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IV. 研究成果の刊行物・別刷（抜粋）

SOCS1 is an inducible host factor during HIV-1 infection and regulates the intracellular trafficking and stability of HIV-1 Gag

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Edited by Robert C. Gallo, University of Maryland, Baltimore, MD, and approved November 19, 2007 (received for review May 24, 2007)

Human immunodeficiency virus type 1 (HIV-1) utilizes the macromolecular machinery of the infected host cell to produce progeny virus. The discovery of cellular factors that participate in HIV-1 replication pathways has provided further insight into the molecular basis of virus–host cell interactions. Here, we report that the suppressor of cytokine signaling 1 (SOCS1) is an inducible host factor during HIV-1 infection and regulates the late stages of the HIV-1 replication pathway. SOCS1 can directly bind to the matrix and nucleocapsid regions of the HIV-1 p55 Gag polypeptide and enhance its stability and trafficking, resulting in the efficient production of HIV-1 particles via an IFN signaling-independent mechanism. The depletion of SOCS1 by siRNA reduces both the targeted trafficking and assembly of HIV-1 Gag, resulting in its accumulation as perinuclear solid aggregates that are eventually subjected to lysosomal degradation. These results together indicate that SOCS1 is a crucial host factor that regulates the intracellular dynamism of HIV-1 Gag and could therefore be a potential new therapeutic target for AIDS and its related disorders.

AIDS | pathogenesis | drug target | lysozyme

Human immunodeficiency virus type 1 (HIV-1) infection is a multistep and multifactorial process mediated by a complex series of virus–host cell interactions (1, 2). The molecular interactions between host cell factors and HIV-1 are vital to our understanding of not only the nature of the resulting viral replication, but also the subsequent cytopathogenesis that occurs in the infected cells (3). The characterization of the genes in the host cells that are up- or down-regulated upon HIV-1 infection could therefore provide a further elucidation of virus–host cell interactions and identify putative molecular targets for the HIV-1 replication pathway (4).

The HIV-1 p55 Gag protein consists of four domains that are cleaved by the viral protease concomitantly with virus release. This action generates the mature Gag protein comprising the matrix (MA/p17), capsid (CA/p24), nucleocapsid (NC/p7), and p6 domains, in addition to two small spacer peptides, SP1 and SP2 (5, 6). The N-terminal portion of MA, which is myristoylated, facilitates the targeting of Gag to the plasma membrane (PM), whereas CA and NC promote Gag multimerization. p6 plays a central role in the release of HIV-1 particles from PM by interacting with the vacuolar sorting protein Tsg101 and AIP1/ALIX (7–9). Several recent studies have implicated the presence of host factors in the control of the intracellular trafficking of Gag. AP-38 is a recently charac-

terized endosomal adaptor protein that binds directly to the MA region of Gag and enhances its targeting to the multivesicular body (MVB) during the early stages of particle assembly (10). The *trans*-Golgi network (TGN)-associated protein hPOSH plays another role in Gag transport by facilitating the egress of Gag cargo vesicles from the TGN, where it assembles with envelope protein (Env) before transport to PM (11). Although the involvement of these host proteins in the regulation of intracellular Gag trafficking has been proposed, the detailed molecular mechanisms underlying this process are still not yet well characterized.

In our current work, we demonstrate that the suppressor of cytokine signaling 1 (SOCS1) directly binds HIV-1 Gag and facilitates the intracellular trafficking and stability of this protein, resulting in the efficient production of HIV-1 particles. These results indicate that SOCS1 is a crucial host factor for efficient HIV-1 production and could be an intriguing molecular target for future treatment of AIDS and related diseases.

Results

SOCS1 Is Induced upon HIV-1 Infection and Facilitates HIV-1 Replication via Posttranscriptional Mechanisms. We and others have shown that HIV-1 infection can alter cellular gene expression patterns, resulting in the modification of viral replication and impaired homeostasis in the host cells (4, 12). Hence, to elucidate further the genes and cellular pathways that participate in HIV-1 replication processes, we performed serial analysis of gene expression (SAGE) using either a HIV-1 or mock-infected human T cell line, MOLT-4 (12). Further detailed analysis of relatively low-abundance SAGE tags identified *SOCS1* as a preferentially up-regulated gene after HIV-1 infection. This finding was validated by both semiquantitative RT-PCR and immunoblotting analysis with anti-SOCS1 anti-

Author contributions: A.R. and N.T. contributed equally to this work; A.R., A.Y., Y.M., and N.Y. designed research; A.R., N.T., K.O., R.K., M.N., H.S., S.H., T.S., I.A., and Y.M. performed research; J.K., S.H., M.Y., J.C., J.-i.M., K.Y., S.M., M.H., and A.Y. contributed new reagents/analytic tools; A.R., N.T., K.O., M.N., H.S., K.P., M.Y., K.Y., S.M., T.S., I.A., Y.M., and N.Y. analyzed data; and A.R., K.P., and N.Y. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

Freely available online through the PNAS open access option.

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This article contains supporting information online at www.pnas.org/cgi/content/full/0704831105/DC1.

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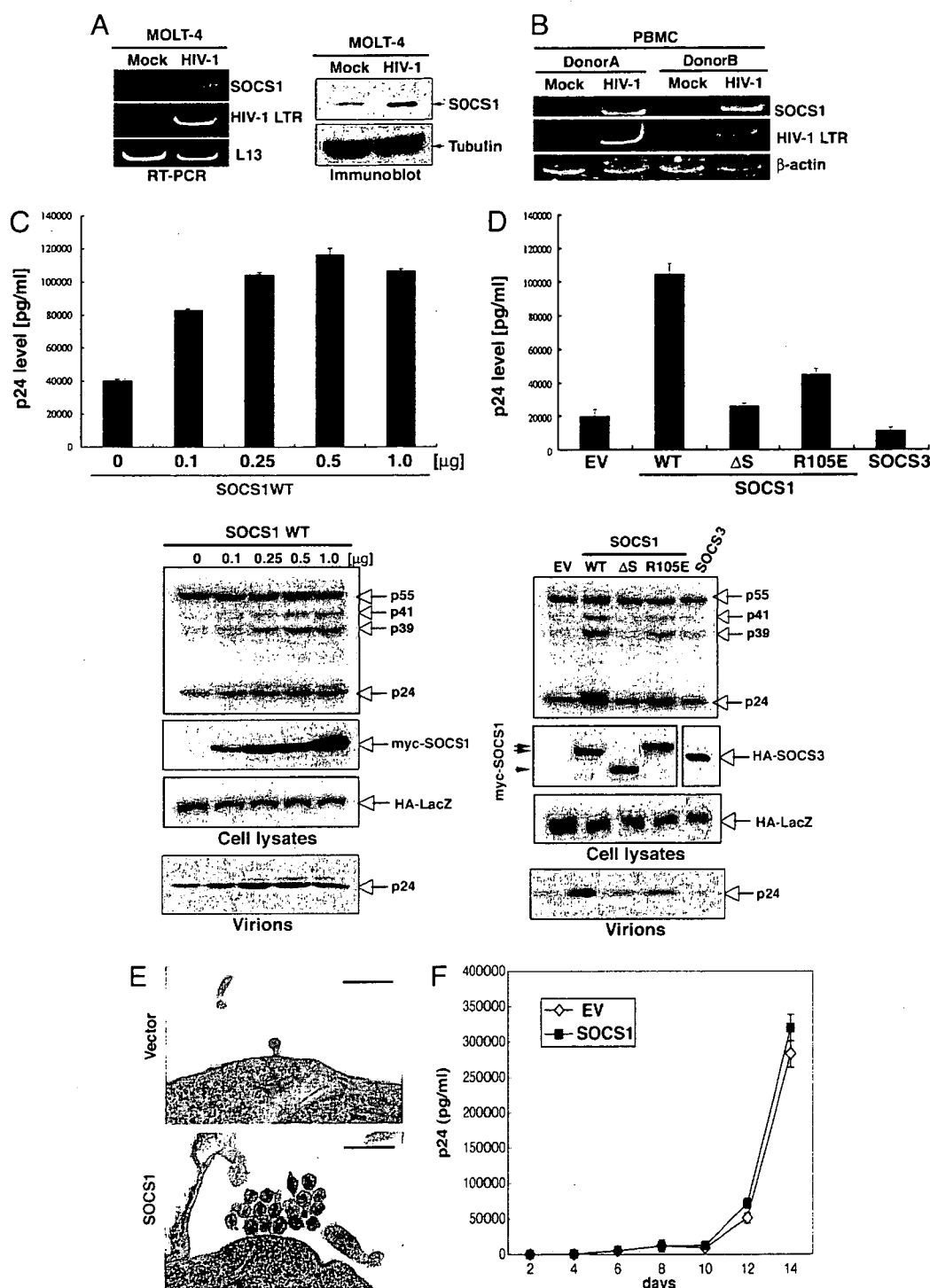


Fig. 1. SOCS1 is induced upon HIV-1 infection and enhances HIV-1 particle production. (A) MOLT-4 cells were mock-infected or infected with HIV-1_{NL4-3}, and then total RNA and protein extracts derived from these cells were subjected to semiquantitative RT-PCR (Left) and immunoblotting (Right), respectively. (B) PBMC from two healthy individuals were infected with HIV-1_{NL4-3} or were mock-infected, and SOCS1 expression was examined by semiquantitative RT-PCR. (C) 293T cells were transfected with pNL4-3 and cotransfected with various amounts of pcDNA-myc-SOCS1. Forty eight hours after transfection, p24 antigen release into the supernatant in each case was measured by antigen-capture ELISA (Upper), and the cell lysates and pelleted viruses were analyzed by immunoblotting (Lower). The data shown represent the mean \pm SD from three independent experiments. HA-LacZ is a transfection control. (D) 293T cells were transfected with pNL4-3 and cotransfected with control vector, SOCS1 (WT), SOCS1 Δ S (ΔSOCS box), SOCS1R105E, or SOCS3. Cell lysates and pelleted viruses were then collected after 48 h and subjected to ELISA (Upper) or immunoblotting (Lower), as described in C. (E) 293T cells cotransfected with either pNL4-3 plus control vector, or pNL4-3 plus myc-tagged SOCS1 were analyzed by TEM. Note that substantial numbers of mature virus particles can be observed in the myc-SOCS1-transfected cells. (Scale bars: 500 nm.) (F) Jurkat cells were infected with virions (adjusted by p24 levels) from either control vector (EV)- or SOCS1-transfected 293T cells. Supernatant p24 levels at the indicated time points were measured by ELISA.

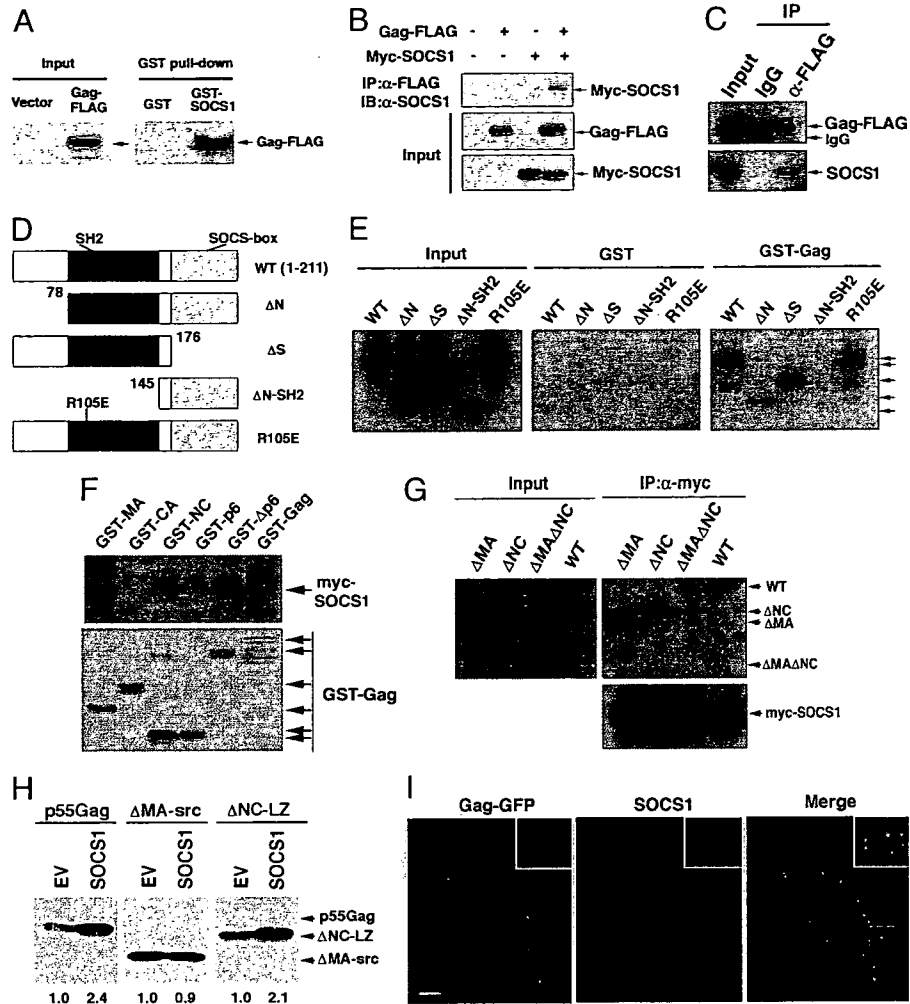
bodies (Fig. 1A). In addition, *SOCS1* was found to be up-regulated also in peripheral blood mononuclear cells (PBMC) from two different individuals (following HIV infection, Fig. 1B).

Our initial findings that SOCS1 is induced upon HIV-1 infection prompted us to examine whether this gene product affects viral replication. We first cotransfected 293T cells with a HIV-1 infectious molecular clone, pNL4-3 (13), and also pcDNA-myc-SOCS1, and then monitored the virus production levels in the resulting supernatant. We then performed ELISA using an anti-p24 antibody and found that wild-type SOCS1 significantly increases the production of HIV-1 in the cell supernatant in a dose-dependent

manner (Fig. 1C Upper). In contrast, neither the SH2 domain-defective mutant (R105E) nor the SOCS box deletion mutant (ΔS) of SOCS1 could promote virus production to the same levels as wild type, indicating that both domains are required for this enhancement (Fig. 1D Upper). Furthermore, another SOCS box protein, SOCS3, failed to augment HIV-1 replication in a parallel experiment (Fig. 1D Upper), indicating that the role of SOCS1 during HIV-1 replication is specific.

We next performed immunoblotting analysis using cell lysates and harvested virus particles in further parallel experiments (Fig. 1C and D Lower). Consistent with our ELISA analysis, the expres-

Fig. 2. SOCS1 interacts with HIV-1 Gag. (A) Extracts of 293T cells transfected with either empty vector or Gag-FLAG were subjected to pull-down analyses using glutathione-agarose beads with GST-SOCS1 in the presence of 10 ng/ml RNase followed by immunoblotting with anti-FLAG antibodies. (B) Extracts of 293T cells transiently expressing myc-SOCS1 and Gag-FLAG were subjected to immunoprecipitation (IP) with anti-FLAG monoclonal antibodies in the presence of 10 ng/ml RNase followed by immunoblotting (IB) analysis with either anti-FLAG or anti-myc polyclonal antibodies. (C) 293T cells were transiently transfected with Gag-FLAG, and cell lysates were then subjected to immunoprecipitation with anti-FLAG antibodies followed by immunoblotting with an antibody directed against endogenous SOCS1. (D and E) 293T cells expressing various myc-tagged SOCS1 mutants (schematically depicted in D) were analyzed by GST pull-down analysis with either GST or GST-Gag recombinant protein (E). (F) GST fusion proteins of the indicated regions of Gag were bound to glutathione beads and incubated with cell lysates from 293T cells expressing myc-SOCS1 in the presence of 10 ng/ml RNase followed by immunoblotting with anti-myc antibodies. (G) SOCS1 binds p55 Gag via either its MA or NC domains. 293T cells were transfected with myc-SOCS1 and cotransfected with Gag-FLAG, GagΔMA-FLAG, GagΔNC-FLAG, or GagΔMAΔNC-FLAG. At 24 h after transfection, cell lysates treated with 10 μg/ml RNase were subjected to coimmunoprecipitation with anti-myc monoclonal antibodies followed by immunoblotting with anti-FLAG or anti-myc polyclonal antibodies. (H) Functional interaction of SOCS1 with MA but not NC. 293T cells were transfected with wild-type Gag, ΔMA-src, or ΔNC-LZ (ZIL-p6) and cotransfected with either control vector or SOCS1. Supernatant virus particles were then collected after 24 h and subjected to immunoblotting with anti-p24 antibody. Numerical values below the blots indicate fold induction of supernatant p55 signal intensities derived by densitometry. (I) Colocalization of SOCS1 with Gag. HeLa cells were transiently transfected with Gag-GFP. After 24 h, the cells were fixed, permeabilized, and immunostained with anti-SOCS1 polyclonal antibody followed by fluorescently labeled secondary antibodies before confocal microscopy. (Scale bar: 10 μm.)



sion of wild-type SOCS1, but neither its SH2 nor SOCS box mutant counterparts, resulted in a marked and dose-dependent increase in the level of intracellular Gag protein, particularly in the case of CA (p24) and intermediate cleavage products corresponding to MA-CA (p41) and CA-NC (p39). This increase was found to be accompanied by an enhanced level of HIV-1 particle production in the supernatant (Fig. 1 C and D Lower). These results together indicated that SOCS1 facilitates HIV-1 particle production in infected cells and that this role of SOCS1 requires the function of both its SH2 and SOCS box domains. For further details about SOCS1 interaction with MA and NC and SOCS1-enhanced particle production, see supporting information (SI) Text.

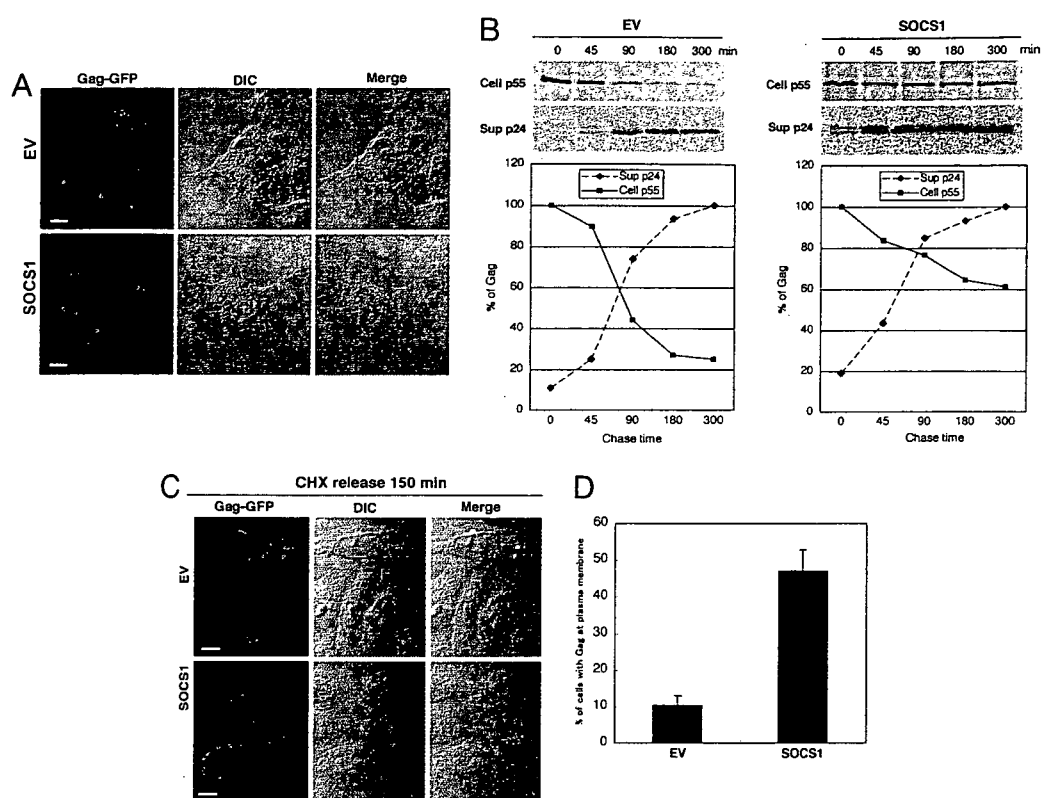
To examine the morphological aspects of HIV-1 particle production, transmission electron microscopy (TEM) was performed. 293T cells that had been cotransfected with pNL4-3, and either a control vector or a SOCS1 expression construct, were subjected to TEM analysis after fixation in glutaraldehyde. In SOCS1-transfected cells, a significantly increased number of mature virus particles was observed on the surfaces of PM compared with the control vector-transfected cells (Fig. 1E). There were also no obvious malformations of the virus particles in SOCS1-expressing cells, such as doublet formation or tethering to PM, which are characteristic of particle budding arrest (14) (Fig. 1E). Consistent with this observation, virions from SOCS1-transfected cells were found to be infectious as control viruses in Jurkat cells when the

same amounts of virus were infected (Fig. 1F). These results together indicate that SOCS1 enhances mature and infectious HIV-1 particle formation.

To elucidate the specific step in HIV-1 production that is enhanced by SOCS1, we next performed gene reporter assays using either luciferase expression constructs under the control of wild-type HIV-LTR (pLTR-luc), or a full-length provirus vector (pNL4-3-luc) (15). Interestingly, SOCS1 overexpression was found not to affect the transcription of these reporter constructs (data not shown), indicating that SOCS1 enhances HIV-1 replication via posttranscriptional mechanisms during virus production.

SOCS1 Interacts with the HIV-1 Gag Protein. The results of our initial experiments indicated that SOCS1 enhances HIV-1 production via a posttranscriptional mechanism. We therefore next tested whether SOCS1 could bind directly to HIV-1 Gag. GST pull-down analysis using C-terminal FLAG-tagged p55 Gag (codon-optimized) and GST-fused SOCS1 revealed that p55 Gag undergoes specific coprecipitation with GST-SOCS1 (Fig. 2A). Furthermore, both ectopically expressed myc-tagged SOCS1 and endogenous SOCS1 were found to undergo coimmunoprecipitation with Gag-FLAG in 293T cells (Fig. 2B and C). Additionally, GST pull-down analysis with various SOCS1 mutants, as depicted in Fig. 2D, further demonstrated that a mutant lacking the both N-terminal and SH2 domain (ΔN-SH2) could not bind

Fig. 3. SOCS1 enhances both the stability and trafficking of HIV-1 Gag. (A) HeLa cells cotransfected with pNL4-3 and either control vector (EV) or SOCS1 were immunostained with antibodies targeting anti-p24 (CA). Confocal microscopy with differential interference contrast (DIC) was then performed. (Scale bars: 10 μ m.) (B) 293T cells were transfected with either a control empty vector (EV) (Left) or myc-SOCS1 (Right) and cotransfected with pNL4-3. After 48 h, cells were pulse-labeled with [³⁵S]methionine or [³⁵S]cysteine for 15 min and chased for the durations indicated. Cell lysates and pelleted supernatant virions were immunoprecipitated with anti-p24 antibodies followed by autoradiography. (C and D) HeLa cells seeded on poly-L-lysine-coated cover slides were transfected with either vector control or SOCS1. After 24 h, cells were again transfected with Gag-GFP for 3 h and then treated with 100 μ g/ml CHX for 5 h to inhibit protein synthesis. This treatment was followed by incubation with fresh medium; then 150 min after the CHX release, cells were fixed and subjected to confocal microscopy (C). (Scale bars: 10 μ m.) Cells with Gag protein on the plasma membrane were scored out of 200 transfected cells (D).



p55 Gag, whereas an N-terminal or a SOCS box deletion did not affect the binding of SOCS1 to Gag in 293T cells (Fig. 2E). This finding indicates that the SH2 domain is important for the interaction of SOCS1 with HIV-1 Gag. Interestingly, the R105E mutant of SOCS1, which disrupts the function of the SH2 domain, still binds Gag (Fig. 2E), indicating that the Gag-SOCS1 association is independent of the tyrosine phosphorylation of Gag, as is the case for both HPV-E7 and Vav (16, 17).

To elucidate the SOCS1-binding region of the Gag protein, GST pull-downs with various GST-fused Gag domain constructs were performed. SOCS1 was detected in glutathione bead precipitates with GST-wild-type Gag, GST- Δ p6, GST-MA, and GST-NC, but not with other domain constructs (Fig. 2F), indicating that SOCS1 interacts with Gag via its MA and NC domains. Consistent with these results, the deletion of both the MA and NC domains of p55 Gag (Δ MA Δ NC) completely abolishes its interaction with SOCS1 in coimmunoprecipitation experiments (Fig. 2G). Furthermore, *in vitro* analysis with purified proteins also demonstrated that SOCS1 can indeed interact with both the MA and NC regions of HIV-1 Gag in the absence of nucleic acids or other proteins (SI Fig. 5).

We next wished to determine the functional interaction domain in HIV-1 Gag through which SOCS1 functions in terms of virus-like particle production. To this end, we used a MA-deleted Gag mutant with an N-terminal myristoyl tag derived from src (Δ MA-src) (18) and also an NC-deleted Gag mutant with a GCN4 leucine zipper in place of NC, which we herein denote as Δ NC-LZ but which has been described as ZIL-p6 (19). Both of these mutants have been shown still to assemble and bud (18, 19). We found that SOCS1 overexpression can still augment the particle formation of both wild-type Gag and Δ NC-LZ but not Δ MA-src (Fig. 2H), indicating that the functional interaction between SOCS1 and HIV-1 Gag is in fact mediated through MA.

To confirm further the direct interaction between SOCS1 and Gag in cells, we examined the intracellular localization of these two proteins. Confocal microscopy revealed that endogenous SOCS1

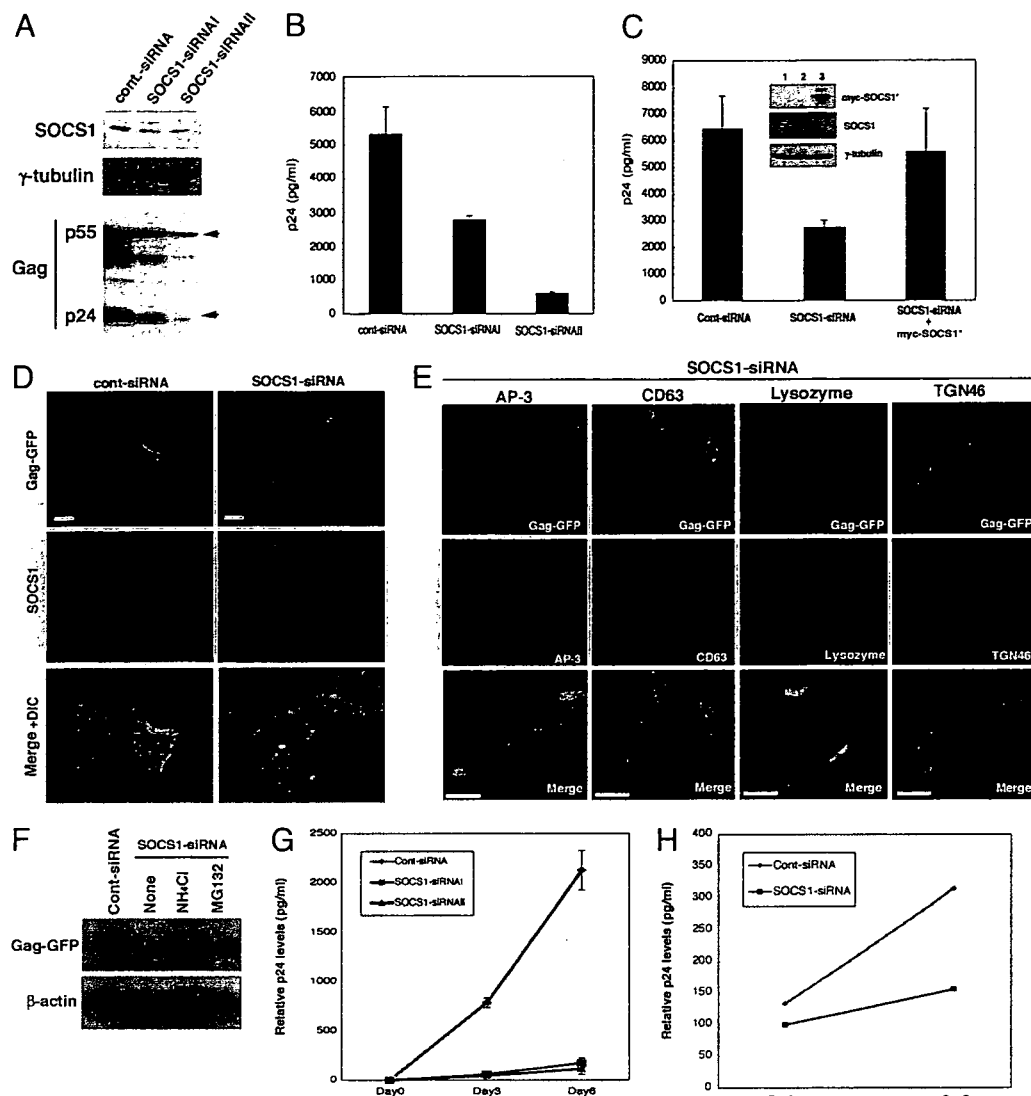
forms dotted filamentous structures in the cytoplasm and that Gag localizes in a very punctate pattern with SOCS1 from the perinuclear regions to the cell periphery (Fig. 2J). These data indicate that SOCS1 interacts with HIV-1 Gag in the cytoplasm during HIV-1 particle production.

SOCS1 Promotes both the Stability of Gag and Its Targeting to the Plasma Membrane. Because we had found from our initial data that SOCS1 increases HIV-1 particle production as a result of its direct interaction with intracellular Gag proteins, we next addressed whether SOCS1 positively regulates Gag stability and subsequent trafficking to PM. Our immunofluorescent analysis with the anti-p24 (CA) antibody initially revealed that SOCS1 overexpression increases the levels of Gag at PM when cotransfected with pNL4-3 at 48 h after transfection, although it was detected at PM in both control and SOCS1-expressing cells (Fig. 3A). Furthermore, the levels of cytoplasmic Gag were found to be much lower in the SOCS1-expressing cells compared with the control cells (Fig. 3A). These results indicate that SOCS1 enhances Gag trafficking to PM.

To examine next whether SOCS1 affects the stability and trafficking of newly synthesized Gag proteins, we performed pulse-chase analysis. This experiment revealed that SOCS1 significantly increases the stability of the intracellular p55 Gag polypeptide as well as the levels of p24 in the supernatant (Fig. 3B). Importantly, p24 was detectable at an earlier time point and reached maximum levels in a shorter period in the cell supernatant of SOCS1-transfected cells compared with control vector-transfected cells (Fig. 3B). This finding again suggests that SOCS1 facilitates the intracellular trafficking of newly synthesized Gag proteins to PM.

To confirm this hypothesis further, we performed cycloheximide (CHX) analysis with HeLa cells transfected using either vector control or SOCS1. After 24 h, cells were again transfected with Gag-GFP for 3 h and treated with CHX for 5 h to inhibit protein synthesis. Cells were then cultured in fresh medium without CHX for an additional 150 min and subjected to confocal microscopy. At

Fig. 4. The targeted inhibition of SOCS1 suppresses Gag trafficking and HIV-1 particle production and enhances Gag degradation in lysosomes. (A and B) 293T cells were transfected with either control siRNA or two different SOCS1-specific siRNAs (I or II) together with pNL4-3. At 48 h after transfection, cell lysates were subjected to immunoblotting analysis with the indicated antibodies (A). Cell supernatants were then subjected to ELISA analysis of p24 levels (B). (C) 293T cells were transfected with pNL4-3 and cotransfected with control-siRNA, SOCS1-siRNAI alone, or SOCS1-siRNAI plus siRNA-resistant myc-SOCS1 (myc-SOCS1*). After 48 h, cell supernatants were collected and subjected to p24 ELISA. (Inset) Immunoblots of the cell lysates. (D) HeLa cells were transfected with control or SOCS1-specific siRNA and cotransfected with GFP-Gag. At 48 h after transfection, the cells were subjected to confocal microscopy. (E) HeLa cells were transfected with Gag-GFP and SOCS1-siRNA constructs for 48 h. Cells were then fixed and subjected to immunofluorescent analysis with indicated antibodies followed by DAPI staining. (Scale bars: 10 μ m.) (F) HeLa cells were transfected with Gag-GFP and cotransfected with either control-siRNA or SOCS1-siRNA. After 36 h, the cells were treated with a mock solution, 10 mM NH_4Cl or 10 μM MG132 for another 16 h. Cells were then harvested and subjected to immunoblotting analysis with anti-GFP or anti- β -actin antibodies. (G) Jurkat cells were infected with a retroviral vector encoding control (Cont) or two different SOCS1-specific siRNAs (I or II). After selection with puromycin, the cells were then infected with HIV-1_{NL4-3} (multiplicity of infection, 0.1), and p24 antigen levels in cell supernatant were measured by ELISA at the indicated time points. (H) Human primary CD4 T cells were separated from healthy donors and infected with lentivirus vectors encoding either control- or SOCS1-siRNAI. The cells were then infected with HIV-1_{NL4-3} (multiplicity of infection, 0.1), and p24 antigen levels in cell supernatant were measured by ELISA at the indicated time points.



this time point, Gag-GFP was found to localize predominantly in a perinuclear region in the control cells (Fig. 3C), whereas almost half of the SOCS1-transfected cells exhibited Gag-GFP localization on PM (Fig. 3D). These results again indicate that SOCS1 efficiently enhances the trafficking of newly synthesized Gag protein to PM.

The Targeted Disruption of SOCS1 Inhibits Gag Trafficking and HIV-1 Particle Production. To delineate further the role of SOCS1 in the trafficking of Gag and in subsequent HIV-1 particle production, we depleted cellular SOCS1 by siRNA. The significant depletion of SOCS1 expression by two different SOCS1-specific siRNA constructs was confirmed by immunoblotting analysis (Fig. 4A and B). Significantly, in cells cotransfected with pNL4-3 and SOCS1-specific siRNAs, both HIV-1 particle release and the levels of intracellular Gag protein are significantly decreased compared with the control cells (Fig. 4A and B). Furthermore, the effects of SOCS1-siRNA on the inhibition of HIV-1 particle production was diminished by reexpression with a codon-optimized SOCS1 construct that is resistant to these siRNAs (Fig. 4C), indicating that the SOCS1 siRNA suppression of HIV-1 particle production depends on the availability of endogenous SOCS1.

Consistent with these observations, immunofluorescent analysis further revealed that the expression of SOCS1-siRNA dramatically inhibits Gag trafficking such that Gag proteins accumulate in the perinuclear regions as large solid aggregates, as has been reported (20) (Fig. 4D). This finding indicates that SOCS1 plays an essential role in the Gag trafficking from perinuclear clusters to PM. Interestingly, these discrete perinuclear clusters of Gag were found to colocalize with lysosome markers, lysozyme, and partly with AP-3, but neither with the late endosome MVB marker CD63 nor the *trans*-Golgi marker TGN46, indicating that Gag is targeted for degradation by lysosomes when the function of SOCS1 is inhibited (Fig. 4E). In support of this notion, the levels of intracellular Gag were found to be significantly increased by treatment with a lysosome inhibitor NH_4Cl but not by a proteasome inhibitor MG132 in SOCS1-siRNA cells (Fig. 4F), further indicating that the perinuclear clusters of Gag will undergo lysosomal degradation rather than proteasomal degradation when optimal Gag transport to PM is suppressed by the inhibition of SOCS1.

We next addressed whether targeted SOCS1 inhibition would affect HIV-1 particle production in human T cells. The effect of SOCS1 depletion was clearly evident in both HIV-1_{NL4-3}-infected

Jurkat cells and human primary CD4⁺ T cells, which demonstrated pronounced decreases in virus particle production in SOCS1-siRNA-expressed cells compared with the controls (Fig. 4 G and H). These results together indicate that the specific inhibition of SOCS1 suppresses the optimal trafficking of Gag to PM, resulting in the degradation of Gag in lysosomes, which in turn leads to the efficient and reproducible inhibition of HIV-1 particle production in various types of human cells.

Discussion

In this work, we report that SOCS1 is an inducible host factor during HIV-1 infection and plays a key role in the late stages of the viral replication pathway via an IFN-independent mechanism (SI Fig. 6). These results represent evidence that SOCS1 is a potent host factor that facilitates HIV-1 particle production via posttranscriptional mechanisms.

SOCS1 has been shown to be a suppressor of several cytokine signaling pathways, and like all SOCS family members it has a central SH2 domain and a conserved C-terminal domain known as the SOCS box (21, 22). Structure-function analyses have further demonstrated that the SOCS1 SH2 domain is required for the efficient binding of its substrates (23, 24). Indeed, our current analyses have also revealed that the SH2 domain of SOCS1 is required for its interaction with the HIV-1 Gag protein. We have shown from our present data that the SOCS box is also required for SOCS1 to function during HIV-1 particle production.

The SOCS box-mediated function of SOCS1 is chiefly exerted via its ubiquitin ligase activity (21, 25). Biochemical binding studies have shown that the SOCS box of SOCS1 interacts with the elongin BC complex, a component of the ubiquitin/proteasome pathway that forms an E3 ligase with Cul2 (or Cul5) and Rbx-1 (21, 26, 27). We show from our current experiments that the SOCS box is required for HIV-1 particle production, indicating the involvement of the ubiquitin/proteasome pathway. However, it is still unknown whether SOCS1 promotes the ubiquitination of Gag and, if so, whether the mono- or poly-ubiquitination of Gag would affect its trafficking and protein stability. Further studies will be necessary to clarify the biological significance of Gag ubiquitination.

Perlman and Resh (20) recently reported that newly synthesized Gag first appears to be diffusely distributed in the cytoplasm,

accumulates in perinuclear clusters, passes transiently through a MVB-like compartment, and then traffics to PM. Consistent with these observations, our current work also shows that Gag is accumulated at perinuclear clusters as solid aggregates when its targeting to PM is impaired because of the SOCS1 inhibition.

Another aspect of SOCS1 function during HIV-1 infection was proposed recently. Song *et al.* (28) reported that SOCS1-silenced dendritic cells broadly induce the enhancement of HIV-1 Env-specific CD8⁺ cytotoxic T lymphocytes and CD4⁺ T helper cells as well as an antibody response. The induction of the SOCS1 gene in HIV-1 infected cells might therefore disrupt a specific intracellular immune response to HIV-1 in infected host cells.

Based on the strong evidence that we present in our current work that SOCS1 positively regulates the late stages of HIV replication, we conclude that SOCS1 is likely to be a valuable therapeutic target not only for future treatments of AIDS and related diseases, but also for a postexposure prophylaxis against disease in HIV-1-infected individuals.

Materials and Methods

Antibodies and Fluorescent Reagents. Antibodies and fluorescent reagents were obtained from the following sources. Anti-CD63, anti-AP-3, anti-myc (A-14), and anti-SOCS1 (H-93) were from Santa Cruz Biotechnology. Anti-SOCS1 was from Zymed Laboratories. Anti-FLAG (M2) and anti-HA (12CA5) were from Sigma and Roche Diagnostics, respectively. Anti-HIV-p24 (Dako; Cytoation), anti-STAT1, and anti-phospho-STAT1 (Y701) were from BD Transduction Laboratories. Sheep polyclonal anti-TGN46 was from GeneTex.

Plasmid Constructs. Expression constructs for SOCS1 have been described in ref. 29. GST fusion constructs with specific regions derived from the codon-optimized gag were generated (MA, CA, NC, p6, Δp6, full-length Gag) by cloning into pGEX-2T (GE Healthcare Bio-Sciences) as described in ref. 30. For retrovirus-mediated siRNA expression, pSUPER.retro.puro vector was digested, as described in ref. 31, with the following sequences: SOCS1-siRNAI, TCGAGCTGCTGGAGCACTA; SOCS1-siRNAII, GGCCAGAACCTCTCTCTCTT; control siRNA, TCGATGTTGTGTGAATT.

Electron Microscopy. Transfected 293T cells were fixed with 2.5% glutaraldehyde and subjected to TEM, as described (14, 32).

ACKNOWLEDGMENTS. We thank Dr. H. Gottlinger (University of Massachusetts) for providing plasmids. This work was supported in part by grants from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and Human Health Science of Japan.

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