Biochemical and Biophysical Research Communications 453 (2014) 527–532

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

COP9 signalosome subunit 6 binds and inhibits avian leukosis virus integrase

Zhanxin Wang [a,b], Aotian Xu [a,b], Xinhui Hou [a,b], Fuyong Chen [b], Weisheng Cao [c], Jieshi Yu [a,b], Ming Liao [c], Jun Tang [a,b,*]

[a] State Key Laboratory of Agrobiotechnology, China Agricultural University, Beijing 100193, China
[b] College of Veterinary Medicine, China Agricultural University, Beijing 100193, China
[c] Key Laboratory of Animal Disease Control and Prevention of the Ministry of Agriculture, College of Veterinary Medicine, South China Agricultural University, Guangzhou 510642, China

ARTICLE INFO

Article history:
Received 12 September 2014
Available online 5 October 2014

Keywords:
Integrase
Retrovirus
Avian leukosis virus
COP9 signalosome subunit 6

ABSTRACT

The retroviral integrase plays an essential role in the integration of reverse-transcribed retroviral cDNA into the host cell genome, and serves as an important target for anti-viral therapeutics. In this study, we identified the COP9 signalosome subunit 6 (CSN6) as a novel avian leukosis virus (ALV) integrase binding protein. Co-immunoprecipitation and GST pull-down assays showed that CSN6 bound to ALV integrase likely through direct interaction of CSN6 to the catalytic core of the integrase. We further demonstrated CSN6 inhibited integrase activity *in vitro*; knockdown of CSN6 in DF-1 promoted ALV production. These results indicated that CSN6 may be a negative regulator of ALV replication by binding to and inhibiting integrase. Our findings provided the insight into the integrase-based host defense system and may have implications in the development of integrase-based anti-viral strategies.

1. Introduction

A critical step in the retroviral life cycle is the integration of reverse-transcribed viral cDNA into the host cell genome [1], which is catalyzed by the viral integrase (IN) [2]. During its path from the cytoplasm to the chromosome, IN utilizes multiple cellular proteins for various assistances, which include but are not limited to, nuclear import [3], prevention of auto-integration [4], access of integration sites [5] and DNA repair to fill the gap between the viral and host DNA [6].

As a key enzyme in the retroviral life cycle, IN may be a target for cellular anti-viral mechanisms. Indeed, the HIV-1 IN has been found to be degraded by the host ubiquitin–proteasome system [7]. Currently, most knowledge about IN and host interactions are from the HIV-1 study, while data for other retroviruses is still emerging, especially for viruses that can be exploited as a delivery vector in human gene therapy.

Avian leukosis virus (ALV) and avian sarcoma virus (ASV) are closely related alpharetroviruses, collectively referred to as avian sarcoma and leukosis virus (ASLV). ASV-derived vectors appear to mediate integration of viral carried DNA into the host genome relatively randomly [8]. This feature may give the ASV vector an advantage in gene therapy because it avoids the risk of inducing tumorigenesis associated with integration site preference [9]. However, the interactions between ASLV and host cells at the molecular level are poorly understood.

In this study, we searched for binding proteins for the IN of ASLV in its natural host, chicken cells. We chose a naturally isolated subgroup, the J avian leukosis virus (ALV-J) strain, as our research target. This virus has been widespread in China in recent years and caused enormous losses in the poultry industry [10]. The IN of ALV-J shares 99% identity to that of a commonly used ASV based vector [11]. We identified the COP9 signalosome subunit 6 (CSN6) as a binding protein for ALV-J IN and provided evidence to suggest that CSN6 hinders ALV-J production by directly binding to and inhibiting IN activity.

2. Materials and methods

2.1. Cell and virus propagation

HEK293T cells were obtained from ATCC and maintained in Gibco Dulbecco's modified Eagle medium (DMEM) supplemented with 10% FBS at 37 °C under 5% CO_2. DF-1 cells were kindly provided by

* Corresponding author at: College of Veterinary Medicine, China Agricultural University, Beijing 100193, China. Fax: +86 10 62732328.
E-mail address: jtang@cau.edu.cn (J. Tang).

http://dx.doi.org/10.1016/j.bbrc.2014.09.116
0006-291X/

Xiaomei Wang (Chinese Academy of Agricultural Sciences) and maintained in the above culture condition. ALV-J used in this study is strain CAUHM01 [GenBank accession number JF932000.1]. The viruses were propagated in DF-1 cells.

2.2. Antibodies and reagents

Antibodies used in this study were Flag (Sigma, M2), GFP (Santa Cruz, sc-996), HA (Santa Cruz, sc-805), β-actin (Santa Cruz, sc-1616-R), β-tubulin (MBL, PM054), CSN6 (ENZO, Q7L5N1), and p27 (kindly gifted by Xiaomei Wang). Dabco 4, 6-diamidino-2-phenylindole (DAPI) was purchased from Beyotime (Shanghai, China). All the restriction enzymes were purchased from Takara (Dalian, China).

2.3. Construction of plasmids and transfection

IN was cloned from ALV-J strain CAUHM01. Chicken CSN6 [GenBank accession number BX936166.1] was cloned from cDNA of DF-1. Plasmids for expressing IN and CSN6 in mammalian cells were constructed in pRK5 with the Flag or HA tag as indicated. GFP-IN and RFP-CSN6 were constructed in pEGFPC1 and pRFPC1 respectively. For bacterial expression, IN-NTD, ALV-IN-CCD and IN-CTD were cloned into pET-28a vector, whereas CSN6-N, CSN6-C and full length CSN6 were cloned into pGEX-6p-1 vector. IN catalytic mutant IN(E157A) was obtained by site directed mutagenesis and constructed into pET-28a vector. All the plasmids were constructed by standard molecular biology techniques with sequences confirmed.

Plasmids were transfected into HEK293T cells using calcium phosphate transfection method. DF-1 cells were transfected using Lipofectamine™ LTX and Plus Reagent following the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA).

2.4. Coimmunoprecipitation and Western blot

Coimmunoprecipitation and Western blot were performed as previously described [12].

2.5. Protein purification and GST pull-down

Bacterial strain BL21 containing plasmids for the GST-fusion and His-tagged proteins were cultured until OD_{600} reached 0.8. Protein expression was induced by addition of 1.0 mM isopropyl-beta-D-thiogalacto-pyranoside (IPTG) for 6 h at 16 °C. The bacteria were disrupted by ultra-sonication on ice. The cleared supernatants were incubated at 4 °C for 12 h with Glutathione-Sepharose beads (GE Healthcare, Piscataway, NJ, USA) or Ni-Agarose Resin beads (CWBIO, Beijing). The beads were washed three times with a wash buffer (100 mM Tris–HCl, 500 mM NaCl) and then eluted with an elution buffer (100 mM Tris–HCl, 500 mM NaCl plus glutathione pH7.5 for GST-fusion proteins or 20 mM Tris–HCl, 500 mM NaCl plus 250 mM imidazole pH7.9 for His-tagged proteins).

For GST pull-down, purified GST, full-length or truncated CSN6-GST fusion proteins were mixed with His-tagged IN proteins individually, and then incubated with Glutathione-Sepharose beads for 0.5 h. After thorough washing, the protein samples were subject to SDS–PAGE and Western blot.

2.6. IN 3′-processing assay

The 3′-processing substrate of ALV-J IN is a synthesized oligonucleotide with the sequence of 5′-aatgaagccatccgcttcatgaagcg gatggcttcatt-3′ and labeled with a fluorophore (carboxyfluorescein, FAM) at the 5′ end and a quencher (4-[4′-dimethylaminophenylazo] benzoic acid, DABCYL) at the 3′ end. The assay was performed as described in [13]. The fluorescence signals were monitored at different time points in a fluorescence plate reader (Spectra Max M5; MD) with 485 nm excitation and 535 nm emission filters.

2.7. siRNA and transfection

The siRNA target sequences for CSN6 (CSN6 and CSN6–1) were 5′-CCAAGGAGGAGCAGUUCAATT-3′ and 5′-CCGUGGCGGAACA UUUAAUTT-3′ respectively, and the control siRNA sequence was 5′-UUCUCCGAACGUGUCACGUTT-3′ (Genepharma, Shanghai, China). DF-1 cells were seeded in six-well plates and transfected with the indicated siRNAs using RNAiMAX reagent (Life Technolo-

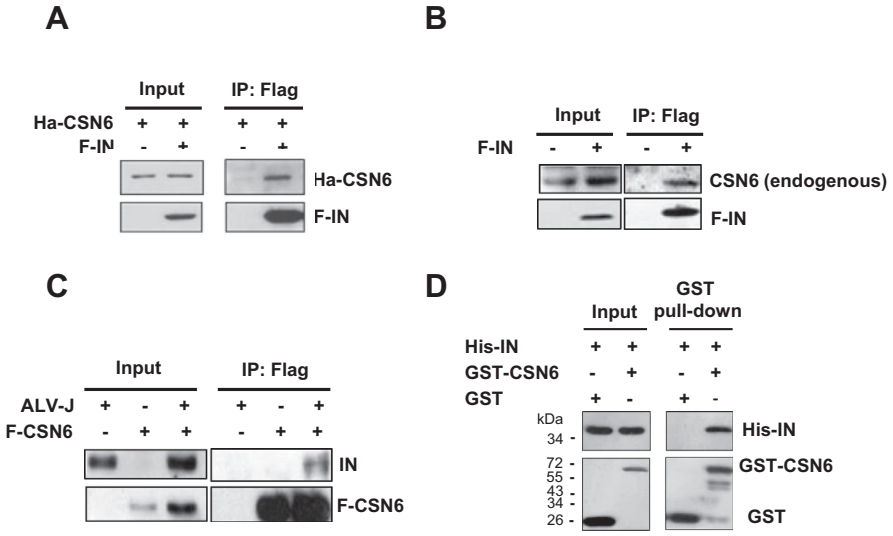

Fig. 1. CSN6 interacts with ALV-J IN. (A) Interaction between Flag-IN and Ha-CSN6 was examined by cotransfection of these two plasmids into HEK293T cells followed by immunoprecipitation and Western blot. (B) Interaction of Flag-IN with endogenous CSN6 was examined by transfection of the Flag-IN plasmid in DF-1 cells followed by immunoprecipitation and Western blot. (C) Interaction between F-CSN6 and IN after ALV-J infection was examined by transfection of Flag-CSN6 into DF-1 cells followed by infection with ALV-J before immunoprecipitation and Western blot with the antibody raised against ALV-J IN and an anti-Flag antibody. (D) Direct interaction between IN and CSN6 *in vitro* was examined by GST pull-down of recombinant proteins and Western analysis with anti-His and anti-GST antibodies.

gies, Carlsbad, CA, USA), according to the manufacturer's instruction.

2.8. RNA isolation and qPCR

RNA isolation and qPCR were performed as previously described [12]. The ALV-J primers are specific to gp85 with forward 5′-TTTGCAGGCATTTCTGACTG-3′ and reverse 5′-CCACGCACACAA GTATCATTTG-3′ [14]. The absolute quantity was calculated by calibration to gp85-pMD19T of known concentrations.

2.9. Measurement of the virus production in DF-1 cells

DF-1 cells were transfected with siRNAs. 24 h later, cells were infected with 0.1 ml of 10^5 $TCID_{50}$/ml (MOI 0.014) of ALV-J for 2 h at 37 °C, followed by three times of washes with PBS to remove the residual viruses in the medium. The cells were then cultured in DMEM with 2% FBS. Cells and culture media were collected at different time points for detection of viral protein p27 by Western blot and quantification of viral RNAs by qPCR.

2.10. Statistical analysis

Results are presented as means ± S.E.M for at least three independent experiments. Statistical analysis was performed using software GraphPad Prism, and a Student's t test value of $p < 0.05$ was considered to be statistically significant.

3. Results

3.1. Interactions between ALV-J IN and chicken CSN6 in cells

To search for the potential binding partners for ALV-J IN from its natural host, chicken cells, we performed a yeast two-hybrid screen of a chicken cDNA library generated from chicken bursa of Fabricius with ALV-J IN as bait. Unexpectedly, only three positive clones were recovered and all contained the same sequence, which encodes the C-terminal region of chicken CSN6. To examine whether ALV-J IN interacts with full length CSN6 in cells, we overexpressed Flag-tagged ALV-J IN (F-IN) and HA-tagged chicken CSN6 (Ha-CSN6) in HEK293T cells, and then performed a co-immunoprecipitation analysis. The result indicated that CSN6 was able to

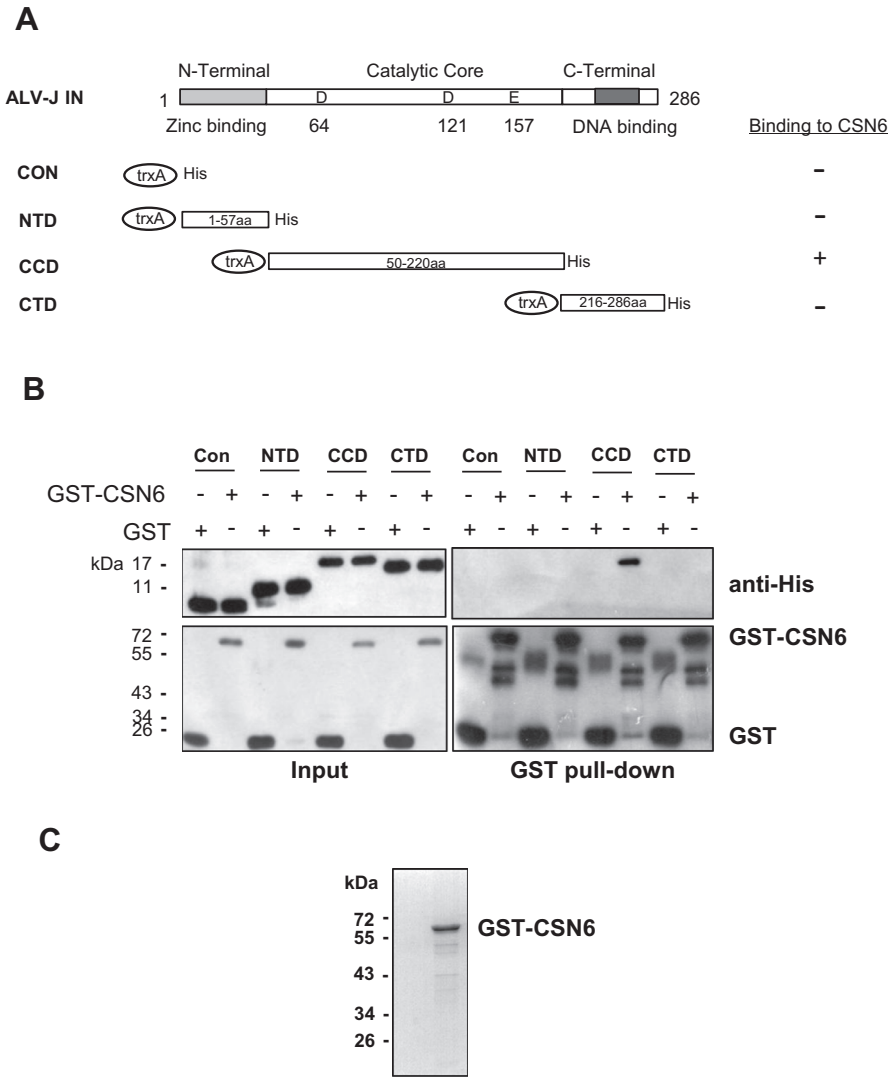

Fig. 2. Catalytic core of IN mediates the interaction between ALV-J IN and CSN6. (A) Schematic diagram illustrating the structural domains and catalytic triad of ALV-J IN as well as IN deletion mutants that were expressed as trxA-His fusion proteins. The binding ability of each mutant to CSN6 is indicated. (B) Purified recombinant GST or GST-CSN6 was mixed individually with equal amounts of recombinant trxA-His or each of trxA-His fused IN deletion mutants, and then subject to GST pull-down assay followed by Western blot with anti-His or anti-GST antibodies. (C) Purified GST-CSN6 was subject to SDS–PAGE followed by Coomassie blue staining.

interact with ALV-J IN (Fig. 1A). We also transfected Flag-tagged ALV-J IN (F-IN) into DF-1, a chicken cell line, and showed that endogenous CSN6 was present in F-IN immunoprecipitated complex (Fig. 1B). To examine whether CSN6 interacted with IN in infected cells, we raised an antibody that can specifically recognize ALV-J IN via Western blot. We infected DF-1 cells with the ALV-J and then transfected with an F-CSN6 plasmid. Western analysis indicated that IN is capable of interacting with F-CSN6 in ALV-J infected cells (Fig. 1C). Taken together, these data suggest that CSN6 is a host protein that can interact with ALV-J integrase after infection.

To examine if ALV-J IN interacts with CSN6 *in vitro*, we performed GST pull-down experiments using bacterially expressed recombinant IN and CSN6, and showed that these two proteins interacted *in vitro* (Fig. 1D).

3.2. The catalytic core of ALV-J interacts with CSN6

Retroviral INs are divided into three functional domains: the N-terminal Zinc binding region, the central catalytic core and the C-terminal DNA binding region. The catalytic core of ALV-J IN contains a triad of three highly conserved residues, D64, D121 and E157 (Fig. 2A). To define the region that CSN6 binds to, we bacterially expressed three deletion mutants of ALV-J as trxA-His fusion proteins, designated as NTD, CCD and CTD, respectively. The trxA-His protein was used as the control (Fig. 2A). We then mixed the same amount of each of the expressed proteins with purified GST or GST-CSN6, followed by a GST pull-down assay (Fig. 2B). Purified GST-CSN6 was relatively stable as shown in Coomassie blue staining (Fig. 2C). We demonstrated that only the catalytic core of ALV-J was pulled down by CSN6, indicating that it was the binding region of ALV-J to CSN6.

3.3. CSN6 inhibits 3′ processing activity of ALV-J IN in vitro

The binding of CSN6 to the catalytic core of ALV-J led us to posit that CSN6 may alter IN activity. IN catalyzes two important reactions: 3′ processing activity and strand transfer activity [1]. A 3′

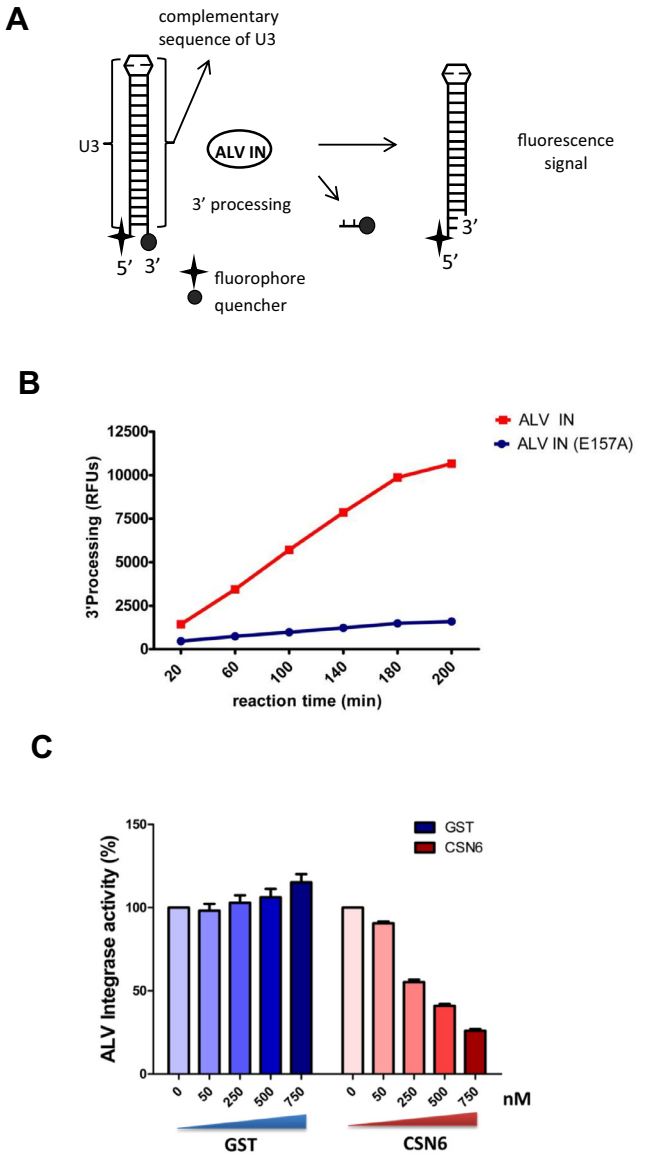

Fig. 3. CSN6 inhibits 3′-processing activity of ALV-J IN *in vitro*. (A) Diagram of the assay to measure 3′-processing activity of IN. (B) The 3′-processing activities of recombinant His-tagged wild-type IN and enzymatic dead mutant E157A were measured. (C) The 3′-processing activities of recombinant His-tagged wild-type IN were measured in the presence of an increasing concentration of purified recombinant GST-CSN6 or GST protein. Representative results from three independent experiments were shown.

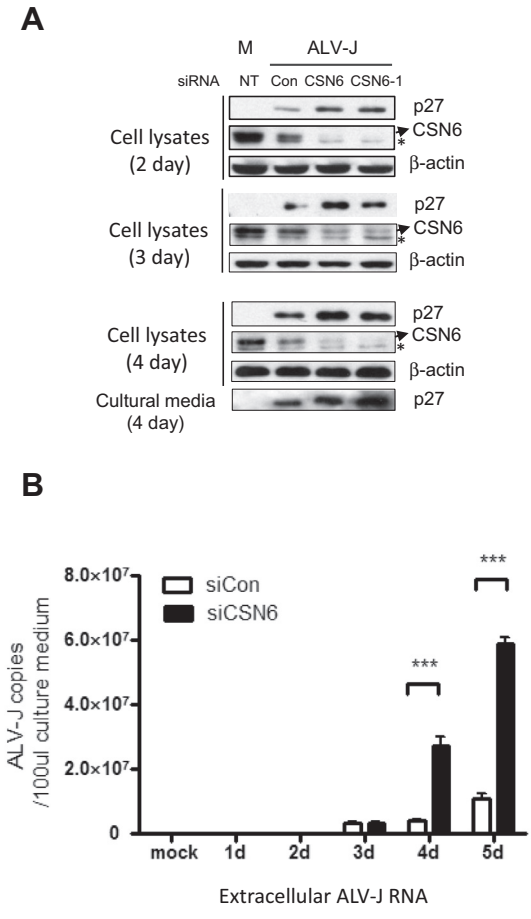

Fig. 4. Knockdown of CSN6 enhances ALV-J production. (A) DF-1 cells untransfected (NT) or transfected with Con siRNA or two individual siRNAs against CSN6 were infected with ALV-J. Cells or culture media were collected and analyzed by Western blot using an anti-p27 antibody. *Indicates a nonspecific band. M means mock infection. (B) The culture media from DF-1 cells treated as (A) were collected and processed for absolute quantification of viral RNA by RT-qPCR. Data are mean ± S.E.M. from three independent experiments. Differences were evaluated by Student's *t* test. (**$p < 0.01$; ***$p < 0.001$.) Representative results from at least three independent experiments were shown.

processing activity based-fluorogenic assay has been widely used to measure HIV-1 IN activity [13]; we therefore developed a similar assay to evaluate ALV-J IN activity. We synthesized a 38 mer long oligonucleotide which contains a 19 nucleotide terminal sequence of the U3 region of the viral long terminal repeats (LTR) of ALV-J cDNA and its complementary sequence. The oligonucleotide was fused with a fluorophore and its quencher at the 5′ and 3′ end, respectively. The pair of complementary sequences allows the oligonucleotide to form a double-stranded DNA structure that mimics the 3′ end of viral cDNA, which also brings the fluorophore and quencher into close proximity. In the presence of active IN, the 3′ end dinucleotide will be cleaved, thereby releasing the quencher and allowing a fluorescence signal to be generated. The diagram of the substrate and the assay was shown in Fig. 3A.

We first demonstrated that we were able to successfully measure ALV-J IN activity specifically using this system, as addition of purified recombinant His-tagged IN generated fluorescent signals, whereas the same amount of purified enzymatic dead mutant (E157A) did not (Fig. 3B). We then examined the effect of increasing concentrations of the purified recombinant GST-CSN6 on IN activity with GST protein as the control and found that GST-CSN6 inhibited IN activity in a dose-dependent manner (Fig. 3C).

3.4. CSN6 inhibits ALV-J production

The inhibition of IN activity by CSN6 in the above-mentioned *in vitro* assay hints that CSN6 may limit ALV-J production. To test this possibility, we treated DF-1 cells with either non-targeting control siRNA or two individual siRNAs against CSN6 prior to ALV-J infection. As expected, we found that knockdown of CSN6 markedly enhanced intracellular p27 levels starting at day 2 post-infection (Fig. 4A). Extracellular p27 can be detected on day 4 post-infection, and was also increased upon CSN6 depletion (Fig. 4A). To further confirm this, we measured extracellular viral RNAs by performing qRT-PCR, and showed that knockdown of CSN6 significantly increased extracellular ALV-J RNAs (Fig. 4B). These results indicated that the endogenous CSN6 plays a role in the inhibition of ALV-J production.

4. Discussion

In this study, we identified CSN6 as an ALV IN binding protein in chicken cells. The interactions between these two proteins were analyzed and confirmed by various approaches including yeast two-hybrid, GST pull-down and co-immunoprecipitation. Interestingly, contrary to the known IN interacting host proteins whose roles in the viral life cycle are to facilitate IN functions [3,15,16], we found CSN6 inhibited ALV-J IN activity *in vitro* (Fig. 3B), and viral production *in vivo* (Fig. 4). These data indicate that CSN6 may be an inhibitory host factor of ALV-J replication likely by directly attacking IN. Our finding provides a fresh insight into host defense mechanisms against retroviral replication.

Cells possess an intrinsic defense system against viral infection. Some of the anti-viral activities occur even before innate anti-viral immune responses, and provide the initial line of defense against virus infection. For HIV-1, several proteins have been identified to inhibit HIV-1 infection, including APOBEC3 family of proteins [17,18], tetherin [19], SAMHD1, some TRIM family members particularly TRIM5α [20]. These proteins act at various key steps in the retrovirus life cycle through distinct mechanisms, ranging from inducing premature disassembly of the viral capsid to preventing enveloped viral particles from releasing [19,21]; however, none of these involve IN. In this study, we identified CSN6 as a negative regulator of ALV-J replication in its host, chicken cells, with IN being its primary target. CSN6 inhibits IN activity through direct interaction with the catalytic core of IN. Our data indicates host cellular factors can impinge on IN to hinder ALV-J replication.

CSN6 is a subunit of the COP9 signalosome, which is a highly conserved protein complex and plays an important role in development and protein degradation [22–24]. Several studies indicate that COP9, especially its component CSN5, has a close relationship with various viruses. On the one hand, viruses such as Geminivirus or HBV can alter cellular functions by interacting with CSN5 for their own advantage [25–27]. On the other hand, CSN5 or COP9 confers resistance to other viral infections including Tobacco mosaic virus and West Nile virus [28,29]. These findings, together with our result that CSN6 has the ability to defend against ALV-J, suggest that COP9 components may contain some innate anti-viral activities. These activities may represent an ancient approach for hosts to hinder virus infection before the innate immune system is evolved.

We showed in the *in vitro* assay that CSN6 inhibited ALV-J IN activity in a dose-dependent manner. At a molar ratio of 1:1, CSN6 confers about 50% inhibition. The direct interaction between CSN6 and the catalytic core of IN suggests that CSN6 may directly impair the structure or function of the IN catalytic core. Detailed molecular and structural analysis is required to decipher the molecular mechanism by which CSN6 inhibits IN, which may also provide a new strategy for anti-retroviral drug development.

Acknowledgments

We thank Dr. Xiaomei Wang for generously providing DF-1 cells and anti-p27 antibody, Dr. Shijun J. Zheng for cDNA library of chicken bursa of Fabricius, Dr. Wenhai Feng for comments, Dr. Miao Sun for technique assistance. This work was supported by NSFC-Guangdong Union Foundation (U0831002), Chinese Universities Scientific Fund (2013YJ017) and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry.

References

[1] P.O. Brown, Integration, in: J.M. Coffin, S.H. Hughes, H.E. Varmus (Eds.), Retroviruses, Cold Spring Harbor, NY, 1997.
[2] O. Delelis, K. Carayon, A. Saib, E. Deprez, J.F. Mouscadet, Integrase and integration: biochemical activities of HIV-1 integrase, Retrovirology 5 (2008) 114.
[3] G. Maertens, P. Cherepanov, W. Pluymers, K. Busschots, E. De Clercq, Z. Debyser, Y. Engelborghs, LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells, J. Biol. Chem. 278 (2003) 33528–33539.
[4] N. Yan, P. Cherepanov, J.E. Daigle, A. Engelman, J. Lieberman, The SET complex acts as a barrier to autointegration of HIV-1, PLoS Pathog. 5 (2009) e1000327.
[5] K.E. Ocwieja, T.L. Brady, K. Ronen, A. Huegel, S.L. Roth, T. Schaller, L.C. James, G.J. Towers, J.A. Young, S.K. Chanda, R. Konig, N. Malani, C.C. Berry, F.D. Bushman, HIV integration targeting: a pathway involving Transportin-3 and the nuclear pore protein RanBP2, PLoS Pathog. 7 (2011) e1001313.
[6] K.E. Yoder, F.D. Bushman, Repair of gaps in retroviral DNA integration intermediates, J. Virol. 74 (2000) 11191–11200.
[7] L.C. Mulder, M.A. Muesing, Degradation of HIV-1 integrase by the N-end rule pathway, J. Biol. Chem. 275 (2000) 29749–29753.
[8] R. Daniel, J.A. Smith, Integration site selection by retroviral vectors: molecular mechanism and clinical consequences, Hum. Gene Ther. 19 (2008) 557–568.
[9] S. Hacein-Bey-Abina, C. Von Kalle, M. Schmidt, M.P. McCormack, N. Wulffraat, P. Leboulch, A. Lim, C.S. Osborne, R. Pawliuk, E. Morillon, R. Sorensen, A. Forster, P. Fraser, J.I. Cohen, G. de Saint Basile, I. Alexander, U. Wintergerst, T. Frebourg, A. Aurias, D. Stoppa-Lyonnet, S. Romana, I. Radford-Weiss, F. Gross, F. Valensi, E. Delabesse, E. Macintyre, F. Sigaux, J. Soulier, L.E. Leiva, M. Wissler, C. Prinz, T.H. Rabbitts, F. Le Deist, A. Fischer, M. Cavazzana-Calvo, LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1, Science 302 (2003) 415–419.
[10] Y.L. Gao, L.T. Qin, W. Pan, Y.Q. Wang, X. Le Qi, H.L. Gao, X.M. Wang, Avian leukosis virus subgroup J in layer chickens, China, Emerg. Infect. Dis. 16 (2010) 1637–1638.
[11] R.S. Mitchell, B.F. Beitzel, A.R. Schroder, P. Shinn, H. Chen, C.C. Berry, J.R. Ecker, F.D. Bushman, Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences, PLoS Biol. 2 (2004) E234.

[12] D. Cui, L. Li, H. Lou, H. Sun, S.M. Ngai, G. Shao, J. Tang, The ribosomal protein S26 regulates p53 activity in response to DNA damage, Oncogene 33 (2014) 2225–2235.
[13] H.Q. He, X.H. Ma, B. Liu, X.Y. Zhang, W.Z. Chen, C.X. Wang, S.H. Cheng, High-throughput real-time assay based on molecular beacons for HIV-1 integrase 3′-processing reaction, Acta Pharmacol. Sin. 28 (2007) 811–817.
[14] Q. Wang, Y. Gao, Y. Wang, L. Qin, X. Qi, Y. Qu, H. Gao, X. Wang, A 205-nucleotide deletion in the 3′ untranslated region of avian leukosis virus subgroup J, currently emergent in China, contributes to its pathogenicity, J. Virol. 86 (2012) 12849–12860.
[15] E. Yung, M. Sorin, E.J. Wang, S. Perumal, D. Ott, G.V. Kalpana, Specificity of interaction of INI1/hSNF5 with retroviral integrases and its functional significance, J. Virol. 78 (2004) 2222–2231.
[16] B. Studamire, S.P. Goff, Host proteins interacting with the Moloney murine leukemia virus integrase: multiple transcriptional regulators and chromatin binding factors, Retrovirology 5 (2008) 48.
[17] F.A. Koning, C. Goujon, H. Bauby, M.H. Malim, Target cell-mediated editing of HIV-1 cDNA by APOBEC3 proteins in human macrophages, J. Virol. 85 (2011) 13448–13452.
[18] S. Jager, D.Y. Kim, J.F. Hultquist, K. Shindo, R.S. LaRue, E. Kwon, M. Li, B.D. Anderson, L. Yen, D. Stanley, C. Mahon, J. Kane, K. Franks-Skiba, P. Cimermancic, A. Burlingame, A. Sali, C.S. Craik, R.S. Harris, J.D. Gross, N.J. Krogan, Vif hijacks CBF-beta to degrade APOBEC3G and promote HIV-1 infection, Nature 481 (2012) 371–375.
[19] D. Perez-Caballero, T. Zang, A. Ebrahimi, M.W. McNatt, D.A. Gregory, M.C. Johnson, P.D. Bieniasz, Tetherin inhibits HIV-1 release by directly tethering virions to cells, Cell 139 (2009) 499–511.
[20] M. Stremlau, C.M. Owens, M.J. Perron, M. Kiessling, P. Autissier, J. Sodroski, The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys, Nature 427 (2004) 848–853.
[21] H. Huthoff, G.J. Towers, Restriction of retroviral replication by APOBEC3G/F and TRIM5alpha, Trends Microbiol. 16 (2008) 612–619.
[22] S.V. Iyer, T. Iwakuma, A novel link between the HER2-Akt and MDM2-p53 pathways via CSN6, Cell Cycle 11 (2012) 4112.
[23] R. Zhao, S.C. Yeung, J. Chen, T. Iwakuma, C.H. Su, B. Chen, C. Qu, F. Zhang, Y.T. Chen, Y.L. Lin, D.F. Lee, F. Jin, R. Zhu, T. Shaikenov, D. Sarbassov, A. Sahin, H. Wang, C.C. Lai, F.J. Tsai, G. Lozano, M.H. Lee, Subunit 6 of the COP9 signalosome promotes tumorigenesis in mice through stabilization of MDM2 and is upregulated in human cancers, J. Clin. Invest. 121 (2011) 851–865.
[24] S. Denti, M.E. Fernandez-Sanchez, L. Rogge, E. Bianchi, The COP9 signalosome regulates Skp2 levels and proliferation of human cells, J. Biol. Chem. 281 (2006) 32188–32196.
[25] R. Lozano-Duran, T. Rosas-Diaz, G. Gusmaroli, A.P. Luna, L. Taconnat, X.W. Deng, E.R. Bejarano, Geminiviruses subvert ubiquitination by altering CSN-mediated derubylation of SCF E3 ligase complexes and inhibit jasmonate signaling in *Arabidopsis thaliana*, Plant Cell 23 (2011) 1014–1032.
[26] J.L. Hsu, W.J. Chuang, I.J. Su, W.J. Gui, Y.Y. Chang, Y.P. Lee, Y.L. Ai, D.T. Chuang, W. Huang, Zinc-dependent interaction between JAB1 and pre-S2 mutant large surface antigen of hepatitis B virus and its implications for viral hepatocarcinogenesis, J. Virol. 87 (2013) 12675–12684.
[27] Y. Tanaka, F. Kanai, T. Ichimura, K. Tateishi, Y. Asaoka, B. Guleng, A. Jazag, M. Ohta, J. Imamura, T. Ikenoue, H. Ijichi, T. Kawabe, T. Isobe, M. Omata, The hepatitis B virus X protein enhances AP-1 activation through interaction with Jab1, Oncogene 25 (2006) 633–642.
[28] W. Oh, M.R. Yang, E.W. Lee, K.M. Park, S. Pyo, J.S. Yang, H.W. Lee, J. Song, Jab1 mediates cytoplasmic localization and degradation of West Nile virus capsid protein, J. Biol. Chem. 281 (2006) 30166–30174.
[29] Y. Liu, M. Schiff, G. Serino, X.W. Deng, S.P. Dinesh-Kumar, Role of SCF ubiquitin-ligase and the COP9 signalosome in the N gene-mediated resistance response to tobacco mosaic virus, Plant Cell 14 (2002) 1483–1496.