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Sp100 interacts with phage ΦC31 integrase to inhibit its recombination activity

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Phage ΦC31 integrase is a potential vector for the insertion of therapeutic genes into specific sites in the human genome. To understand the mechanism involved in ΦC31 integrase-mediated recombination, it is important to understand the interaction between the integrase and cellular proteins. Using a yeast two-hybrid system with pLexA-ΦC31 integrase as bait, we screened a pB42AD human fetal brain cDNA library for potential interacting cellular proteins. From the 10^6 independent clones that were screened, 11 potential interacting clones were isolated, of which one encoded C-terminal fragment of Sp100. The interaction between Sp100 and ΦC31 integrase was further confirmed by yeast mating and co-immunoprecipitation assays. The hybridization between a ΦC31 integrase peptide array and an HEK293 cell extract revealed that residues 81RILN84 in the N-terminus of ΦC31 integrase are responsible for the interaction with Sp100. Knocking down endogenous Sp100 with Sp100-specific siRNA increased ΦC31 integrase-mediated recombination but did not impact reporter gene expression. Therefore, endogenous Sp100 may interact with ΦC31 integrase and inhibit the efficiency of ΦC31 integrase-mediated recombination.

Keywords: ΦC31 integrase, Sp100, recombination

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INTRODUCTION

Safety concerns related to retrovirus-mediated gene therapy have been raised due to clinical incidences of insertional mutagenesis (Hacein-Bey-Abina *et al.*, 2003). In order to minimize this risk, improved means of integrating a therapeutic gene into the genome at specific sites must be developed and proven to be safe. The *Streptomyces* phage ΦC31 integrase is a member of the serine recombinase family and can mediate site-specific recombination between two short recognition sites, *attB* and *attP* (Kuhstoss & Rao, 1991; Thorpe & Smith, 1998; Groth *et al.*, 2000). The recombination reaction does not require cofactors, and its hybrid products (*attL* and *attR*) are not substrates for ΦC31 integrase. Therefore, the integration activity is unidirectional and has a high net integration frequency (Thorpe & Smith, 1998). Importantly, the sites at which ΦC31 integrase mediates recombination are limited in number in the human genome, and are orders of magnitude fewer than those in retroviral or transposase-mediated integration systems, and are mostly located away from cancer genes (Chalberg *et al.*, 2006). Therefore, the ΦC31 integrase-based system has been proposed as a promising technology for site-specific, non-virus mediated gene therapy.

The first study to use the ΦC31 integrase system for gene therapy was published in 2002. Using *in vivo* hydrodynamic injection of factor IX-attB plasmid with a ΦC31 integrase expression plasmid into the mouse liver, the authors obtained stable long term expression of factor IX at a level equal to normal serum levels (Olivares *et al.*, 2002). Further studies have been carried out in liver, skin, muscle, eye, joint, neural progenitor cells, a T cell line, and embryonic stem cell lines and have demonstrated the feasibility of using the ΦC31 integrase system for gene therapy (Calos, 2006; Quenneville *et al.*, 2007; Keravala *et al.*, 2008; Liu *et al.*, 2009). However, the recombination mediated by ΦC31 integrase is much less efficient than that in bacteria (Held *et al.*, 2005). In order to safely and efficiently use ΦC31 integrase as a tool for gene therapy, potential interactions between ΦC31 integrase and proteins in mammalian host cells should be studied.

We previously reported that ΦC31 integrase can interact with the cellular protein DAXX (Chen *et al.*, 2006). A later report demonstrated that in different cell types the efficiency of ΦC31 integrase-mediated recombination differs with varying DAXX expression levels (Maucksch *et al.*, 2008). Here we report that Sp100 can directly bind to the catalytic N-terminus of ΦC31 integrase; this is different from DAXX, which binds to the C-terminal region of ΦC31 integrase. We also demonstrate that the interaction with Sp100 can influence the activity of ΦC31 integrase.

MATERIAL AND METHODS

Plasmid construction. The ΦC31 integrase-expressing plasmid, pCMV-Int, was kindly provided by Professor M. P. Calos. The plasmids pLexA-ΦC31, pLexA-ΦC31(1–257), pLexA-ΦC31(259–613), pEGFP-ΦC31 and pΦC31-EGFP have been described previously (Chen *et al.*, 2006).

The ORF of Sp100 (AAA35537) was amplified from a human fetal brain cDNA library (Clontech) and was

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Abbreviations: DAPI, 4′,6-diamidino-2-phenylindole; GFP, green fluorescent protein; PMSF, phenylmethylsulfonyl fluoride; RFP, red fluorescent protein

inserted into plasmid pB42AD between the *Eco*RI and *Xho*I sites to generate pB42AD-Sp100. Using overlap PCR method, four deletion mutants, Sp100Δ29–152, Sp100Δ153–286, Sp100Δ287–333 and Sp100Δ334–407, were inserted into pB42AD between the *Eco*RI and *Xho*I sites to generate pB42AD–Sp100Δ29–152, pB42AD–Sp100Δ153–286, pB42AD–Sp100Δ287–333 and pB42AD–Sp100Δ334–407. The N- or C-terminus of Sp100 was amplified from pB42AD–Sp100 and inserted into pB42AD between the *Eco*RI and *Xho*I sites to generate pB42AD-Sp100(1–300) and pB42AD-Sp100(300–480). The ORF of Sp100 was amplified from pB42ADSp100 and inserted into the plasmid pDsRed-N1 (Clontech) between the *Eco*RI and *Kpn*I sites to generate pSp100-DsRed that expresses an Sp100-DsRed fusion protein. The primers used to generate the plasmids are listed in Table 1.

Yeast two-hybrid assay. The MATCHMAKER LexA two-hybrid system was purchased from Clontech. The yeast two-hybrid assay was performed using a standard two-step protocol provided by the manufacturer. All of the isolated positive clones were amplified with the universal pB42AD sequence primers (5'-ccagcctcttgctgagtggagatg-3'; 5'-ggagacttgaccaaacctctggcg-3'), and the PCR products were sequenced with the same primers.

The interaction between Sp100 and ΦC31 integrase and their interacting domain were also detected with a yeast mating assay. Using strain EGY48 [p8opLacZ] transformed with eight pB42AD-based constructs of Sp100 (pB42AD-Sp100, pB42AD-Sp100Δ29–152, pB42AD-Sp100Δ153–286, pB42AD-Sp100Δ287–333, pB42AD-Sp100Δ334–407, pB42AD-Sp100(1–300), pB42AD-Sp100(300–480) and pB42AD-Sp100(300–434)), and strain YM4271 transformed with three pLexA-based constructs of ΦC31 integrase (pLexA-ΦC31, pLexA-ΦC31(1–257) and pLexA-ΦC31(259–613)), the interaction was tested according to the standard yeast mating protocol provided by the manufacturer.

Cell culture. The HEK293 cells were maintained in DMEM (Gibco) medium with 10% fetal calf serum, 100 U/ml penicillin, and 100 U/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

Co-immunoprecipitation. Co-immunoprecipitation was employed to verify the interaction between ΦC31 integrase and the cellular protein Sp100. The cells were transfected with pEGFP-ΦC31 using Lipofectamine2000 (Invitrogen). At 48 h post-transfection, the cells were harvested and lysed with 1 ml lysis buffer (20 mM Tris pH 7.5, 100 mM NaCl, 0.5% NP-40, 0.5 mM EDTA, 0.5 mM PMSF, 0.5% protease inhibitor cocktail (Sigma)). Ten micrograms of rabbit anti-Sp100 polyclonal antibody (Santa Cruz) were added to 1 ml of each cell lysate. After incubating with end-over-end mixing for 2 h at 4 °C, 10 µl of pre-washed protein A/G Sepharose (Santa Cruz) was added to each extract and shaken overnight at 4 °C. The immunoprecipitates were washed three times with the lysis buffer. The pellets were then separated by SDS/PAGE and transferred to a nitrocellulose membrane. The blot was probed using a mouse anti-GFP monoclonal antibody (Santa Cruz) followed by a horseradish peroxidase-conjugated anti-mouse antibody (Sigma), and the bands were visualized using an enhanced chemiluminescent immunoblotting detection kit (Amersham).

ΦC31 integrase peptide array analysis. Three ΦC31 integrase peptide arrays (12, 15 and 20 mers) were generated and probed with Sp100 to determine the Sp100 binding region in ΦC31 integrase. Cellulose-bound overlapping peptides derived from ΦC31 integrase were synthesized using an AutoSpot robot (Intavis Bioanalytical Instruments).

The basic lengths of the spotted fragments for the three arrays were 12, 15 and 20 residues, respectively. Each neighboring peptide shifted two residues towards the C-terminus. The arrays were incubated with a cell lysate of 5×10^7 HEK293 cells in 10 ml of lysis buffer at 4 °C overnight. The membranes were then incubated with a rabbit anti-human Sp100 polyclonal antibody (Santa Cruz) followed by a horseradish peroxidase-conjugated anti-rabbit antibody (Sigma). The bound proteins were visualized by using enhanced chemiluminescent immunoblotting detection reagents (Amersham). For controls, the membranes were processed identically, but incubation with the cell lysate was omitted.

Intracellular localization of Sp100 and ΦC31 integrase. The subcellular locations of Sp100 and ΦC31 integrase were determined in HEK293 cells. The fusion plasmids pSp100-DsRed, pEGFP-ΦC31 and pΦC31-EGFP were used to identify the location of Sp100 and ΦC31 integrase, respectively. The cells were transfected with the plasmids above using Lipofectamine2000 (Invitrogen). At 24 h post-transfection, the cells were observed under a confocal fluorescent microscope (TSC SP2, Leica). The nuclei were stained with DAPI as a control. The fluorescence of GFP, RFP and DAPI was observed at 510, 582 and 461 nm, respectively, with excitation at 488, 543 and 405 nm.

Sp100 knock-down assay. Endogenous expression of Sp100 was knocked-down by transfection with two Sp100-specific duplex RNAs: 5'-AACCATGGAATCCAAATTAAT-3' (Qiagen) and 5'-TGCGACTGGTGGATATAAA-3' (Milovic-Holm *et al.*, 2007). Both siRNA sequences were verified to confirm their specificity to Sp100, as estimated by RT-PCR and Western blot. As a control, a human non-silencing duplex RNA was used: 5'-UUAAGUAGCUUGGCCUUGAdTdT-3' (GeneChem, China).

ΦC31 integrase activity assay. ΦC31 integrase-mediated integration in mammalian cells was measured using a ΦC31 integrase reporter plasmid, PB[EGFP] (Zhang *et al.*, 2009). PB[EGFP] contains a single copy structure pCMV-attB-BGH-poly(A)-attP-EGFP. ΦC31 integrase-mediated recombination between *attB* and *attP* sites removes the BGH poly(A) from the inserted structure, resulting in the expression of EGFP. For the integration assay, HEK293 cells were plated in a 12-well plate and transfected with 50 ng of CMV-Int together with 1 µg of PB[EGFP] per well. The cells were harvested at 48 h post-transfection. The percentage of EGFP-expressing cells, which represents the recombination efficiency, was determined by flow cytometry (Becton Dickinson).

Reporter gene expression assay. HEK293 cells were plated in a 12-well plate and transfected with 50 ng of CMV-Int together with 1 µg of PB[EGFP] per well. The cells were harvested at 48 h after transfection. The mean GFP intensity in EGFP-expressing cells was determined by flow cytometry (Becton Dickinson). The GFP mRNA level was also determined by quantitative real-time RT-PCR.

Quantitative real-time RT-PCR. Cellular genomic DNA was extracted with a genomic DNA purification kit provided by QIAGEN. To measure ΦC31 integrase-mediated integration, the integrated DNA

plasmids were quantified by real-time PCR using Sybr Green-based detection (TOYOBO Code No. QPK-201, 201T).

In the presence of ΦC31 integrase, the recombination takes place between the *attB* and *attP* sites, removes the BGH poly(A) and generates a hybrid *attL* site in the PB[EGFP] plasmid (Zhang *et al.*, 2009). In order to detect the integrated plasmid, we designed a pair of primers 5'-CTACGCCCCCAACTGAGAGAAC-3' and 5'-GCGGTACCGTCGACGATGTAG-3'. The upstream primer was in the *attL* sequence and the downstream primer was in the downstream GFP gene sequence in the plasmid backbone. This pair of primers can specifically amplify a 100 bp band from the integrated plasmid but not an unintegrated one. We designed another pair of primers, 5'-CGTAACAACTCCGCCCCATTGAC-3' and 5'-GCTGAACTTGTGGCCGTTTACGTC-3', which can amplify a 100 bp GFP sequence on the plasmid backbone and thus can represent the PB[EGFP] plasmid. Both pairs of primers cannot amplify bands from normal cellular genomic DNA. The ratio of the attL containing PCR products to the GFP specific PCR products was used to represent the relative recombination efficiency.

Total RNA was extracted with Trizol reagent (Invitrogen, USA) using the standard method provided by the manufacturer. Reverse-transcribed cDNA was quantified by real-time PCR using Sybr Green-based detection. The primers used were the following: 5'-CGTAACAACTCCGCCCCATTGAC-3' and 5'-GCTGAACTTGTGGCCGTTTACGTC-3' for GFP, and 5'-ACGAGGCCCAGAGCAAGAG-3' and 5'-TCTCCATGTCGTCCCAGTTG-3' for β-actin as a control (Pfafl, 2001).

RESULTS

Interaction between ΦC31 integrase and Sp100

We used pLexA-ΦC31 as a bait to screen a pB42AD-based human fetal brain cDNA library for potential

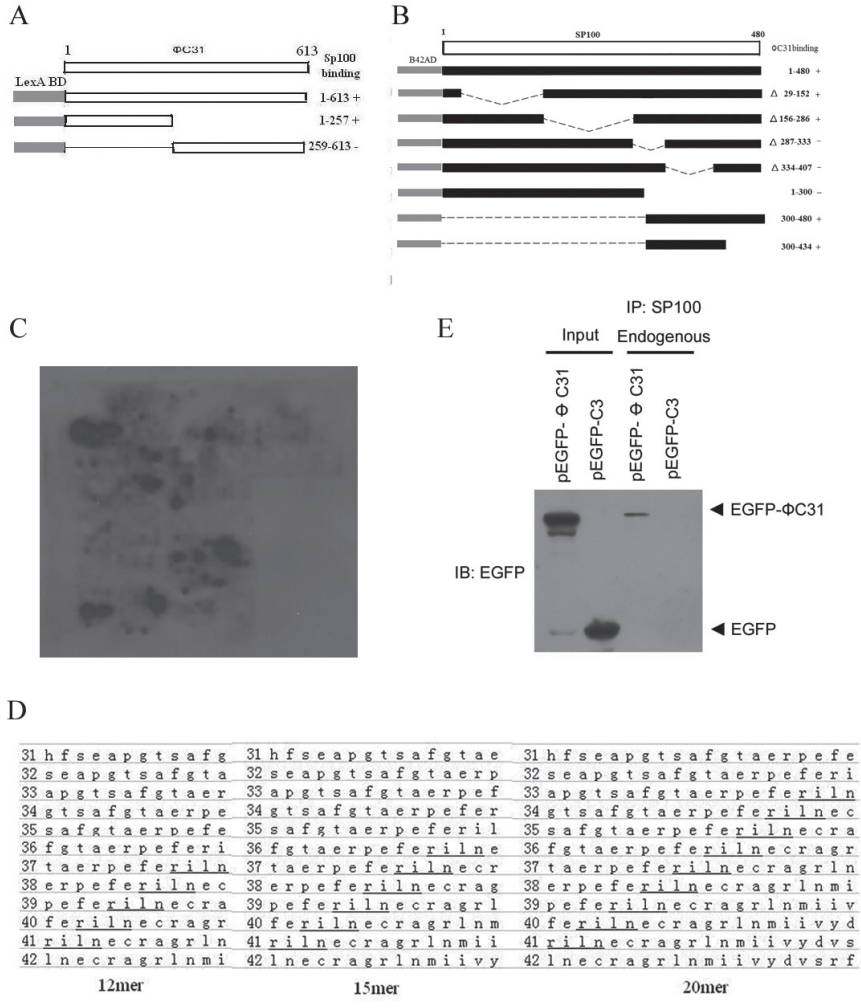

Figure 1. Interaction between ΦC31 integrase and Sp100
(A) Construction of ΦC31 integrase and its truncation mutants as pLexA fusion plasmids for the yeast mating assay. **(B)** Construction of Sp100 and its mutants as pB42AD fusion plasmids for the yeast mating assay. **(C)** Three ΦC31 integrase overlapping peptide arrays with different lengths of peptides (12, 15 and 20 mers) for localization of the Sp100 interacting region of ΦC31 integrase. **(D)** Amino-acid sequences of peptide spots corresponding to the array shown in C; the Sp100 binding tetramer is indicated by an underline. **(E)** Lysates of pEGFP-ΦC31-transfected and pEGFP-C3-transfected HEK293 cells were immunoprecipitated (IP) with a rabbit anti-Sp100 antibody on A/G agarose beads. Samples were detected by immunoblotting (IB) with a mouse anti-EGFP antibody.

Table 1. Primers for constructing plasmids mentioned in Materials and Methods

Plasmid	Sense primer	Antisense primer
pB42AD-Sp100	5′-CGGAATTCATGGCAGGTGGGGGCGGC-3′	5′-GCGGTCGACCTAATCTTCTTTACCTGACCCTC-3′
pB42AD-Sp100 (Δ29–152)up	5′-CGGAATTCATGGCAGGTGGGGGCGGC-3′	5′-GGAGAGGAGGAAGATGGTTCATCTCATTTG-3′
pB42AD-Sp100 (Δ29–152)down	5′-CATCTTCCTCCTCTCCAAGAAAGTGAAGAAG-3′	5′-GCGGTGACCTAATCTTCTTTACCTGACCCTC-3′
pB42AD-Sp100 (Δ153–286)up	5′-CGGAATTCATGGCAGGTGGGGGCGGC-3′	5′-CAGGAATTCAATTTGTCATGGATTACATTTTC-3′
pB42AD-Sp100 (Δ153–286)down	5′-GACAAATTGAATTCCTGTTCTGTGCGACTG-3′	5′-GCGGTGACCTAATCTTCTTTACCTGACCCTC-3′
pB42AD-Sp100 (Δ287–333)up	5′-CGGAATTCATGGCAGGTGGGGGCGGC-3′	5′-CAGTGGAAATTTGGATTCCATGGTTGTGTAG-3′
pB42AD-Sp100 (Δ287–333)down	5′-CCAAATTTCCACTGACGTTGATGAGCCC-3′	5′-GCGGTGACCTAATCTTCTTTACCTGACCCTC-3′
pB42AD-Sp100 (Δ334–407)up	5′-CGGAATTCATGGCAGGTGGGGGCGGC-3′	5′-CTGGATTCTCCTTCAGAGTCCTCACTGC-3′
pB42AD-Sp100 (Δ334–407)down	5′-CTGAAGGAGAATCCAGTGAGGAGGAGGC-3′	5′-GCGGTGACCTAATCTTCTTTACCTGACCCTC-3′
pB42AD-Sp100 1–300	5′-CGGAATTCATGGCAGGTGGGGGCGGC-3′	5′-GCGGTCGACCTTTTCCTTTTTTATATCCACC-3′
pB42AD-Sp100 300–480	5′-CGGAATTCAAGCCATTTTCTAATTCAAAAG-3′	5′-GCGGTCGACCTAATCTTCTTTACCTGACCCTC-3′
pDsRed-Sp100	5′-CGGAATTCTGATGGCAGGTGGGGGCGGC-3′	5′-GCGGTACCACATCTTCTTTACCTGACCCTCTTC-3′

ΦC31 integrase interacting proteins. Eleven positives were isolated from 10^6 independently screened clones. All positive clones were mated with pLexA-ΦC31 N-terminus and pLexA-ΦC31 C-terminus (Table 2). One of the 11 positive clones corresponded to the C-terminus of the Sp100 sequence, from residues 300 to 434. The interacting fragment in ΦC31 integrase is in its N-terminus (Fig. 1A). As for determination of the interaction region of Sp100, eight EGY48[p8opLacZ] yeast transformants containing pB42AD-based constructs with different fragments of Sp100 were mated with YM4271 carrying pLexA-ΦC31 integrase. All matings with the plasmids containing Sp100(300–434) activated the reporter genes expression, and none with plasmids lacking this region did. This result revealed that Sp100(300–434) is both necessary and sufficient for the interaction with ΦC31 integrase (Fig. 1B).

To further narrow the exact region of interaction in the N-terminus of ΦC31 integrase, peptide arrays displaying overlapping fragments of ΦC31 integrase that covered the entire sequence of the ΦC31 integrase protein were utilized for the binding of endogenous Sp100 protein from an HEK293 cell lysate. Following incubation with the lysate, bound Sp100 was detected with an anti-Sp100 antibody. The strongest signal was found predominantly at spot 37–41 on the 12-residue arrays, spot 36–41 on the 15-residue arrays and spot 33–41 on the 20-residue arrays (Fig. 1C). The positive spots from the three ΦC31 integrase arrays shared a common corresponding region of a tetramer, 81RILN84, indicating that this fragment in ΦC31 integrase is probably the site of Sp100 binding (Fig. 1D).

The interaction between ΦC31 integrase and Sp100 was further verified by co-immunoprecipitation analysis. HEK293 cells were transfected with either pEGFP-ΦC31 encoding an EGFP-ΦC31 fusion protein or a control plasmid pEGFP-C3 encoding EGFP only.

Table 2. Cellular proteins that interact with ΦC31 integrase

GenBank number	Gene Identity	Integrase:		
		Full length	N-terminal	C-terminal
NM-001350	Death-associated protein (DAXX)	+		+
NM-016614	TRAF and TNF receptor associated protein (TTRAP)	+		+
NM-003113	Nuclear antigen sp100 (Sp100)	+	+	
NM-032308	RPA interaction protein gamma (RIP)	+	+	
NM-013263	Bromodomain containing 7 (BRD7)	+		+
NM-024835	Zinc finger protein 403 (ZNF403)	+		+
NM-003973	Ribosomal protein L14 (RPL14)	+		+
NM-005499	SUMO-1 activating enzyme subunit 2 (UBA2)	+		+
NM-007375	TAR DNA binding protein (TARDBP)	+		+
NM-006904	Protein kinase, DNA-activated, catalytic polypeptide (PRKDC)	+		+
NM-014302	Sec61 gamma subunit (SEC61G)	+		+

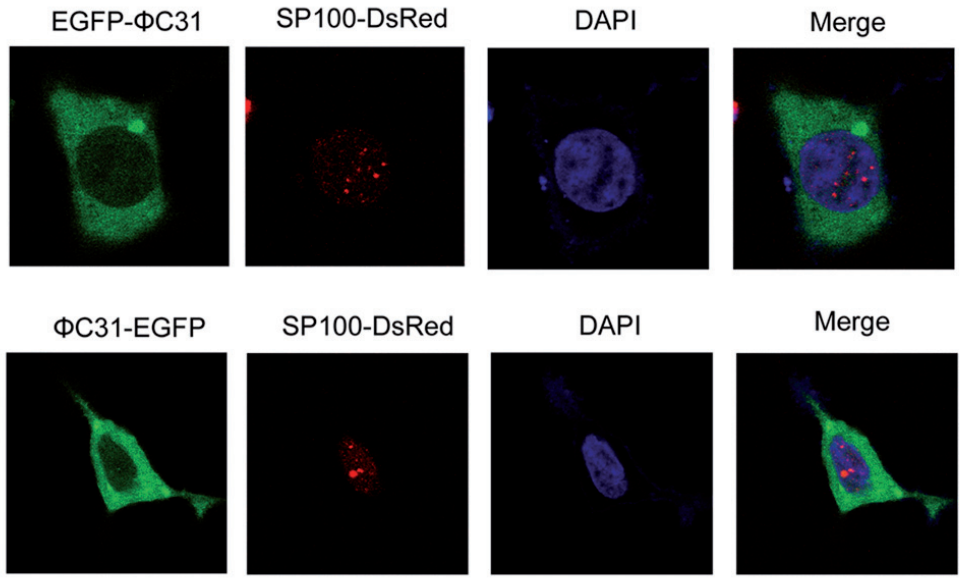

Figure 2. Subcellular localization of ΦC31 integrase and Sp100
pSp100-DsRed and N- or C-terminus EGFP-fused ΦC31 integrase constructs were co-transfected into HEK293 cells.

The rabbit anti-Sp100 antibody was able to precipitate EGFP-ΦC31 but not EGFP (Fig. 1E).

Subcellular localization of ΦC31 integrase and Sp100

To localize ΦC31 integrase in relationship to Sp100 within the cell, Sp100-DsRed and ΦC31-EGFP fusion proteins were expressed in HEK293 cells by co-transfection of the plasmids pSp100-DsRed and ΦC31-expressing plasmids with EGFP fused to the N- or C-terminus of ΦC31 integrase (pEGFP-ΦC31 or pΦC31-EGFP). As shown in Fig. 2, puncta of red fluorescent Sp100-DsRed fusion proteins were predominantly located within the nucleus. Apart from low EGFP fluorescence of both the N- and C-terminus-fused EGFP-ΦC31 integrase that was seen in the nuclei of some cells, the fusion protein was mainly dispersed throughout the cytoplasm.

Endogenous Sp100 inhibits ΦC31 integrase-mediated recombination

Our previous study demonstrated that endogenous DAXX interacted with ΦC31 integrase and inhibited the recombination mediated by this integrase (Chen *et al.*, 2006). We wondered if the interaction between ΦC31 integrase and Sp100 also had some effect on the recombination efficiency. We designed two Sp100-specific siRNAs (siSp100a and siSp100b). Real-time RT-PCR and Western blotting (Fig. 3A) showed that they can dramatically knock down the endogenous Sp100 expression in HEK293 cells. We co-transfected the reporter plasmid PB[EGFP] and the ΦC31 integrase expression plasmid pCMV-Int. The percentage of EGFP positive cells, which represents the rate of recombination events, was measured. Expression of ΦC31 integrase by pCMV-Int together with a human non-silencing siRNA gave rise to $21.72 \pm 1.75\%$ EGFP-positive cells. Notably, the percentages of EGFP-positive cells increased to $44.79 \pm 1.14\%$ ($P < 0.01$) and $43.25 \pm 1.12\%$ ($P < 0.01$) when endogenous Sp100 was knocked down by siSp100a and siSp100b, respectively (Fig. 3B). To further confirm the above conclusion, we designed a pair of primers which can detect the plasmid recombined in the presence of ΦC31 integrase. Cell genomic DNA was extracted, and a real-time RT-PCR assay, using the GFP sequence on the plasmid backbone as a control, demonstrated that percentage of recombination of the plasmid into Sp100 knock-down cells was increased 1.7 ± 0.11 and 1.6 ± 0.09 fold, respectively, ($P < 0.01$) compared to the control cells (Fig. 3C). Thus, both of the results showed that, similar to DAXX, reducing the levels of intracellular Sp100 indeed resulted in an increased efficiency of ΦC31 integrase-mediated recombination.

As Sp100 possesses transcriptional regulatory properties (Seeler *et al.*, 1998; Möller *et al.*, 2003; Yordy *et al.*, 2004), we also determined the impact of Sp100 on ΦC31 integrase-mediated reporter gene expression. Both the GFP mean intensity that was examined by a fluorescence-activated cell sorter (FACS) and the relative GFP mRNA level that was examined by using a real-time RT-PCR assay showed only slight differences between the Sp100 knockdown cells and the control cells. This result suggests that Sp100 had little influence on ΦC31 integrase-mediated reporter gene expression (not shown).

DISCUSSION

Sp100 was first identified as an autoantigen from patients suffering from primary biliary cirrhosis and is a constitutive component of ND10 (Szostecki *et al.*, 1990; Negorev & Maul, 2001). Like other ND10 proteins, Sp100 participates in many cellular processes (Negorev & Maul, 2001). Our study demonstrated that Sp100 interacts with ΦC31 integrase and inhibits ΦC31 integrase-mediated recombination.

Although Sp100 has been reported to interact with a few human and virus proteins (Seeler *et al.*, 1998;

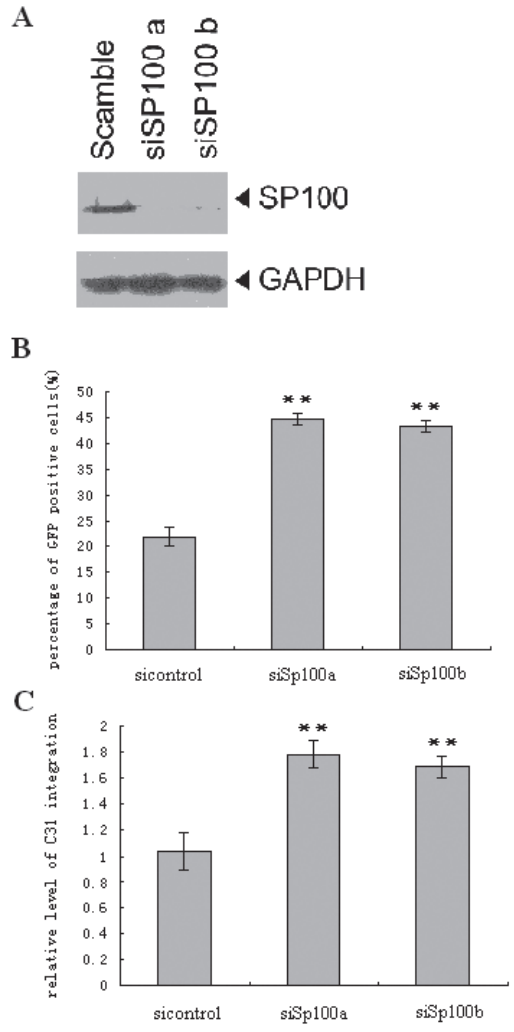

Figure 3. Endogenous Sp100 inhibits ΦC31 integrase-mediated recombination

(A) Knock-down efficiency of Sp100 was determined by Western blotting. **(B)** A ΦC31 integrase reporter plasmid, PB[EGFP], together with pCMV-Int and indicated siRNAs, were cotransfected into HEK293 cells. The percentage of EGFP positive cells was measured by flow cytometry. $^{**}P < 0.01$ and $^{*}P < 0.05$. **(C)** A ΦC31 integrase reporter plasmid, PB[EGFP], together with pCMV-Int and indicated siRNAs, were cotransfected into HEK293 cells. Relative recombination efficiency was measured by real-time RT-PCR. $^{**}P < 0.01$ and $^{*}P < 0.05$.

Möller *et al*., 2003; Yordy *et al*., 2004; Tavalai & Stamminger, 2008), the present study is the first report that Sp100 interacts with a phage integrase, ΦC31 integrase. The interaction between ΦC31 integrase and Sp100 is strongly supported by results of the yeast two-hybrid and co-immunoprecipitation assays. Moreover, our yeast two-hybrid and yeast mating tests showed that only constructs containing the Sp100 300–434 fragment interacted with ΦC31 integrase, suggesting that this 300–434 amino-acid region is crucial for the Sp100–ΦC31 interaction. This Sp100 region includes an activation domain (AD), which has been suggested to be responsible for Sp100 activated transcription (Xie *et al*., 1993); little is known about this domain from searching Sp100 properties and functions even though Sp100 has been reported in many papers. Our yeast mating and ΦC31 integrase peptide array demonstrated that the interacting region of ΦC31 integrase with Sp100 is in its N-terminus. This is unique to Sp100 when compared with other ND10 proteins, including DAXX and TTRAP, which interact with the C-terminus of ΦC31 integrase (Chen *et al*., 2006; Wang *et al*., 2009).

However, despite strong evidence provided by the yeast mating and co-immunoprecipitation assays that supports a direct interaction between ΦC31 integrase and Sp100, these two proteins co-localized poorly in cells. Consistent with other reports, we found that the punctate Sp100-DsRed fluorescence was mainly localized to the nucleus (Seeler *et al*., 1998; Möller *et al*., 2003; Yordy *et al*., 2004). Meanwhile, the GFP-ΦC31 integrase fusion protein was predominantly located in the cytoplasm. Its presence in the nucleus was barely observable (Fig. 2), which is consistent with our earlier results (Chen *et al*., 2006; Zhang *et al*., 2009), as well as those of others (Andreas *et al*., 2002). The seemingly contradictory evidence between the Sp100–ΦC31 integrase interaction and their localizations may be attributed to the insufficient sensitivity of fluorescence microscopy and/or low amounts of the co-localized proteins. This argument is also supported by the fact that, despite the low visibility of EGFP-ΦC31 integrase localization in the nucleus, a significant percentage of ΦC31 integrase reporter cell lines showed ΦC31 integrase-mediated site-specific recombination, which only takes place in the cellular genome, within the nucleus of the cells. Furthermore, functional interactions between ΦC31 integrase and Sp100 were also confirmed by the fact that knockdown of endogenous Sp100 improved ΦC31 integrase-mediated recombination.

Until now, little has been known about the relationship between the structure and function of the ΦC31 integrase. It has only been reported that the N-terminus of ΦC31 integrase is responsible for its catalytical function, and the C-terminal domain of ΦC31 integrase controls the directionality of recombination (Rowley & Smith, 2008; Rowley *et al*., 2008; McEwan *et al*., 2009). In our research, we find that Sp100 can directly interact with the N-terminal region of ΦC31 integrase, which strongly supports the fact that endogenous Sp100 inhibits the recombination efficiency of the ΦC31 integrase. Sp100 can interact and colocalize with the heterochromatin protein HP1, and SUMO modification of Sp100 enhances the stability of Sp100–HP1 complexes, which suggests that Sp100 may function in chromatin pathways and influence chromatin dynamics (Seeler *et al*., 1998; 2001). *Via* its interaction with NBS1, Sp100 may also be involved in genomic stability and telomere maintenance (Naka *et al*., 2002). As ΦC31 integrase-mediated recombination may destroy the genomic stability of host cells (Ehrhardt *et al*., 2006), we suggest that Sp100 may inhibit ΦC31 integrase-mediated recombination, and that this Sp100-mediated inhibition may reveal that the function of Sp100 is to maintain cellular genomic integrity.

As Sp100 can interact with specific DNA-binding proteins and participates in transcription regulation (Seeler *et al*., 1998; Möller *et al*., 2003; Yordy *et al*., 2004), we asked if Sp100 could also impact ΦC31 integrase-mediated gene expression. The results of the assays of GFP mean intensity and relative GFP mRNA levels both indicated little differences between the Sp100 knock-down cells and control cells. Since the GFP expression was observed only in those cells that did undergo ΦC31 integrase-mediated reporter gene activation, our research indicated that Sp100 has no direct effect on the reporter gene expression at either transcriptional or post-transcriptional level.

ND10 is a discrete nuclear body. It has been implicated in a variety of cellular processes (Negorev & Maul, 2001). Previously, we reported that ΦC31 integrase interacted with the ND10 protein DAXX/TTRAP and this interaction influenced the integrase recombination efficiency (Chen *et al*., 2006; Wang *et al*., 2009). Our present study revealed that ΦC31 integrase interacts with another important ND10 protein, Sp100, which inhibits its recombination efficiency. The relationship between ΦC31 integrase and ND10 proteins indicates the importance of understanding potential interactions between the integrase and cellular proteins in mammalian cells. This information may be important in developing a gene therapy strategy for efficient and site-specific genomic insertion.

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