular factors for EBOV entry (7–9). Several host proteins, such as Tyro3 receptor tyrosine kinase family Axl, Dtk, Mer, and cathepsin L/B, are also involved in the entry step (10–12). Furthermore, cellular proteins such as Nedd4, VPS4, and Tsg101 play important roles in assembly and budding (13–15). However, the cellular factors that participate in EBOV transcription and replication remain unknown.

In this study, to identify cellular proteins that interact with EBOV L (EBOL) and contribute to the transcription and replication of the EBOV genome, we coimmunoprecipitated cellular proteins with EBOL and identified the precipitants by using mass spectrometry. Of the 65 candidates identified, we focused on DNA topoisomerase 1 (TOP1) as a binding partner of EBOL and investigated its biological significance in EBOV replication.

## MATERIALS AND METHODS

**Cells and antibodies.** Human embryonic kidney cells (HEK293 cells) and baby hamster kidney cells (BHK cells) were maintained in Dulbecco's modified Eagle's medium (Sigma, St. Louis, MO) supplemented with 10% fetal calf serum and a penicillin-streptomycin solution. Madin-Darby canine kidney cells (MDCK cells) were maintained in Eagle's minimal essential medium (MEM; Gibco) with 5% newborn calf serum. The cells were maintained at 37°C under 5% $CO_2$. The anti-NP monoclonal antibodies used were described previously (16). An affinity-purified monoclonal anti-FLAG antibody (Sigma, St. Louis, MO) and a rabbit polyclonal anti-TOP1 antibody (Abcam, Cambridge, United Kingdom) were commercially available. A chicken anti-rabbit–Alexa Fluor 594 antibody (Lifescience Technologies, Japan), mouse TrueBlot Ultra, and horseradish peroxidase-conjugated anti-mouse IgG (eBioscience, San Diego, CA) were also commercially available.

**Plasmids and transfection reagents.** Plasmids for the expression of Zaire EBOV NP, VP35, VP30, and L, a plasmid for the expression of T7 polymerase, and a plasmid for the expression of an EBOV minigenome containing a firefly luciferase gene between the leader and trailer regions have been described elsewhere (17). Plasmids encoding L fused with FLAG or Venus, a derivative of green fluorescent protein (18), at the N terminus were constructed by PCR and designated FLAG-EBOL and Venus-EBOL, respectively. To generate a plasmid for the expression of TOP1, we extracted total RNA from HEK293 cells and amplified it by reverse transcription-PCR (RT-PCR) with TOP1-specific primers. The PCR product was cloned into pCAGGS/MCS. A plasmid for the expression of RNA interference (RNAi)-resistant TOP1 [TOP1(WT)-R] or a derivative mutant containing a T723F mutation [TOP1(Y723F)-R] was generated by PCR. For protein expression, HEK293 cells were transfected with plasmids by use of TransIT 293 reagent (Mirus, Madison, WI) in accordance with the manufacturer's instructions.

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**Pulldown assay.** A FLAG tag-based pulldown assay was performed as described elsewhere (19), with some modifications. HEK293 cells transfected with a plasmid expressing FLAG-EBOL were collected at 48 h posttransfection and were lysed in a lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, and Complete Mini EDTA(−) protease inhibitor cocktail (Roche, Basel, Switzerland)] for 1 h at 4°C. After centrifugation at 20,000 × *g* for 10 min at 4°C, the supernatants were incubated with an anti-FLAG affinity gel (Sigma, St. Louis, MO) overnight at 4°C. The FLAG-agarose beads were washed 4 times with the lysis buffer, and proteins were eluted with a FLAG elution buffer (50 mM Tris-HCl, 150 mM NaCl, and 0.5 mg/ml FLAG peptide [Sigma, St. Louis, MO]) for 1 h at 4°C. The final eluted mixtures were then removed from the FLAG-agarose by centrifugation. The eluted fractions were mixed with Tris-glycine-sodium dodecyl sulfate (SDS) sample buffer (Lifescience Technologies, Japan), incubated for 5 min at 95°C, and then subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Proteins were detected using silver staining. An aliquot of the eluted mixture was digested with trypsin and subjected to nano-liquid chromatography–electrospray ionization–quadrupole time of flight (LC-ESI-Q-TOF) mass spectrometry (LC-MS/MS) (Q-Star Elite; Applied Biosystems) to identify the coimmunoprecipitated cellular proteins.

**Immunoprecipitation.** HEK293 cells transfected with an empty plasmid (pCAGGS/MCS) or a plasmid expressing FLAG-EBOL were lysed in lysis buffer for 1 h at 4°C. Part of the cell lysate was retained and mixed with Tris-glycine-SDS sample buffer to serve as a whole-cell lysate sample. After clarification by centrifugation, the supernatants were incubated with an anti-FLAG affinity gel for 1 h at 4°C. The beads were then washed 4 times with the lysis buffer, suspended in Tris-glycine-SDS sample buffer, and then incubated for 5 min at 95°C. After the FLAG beads were removed by centrifugation, the samples were subjected to SDS-PAGE followed by Western blotting with an anti-DDDDK-tag antibody (MBL, Japan) and a rabbit anti-TOP1 antibody.

**Immunofluorescence assay.** HEK293 cells were transfected with plasmids expressing Venus-EBOL alone or a combination of Venus-EBOL, VP35, VP30, and NP. Twenty-four hours after transfection, the cells were fixed with 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) at room temperature for 15 min before immunostaining. TOP1 was detected by using a rabbit anti-TOP1 antibody as the primary antibody, followed by Alexa Fluor 594–chicken anti-rabbit IgG as the secondary antibody. Nuclei were stained with Hoechst 33342 (Lifescience Technologies, Japan). Slides were imaged using confocal microscopy on an LSM510 Meta system (Carl Zeiss, Oberkochen, Germany).

**siRNA treatment of cells.** HEK293 cells were transfected with small interfering RNA (siRNA) at a final concentration of 30 nmol by using Lipofectamine RNAiMAX (Lifescience Technologies, Japan) and were then incubated for 24 h posttransfection, according to the manufacturer's instructions. AllStar negative-control siRNA (Qiagen, Hilden, Germany) was used as the nontargeted siRNA. The siRNA against TOP1 used here was Hs_TOP1 FlexiTube siRNA (SI02662366). The effect of the siRNA was evaluated by Western blotting.

**EbolaΔVP30 virus replication.** EbolaΔVP30 virus was prepared as described elsewhere (20). 293T cells stably expressing EBOV VP30 were treated with siRNA against TOP1 for 48 h before infection. The cells were then infected with EbolaΔVP30 virus at a multiplicity of infection of 0.1. The virus titers were determined by plaque assay 3 and 6 days after infection. All work with EbolaΔVP30 virus was performed in a biosafety level 3 laboratory at the University of Wisconsin—Madison.

**Minigenome assay.** EBOV RNA polymerase activity was evaluated by using a minigenome assay as described elsewhere (17). Briefly, HEK293 cells were transfected with plasmids expressing EBOV L, VP30, VP35, and NP, an EBOV minigenome encoding the firefly luciferase in the antisense orientation, T7 polymerase, and *Renilla* luciferase. At 48 h posttransfection, the cells were lysed, and the luciferase activity was measured using a Glomax 96 microplate luminometer with a dual-luciferase reporter assay system (Promega, Madison, WI) according to the manufacturer's instructions. Relative firefly luciferase activity, normalized to *Renilla* luciferase activity, is shown.

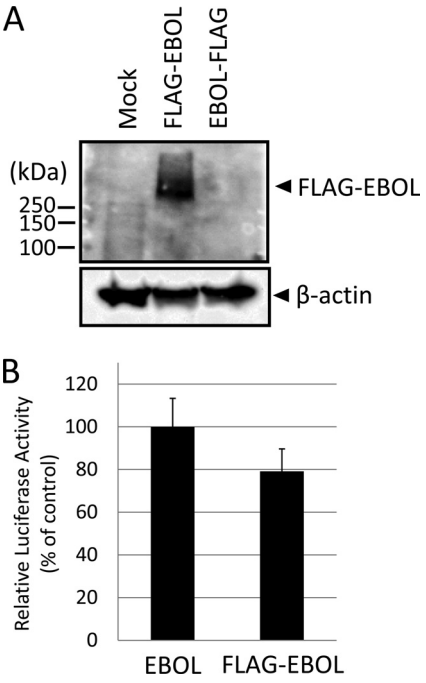


**FIG 1** Characterization of FLAG-tagged EBOL. (A) Expression levels of full-length EBOL fused with a FLAG tag at its N terminus (FLAG-EBOL) or C terminus (EBOL-FLAG). At 48 h posttransfection, cells were lysed and subjected to Western blotting with anti-FLAG and anti-β-actin antibodies. (B) Polymerase activity of FLAG-EBOL. The polymerase activity was assessed by using a minigenome assay. Each luciferase activity value is the average for three independent experiments. Error bars indicate standard deviations for triplicate samples.

**Real-time PCR.** The mRNA synthesis of the EBOV minigenome was examined by using real-time PCR as described elsewhere (21), with some modifications. Briefly, HEK293 cells were transfected with plasmids expressing EBOV L, VP30, VP35, and NP. At 6 h posttransfection, the cells were transfected with recombinant RNAs encoding the EBOV minigenome, which was transcribed *in vitro* by using a RiboMAX T7 large-scale RNA production system (Promega, Madison, WI). Forty-eight hours after transfection of the recombinant RNAs, the RNAs were extracted from the cells by using a Maxwell 16 LEV Simply RNA purification kit (Promega, Madison, WI). Reverse transcription was performed with a specific sequence-tagged oligo(dT) primer, and real-time PCR was conducted by using the tag sequence-specific primer and a reporter gene-specific primer with SYBR GreenER qPCR SuperMix for ABI Prism (Lifescience Technologies, Japan) on an ABI Prism 7900HT instrument.

**TOP1 inhibitor treatment.** HEK293 cells were transfected with plasmids as described above. At 6 h posttransfection, the cells were treated with different concentrations of the TOP1-specific inhibitor irinotecan (CPT-11) or water-soluble topotecan (Sigma, St. Louis, MO). At 48 h posttransfection, TOP1 inhibitor-treated cells were lysed and subjected to the minigenome assay. This minigenome assay system was described previously (17, 22). Cell viability was evaluated by measuring ATP levels in the cells by using CellTiter-Glo (Promega, Madison, WI).

## RESULTS

**Identification of host factors that interact with EBOL.** To perform the epitope tag-based immunoprecipitation assay, we constructed plasmids expressing EBOL fused with a FLAG tag at its N terminus (FLAG-EBOL) or C terminus (EBOL-FLAG) and exam-

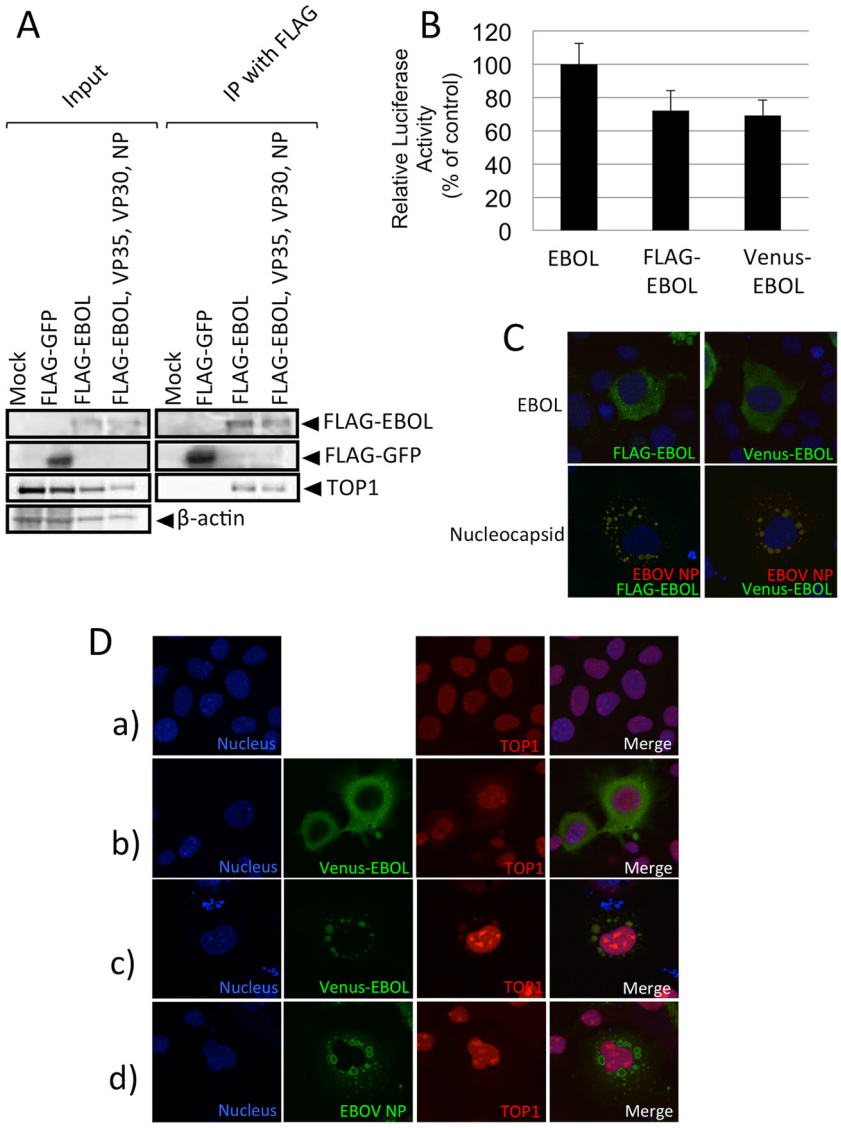


**FIG 2** Interaction between EBOL and TOP1. (A) Coimmunoprecipitation of EBOL and TOP1. Lysates from cells expressing FLAG-EBOL were immunoprecipitated by an anti-FLAG antibody and Western blotted for FLAG and TOP1. FLAG-GFP (green fluorescent protein fused with FLAG at the N terminus) served as a control. IP, immunoprecipitation. (B) The polymerase activity of Venus-EBOL was compared with that of EBOL and FLAG-EBOL by using the minigenome assay system. (C) Intracellular localization of Venus-EBOL. The localization of FLAG-EBOL is shown as a control. Localization of EBOL is shown in the absence (upper panels) or presence (lower panels) of VP35, VP30, and NP. (D) Intracellular localization of TOP1 in EBOV protein-expressing cells. Endogenous TOP1 was detected by an anti-TOP1 antibody (red). Nuclei were stained with Hoechst stain (blue). Mock-transfected cells (a), cells expressing Venus-EBOL alone (b), cells expressing Venus-EBOL, NP, VP30, and VP35 (c), and cells expressing NP, VP30, and VP35 (d) are shown. In panel d, NP was detected with an anti-NP antibody.

ined the expression levels in plasmid-transfected cells. FLAG-EBOL, with a molecular mass of approximately 270 kDa, was detected in the cells, but EBOL-FLAG was not detected, for an unknown reason (Fig. 1A). Next, we assessed the polymerase activity of FLAG-EBOL by using a minigenome assay. FLAG-EBOL showed polymerase activity similar to that of wild-type EBOL (Fig. 1B), indicating that FLAG-EBOL can mediate transcription and replication of the EBOV genome. Hence, we decided to use FLAG-EBOL as "bait" for the coimmunoprecipitation assay. Lysates from FLAG-EBOL-expressing cells were immunoprecipitated with an anti-FLAG antibody, and the EBOL-interacting proteins were analyzed by LC-MS/MS. We identified a total of 65 cellular proteins, including RNA-binding proteins, chaperone proteins, and cytoskeleton-related proteins, after excluding the host proteins that were coprecipitated in the empty plasmid sample (see Table S1 in the supplemental material). Of these 65 cellular proteins, we selected DNA topoisomerase 1 (TOP1) for further study, because TOP1 binds to RNA as well as DNA (23, 24), suggesting its potential involvement in the transcription and replication of the EBOV RNA genome.

**Interaction between EBOL and TOP1.** To confirm the interaction between EBOL and TOP1, we performed an immunoprecipitation assay and Western blotting. Because EBOL functions as an RNA-dependent RNA polymerase as part of the nucleocapsid,

we also examined whether TOP1 could be coimmunoprecipitated with FLAG-EBOL in the presence of the other nucleocapsid components. Under such conditions, TOP1 was coimmunoprecipitated with FLAG-EBOL (Fig. 2A), indicating that EBOL interacts with endogenous TOP1. Next, to examine where in the cell EBOL interacts with TOP1, we constructed a plasmid expressing Venus-EBOL, which showed similar properties to FLAG-EBOL in terms of its polymerase activity and intracellular localization (Fig. 2B and C). TOP1 was detected exclusively in the nucleus in mock-treated cells (Fig. 2D, panel a); however, in cells expressing Venus-EBOL, it was detected in both the nucleus and the cytoplasm, where it partly colocalized with Venus-EBOL (Fig. 2D, panel b). When Venus-EBOL was expressed together with VP30, VP35, and NP, it was detected in inclusion bodies, as reported recently (25), where it colocalized with endogenous TOP1 (Fig. 2D, panel c). However, TOP1 was not observed in the cytoplasm of cells transfected with VP30, VP35, and NP (Fig. 2D, panel d). These results indicate that in the presence of EBOL, TOP1 partly localizes in the cytoplasm, where it colocalizes with EBOL, and that EBOL interacts with TOP1 in inclusion bodies in the presence of NP, VP30, and VP35.

**Involvement of TOP1 in EbolaΔVP30 virus replication.** To examine the biological significance of TOP1 to the EBOV life cycle, we examined the growth properties of EBOV in TOP1-knockdown cells by using an EBOV strain lacking the VP30 gene (EbolaΔVP30 virus) (20). Cells stably expressing the VP30 protein were treated with siRNA against a nontarget (siNC) or TOP1 (siTOP1), followed by infection with the EbolaΔVP30 virus at a multiplicity of infection (MOI) of 0.1. As controls, HEK293 cells treated with either siNC or siTOP1 were infected with vesicular stomatitis virus (VSV) or influenza virus (A/WSN/33 [H1N1]) at an MOI of 0.001. Although treatment of the cells with siTOP1 caused about a 10-fold decrease of TOP1 expression levels (Fig. 3A), it caused no significant cell toxicity as assessed in a cell viability assay (Fig. 3B). Six days after infection, we harvested supernatants of EbolaΔVP30 virus-infected cells and determined the virus titers. Downregulation of TOP1 significantly reduced EbolaΔVP30 virus replication, by approximately 1 log unit ($P < 0.05$) (Fig. 3C). However, downregulation of TOP1 expression had little effect on the growth of VSV and influenza virus on days 1 and 2 postinfection, respectively (Fig. 3C), confirming that cellular proliferation was not inhibited under these conditions. These findings strongly suggest that TOP1 contributes to the EBOV life cycle.

**Contribution of TOP1 to EBOV transcription and replication.** Because EBOL is an RNA-dependent RNA polymerase, we hypothesized that TOP1 would be involved in the transcription and replication of the EBOV genome by interacting with EBOL. To examine the effect of TOP1 on EBOL polymerase activity, we employed minigenome assays of EBOV and influenza virus. Knockdown of TOP1 caused an approximately 80% reduction in EBOV polymerase activity but not influenza virus polymerase activity (Fig. 4A); influenza virus polymerase activity was enhanced by siTOP1 treatment, for an unknown reason. Furthermore, downregulation of TOP1 significantly inhibited the mRNA synthesis of the EBOV minigenome (Fig. 4B). The expression level of the viral protein was not affected by the knockdown of TOP1 (Fig. 4C), suggesting that downregulation of TOP1 specifically reduces the EBOV polymerase activity. To confirm that the attenuation of the EBOL polymerase activity was caused specifically by TOP1

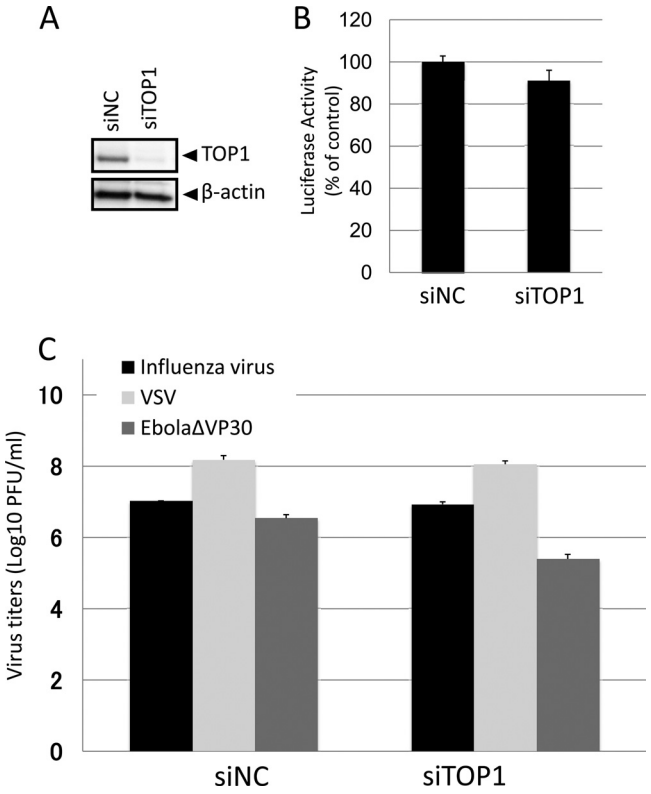


**FIG 3** Downregulation of TOP1 attenuates EBOV growth. (A) Western blotting with an anti-TOP1 or anti-β-actin antibody. The cells were treated with siRNA against a nonhomologous sequence of the mammalian genome (siNC) or TOP1 (siTOP1). (B) Cell viability assay. The amount of ATP in the respective siRNA-treated cells was measured. Each luciferase activity value is the average for three independent experiments. Error bars indicate standard deviations for triplicate samples. (C) Virus growth in siRNA-treated cells. Cells treated with siNC or siTOP1 were infected with influenza virus or VSV at an MOI of 0.001 or with EbolaΔ30 virus at an MOI of 0.1. Virus titers were determined by plaque assay.

depletion, we constructed a plasmid encoding RNA interference-resistant TOP1 [TOP1(WT)-R] by introducing six silent mutations into the siRNA target sequence of TOP1. When TOP1 was downregulated by siTOP1, exogenous expression of TOP1(WT)-R restored the expression level of TOP1 to that of endogenous TOP1, whereas overexpression of RNAi-susceptible TOP1 did not (Fig. 4D). Under these conditions, EBOL polymerase activity was restored to approximately 80% of the control level by the exogenous expression of TOP1(WT)-R (Fig. 4E), but it was not restored by the expression of RNAi-susceptible TOP1 (Fig. 4E). Taken together, these results demonstrate that TOP1 specifically contributes to the transcription and replication of the EBOV genome via its interaction with EBOL.

**Phosphodiester bridge-cleaving and recombination activities of TOP1.** TOP1 unwinds the helical structure of DNA and RNA by cleaving the phosphodiester bridges of the nucleotide chain (26, 27). To examine whether the phosphodiester bridge-cleaving activity of TOP1 is involved in EBOV transcription and replication, a plasmid expressing TOP1(Y723F)-R, which lacks the phosphodiester bridge-cleaving activity, was constructed by introducing a point mutation into the active site of TOP1 in TOP1(WT)-R, and an EBOV minigenome assay was performed.

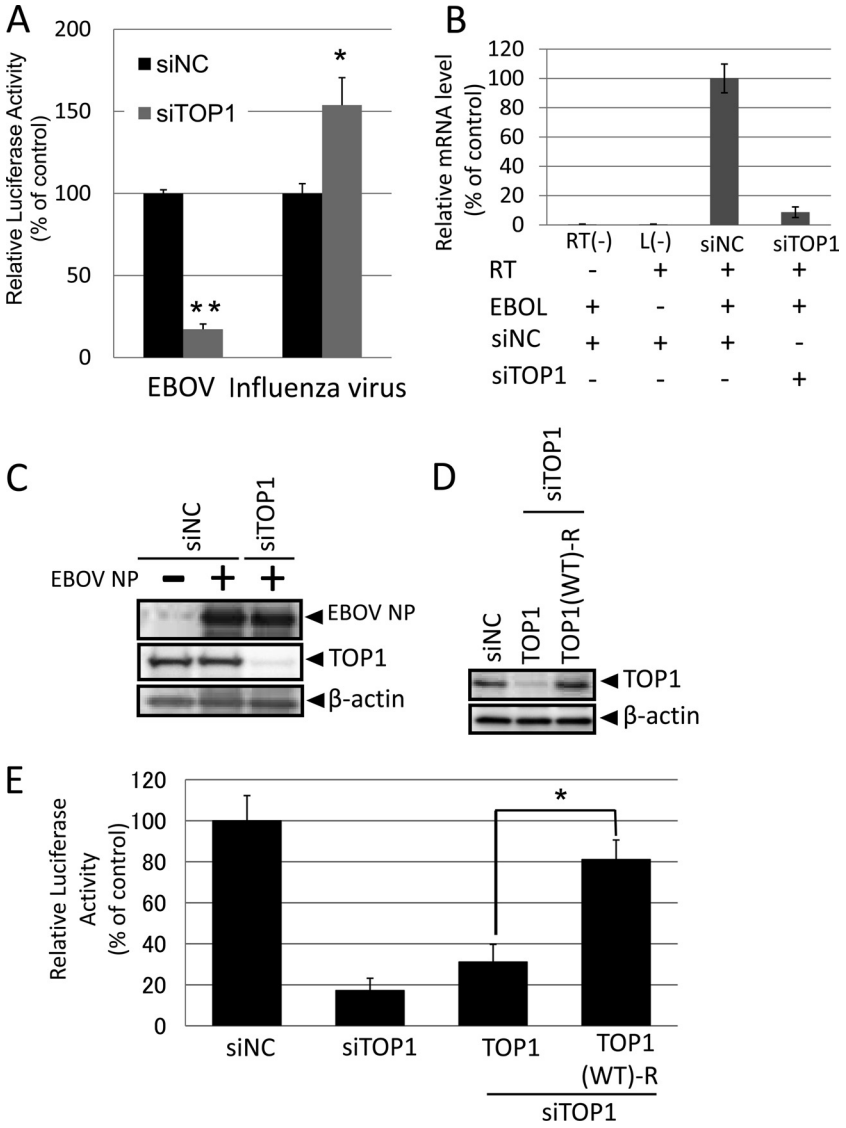


**FIG 4** TOP1 is involved in EBOV transcription and replication. (A) EBOV polymerase activity in TOP1-knockdown cells. Cells treated with siRNA were transfected with a plasmid for the expression of a virus-like RNA encoding firefly luciferase and with plasmids for the expression of viral proteins responsible for the transcription and replication of the virus-like RNA. Each luciferase activity value is the average for three independent experiments. *, $P < 0.05$; **, $P < 0.001$, based on one-sample Student's *t* test. (B) The mRNA synthesis level of the EBOV minigenome in cells treated with siRNA against TOP1 was evaluated by using real-time PCR. Each RNA synthesis level is the average for three independent experiments. RT, reverse transcription; EBOL, expression of EBOL; siNC, siRNA treatment against a nontarget sequence; siTOP1, siRNA treatment against TOP1. (C) Western blotting with an anti-NP, anti-TOP1, or anti-β-actin antibody in siRNA-treated cells. (D) Western blotting with an anti-TOP1 antibody in siTOP1-treated cells. After treatment with siRNA specific to TOP1, the cells were transfected with a plasmid encoding TOP1 or RNA interference-resistant TOP1. (E) Recovery of EBOV polymerase activity by exogenous expression of RNA interference-resistant TOP1. After treatment with siRNA specific to TOP1, the cells were transfected with a plasmid encoding TOP1 or TOP1(WT)-R, and luciferase activities were measured. Each luciferase activity value is the average for three independent experiments. *, $P < 0.05$, based on one-sample Student's *t* test.

Under conditions in which endogenous TOP1 was downregulated by siRNA, the exogenous expression of TOP1(Y723F)-R restored TOP1 expression levels to those obtained with siNC treatment (Fig. 5A). While exogenous expression of TOP1(WT)-R recovered the polymerase activity to approximately 80% of the control level, the polymerase activity was restored to only approximately 30% of the control level by the exogenous expression of TOP1(Y723F)-R (Fig. 5B). These results demonstrate that the phosphodiester bridge-cleaving activity of TOP1 is necessary for EBOV transcription and replication.

Lastly, to determine whether the DNA recombination activity of TOP1 is involved in EBOV transcription and replication, we examined the effect of the TOP1-specific inhibitor irinotecan (CPT-11) on the polymerase activity of EBOL. CPT-11 is an anticancer chemotherapy drug that inhibits the rejoining activity of TOP1 (28, 29). When HEK293 cells were treated with CPT-11, the EBOL polymerase activity was reduced in a dose-dependent manner, whereas influenza virus polymerase activity was not (Fig. 5C). With 50 μM CPT-11, the EBOL polymerase activity was reduced to approximately 40% of the non-CPT-11-treated control level (Fig. 5C), and mRNA synthesis was also reduced in a dose-dependent manner (Fig. 5D). Yet the expression level of the viral protein, as well as that of the host protein,

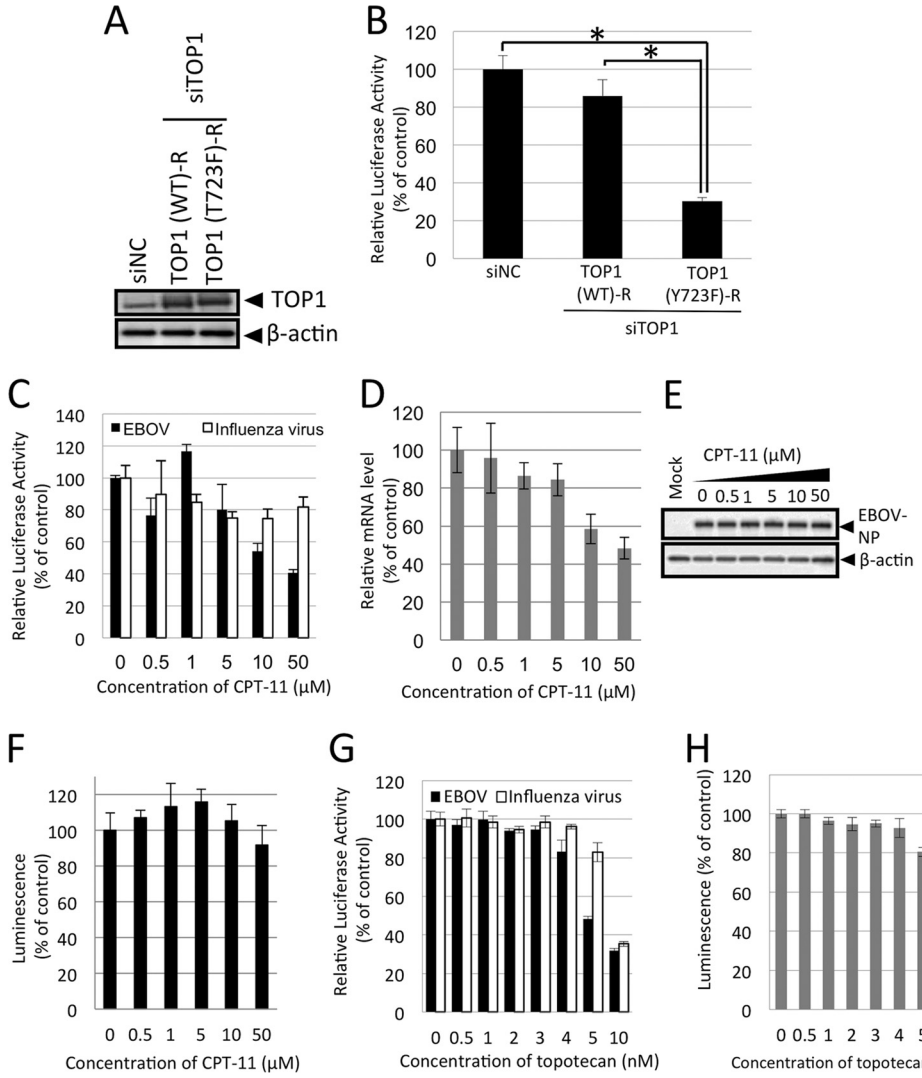


**FIG 5** Intrinsic activities of TOP1 are important for EBOV genome expression. (A) Expression levels of TOP1(WT)-R and TOP1(Y723F)-R in siTOP1-transfected cells. TOP1 was detected with an anti-TOP antibody. (B) EBOV polymerase activity. TOP1-knockdown cells were transfected with TOP1(WT)-R or TOP1(Y723F)-R, and luciferase activities were measured. Each luciferase activity value is the average for three independent experiments. Error bars indicate standard deviations for triplicate samples. *, $P < 0.05$, based on one-sample Student's *t* test. (C) Effects of CPT-11 treatment on EBOV polymerase activity. Cells treated with different concentrations of CPT-11 were subjected to the minigenome assay. The solid boxes indicate EBOV polymerase activity, and the open boxes indicate influenza virus polymerase activity. Each luciferase activity value is the average for three independent experiments. (D) mRNA synthesis levels of the EBOV minigenome in CPT-11-treated cells. Each mRNA synthesis level is the average for three independent experiments. (E) Western blotting of CPT-11-treated cells with an anti-NP antibody. (F) Cell viability assay. The amount of ATP in the CPT-11-treated cells was measured. Each luciferase activity value is the average for three independent experiments. Error bars indicate standard deviations for triplicate samples. (G) EBOV polymerase activity in topotecan-treated cells. Cells treated with different concentrations of topotecan were subjected to the minigenome assay. The solid bars indicate EBOV polymerase activity, and the open bars indicate influenza virus polymerase activity. Each luciferase activity value is the average for three independent experiments. (H) Cell viability assay. The amount of ATP in the topotecan-treated cells was measured. Each luciferase activity value is the average for three independent experiments. Error bars indicate standard deviations for triplicate samples.

was not affected (Fig. 5E). Moreover, we saw no notable CPT-11-induced cytotoxicity as assessed by use of a cell viability assay (Fig. 5F). In addition, another TOP1-specific inhibitor, topotecan, which inhibits the rejoining activity of TOP1, also reduced the EBOL polymerase activity in a dose-dependent manner, without causing any apparent cytotoxicity (Fig. 5G and H). These results indicate that both the DNA recombination activity and the phosphodiester bridge-cleaving activity of TOP1 are important for the transcription and replication of the EBOV genome.

## DISCUSSION

The host factors that are required for EBOV transcription and replication remain largely unknown. In this study, by using coimmunoprecipitation and mass spectrometry, we are the first to identify a cellular factor, TOP1, that contributes to the transcription and replication of the EBOV genome mediated by EBOL. The expression of EBOL partly relocates TOP1 from the nucleus to the cytoplasm, where TOP1 colocalizes and interacts with EBOL. The downregulation of TOP1 specifically and significantly reduced EBOV growth as

well as the transcription and replication of the EBOV genome, demonstrating its biological importance in the EBOV life cycle.

TOP1 is a nuclear protein that binds to helical structures of double-stranded DNA to unwind the helices for transcription and replication (26). In addition, TOP1 recognizes stem-loop RNA structures and is responsible for RNA processing via its endoribonuclease activity (23). Some DNA viruses, such as simian virus 40 (SV40) and herpes simplex virus type 2 (HSV2), utilize TOP1 for their DNA genome replication (30–33). In addition, it has been demonstrated that some retroviruses with RNA genomes also utilize TOP1. In the replication of HIV-1 and Rous sarcoma virus, the Gag and reverse transcriptase proteins, respectively, are associated with TOP1 (34–36). In these cases, TOP1 interacts with the viral genome through a stem-loop RNA structure in the p7 region of the viral genome (37), suggesting that TOP1 interacts directly with the stem-loop structures of viral genomic RNA to regulate the transcription and replication of the retrovirus genome. However, it remains unclear whether TOP1 interacts directly with the EBOV genomic RNA. The transcription start signals on the EBOV genome are thought to contain well-conserved stem-loop RNA structures upstream of each open reading frame (38) that are involved in regulating the transcription of the EBOV genome (39). Importantly, in the consensus stem-loop of the viral RNA sequences of the EBOV NP and L genes, there is a potential target sequence, namely, TCCTT, for poxvirus TOP1, which is classified together with human TOP1 as a type 1B topoisomerase (26), implying that human TOP1 may also recognize this sequence. In addition, given that the phosphodiester bridge-cleaving activity and the rejoining activity are necessary for the transcription and replication of the EBOV genome, as shown in experiments with the TOP1(Y723F) mutant and TOP1 inhibitors, respectively (Fig. 5), TOP1 may directly recognize and bind to the EBOV genomic RNA.

Interestingly, TOP1 was found in the cytoplasm in the presence of EBOL. Endogenous TOP1 colocalizes and interacts with EBOL in the cytoplasm. However, in the absence of EBOL, TOP1 is present exclusively in the nucleus (Fig. 2A and D), suggesting that the expression of EBOL causes the relocation of TOP1 from the nucleus to the cytoplasm. The mechanism by which EBOL alters the intracellular distribution of TOP1 remains unknown. One possibility is that EBOL associates with the newly synthesized TOP1 in the cytoplasm and prevents its nuclear import. It will be interesting to reveal the mechanisms behind the intracellular transport of TOP1.

Shabman et al. reported that a nuclear protein, double-stranded RNA binding protein 76 (DRBP76), interacts with EBOV VP35 (40). Overexpression of DRBP76 disturbs the interactions between VP35 and NP, which results in the suppression of the EBOV polymerase activity, suggesting that DRBP76 might exert an anti-EBOV function via its association with VP35. DRBP76 was also detected in our mass spectrometry analysis (see Table S1 in the supplemental material). Considering that EBOL and VP35 form the functional polymerase complex by interacting with each other and function cooperatively in EBOV transcription and replication (41), it is likely that EBOL and VP35 share additional host factors.

In summary, we identified a novel cellular factor, TOP1, that interacts with EBOL and plays important roles in the EBOV life cycle. Further studies are needed to better understand EBOV transcription and replication, which in turn will help us to develop novel drugs to combat Ebola hemorrhagic fever.

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