

Contribution of Sec61 α to the Life Cycle of Ebola Virus

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Background. Similar to other viruses, the viral proteins of Ebola virus (EBOV) interact with a variety of host proteins for its replication. Of the 7 structural proteins encoded in the EBOV genome, VP24 is the smallest and is multifunctional.

Methods. To identify host factors that interact with VP24 and are required for EBOV replication, we transfected 293 cells with plasmid expressing FLAG- and HA-tagged VP24, immunoprecipitated the host proteins that bound to VP24, and analyzed the immunoprecipitants with use of mass spectrometry.

Results. Of the 68 candidate host proteins identified, we selected Sec61 α because of its similar intracellular localization to that of VP24 (ie, perinuclear region), its involvement in various biological functions, and its roles in pathogenesis, such as type 2 diabetes and hepatosteatosis, and investigated its possible role in the EBOV life cycle. Our results suggest that Sec61 α is not involved in EBOV entry, interferon antagonism by VP24, nucleocapsid formation, or budding. However, Sec61 α colocalized with VP24 contributed to the ability of VP24 to inhibit EBOV genome transcription and reduced the polymerase activity of EBOV.

Conclusions. The present study indicates that Sec61 α is a host protein involved in EBOV replication, specifically in EBOV genome transcription and replication.

VP24, the smallest of the 7 structural proteins of Ebola virus (EBOV) [1], is membrane associated [2]. Although the function of VP24 is not fully understood, it is multifunctional, because (1) together with NP and VP35, it is necessary for the formation of nucleocapsid-like structures [3]; (2) it is necessary for the formation of a functional viral ribonucleoprotein (vRNP) complex [4]; (3) it has an inhibitory effect on the transcription and replication of the EBOV genome through its association with the vRNP complex [5]; and (4) it functions as an interferon antagonist by binding to karyopherin α , which is required for nuclear import of phosphorylated

STAT1, a component of the Jak-STAT signal transduction pathway [6, 7].

In the present study, to better understand the function of VP24, we first identified host proteins that interact with VP24 by using coimmunoprecipitation and mass spectrometry. Of the 68 candidate host proteins identified, we selected and investigated the role in EBOV replication of Sec61 α , a component of the heterotrimeric Sec61 complex that plays a critical role in promoting protein translocation at the ER membrane [8–10]. Sec61 α was chosen because its intracellular distribution is similar to that of VP24 (ie at the perinuclear region) and because of its involvement in various biological functions [11] and its roles in the pathogenesis of type 2 diabetes and hepatosteatosis [12–14].

MATERIALS AND METHODS

Cells and Antibodies

Human embryonic kidney cells (293 and 293T cells) were maintained in Dulbecco's modified Eagle's medium

Potential conflicts of interest: none reported.

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The Journal of Infectious Diseases 2011;204:S919–S926

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0022-1899 (print)/1537-6613 (online)/2011/204S3-0024\$14.00

DOI: 10.1093/infdis/jir324

(Sigma) supplemented with 10% fetal calf serum and a penicillin-streptomycin solution. The cells were maintained at 37°C under 5% carbon dioxide.

The anti-GP monoclonal, the mixture of anti-NP monoclonal, and the anti-VP40 polyclonal antibodies are described elsewhere [15–17]. Monoclonal anti-VP24 mouse ascites fluid, rabbit anti-VP24 antibodies produced by immunization of a rabbit with peptides (amino acids 6–21 or 196–212), a mixture of anti-VP35 peptide antibodies prepared by immunization of a rabbit with peptides (amino acids 54–73, 182–200, or 259–275), and an anti-VP30 peptide antibody prepared by immunization of a rabbit with a peptide (amino acids 5–21) were also used. An affinity purified monoclonal anti-FLAG M2 (Sigma), a goat polyclonal anti-Sec61 α (G-20; Santa Cruz), a monoclonal anti-disulfide isomerase (PDI; 1D3; Enzo Life Sciences) antibody, and monoclonal anti-HA clone HA-7 mouse ascites fluid (Sigma) were commercially available. Donkey anti-goat Alexa Fluor488, chicken anti-mouse Alexa Fluor 594, chicken anti-rabbit Alexa Fluor 594 antibodies, and Hoechst 33342 (Invitrogen) were also commercially available. Mouse TrueBlot ULTRA: horseradish peroxidase-conjugated anti-mouse IgG (eBioscience), anti-goat antibody, and the universal antibody in the VECTASTain ABC kit (Vector Laboratories) were purchased.

Plasmids and Transfection Reagents

To express the Mayinga strain of Zaire Ebolavirus (ZEBOV) NP, VP35, VP40, glycoprotein (GP), VP30, VP24, and L proteins, their respective genes were cloned into a protein expression vector, pCAGGS/MCS [18, 19], as described elsewhere [17, 20–22]. Plasmids for the expression of the T7 polymerase and M1 of influenza A virus (A/WSN/33) are also described elsewhere [22, 23]. The EBOV mini genome containing the firefly luciferase gene was used [5]. A plasmid encoding VP24 with both a FLAG and an HA tag at the N-terminus of the open reading frame (ORF) was constructed (FLAG HA-VP24). A plasmid encoding L with a FLAG tag at the N-terminus of the ORF was also constructed (FLAG-L). Total 293 cell RNA was obtained using the RNeasy Mini Kit (Qiagen). cDNA from total 293 cell RNA was prepared using reverse-transcription polymerase chain reaction (RT-PCR) with an Oligo(dT)15 primer and SuperScript III (Invitrogen). The cDNA of Sec61 α was prepared with the Sec61 α -specific primers EcoRI-Sec61 α (CACGAATTCAC-CATGGCAATCAAATTTCTGGAAG) and Sec61 α -XhoI (CA-CCTCGAGTCAGAAGAGCAGGGCCCCATGC) and with Phusion DNA High-Fidelity DNA Polymerases (Finnzymes) and was introduced into pCAGGS/MCS. A plasmid expressing Sec61 α with a FLAG tag at the N-terminus of the ORF (FLAG-Sec61 α) and a plasmid expressing Sec61 α with eGFP at the N-terminus of the ORF (eGFP-Sec61 α) were prepared using PCR and standard cloning techniques. Untranslated regions of the ZEBOV genes were deleted to minimize unforeseen effects, such as mRNA splicing or the regulation of translation by these

regions. Cells were transfected with plasmids with use of the Trans IT 293 reagent (Mirus) in accordance with the manufacturer's instructions.

A Tandem-Tag-Based Pull-Down Assay

A tandem-tag-based pull-down assay was performed as reported elsewhere [24], with some modifications. 293 cells transfected with plasmids expressing FLAG HA-VP24 or VP24 were collected at 24 hours after transfection, were lysed in lysis buffer (50 mmol/L Tris-HCl [pH, 7.5], 150 mmol/L NaCl, 1 mmol/L EDTA, 0.5% Nonidet P-40, and protease inhibitor cocktail complete mini [Roche]), and were incubated for 1 hour at 4°C. After clarification with use of low-speed centrifugation, the supernatants were incubated with anti-HA agarose conjugated with clone HA-7 (Sigma) overnight at 4°C and then eluted with HA elution buffer (50 mmol/L Tris-HCl, 1 mol/L NaCl, and 0.5 mg/mL HA peptide [Sigma]) for 6 hours at 4°C. After the HA-agarose beads were removed by centrifugation, the supernatants were incubated with anti-FLAG M2 Affinity gel (Sigma) overnight at 4°C. The FLAG-agarose beads were washed 3 times with lysis buffer, and the bound proteins were eluted with FLAG elution buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, and 0.5 mg/mL FLAG peptide [Sigma]) for 1 hour 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 (Invitrogen), incubated for 5 minutes at 95°C, and then subjected to SDS-polyacrylamide gel electrophoresis (PAGE). Protein expression was detected using silver staining. The remainder of the mixtures was digested with trypsin and was subjected to nanoLC-ESI-Q-TOF mass spectrometry (Q-STAR Elite; Applied Biosystems) for identification of co-immunoprecipitated host proteins.

Immunoprecipitation

293 cells transfected with an empty plasmid (pCAGGS/MCS) or a plasmid expressing FLAG/HA-tagged VP24 or non-tagged VP24 were lysed in lysis buffer and maintained 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 anti-FLAG M2 Affinity gel overnight at 4°C. The beads were washed 3 times with lysis buffer, suspended in Tris-Glycine SDS 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 blot analysis with a rabbit anti-VP24 antibody and goat anti-Sec61 α antibody.

Immunofluorescence Assay

293 cells were transfected with plasmids expressing VP24, a combination of VP24 and FLAG-Sec61 α , or a combination of VP24 and eGFP-Sec61 α . Similarly, 293 cells were co-transfected with plasmids expressing FLAG-L and eGFP-Sec61 α , NP and eGFP-Sec61 α , VP30 and eGFP-Sec61 α , or VP35 and

eGFP-Sec61 α , respectively. After 24 hours, the cells were fixed in -20°C methanol and incubated in PBS for 1 hour before staining. Antigens were detected using a rabbit anti-VP24 antibody, an anti-Sec61 α antibody, an anti-PDI antibody, an anti-FLAG antibody, anti-NP antibodies, an anti-VP30 antibody, or anti-VP35 antibodies as the primary antibody, followed by Alexa Fluor 488 donkey anti-goat IgG, Alexa Fluor 594 chicken anti-mouse IgG, or Alexa Fluor 594 chicken anti-rabbit IgG. Nuclei were stained with Hoechst 33342 (Invitrogen). Slides were imaged using confocal microscopy with an LSM510 META system (Carl Zeiss).

siRNA Treatment of Mammalian Cells

293 cells were transfected with siRNA (to a final concentration of 40 nmol) with use of the HiPerfect transfection reagent (Qiagen) and then incubated for 48 h after transfection, according to the manufacturer's instructions. AllStars Negative Control siRNA (Qiagen) was used as the nontargeted siRNA. The siRNA against Sec61 α used was Hs_SEC61A1_6 FlexiTube siRNA (target sequence: CCCGGTCCATGCAGTTGTATA). The effect of the siRNA was evaluated using RT-PCR and Western blot. The siRNA against the L gene of vesicular stomatitis virus (VSV), described elsewhere [25], served as a control

Ebola Δ VP30-eGFP Virus Replication

Ebola Δ VP30-eGFP virus was prepared as described elsewhere [26]. Vero cells stably expressing Ebola VP30 (VeroVP30) were treated with the siRNA (final concentration, 35 nmol) against Sec61 α and the HiPerfect transfection reagent (Qiagen) for 48 hours before infection. The cells were then infected with Ebola Δ VP30-eGFP virus [26] at a multiplicity of infection of 0.02. The titers were determined by counting the eGFP fluorescence under a fluorescence microscope 5 days after infection. All work with Ebola Δ VP30-eGFP virus was performed in a biosafety level 3 laboratory at the University of Wisconsin, Madison.

VSV Pseudovirion Infectivity Assay

A VSV pseudotyped system was previously developed to study the function of the EBOV GP without the need for biosafety level 4 containment [27]. VSV Δ G* pseudotyped with EBOV glycoproteins (VSV Δ G*GP) were prepared [27] and treated with VSV-G-neutralizing antibody I1 before inoculation to reduce background infection mediated by residual VSV Δ G* possessing VSV-G, which may have been carried over during the preparation of the pseudotype virus, as described elsewhere [28].

293 cells were transfected with nontarget siRNA or Sec61 α siRNA 48 hours before infection. The cells were infected with VSV Δ G*GP at a multiplicity of infection of 0.01. Twenty-four hours after infection, the number of GFP-positive cells was counted to determine the titers of the VSV Δ G*GP in the 293 cells treated with siRNA.

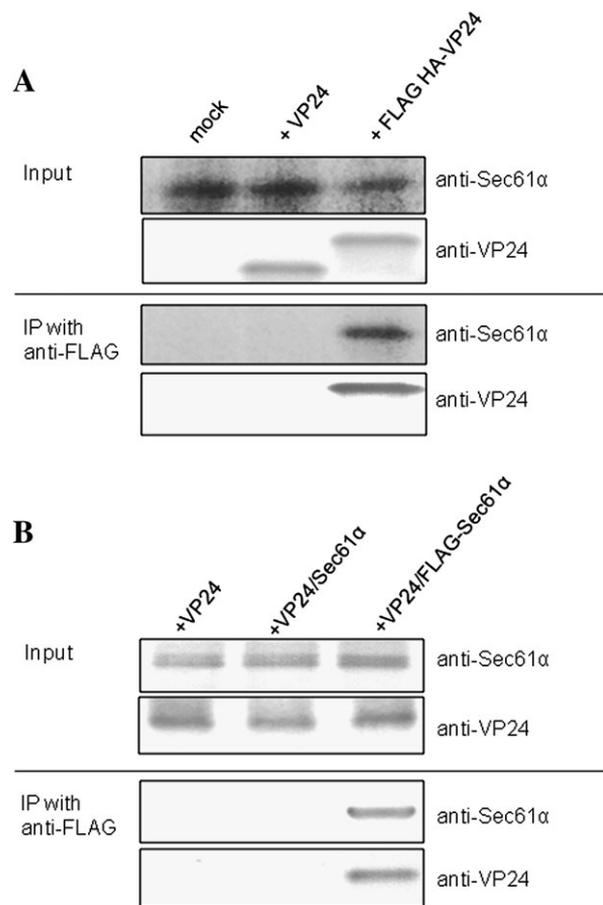


Figure 1. Co-immunoprecipitation of Sec61 α and VP24. 293 cells were transfected with pCAGGS/MCS, authentic VP24, and FLAG HA-VP24 (A). The cells were harvested, and lysed (A, top panel and second panel). The lysates were incubated with anti-FLAG agarose, and Sec61 α and VP24 eluted from anti-FLAG agarose were detected with an anti-Sec61 α antibody and an anti-VP24 antibody, respectively (A, third panel and bottom panel). Conversely, 293 cells were transfected with authentic VP24 and pCAGGS/MCS, authentic Sec61 α , or FLAG-Sec61 α . The cells were then harvested, lysed, and incubated with anti-FLAG beads (B). Sec61 α and VP24 eluted from anti-FLAG beads were detected with an anti-Sec61 α antibody and an anti-VP24 antibody, respectively (B, third panel and bottom panel).

Minigenome Assay

The contribution of Sec61 α to EBOV replication was evaluated using the EBOV minigenome assay, as described elsewhere [5]. First, 293 cells were transfected with Sec61 α siRNA or nontargeting siRNA (final concentration, 40 nmol) in 24-well plates. Then, the cells were transfected with different amounts of plasmids (0.0625, 0.125, and 0.25 μg) encoding VP24 or influenza virus M1, in addition to plasmids for the expression of ZEBOV NP (0.125 μg), VP35 (0.125 μg), VP30 (0.075 μg), L (1 μg), the EBOV minigenome encoding firefly luciferase in the antisense orientation (0.25 μg) [29], T7 polymerase (0.25 μg), and Renilla luciferase (0.0025 μg) [5]. Forty-eight hours later, the cells were lysed, and the luciferase activity was measured using a Glomax 96 microplate luminometer with the dual-

luciferase reporter assay system (Promega), according to the manufacturer's instructions.

Interferon Bioassay

In 48-well plates, 293 cells were transfected with siRNA (final concentration, 40 nmol) against a nontarget, Sec61 α , and VSV-L (as a positive control) with HiPerfect reagent (Qiagen) and were incubated for 48 hours. The cells were then transfected with a plasmid expressing VP24 and incubated for 24 hours. They were then treated with 10² units/mL of human interferon β , 1 α (PBL Interferon Source) and, 24 hours after this treatment, were infected with VSV Δ G*G at a multiplicity of infection (as titrated with 293 cells) of 0.01. Infection efficiencies for VSV Δ G*G were determined by measuring the number of GFP-positive cells under a conventional fluorescence microscope.

Electron Microscopy

293 cells were transfected with nontarget or Sec61 α siRNA in 6-well plates. The cells were then transfected with plasmids for the expression of NP (1 μ g), VP35 (0.5 μ g), and VP24 (0.5 μ g) 48 hours after the siRNA transfection [3]. Ultrathin-section electron microscopy was performed as described elsewhere [30, 31]. The samples were examined with a Tecnai G2 F20 electron microscope (FEI Company) at 200 kV.

Budding Efficiency Assay

In 6-well plates, 293T cells were transfected with nontarget or Sec61 α siRNA. Twenty-four hours later, the siRNA-treated cells were transfected with plasmids to produce virus-like particles (VLPs) [4]. The ratio of each plasmid was determined as described elsewhere [4]. Three days after the transfection, the cell supernatant containing released VLPs was harvested and clarified using brief centrifugation. VLPs were then concentrated through a 30% sucrose cushion for 1 hour at 80 000 g. The concentrated VLPs were suspended in PBS and mixed with Tris-Glycine SDS sample buffer (Invitrogen), incubated for 5 minutes at 95°C, and then subjected SDS-PAGE; proteins were detected using Western blot.

RESULTS

Tandem-Tag-Based Pull-Down Analysis Reveals Host Factor Candidates That Interact with EBOV VP24

To identify host factors that interact with EBOV VP24, we used a tandem-tag-based pull-down assay. For this purpose, we constructed a plasmid expressing VP24 with both a FLAG and an HA tag at its N-terminus (FLAG HA-VP24) and examined the properties of this tagged-VP24, including its expression level and reactivity with a panel of monoclonal and polyclonal antibodies. There were no appreciable differences between FLAG HA-VP24 and authentic VP24, except for the reactivity of the tandem-tagged VP24 with the anti-FLAG and anti-HA

antibodies and the apparent molecular weight resulting from the tags (data not shown). The localization of FLAG HA-VP24 was more diffuse in the cytoplasm, compared with that of authentic VP24; however, the characteristic distribution of VP24 to the perinuclear region and plasma membrane was maintained to some extent (data not shown). Next, we applied FLAG HA-VP24 to a tandem-tag-based pull-down assay and identified 68 host proteins that interacted with VP24. These proteins were categorized on the basis of their functions as follows: transport, 16 proteins; cytoskeleton, 12 proteins; metabolism, 11 proteins; chaperone, 10 proteins; translation, 6 proteins; apoptosis, 2 proteins; immunity, 1 protein; and others, 10 proteins. Of these, we selected Sec61 α for further study, because its intracellular distribution is similar to that of VP24 (ie at the perinuclear region) [2, 11] and it is involved in various biological functions and pathogenesis [14–18].

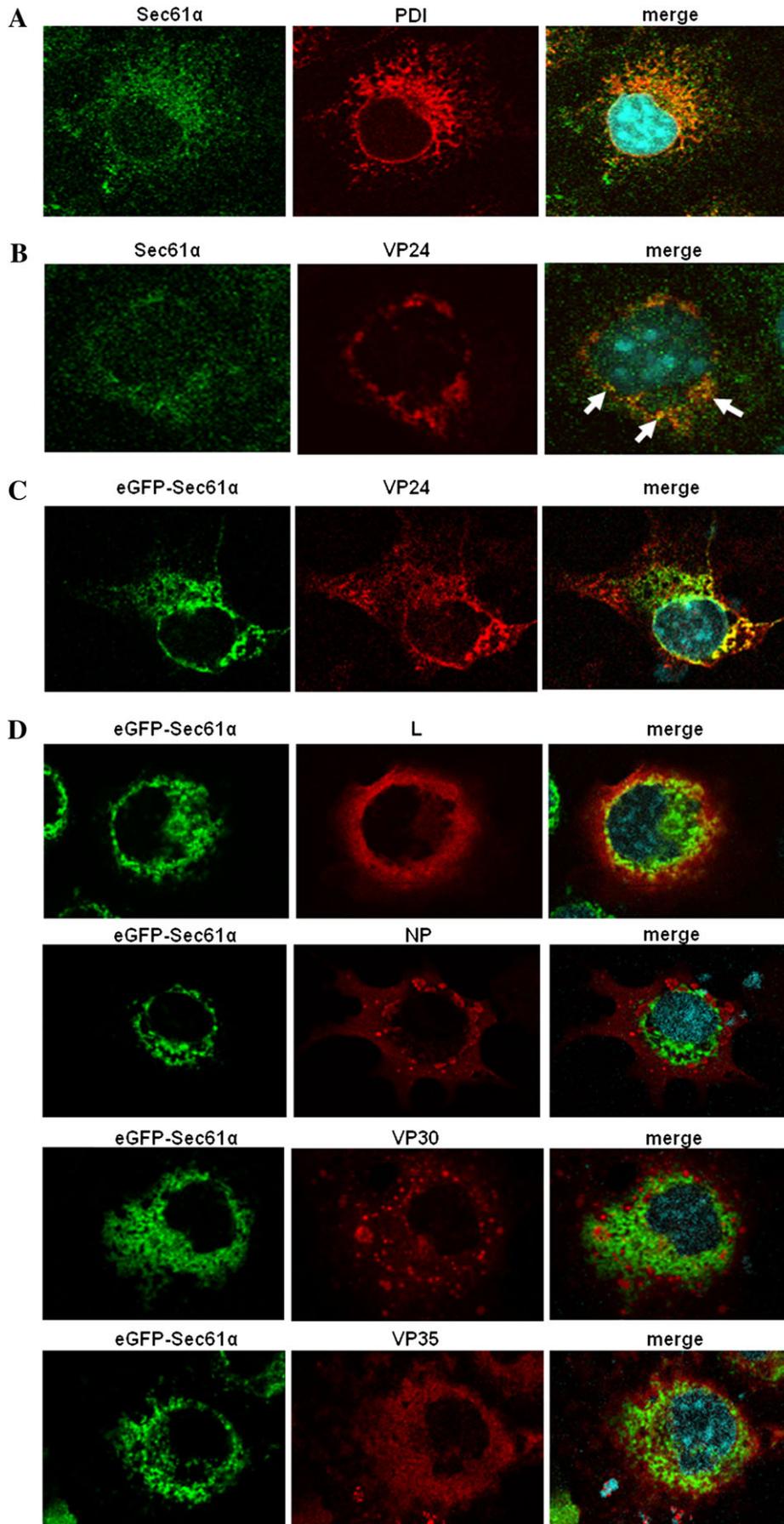
Sec61 α Co-immunoprecipitates and Colocalizes With EBOV VP24

To demonstrate an interaction between VP24 and Sec61 α , we performed immunoprecipitation and Western blot analysis with authentic VP24 and FLAG HA-VP24. As shown in Figure 1A by using an anti-FLAG antibody, endogenous Sec61 α co-immunoprecipitated with FLAG HA-VP24. We also examined whether FLAG-Sec61 α co-immunoprecipitated with VP24 by using an anti-FLAG antibody and found that VP24 co-immunoprecipitated with FLAG-Sec61 α (Figure 1B). These results indicate that VP24 specifically interacts with endogenous and exogenous and/or transiently expressed Sec61 α .

Next, we examined the intracellular localization of Sec61 α and VP24. To confirm Sec61 α localization in the ER, 293 cells were incubated with antibodies to Sec61 α and PDI, an ER marker. Sec61 α colocalized with PDI, confirming its localization in the ER (Figure 2A). VP24 partially localized in the perinuclear region (Figure 2B). When 293 cells expressing VP24 were stained with an anti-VP24 antibody and with anti-Sec61 α , VP24 and Sec61 α partially colocalized (Figure 2B). VP24 also colocalized with eGFP-Sec61 α in the perinuclear region (Figure 2C). Similar experiments were performed to examine the colocalization of Sec61 α and FLAG-L, NP, VP30, and VP35. The results suggest that L, but not NP, VP30, and VP35, weakly colocalizes with Sec61 α (Figure 2D).

Knock Down of Sec61 α Reduces Ebola Δ VP30-eGFP Virus Replication

In the Ebola Δ VP30 virus, the gene encoding the VP30 protein, which is an indispensable EBOV-specific transcription factor in the EBOV genome, was replaced with the eGFP reporter gene [26]. In this study, VeroVP30 cells were treated with siRNA against a nontarget, Sec61 α , or Tyro3, which is known to mediate EBOV cell entry [32], followed by infection with Ebola Δ VP30-eGFP virus or VSV Δ G*G. We found that depletion of Sec61 α or Tyro3 by siRNA reduced Ebola Δ VP30-eGFP virus



titers by >2 logs, compared with the nontarget siRNA control (Figure 3A). However, similar experiments using siRNA against Sec61 α had no effect on VSV replication, unlike siRNA against VSV-L (Figure 3B). These findings suggest the potential involvement of Sec61 α in EBOV replication.

Sec61 α Is Not Involved in EBOV Entry

To examine whether Sec61 α is involved in EBOV entry, we used VSV Δ G*GP, described elsewhere [31]. 293 cells were transfected with siRNA against a nontarget or Sec61 α . At 48 hours after transfection, the siRNA-treated cells were infected with VSV Δ G*GP. The pseudotype virus titer in Sec61 α siRNA-treated cells was comparable to that in nontarget siRNA-treated cells (data not shown). These results suggest that Sec61 α is not involved in virus entry.

Sec61 α Does Not Affect the Interferon Antagonism of VP24

VP24 functions as a type I interferon antagonist [6, 7]. To investigate the effect of Sec61 α on the type I interferon antagonism by VP24, we performed an interferon bioassay using VSV Δ G*G. When VP24 was expressed in 293 cells, the infectivity of VSV Δ G*G increased by \sim 100-fold (data not shown), suggesting that VP24 inhibited the induction of interferon, as reported elsewhere [6]. When cells were treated with nontargeting siRNA or Sec61 α siRNA, no statistically significant difference in pseudotype virus titer was found (data not shown). Therefore, these data suggest that Sec61 α is not involved in the inhibition of interferon signaling by VP24.

Sec61 α May Be Involved in EBOV Polymerase Activity and the Inhibitory Effect of VP24 on the Transcription of the EBOV Genome

VP24 has an inhibitory effect on the transcription and replication of the EBOV genome through its association with the vRNP complex [5]. To examine the effect of Sec61 α on this function of VP24, we used the EBOV mini-genome system [20, 33]. As shown in Figure 4, the expression of VP24 caused a dose-dependent decrease in luciferase activity, whereas the influenza virus M1 protein had no effect on luciferase activity, confirming the inhibitory effect of VP24 on EBOV genome transcription and replication [5]. However, when the expression of Sec61 α was knocked down by the siRNA, the level of inhibition decreased (Figure 4). This result suggests that Sec61 α is involved in VP24-mediated inhibition of EBOV genome transcription and

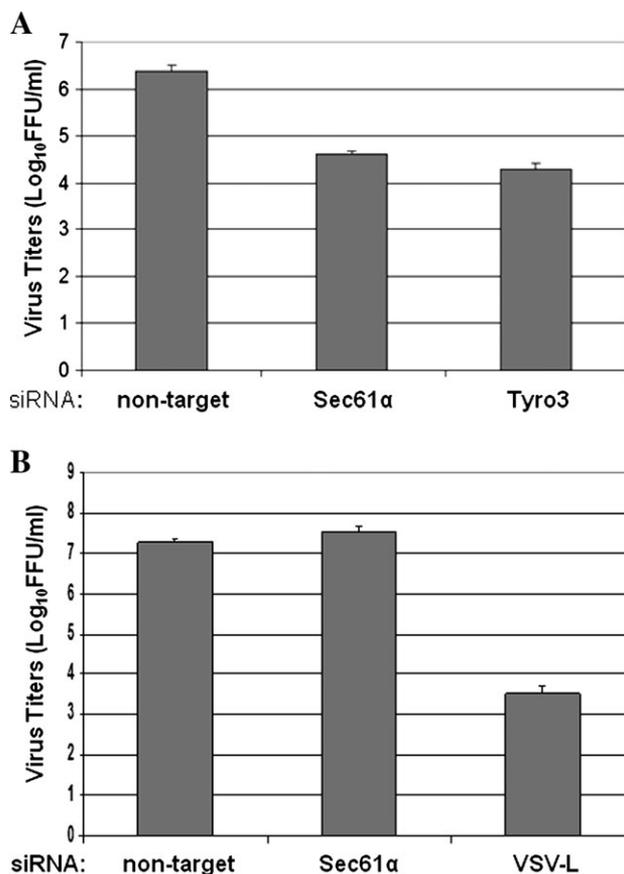


Figure 3. The effect of Sec61 α on the infectivity of Ebola Δ VP30-eGFP virus. Vero E6 cells expressing VP30 were transfected with siRNA (negative control, Sec61 α , or Tyro3 as a positive control) and infected with Ebola Δ VP30-eGFP virus (A). To confirm that the reduction in infectivity by Sec61 α was specific to Ebola virus, the infectivity of VSV was tested after the siRNA transfection (negative control, Sec61 α , or VSV-L as a positive control) (B).

replication. Of interest, in the absence of VP24, Sec61 α depletion reduced the polymerase activity (ie, there was an \sim 50% reduction relative to the nontarget siRNA control) (Figure 4), suggesting the potential involvement of Sec61 α in EBOV polymerase activity.

Sec61 α Does Not Affect EBOV Nucleocapsid Formation

VP24, together with NP and VP35, is necessary and sufficient for assembly of the EBOV nucleocapsid [3, 30]. We next tested whether Sec61 α is involved in nucleocapsid formation. At

Figure 2. Colocalization of Sec61 α and Ebola virus proteins. Because Sec61 α localizes to the ER and ER-golgi intermediate, colocalization of Sec61 α and protein disulfide isomerase (PDI, an ER-specific protein) was evaluated by using an anti-Sec61 α antibody (green) and an anti-PDI antibody (red; A). Colocalization of endogenous Sec61 α and VP24 was tested by using an anti-Sec61 α antibody (green) and an anti-VP24 antibody (red; B). In the presence of eGFP-Sec61 α (green), coexpressed VP24 was detected with an anti-VP24 antibody (red; C). 293 cells coexpressed with eGFP-Sec61 α and FLAG-L, NP, VP30, or VP35 were detected with an anti-FLAG antibody (D, top panel), an anti-NP antibody (D, second panel), an anti-VP30 antibody (D, third panel), or an anti-VP35 antibody (D, bottom panel), respectively. Nuclei were stained with Hoechst 33342.

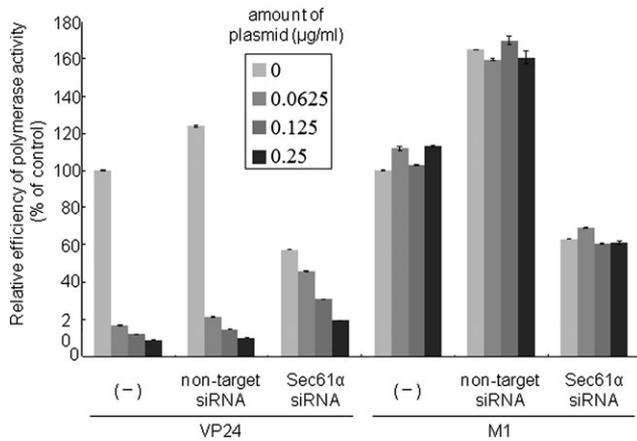


Figure 4. EBOV minigenome assay. 293 cells were transfected with siRNA against the nontarget or Sec61 α . Forty-eight hours later, the cells were transfected with various amounts of plasmid for the expression of VP24 or influenza virus M1 (M1), along with plasmids for the expression of NP, VP35, VP30, L, the T7 polymerase, Renilla luciferase, and an EBOV minigenome possessing the firefly luciferase gene. At 48 hours after transfection, the cells were lysed and the luciferase activity was measured.

24 hours after transfection, these cells were fixed and subjected to thin-section electron microscopy. There was no appreciable difference in the formation efficiency of the nucleocapsid-like structures in cells treated with nontarget siRNA and those treated with Sec61 α siRNA (Figure 5). This observation suggests that Sec61 α has no role in the nucleocapsid assembly of EBOV.

Sec61 α Does Not Affect EBOV Budding

Finally, we tested the effect of Sec61 α on virus budding by using a VLP system. To determine the efficiency of VLP production, the levels of VP40 and GP in the culture supernatant were analyzed using Western blot. The amounts of VLPs, as determined by the amounts of both VP40 and GP, were comparable among all test samples (Figure 6). There were no statistically significant differences in the amounts of VLPs produced when the cells were treated with siRNA against Sec61 α and when they were left untreated, indicating that Sec61 α does not affect the budding efficiency of EBOV.

DISCUSSION

In the present study, 68 cellular proteins that may interact with VP24 were identified. Among them, Sec61 α was shown to interact with VP24. The present study suggests that Sec61 α contributes to the inhibitory effect of VP24 on EBOV genome transcription and replication. In addition, in the absence of VP24, Sec61 α depletion reduced the polymerase activity (Figure 4), suggesting the potential involvement of Sec61 α in EBOV polymerase activity. These results indicate that Sec61 α is involved in the transcription and replication of the EBOV genome.

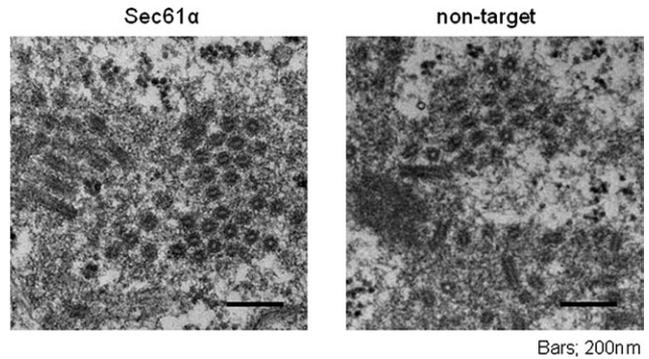


Figure 5. Electron microscopic analysis of nucleocapsid formation. 293 cells were transfected with siRNA against the nontarget or Sec61 α . Forty-eight hours later, the cells were transfected with plasmids for the expression of NP (1 μ g), VP35 (0.5 μ g), and VP24 (0.5 μ g). Ultrathin sections were prepared and observed using electron microscopy.

The precise mechanisms by which Sec61 α contributes to the transcription and replication of the EBOV genome were not addressed in this study. The inhibitory effect of VP24 on viral transcription and replication is suggested by its direct interaction with NP [3, 5]. Sec61 α may somehow interfere with this interaction, hampering the functions of both proteins. The reduced polymerase activity caused by Sec61 α depletion indicates that Sec61 α is important for EBOV genome transcription and replication. The L protein, with which Sec61 α partially colocalized (Figure 2D), transcribes and replicates the viral genome [32]. Sec61 α may affect either or both of these functions. The viral nucleocapsid is composed of NP and VP30, together

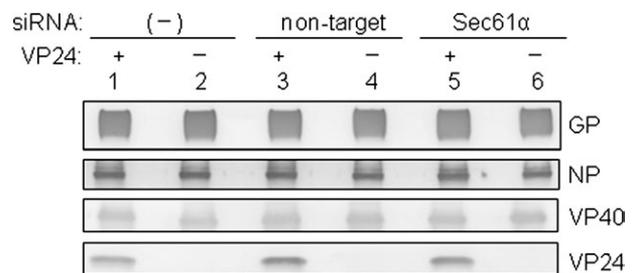


Figure 6. Budding efficiency assessment with use of the VLP system. 293T cells were transfected with siRNA against the nontarget or Sec61 α . Twenty-four hours after transfection, siRNA-treated cells were transfected with plasmids encoding the EBOV proteins required for VLP production in the presence or absence of VP24, a plasmid for the expression of T7 polymerase, and the T7-driven EBOV minigenome encoding the firefly luciferase reporter. Three days after the transfection, VLPs were harvested and purified. The purified VLPs were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and the viral proteins were detected by using Western blot. Lanes 1 and 2, mock-treated; lanes 3 and 4, nontarget siRNA-treated; lanes 5 and 6, Sec61 α siRNA-treated. Lanes 1, 3, 5, pCVP24 transfected; lanes 2, 4, 6, pCVP24 nontransfected.

with VP35 and L [32]. A preliminary model predicts that VP30 and VP35 bridge NP and L [33, 34]. Sec61 α may promote the proper interaction(s) of these proteins. Thus, the role of Sec61 α in EBOV replication might differ from its known function as a translocon protein.

In conclusion, our study identifies Sec61 α as a novel host factor for EBOV. Further investigation is required to elucidate the mechanisms by which this host factor contributes to EBOV replication.

Funding

This work was supported by Grants-in-Aid for Specially Promoted Research and for Scientific Research, by a Contract Research Fund for the Program of Founding Research Centers for Emerging and Reemerging Infectious Diseases, by Exploratory Research for Advanced Technology (ERATO), by the Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science, and Technology of Japan, by the Region V "Great Lakes" Regional Center of Excellence (GLRCE), and by National Institute of Allergy and Infectious Diseases Public Health Service research grants.

Acknowledgments

We thank Susan Watson for editing the manuscript.

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