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Cellular Proteins Hsp60 and SAHH as Negative Regulators of the Early Stages of HIV-1 Replication

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Abstract—Increasing resistance of human immunodeficiency virus type 1 (HIV-1) to the drugs targeting viral proteins stimulates the search for new therapeutic targets, among which are blockers of virus–host protein interactions. For two cellular proteins (LEDGF/p75 and Ku70) that interact with viral integrase, binding inhibitors have already been identified that reduce replication efficiency. Previously, using the methods of cross-linking and co-immunoprecipitation followed by mass spectrometry, several novel potential cellular partners of HIV-1 integrase were identified, including the Hsp60 chaperonin and S-adenosylhomocysteine hydrolase (SAHH). In the present study, we demonstrate that these purified recombinant proteins co-precipitate *in vitro* with integrase, indicating their ability to directly interact with the enzyme. Knockdown of Hsp60 and SAHH in the human cells was found to stimulate transduction efficiency by the HIV-1-based pseudovirus. This effect occurs specifically at the early stages of HIV-1 replication, not at the stage of proviral transcription. Furthermore, we were able to determine the stage of HIV-1 replication influenced by these proteins. It was revealed that the Hsp60 knockdown stimulates integration, while the SAHH knockdown enhances efficiency of the viral reverse transcription, in which integrase is also involved.

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Keywords: SAHH, Hsp60, reverse transcriptase, integrase, HIV-1

INTRODUCTION

During a replicative cycle, viruses inevitably encounter host cell proteins. Some of these interactions represent cellular defense mechanisms against a virus, whereas others enable a virus to exploit host systems for its own replication [1]. Each viral protein has a specific set of cellular protein partners, constituting its interactome. The number of such partners can be substantial, reaching several dozen cellular proteins per single viral protein and more than a hundred for the entire viral proteome [2]. Information on these interactions could be valuable for the development of new therapeutic strategies against viral infections, particularly in the case of rapidly evolving viruses, such as human immunodeficiency virus type 1 (HIV-1) that acquires resistance to antiviral medications [3, 4].

Most antiretroviral drugs used in the HIV-1 therapy are inhibitors of one of the three viral enzymes: reverse transcriptase (RT), integrase (IN), and protease. Due to the rapid emergence of resistance to individual drugs, current treatment standards recommend the simultaneous use of at least three inhibitors targeting two or more viral enzymes [5]. Nevertheless, the multidrug-resistant HIV-1 strains are regularly detected in patients, and these strains are often difficult to combat using traditional approaches [6, 7]. Recent studies have shown that in such cases, the following

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inhibitors of protein–protein interactions can be effective: fostemsavir, which blocks interaction between the gp120 and the CD4 receptor [8, 9]; ibalizumab, targeting the same interaction [10, 11]; and lenacapavir, blocking interaction between subunits of the capsid protein [12]. Efficacy of such drugs demonstrates that studying these interactions is important not only for advancing theoretical understanding of the HIV-1 life cycle but also for the development of novel therapeutic strategies.

HIV-1 integrase (IN) is a promising subject for investigating protein–protein interaction between a virus and a host cell for two reasons. First, it is required for the successful completion of three major early stages of the viral replicative cycle: reverse transcription, integration, and post-integration repair; disrupting any of them leads to inhibition of replication [13-15]. Second, more than 20 cellular partners of IN have been described to date, while this list is steadily growing; their roles and mechanisms of action are diverse, providing potentials for novel approaches to targeting viral replication [16].

In the 2025 study using cross-linking and co-immunoprecipitation followed by mass spectrometry, a number of potential cellular partners of HIV-1 IN were identified, including the heat shock protein 60 (Hsp60) chaperonin and S-adenosylhomocysteine hydrolase (SAHH, of which two isoforms – SAHH1 and SAHH2 – are known, differing by the presence of a 29-amino acid N-terminal fragment in the former) [17]. In the present work, we demonstrate for the first time that both isoforms of SAHH are capable of interacting with IN *in vitro*, and we also confirm the previously reported *in vitro* interaction between Hsp60 and integrase [18]. Using HIV-1-based pseudotyped viral particles, we characterized these proteins as negative HIV-1 factors acting at the early stages of viral replication. Furthermore, by quantifying different forms of viral DNA using qPCR, we determined that the effect of SAHH is due to its negative influence on reverse transcription, whereas Hsp60 acts at the integration step.

MATERIALS AND METHODS

Oligonucleotides were synthesized by the phosphoramidite method by Evrogen (Russia) (Table 1).

Small interfering RNAs (siRNAs) were synthesized by the phosphoramidite method by Genterra (Russia) (Table 2).

Plasmids. Two commercial plasmids were used in this study: pNL4-3.Luc.R-E (NovoPro, China) and pCMV-VSVG (Addgene, USA), encoding the genome of pseudoviral particles with the firefly luciferase reporter gene and the surface glycoprotein G of the vesicular stomatitis virus, respectively. For prokaryotic expression of the GST-tagged recombinant human proteins SAHH1, SAHH2, and Hsp60, plasmids were constructed on the basis of the pGEX vector (Addgene). For this purpose, PCR was performed with total cellular cDNA using primers Hsp60_s and Hsp60_as; SAHH1_s and SAHH1_as; SAHH2_s and SAHH2_as (Table 1), and Q5 High-Fidelity Polymerases (New England Biolabs, USA), according to the manufacturer's instructions. The resulting PCR products were digested with the restriction endonucleases FastDigest NdeI and FastDigest XhoI (Thermo Fisher Scientific, USA) and cloned into the pGEX vector. Sequences of the resulting pGEX_Hsp60, pGEX_SAHH1, and pGEX_SAHH2 vectors were confirmed by sequencing.

Production, isolation, and purification of recombinant proteins. Recombinant HIV-1 IN was obtained as described previously [19]. For the production of GST-Hsp60, GST-SAHH1, and GST-SAHH2 proteins, competent *Escherichia coli* BL21-Codon Plus cells (Agilent, USA) were transformed with 10 ng of pGEX_Hsp60, pGEX_SAHH1, and pGEX_SAHH2 plasmids, respectively. Selected clones were grown in a 2.5% LB medium (Amresco, USA) supplemented with 100 μg/ml ampicillin in 1-liter flasks at 16°C. When OD_{600} reached 0.8, expression was induced by addition of 0.1 mM IPTG, followed by cultivation for 18 h. Cells were harvested by centrifugation for 30 min at 3000 rpm. Cell pellets were resuspended on ice in a buffer (20 mM Tris-HCl (pH 8.0), 500 mM NaCl, 2 mM 2-mercaptoethanol, 1 mM PMSF) at a ratio 10 ml buffer per 1 g of pellet, and exposed to ultrasonication (3-5 kJ of transmitted energy per 1 g of resuspended

Table 1. Primer sequences used in this study

Primers	5′→3′ primer sequences
Hsp60_s	d(AGACTCCATATGCTTCGGTTACCC)
Hsp60_as	d(GATCCTCGAGTTATCCGAACATGCCAC)
SAHH1_s	d(GCCGATCCATATGTCTGACAAACTGC)
SAHH1_as	d(GATCCTCGAGTTATCCGTAGCGGTAG)
SAHH2_s	d(GGATCCATATGCCGGGCC)
SAHH2_as	d(GATCCTCGAGTTATCCGTAGCGGTAG)
GAPDH_fw	d(CCACTCCTCCACCTTTGAC)
GAPDH_rv	d(ACCCTGTTGCTGTAGCCA)
Hsp60_fw	d(AGCCTTGGACTCATTGAC)
SAHH_fw	d(GTGGAGATCGATGTCAAGTG)
SAHH_rv	d(CTGGTTGGTGAAGGAGTTAC)

Table 2. siRNA sequences used in this study

siRNAs	5′→3′ oligonucleotide sequences
siC	S: rArGrGrUrCrGrArArCrUrArCrGrGrGrUrCrArAdTdT
	AS: rUrUrGrArCrCrCrGrUrArGrUrUrCrGrArCrCrUdTdT
siHsp60	S: rUrGrUrUrGrArArGrGrArUrCrUrUrUrGrArUrAdTdT
	AS: rUrArUrCrArArArGrArUrCrCrUrUrCrArArCrAdTdT
siSAHH	S: rCrArGrGrCrUrGrUrArUrUrGrArCrArUrCrArUdTdT
	AS: rArUrGrArUrGrUrCrArArUrArCrArGrCrCrUrGdTdT

pellet). Insoluble material was removed by centrifugation. Lysates were incubated with a glutathione-agarose (Thermo Fisher Scientific, USA) pre-equilibrated with a buffer for 3 h at 4°C. Glutathione-agarose was washed with 25 ml of a resuspension buffer. Elution was performed in two steps: glutathione-agarose was incubated with an elution buffer (20 mM Tris-HCl (pH 7.5), 500 mM NaCl, 2 mM 2-mercaptoethanol, 50 mM glutathione) for 30 min at room temperature, the eluate was collected, and the procedure was repeated. Dialysis against a dialysis buffer (20 mM Tris-HCl (pH 7.6), 500 mM NaCl, 5% glycerol, 2 mM 2-mercaptoethanol) was performed overnight. For storage, 20% glycerol was added to the buffer, and protein solutions were stored at −80°C. Protein concentration in the final preparations was quantified by the Bradford assay [20].

Protein co-precipitation. Interaction between the GST-tagged human proteins and 6×His-tagged IN was analyzed using protein co-precipitation on glutathione-agarose. Protein pairs were incubated in 200 µl of a co-precipitation buffer (20 mM HEPES (pH 7.5), 100 mM NaCl, 7.5 mM $MgCl_2$, 2 mM 2-mercaptoethanol, 50 µg/ml BSA, 0.1% NP-40) for 1 h at 25°C, followed by addition of 30 µl glutathione-agarose pre-equilibrated in the same buffer supplemented with BSA, and incubation for another hour. Supernatants were removed, and the agarose was washed twice with 600 µl of the co-precipitation buffer. Precipitated proteins were eluted from the agarose by its incubation in a buffer containing 50 mM Tris-HCl (pH 6.8), 1% SDS, 10% glycerol, 100 mM 2-mercaptoethanol, ~0.0025% bromophenol blue at 95°C for 10 min. Proteins were separated with 12% SDS-PAGE according to Laemmli [21] and analyzed by Western blot analysis.

Western blot analysis. After SDS-PAGE separation, proteins were transferred to Immobilon®-PVDF membranes (Bio-Rad, USA) using the Trans-Blot Turbo Transfer System (Bio-Rad), (semi-dry transfer). GST- and 6×His-tagged proteins were detected with rabbit anti-GST and mouse anti-His antibodies (Sigma, USA), respectively. Visualization was performed with HRP-conjugated secondary antibodies: mouse anti-rabbit and goat anti-mouse (Sigma, USA). Detection was carried out using an HRP Clarity Western ECL substrate kit (Bio-Rad) and a ChemiDoc MP system (Bio-Rad).

Culturing human cells. All experiments with human cells were carried out using the HEK 293T cell line. Cells were cultured in DMEM medium (PanEco, Russia) supplemented with 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin (Invitrogen, USA). To obtain HEK 293T cells with a stably integrated pseudoprovirus, cells were transduced with pseudoviral particles and maintained for one week.

Liposome-mediated siRNA transfection. HEK 293T cells were transfected with siHsp60 and siSAHH siRNAs, targeting Hsp60 and SAHH mRNAs, respectively, in the presence of GenJect-40 (Molekta, Russia) according to the manufacturer's instructions. As a control, a nonspecific siRNA (siC) was used. Efficiency of siRNA action was analyzed 48 h after transfection by qPCR with total cellular cDNA using a 5X qPCRmix-HS SYBR mix (Evrogen), a Gentier 96 Real-Time Quantitative PCR system (Drawell Scientific, China), and Hsp60_fw/Hsp60_rv; SAHH_fw/SAHH_rv primers (Table 1) to assess Hsp60 and SAHH siRNA levels, respectively. GAPDH_fw and GAPDH_rv primers (Table 1) were used as an internal control to assess mRNA levels of the GAPDH household gene.

Production of VSV-G-pseudotyped viral particles. To produce pseudoviral particles, HEK 293T cells were transfected using the calcium phosphate method with two plasmids: pNL4-3.Luc.R-E and pCMV-VSVG (at a 5 : 1 mass ratio). For transfection of one T-175 flask (179.5 cm^2), a total of 90 µg of plasmid DNA was used, dissolved in 1969 µl of buffer (10 mM Tris-HCl (pH 8.0), 1 mM EDTA), 2250 µl of 2×HBS buffer (10 mM HEPES (pH 7.4), 150 mM NaCl), and 281 µl of 2 M $CaCl_2$. After 48 h, the culture medium was collected and replaced with a fresh medium.

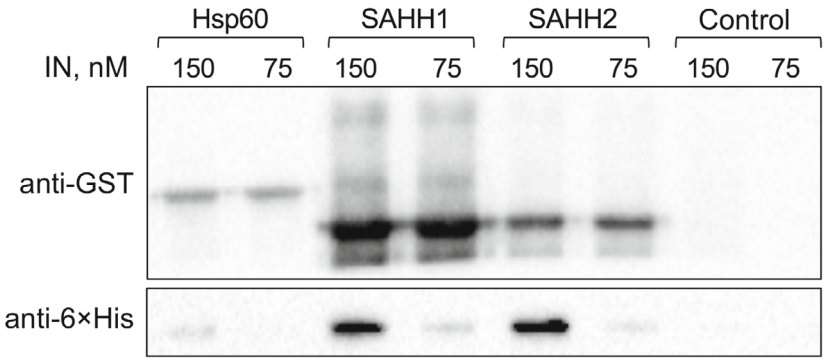

Fig. 1. Co-precipitation of recombinant GST-tagged Hsp60, SAHH1, and SAHH2 proteins (150 nM) with 6×His-tagged HIV-1 IN (75 or 150 nM) on glutathione–agarose.

The harvested medium was centrifuged at 3000 rpm and supernatants were filtered through a 0.45 μm filter (Membrane Solutions, USA). The filtrate was centrifuged at 56,000*g* for 1.5 h at 4°C under vacuum. The resulting pellet was resuspended in PBS buffer (Gibco, USA) and stored at −80°C. After another 24 h (72 h after transfection), the medium was collected again and processed as described above.

Luciferase activity assay in transduced cells. HEK 293T cells were transduced with pseudoviral particles 48 h after siRNA transfection. 24 h post-transduction, *Photinus pyralis* firefly luciferase activity was measured using the Luciferase Assay System (Promega, USA) according to the manufacturer's protocol. Luminescence was analyzed using the BioTek SYNERGY H1 multimode reader (Agilent Technologies, USA). Luminescence in HEK 293T cells with stably integrated pseudoprovirus was analyzed 48 h after siRNA transfection.

Relative quantification of HIV-1 DNA forms. Relative levels of total and integrated HIV-1 DNA were determined as described previously [22]. Relative efficiency of post-integration repair of HIV-1 DNA was assessed as described by Anisenko et al. [23].

Data visualization. Data analysis was performed using the Microsoft Excel software. Luciferase assay and MTT assay results were visualized using the GraphPad Prism 9.5.1 software.

Statistical analysis. All experiments were performed in at least three biological replicates. One-way ANOVA followed by Dunnett's test was used for multiple comparisons.

RESULTS

Interaction of HIV-1 integrase with recombinant Hsp60 and SAHH proteins *in vitro*. Interaction of Hsp60 and SAHH with HIV-1 integrase (IN) observed by Agapkina et al. [17] could have resulted from either direct or indirect protein interactions due to the specifics of the method, which was based on formaldehyde treatment of a cell lysate containing overexpressed HIV-1 IN, followed by co-immunoprecipitation of protein complexes and mass spectrometric detection of IN-bound proteins. To determine whether the potential partners interact directly with IN, we expressed the GST-tagged human proteins (Hsp60 or one of the two SAHH isoforms) and incubated them with the 6×His-tagged HIV-1 IN, followed by analysis of their interaction using an *in vitro* co-precipitation assay. For each of the proteins tested, we observed co-precipitation of IN at least at one of the concentrations used (Fig. 1). In addition, the amount of co-precipitated IN increased with increasing concentration of this protein in solution. No nonspecific binding of IN to the sorbent was observed in the control sample, where IN alone was incubated with the resin without any human protein. These data indicate that the human Hsp60, SAHH1, and SAHH2 proteins specifically bind to the HIV-1 IN *in vitro*.

Effect of Hsp60 and SAHH knockdown on the HIV-1 replication. Having established that the tested proteins can directly interact with IN, we next investigated their effect on the HIV-1 replication. To this end, we performed knockdown of Hsp60 and SAHH in the HEK 293T cells using siRNA, followed by transduction with the replication-defective HIV-1-based pseudovirus particles carrying a firefly luciferase reporter gene. This system allows assessment of the knockdown effects on early stages of replication (reverse transcription, integration, and post-integration repair), as well as transcription of the integrated provirus from the HIV-1 LTR promoter. According to our data, a statistically significant difference was observed between the reporter activity in the cells transfected with the control non-specific siRNA (siC) and the cells transfected with the siRNAs targeting the mRNAs of the studied proteins (Fig. 2a). Knockdown of Hsp60 led to the 1.9 ± 0.4-fold increase in the luciferase signal, while knockdown of SAHH resulted in the 1.65 ± 0.11-fold increase. Thus, since knockdown

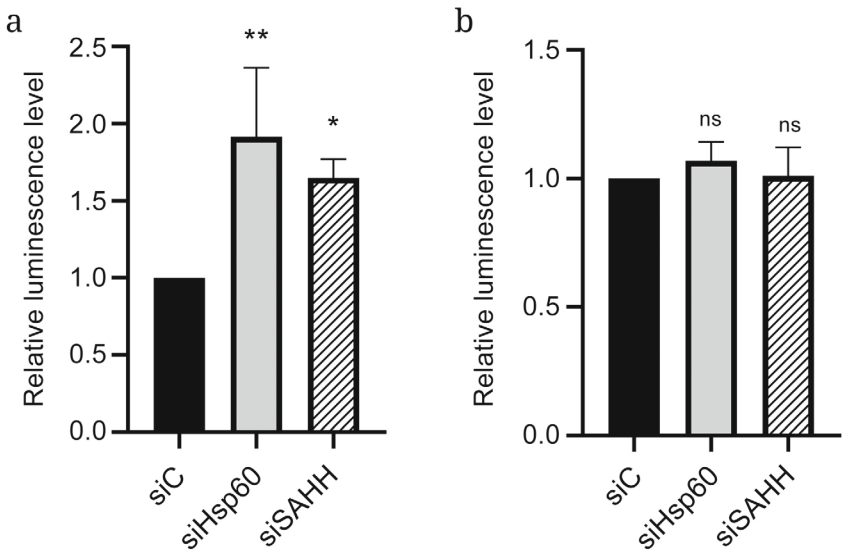

Fig. 2. Effect of Hsp60 or SAHH knockdown on HIV-1 replication in the cells transduced with HIV-1-based pseudoviral particles (a) and on transcription of the integrated pseudoprovirus (b). Changes in the signal intensity of the luciferase reporter protein were measured. Statistical significance was determined using Dunnett's method for multiple comparisons. All experiments were performed in at least four biological replicates. *p*-value: ns > 0.05 ≥ * > 0.01 ≥ ** > 0.005; ns, non-significant.

of either protein enhanced reporter activity, it could be concluded that Hsp60 and SAHH act as negative regulators of the HIV-1 replication cycle.

Effect of Hsp60 and SAHH knockdown on provirus transcription. Since the signal in our pseudoviral system depends not only on the early stages of HIV-1 replication, but also on the proviral transcription efficiency, we performed an additional experiment in which cells were first transduced with pseudoviral particles and, after the completion of all early replication stages, knockdown of SAHH and Hsp60 was carried out and reporter signal was measured. This approach allows identification of the knockdown effect separately on the stage of proviral transcription from the HIV-1 LTR promoter. According to our data, knockdown of these proteins had no statistically significant effect on pseudoprovial transcription (Fig. 2b). Thus, the reduced intracellular levels of Hsp60 and SAHH affect specifically the early stages of HIV-1 replication, highlighting importance of their interaction specifically with IN during these stages.

Relative quantification of HIV-1 DNA forms. To further investigate the mechanism through which Hsp60 and SAHH impact the HIV-1 life cycle, we examined the effect of their knockdown on reverse transcription, integration, and post-integration repair by determining the relative levels of different HIV-1 DNA forms [22, 23]. This method is based on quantification of the total, integrated, and repaired forms of viral DNA produced as a result of reverse transcription, integration, and post-integration repair, respectively. The DNA levels were measured using quantitative PCR (Fig. 3). The knockdown of SAHH led to the 1.47 ± 0.14-fold increase in total HIV-1 DNA, whereas the knockdown of Hsp60 had no visible effect (Fig. 3a). At the same time, knockdown of both Hsp60 and SAHH equally affected the level of integrated DNA, resulting in the 1.6 ± 0.3-fold increase (Fig. 3b). No statistically significant effects of the protein knockdown on relative post-integration repair efficiency were observed (Fig. 3c).

DISCUSSION

Hsp60 is the chaperone family protein, its primary function is to partially unfold proteins in an ATP-dependent manner, and to facilitate their refolding into the correct native conformation [24]. It is well established that under stress conditions the level of cellular expression of Hsp60 is markedly increased, accompanied by the parallel rise in the number of cell proteins associated with it [25]. Our findings on the direct interaction of Hsp60 with IN are consistent with the previously published data obtained with the yeast model [18]. Notably, Hsp60 is a predominantly mitochondrial protein (up to 85% of the total pool is normally localized in mitochondria), but it has also been detected in the cytoplasm, on the outer mitochondrial membrane, within cytoplasmic vesicles and secretory granules, in the nucleus, and even on the outer surface of the plasma membrane [26, 27]. Since the HIV-1 replication does not involve mitochondria, it is reasonable to assume that viral replication is affected precisely by this minor non-mitochondrial fraction of Hsp60. It has been reported that in certain

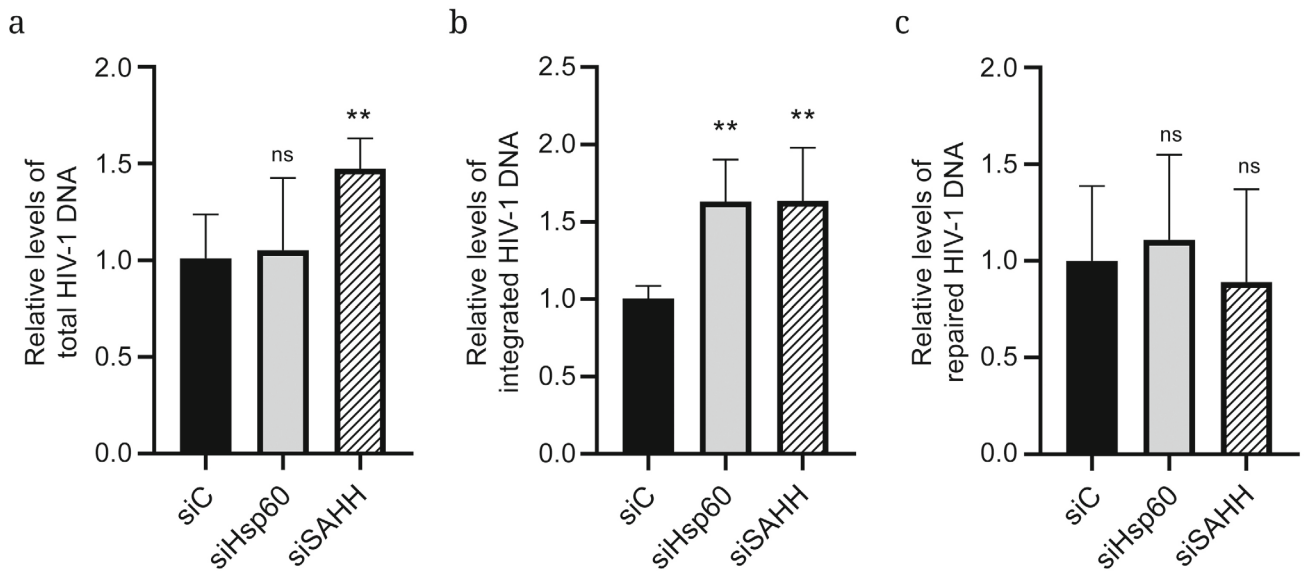

Fig. 3. Effect of Hsp60 or SAHH knockdown on the relative levels of different HIV-1 DNA forms: total (a), integrated (b), and repaired (c). Statistical significance was determined using Dunnett's method for multiple comparisons. All experiments were performed in at least three biological replicates. *p*-value: ns > 0.05; 0.01 ≥ ** > 0.005.

infectious diseases the fraction of non-mitochondrial Hsp60 may increase substantially and play an important role in the immune response [28], although such an effect has not been demonstrated for HIV-1.

As reported by Parissi et al. [18], the Hsp60 binding site was identified between the residues 48 and 212 of IN, i.e., within its central catalytic domain. Moreover, experiments with the recombinant proteins revealed that the addition of small amounts of Hsp60 stimulated the catalytic activity of IN in both 3′-processing and strand transfer reactions [18]. The strongest stimulatory effect was observed at a 10-20-fold excess of IN relative to Hsp60; increasing Hsp60 concentration reduced its stimulatory effect, and at the Hsp60/IN ratio of 1 : 3.5, complete inhibition of both IN catalytic activities was observed, most likely due to excessively strong binding of Hsp60 to the catalytic domain of IN. We suggest that the negative effect of Hsp60 on HIV-1 integration observed in our more physiologically relevant cell system (Fig. 3b) could be explained by functional inhibition of IN through its interaction with Hsp60.

S-adenosylhomocysteine hydrolase (SAHH) is the only mammalian enzyme catalyzing hydrolysis of S-adenosylhomocysteine into L-homocysteine and adenosine [29]. It is one of the most conserved enzymes across living organisms (found in eukaryotes, archaea, and bacteria) [30, 31]. Deletion of the corresponding gene is embryonically lethal in many species [32, 33], while mutations in the human ortholog often result in the severe disorders and early death [33, 34]. SAHH was initially considered to be a cytoplasmic enzyme, but later was shown to accumulate in significant amounts in the nucleus [35], predominantly in the actively transcribed chromatin regions, where it colocalizes with RNA polymerase II.

Several examples of the influence of SAHH inhibitors on viral replication have been documented: negative effect on the Rous sarcoma virus in chicken embryo cells [36], suppression of the human cytomegalovirus replication in embryonic lung fibroblasts [37], as well as reduced cytopathic effects of the cowpox virus, vesicular stomatitis virus, parainfluenza virus, reovirus type 1, herpes simplex virus, and others [38]. Evidence also exists for a negative effect of SAHH inhibitors on HIV-1 transcription in HeLa cells, whereas no similar effect was observed in human lymphocyte and macrophage cultures [39]. Conversely, another study demonstrated that 3-deazaadenosine analogs (SAHH inhibitors) effectively suppressed HIV-1 replication in peripheral blood mononuclear cells [40]. Despite these pronounced antiviral properties of SAHH inhibitors, such findings should be interpreted with caution for two following reasons. First, SAHH inhibitors exert systemic effects on the cell by altering both DNA and RNA methylation profiles, complicating interpretation and often leading to inconsistent results across different cell lines. Second, their broad spectrum of activity most likely reflects a low specificity of their influence on HIV-1 replication. The effect we observed is opposite in nature: the SAHH knockdown enhances pseudovirus replication efficiency, and this effect is unrelated to the transcription stage. This finding indicates importance of the interaction between HIV-1 IN and SAHH, since IN functions at the early stages of the replication cycle

but not in the stage of proviral transcription. Direct interaction between HIV-1 IN and RT was reported [41], in addition, certain IN mutations that disrupt this interaction negatively affected reverse transcription [42, 43]. SAHH interaction with IN is assumed to interfere with the proper IN–RT interaction, which explains the effect of SAHH knockdown on the total HIV-1 DNA level observed in our experiments. Notably, the increase in the integrated DNA level upon the SAHH knockdown was proportional to the increase in the total viral DNA produced during reverse transcription, indicating that the SAHH knockdown affects specifically reverse transcription, but not integration.

CONCLUSION

Integrase is one of the key enzymes of HIV-1 required for successful completion of the early stages of the viral replicative cycle. During replication, IN interacts with numerous host cellular factors that influence replication efficiency. We demonstrated that the previously identified potential partners of IN – Hsp60, SAHH1, and SAHH2 – indeed interact directly with it *in vitro*. Furthermore, we established that these proteins act as negative regulators of the viral replication cycle, since their depletion results in the enhanced replication efficiency. Our data indicate that the observed effects occur at the early stages of viral replication in which IN is involved, and do not affect transcription of the integrated provirus. Moreover, we found that the role of Hsp60 is critical at the integration stage of HIV-1, whereas SAHH exerts its influence at the reverse transcription stage. Further studies of the mechanisms by which these proteins affect HIV-1 replication could be valuable both for advancing our understanding of the virus–host interactions and for developing novel approaches to HIV therapy.

Abbreviations

HIV-1	human immunodeficiency virus type 1
IN	HIV-1 integrase
siRNA	small interfering RNAs
RT	reverse transcriptase
Hsp60	Heat Shock Protein 60
SAHH	S-adenosylhomocysteine hydrolase

Acknowledgments
Isolation and analysis of the relative amounts of different forms of HIV-1 DNA were performed by quantitative PCR using a Gentier 96 Real-Time Quantitative PCR amplifier, an AllSheng Fluo-800 fluorimeter, and a Bioer GenePure Pro automated nucleic acid extraction and purification station, acquired under the Development Program of Lomonosov Moscow State University (PNR 5.13).

Contributions
M.B.G. and S.P.K. developed the concept and supervised the study; T.F.K., S.P.K., and S.E.F. conducted the experiments; Yu.Yu.A., A.N.A., M.B.G., and S.P.K. discussed the study results; S.P.K. and S.E.F. prepared the manuscript; M.B.G. edited the manuscript.

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Ethics approval and consent to participate
This work does not contain any studies involving human and animal subjects.

Conflict of interest
The authors of this work declare that they have no conflicts of interest.

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