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# DAXX interacts with phage ΦC31 integrase and inhibits recombination

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## ABSTRACT

**Phage ΦC31 integrase has potential as a means of inserting therapeutic genes into specific sites in the human genome. However, the possible interactions between ΦC31 integrase and cellular proteins have never been investigated. Using pLexA-ΦC31 integrase as bait, we screened a pB42AD-human fetal brain cDNA library for potential interacting cellular proteins. Among 61 positives isolated from $10^6$ independent clones, 51 contained DAXX C-terminal fragments. The strong interaction between DAXX and ΦC31 was further confirmed by co-immunoprecipitation. Deletion analysis revealed that the fas-binding domain of DAXX is also the region for ΦC31 binding. Hybridization between a ΦC31 integrase peptide array and an HEK293 cell extract revealed that a tetramer, 451RFGK454, in the C-terminus of ΦC31 is responsible for the interaction with DAXX. This tetramer is also necessary for ΦC31 integrase activity as removal of this tetramer resulted in a complete loss of integrase activity. Co-expression of DAXX with ΦC31 integrase in a HEK293-derived ΦC31 integrase activity reporter cell line significantly reduced the ΦC31-mediated recombination rate. Knocking down DAXX with a DAXX-specific duplex RNA resulted in increased recombination efficiency. Therefore, endogenous DAXX may interact with ΦC31 causing a mild inhibition in the integration efficiency. This is the first time that ΦC31 was shown to interact with an important cellular protein and the potential effect of this interaction should be further studied.**

## INTRODUCTION

Gene therapy remains the most promising if not the only approach to treating genetic diseases. The safety concerns of gene therapy have been heightened by the discovery of a leukemia-like disorder in several patients treated with a retrovirus vector, due to an insertional mutagenesis (1). To minimize the potential risk of gene therapy, therapeutic genes should be integrated at specific sites proven to be safe.

While homologous recombination gives rise to precise site-specificity, the low efficiency limits its clinical application (2). Alternatively, integrases mediate site-specific insertions into the genome by recognizing specific sequences. Site-specific recombination systems are ubiquitous throughout prokaryotes but are rare in mammals (3). Some phage recombinases such as Cre are able to insert a gene into a specific site (loxP) and have been employed in engineering mammalian cells (4,5). However, these recombination systems require pre-insertion of their substrate DNA sequences, such as the loxP site for Cre, located in the target region in mammalian genomes, which limits their potential clinical applications. Phage ΦC31 integrase belongs to the serine recombinase family that mediates site-specific recombination between two short recognition sites, attB and attP (3,6). Recombination occurs when these two att sites are present in two different DNA molecules and the attB serves as the donor to be inserted into the attP (3). Interestingly, it has been discovered that ΦC31 integrase can also mediate recombination at a limited number of sites in mammalian genomes (7). These 'pseudo-att' sites have been proposed as potential targets for site-specific gene insertion using the ΦC31 integrase-based system (8–11). Several studies have demonstrated the usefulness of the ΦC31 integrase system in gene therapy. A ΦC31-integrase-mediated gene insertion into a human myoblast genome resulted in a 15-fold increase in stable expression compared to that without integrase (12). Similarly, coinjection of plasmid-expressing ΦC31 in the mdx mouse resulted in 5- to 10-fold higher levels of sustained luciferase expression as well as dystrophin expression (13). However, potential interactions between ΦC31 and proteins in mammalian host cells have never been studied carefully. This issue has become particularly important as it has been reported that ΦC31 integrase induced a high frequency of hepatocyte dysplasia after the integrase system was used to

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**Table 1.** The primers designed for point specific mutation of ΦC31 integrase

| Mutants | Uniform ORF primers | Mutation primers |
|---|---|---|
| Del451–454 | 5′-gaggatcctgacacaaggggttgtgac-3′;<br>5′-ccgctcgagcgccgctacgtcttccgtg-3′ | 5′-ctgtgggaagccgcccgactcactgaggcgcctgag-3′;<br>5′-ctcaggcgcctcagtgagtcgggcggcttcccacag-3′ |
| K454N | 5′-gaggatcctgacacaaggggttgtgac-3′;<br>5′-ccgctcgagcgccgctacgtcttccgtg-3′ | 5′-ctgtgggaagccgcccgacgcttcggcaacctcactgaggcgcctgag-3′;<br>5′-ctcaggcgcctcagtgaggttgccgaagcgtcgggcggcttcccacag-3′ |
| R451H | 5′-gaggatcctgacacaaggggttgtgac-3′;<br>5′-ccgctcgagcgccgctacgtcttccgtg-3′ | 5′-ctgtgggaagccgcccgacacttcggcaagctcactgaggcgcctgag-3′;<br>5′-ctcaggcgcctcagtgagcttgccgaagtgtcgggcggcttcccacag-3′ |

transfer the fumarylacetoacetate hydrolase (FAH) gene into the livers of mice affected with hereditary tyrosinemia type 1 (14).

To test the hypothesis that ΦC31 integrase may interact with cellular proteins, we employed a yeast-two-hybridization assay to detect cellular proteins that interact with ΦC31 integrase. We report here that ΦC31 can strongly bind to an important cellular protein, DAXX, as evidenced by the results of yeast-two-hybridization and co-immunoprecipitation. We have identified the binding regions in both proteins and the functional interaction between DAXX and ΦC31 were also demonstrated by reduced ΦC31 integrase activity in DAXX-overexpressing cells.

## MATERIAL AND METHODS

### Plasmid construction

To construct a pLexA-ΦC31 bait plasmid, the open reading frame (ORF) of ΦC31 integrase was amplified from a pCMVInt plasmid (kindly provided by Prof. M. P. Calos) with a pair of primers: 5′-gaggatcctgacacaaggggttgtgac-3′ and 5′-ccgctcgagcgccgctacgtcttccgtg-3′. The PCR product was inserted into the plasmid pLexA (Clontech) between the BamHI and XhoI sites. The N-terminal catalytic-activity-containing fragment (1–235 amino acid toward CAC93948) and C-terminal fragment (237–613 amino acid towards CAC93948) were amplified with primers 5′-gaggatcctgacacaaggggttgtgac-3′, 5′-ccgctcgagcgcttacaaagccccgtgatgctg-3′ and 5′-gaggatcctggacgctgacgccgtgccg-3′, 5′-ccgctcgagcgccgctacgtcttccgtg-3′. The fragments were then inserted into the pLexA plasmid between BamHI and XhoI to produce the plasmids pLexA-ΦC31 (1–235) and pLexA-ΦC31 (237–613). Both these plasmids were used in a yeast-mating test for locating the interacting fragment of ΦC31 integrase.

pB42AD-DAXX(572–625) and pB42AD-DAXX(625–740) were used for determining the ΦC31-interacting fragment on DAXX. These fragments were amplified from a pB42AD fetal brain cDNA library (Clontech) with the primers 5′-gcgaattcaacatggaagctttgcccctggatac-3′, 5′-ccgctcgagcggaccagaatctccccagttgtg-3′ and 5′-gcgaattcaacatgtctggtcccccctgcaaaaaatc-3′, 5′-ggagacttgaccaaacctctggcg-3′. The PCR products were inserted into the site between EcoRI and XhoI in the pB42AD plasmid (Clontech).

The above obtained ΦC31 ORF was also subcloned into pEGFP-C2 (Clontech) between BamHI and XhoI sites in the frame with the C-terminus of EGFP to generate pEGFP-ΦC31 that expresses EGFP-ΦC31 fusion protein. A construct pΦC31-EGFP expressing C-terminal fused ΦC31 fusion protein was produced similarly.

The ORF of human DAXX was obtained from a human fetal brain cDNA library (Clontech) by amplification with primers 5′-gagaattcacaatggccaccgctaacagc-3′ and 5′-gaggatcctcagagtctgagagcacg-3′. The cDNA was inserted into pDsRed-N1 (Clontech) between EcoRI and BamHI to generate pDAXX-DsRed that expresses DAXX-DsRed fusion protein. Plasmids that express mutant ΦC31s, namely, pLexA-ΦC31(Del451–454), pLexA-ΦC31(R451H) and pLexA-ΦC31 (K454N), were generated by a PCR-based point specific mutation strategy with the primers shown in Table 1.

The above mutant ΦC31s were also amplified with primers 5′-gaggatcctatgacacaaggggttgtgac-3′ and 5′-ccgctcgagcgccgctacgtcttccgtg-3′ and inserted into pcDNA3.0 (Invitrogen) between BamHI and XhoI for expression and determination of the catalytic activity of mutant ΦC31.

The plasmids mentioned above were all verified by sequencing analysis.

### Yeast-two-hybridization assay

The MATCHMARKER LexA two-hybrid system was purchased from Clontech. Yeast-two-hybridization was performed in a standard two-step protocol provided by the manufacturer. All isolated positive clones were amplified with the uniform pB42AD sequence primers (5′-ccagcctcttgctgagtggagatg-3′; 5′-ggagacttgaccaaacctctggcg-3′) and the PCR products were sequenced with the same primers.

For locating the interacting fragment on DAXX, yeast-mating tests between pB42AD-DAXX(572–625)/pB42AD-DAXX(627–740)-transferred EGY48[p8opLacZ] and pLexA-ΦC31-transferred YM4271 were performed according to a standard yeast-mating protocol provided by the manufacturer.

The yeast-mating test was also used to determine the interacting fragment on ΦC31 integrase. Either pLexA-ΦC31(1–235), pLexA-ΦC31(237–613), pLexA-ΦC31(Del451–454), pLexA-ΦC31(R451H) or pLexA-ΦC31(K454N) was used to correspondingly transfect YM4271, which was then mated with pB42AD-DAXX(527–740) EGY48[p8opLacZ] yeast cells.

### Co-immunoprecipitation

Co-immunoprecipitation was employed to verify the interaction between ΦC31 integrase and cellular protein DAXX. 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% $CO_2$. The cells were transfected with pEGFP-ΦC31 or a control plasmid pEGFP-N3 (Clontech)

using Lipofectamine (Invitrogen). At 24 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 mouse anti-GFP monoclonal antibody (NeoMarkers) were added to 1 ml of each cell lysate. After incubation with end-over-end mixing for 2 h at 4°C, 10 μl of pre-washed protein A/G Sepharose (Santa Cruz) were added to each extract and shaken for 1 h at 4°C. Next, the immunoprecipitates were washed three times with the lysis buffer. The pellets were then separated on SDS–PAGE and transferred to a nitrocellulose membrane. The blot was detected using rabbit anti-human DAXX antibody (Santa Cruz) followed by horseradish-peroxidase-conjugated anti-rabbit antibody (Sigma) and the bands were visualized using an enhanced chemiluminescence immunoblotting detection kit (Amersham).

### ΦC31 peptide array analysis

Three ΦC31 peptide arrays (12, 15 and 20 mers) were generated and probed with DAXX to determine the DAXX binding region in ΦC31. 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 $5 \times 10^7$ HEK293 cells in 10 ml of lysis buffer at 4°C overnight. The membranes were then incubated with a rabbit anti-human DAXX polyclonal antibody (Santa Cruz) followed by horseradish-peroxidase-conjugated anti-rabbit antibody (Sigma). The bound proteins were visualized using enhanced chemiluminescence immunoblotting detection reagents (Amersham). For controls, the membranes were processed identically but incubation with the cell lysate was omitted.

### Intracellular localization of DAXX and ΦC31 integrase

The subcellular locations of DAXX and ΦC31 integrase were determined in HEK293 cells. Fusion plasmids, pDAXX-DsRed, pEGFP-ΦC31 and pΦC31-EGFP were used to transfer HEK293 for the location of ΦC31 integrase and DAXX, respectively. The cells were transfected with the plasmids above using Lipofectamine (Invitrogen). At 24 h post-transfection, the cells were observed under a confocal fluorescent microscope (TSC SP2, Leica). The nuclei were strained with DAPI as a control. The fluorescence of GFP, RFP and DAPI was observed at wavelengths of 488, 543 and 405 for excitation and 510, 582 and 461 for emission, respectively.

### ΦC31 integrase activity assay

ΦC31 integrase activity in *Escherichia coli* was determined using a pBCPB-(kindly provided by Prof. M.P. Calos) based system (8). Briefly, plasmid containing ΦC31 integrase or its mutant cDNAs was co-transformed with pBCPB into *E.coli* DH5α-competent cells. The cells were plated on an LB agar medium with ampicillin (50 μg/ml), chloramphenicol (50 μg/ml) and X-gal (40 μg/ml). Recombination through active ΦC31 integrase causes deletion of the beta-glactosidase gene from pBCPB and gives rise to white colonies. The ratio of the number of white colonies to that of total colonies was calculated 24 h later as an index of integrase activity.

ΦC31-mediated integration in mammalian cells was measured using an HEK293-derived ΦC31 integration reporter cell line, PB[EGFP], which was created in our laboratory. PB[EGFP] contains a genomically inserted single copy structure of pCMV-attB-BGH poly A-attP-EGFP. In the presence of ΦC31, integrase-mediated recombination between attB and attP sites removes bGH poly A from the inserted structure, resulting in EGFP expression. At 48 h post-transfection, the cells were harvested and percentages of EGFP-expressing cells were determined by flow cytometery. Overexpression of DAXX was accomplished by co-transfecting pDAXX-DsRed1 with pCMVInt. The plasmid pDsRed1-N1 that contains no DAXX was used as a control construct. Meanwhile, Endogenous expression of DAXX was knocked down by transfection with a DAXX-specific duplex RNA (5′-GGAGUUGGAUCUCUCAGAAdTdT-3′; 5′-UUCUGA-GAGAUCCAACUCCdTdT-3′) (15), which reduced the DAXX protein expression level by ~75%, as estimated by western blot analysis with a human DAXX-specific antibody. As a control, a human non-silencing duplex RNA (5′-UUAA-GUAGCUUGGCCUUGAdTdT-3′; 5′-UCAAGGCCAAGC-UACUUAAdTdT-3′)(GeneChem, China) was used.

## RESULTS

### Results of yeast-two-hybridization and co-immunoprecipitation indicated a strong interaction between ΦC31 and DAXX

We initially used pLexA-ΦC31 as a bait to screen a pB42AD-based human fetal brain cDNA library for potential ΦC31 integrase interacting proteins. Sixty-one positives were isolated from $10^6$ independently screened clones and sequenced. Fifty-one out of the 61 positive clones corresponded with the C-terminus of DAXX sequence, from 527 to 730 amino acid (Figure 1A).

The interaction between ΦC31 integrase and DAXX was verified with co-immunoprecipitation analysis. HEK293 cells were transfected with either pEGFP-ΦC31 that expressed an EGFP-ΦC31 fusion protein or a control construct pEGFP-N3 containing the EGFP only. A mouse anti-GFP antibody was able to precipitate DAXX from the cell lysate of HEK293 transfected with pEGFP-ΦC31 but not with pEGFP-N3 (Figure 1B).

### Characterization of interacting fragments of ΦC31 integrase and DAXX

We then attempted to locate the key fragment that mediates the interaction between ΦC31 integrase and DAXX. Both the C-terminus (237–613) and N-terminus (1–235) of ΦC31 protein were fused to the LexA DNA binding domain. The yeast-mating test revealed that the C- but not the N-terminus of the integrase is necessary for the interaction. To further narrow down the exact region in the C-terminus of ΦC31, peptide arrays displaying overlapping fragments of ΦC31 that covered the entire sequence of the ΦC31 protein were utilized for the binding of endogenous DAXX from the

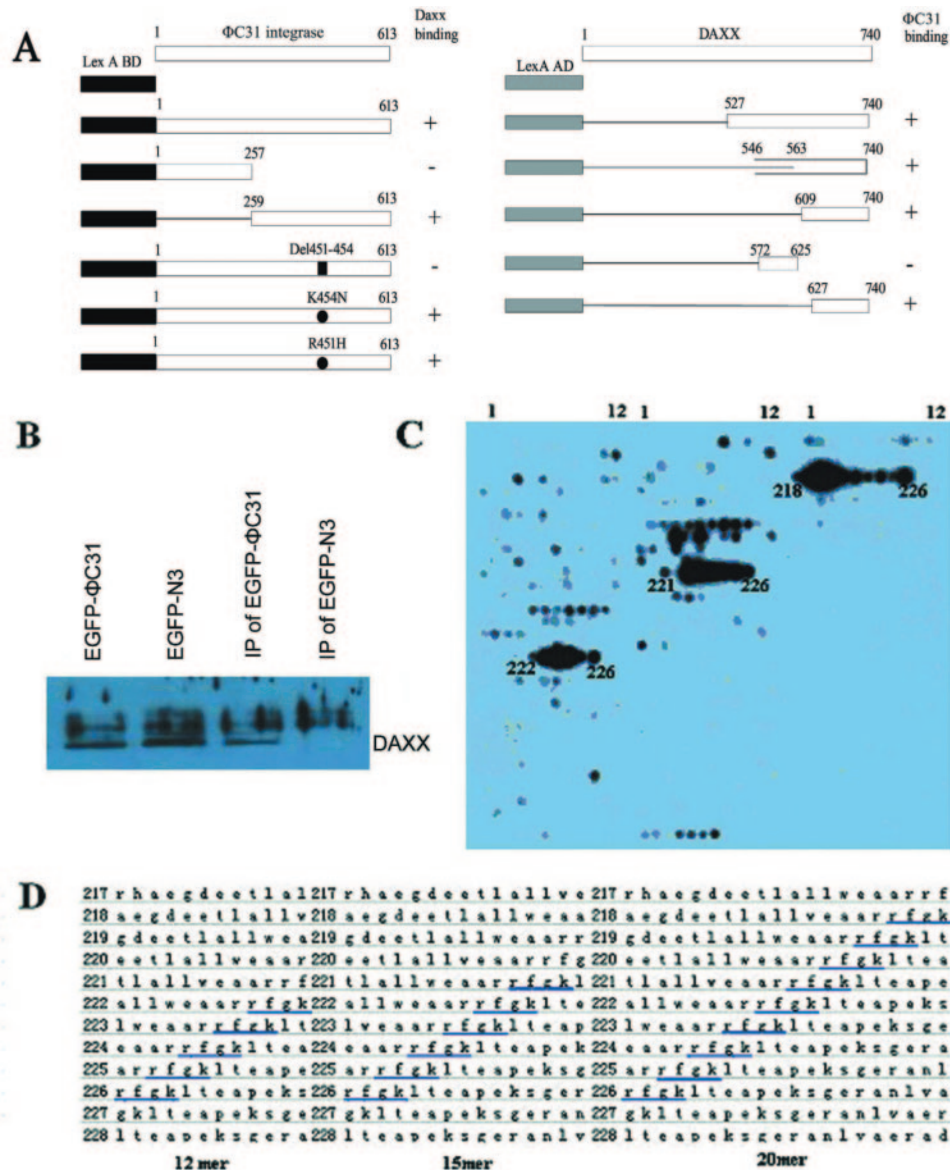


**Figure 1.** Interaction between ΦC31 integrase and human DAXX. (**A**) Yeast two-hybrid analysis was conducted using ΦC31-integrase-derived pLexA fusion plasmids as bait, a human fetal cDNA-derived pB42AD library and two human DAXX-derived pB42AD fusion plasmids as prey. The overlapping open reading frames correspond to human DAXX. (**B**) Cell lysates of pEGFP-ΦC31-transfected and pEGFP-N3-transfected HEK293 cells were each incubated with a mouse anti-GFP binding protein on A/G agarose beads. Samples were analyzed by western blotting with a rabbit anti-human DAXX antibody. An unknown protein with higher molecular weight was also recognized by the anti-DAXX antibody. However, it is probably non-specific by a cross-reaction of the polyclonal antibody as it is present in all the samples. (**C**) Three ΦC31 integrase overlapping peptide arrays with different lengths of peptide (12, 15 and 20mers) for localization of the DAXX interacting region on ΦC31. Serial numbers of spots that were positive for DAXX binding were labeled. (**D**) Amino acid sequences of the peptide spots corresponding to the array shown in C. The DAXX binding tetramer is indicated by underlining.

HEK293 cell lysate. Following incubation with the lysate, the bound DAXX was detected with an anti-DAXX antibody. The strongest immunoreactivity was found predominantly at spot 222–226 on 12-residue arrays, spot 221–226 on 15-residue arrays and spot 218–226 on 20-residue arrays (Figure 1C). Interestingly, the positive spots from the three ΦC31 arrays shared a common corresponding region of a tetramer, 451RFGK454, indicating that it was probably the fragment in ΦC31 responsible for DAXX binding (Figure 1D).

For further characterizing the binding site of ΦC31, we generated three mutant ΦC31 constructs, including an $Arg^{451}$–$His^{451}$ point mutation (pLexA-ΦC31(R451H)), a $Lys^{454}$–$Asn^{454}$ point mutation (pLexA-ΦC31(K454N)) and one with a deletion of the tetramer 451–454 (pLexA-ΦC31(Del451–454)). The yeast-two-hybrid assay showed no interaction between pLexA-ΦC31(Del451–454) and DAXX. However, mutations at the $Arg^{451}$ (pLexA-ΦC31(R451H)) or $Lys^{454}$ (pLexA-ΦC31(K454N)) residue of ΦC31 did not alter its binding to DAXX. (Figure 1A).

To localize the region in DAXX that binds to ΦC31, a pB42AD construct containing a DAXX with a deletion of amino acid 627–740 was generated for the yeast-mating

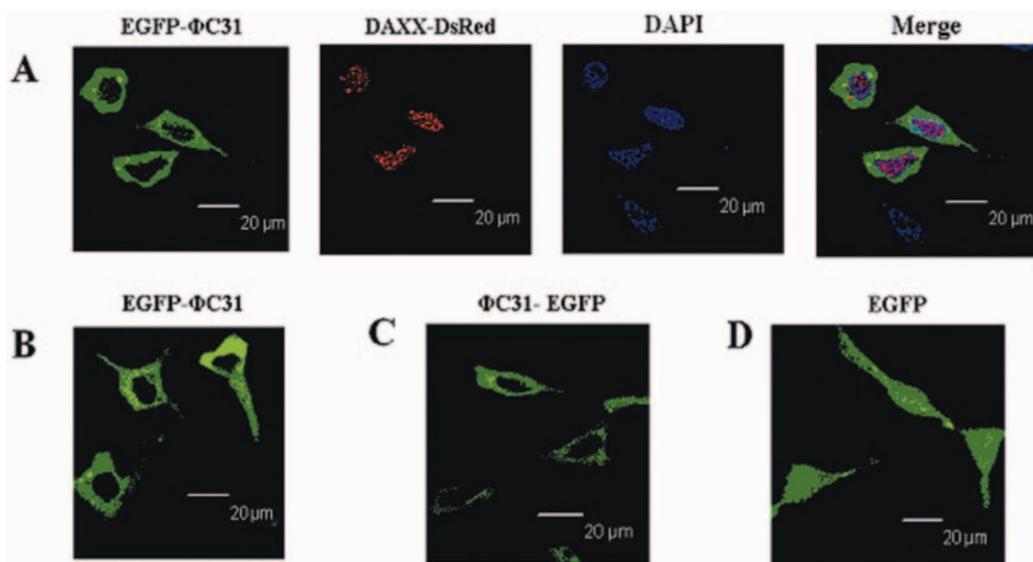


**Figure 2.** Subcellular localization of DAXX and ΦC31 integrase. (**A**) pDAXX-DsRed and pEGFP-ΦC31 fusion protein constructs were co-transfected into HEK293 cells. (**B**) HEK293 cells transfected with pEGFP-ΦC31 alone. (**C**) HEK293 cells transfected with a ΦC31 construct with C-terminal fused EGFP (pΦC31-EGFP) alone. (**D**) Cells transfected with pEGFP alone. Expressed fluorescent GFP and DsRed were observed under a confocal microscope. The nuclei were stained by DAPI (40X).

test. Deletion of the 627–740 amino acid fragment in DAXX completely abolished its binding to ΦC31 (Figure 1A). Furthermore, a construct containing a fragment of DAXX (627–740) (pB42AD-DAXX (627–740), Figure 1A) was sufficient for binding to ΦC31. These results clearly demonstrated that the tetramer 451RFGK454 in the ΦC31 integrase and the region between 627 and 740 amino acid in DAXX are crucial for the interaction between the two proteins.

### Intracellular localization of ΦC31 and DAXX

Plasmids of pDAXX-DsRed, pEGFP-ΦC31, pΦC31-EGFP and pEGFP that express DAXX-DsRed, N- or C-terminal fused EGFP/ΦC31 fusion proteins and EGFP alone, respectively, were used for intracellular localization of ΦC31 integrase and DAXX. As shown in Figure 2A, punctates of red fluorescent DAXX-DsRed fusion protein were present predominantly in the nucleus. In contrast, both N- and C-terminal fused EGFP/ΦC31 fusion protein were mainly localized in the cytoplasm (Figure 2A–C). Meanwhile, EGFP alone was distributed evenly in both the cytoplasm and nucleus, suggesting that ΦC31 dominated the intracellular localization of the fusion proteins.

### The 451RFGK454 site is essential for ΦC31 integrase activity

To determine whether the DAXX binding site, 451RFGK454, on ΦC31 is involved in the activity of the integrase, pcDNA-ΦC31, pcDNA-ΦC31(Del451–454), pcDNA-ΦC31(R451H) or pcDNA-ΦC31(K454N) was co-transformed with pBCPB into *E.coli* DH5α-competent cells. Twenty-four hours later, pcDNA-ΦC31(R451H) gave rise to a similar number of positive colonies as that of pcDNA-ΦC31, (51.20% ± 1.60% versus 49.98% ± 2.63%). Thus, mutation from $Arg^{451}$ to $His^{451}$ in ΦC31 did not affect the integrase activity. In contrast, no white colonies were seen in samples transformed with pcDNA-ΦC31(Del451–454) and pcDNA-ΦC31(K454N), indicating that 451RFGK454 and $Lys^{454}$ are essential for the activity of ΦC31 integrase.

### DAXX inhibits ΦC31-mediated integration

The ΦC31 activity reporter cells PB[EGFP] transfected with pCMVInt gave rise to a recombination rate in the total transfectants of 20.30% ± 1.04% when the transfection efficiency averaged 47.50% ± 3.04%. Co-expression of pDAXX-DsRed but not a control plasmid pDsRed1 significantly reduced the recombination rate by 39.40 ± 0.98% ($P < 0.01$). On the other hand, co-transfection with pCMTInt and a DAXX-specific duplex small RNA resulted in an increase in GFP-expressing cells of 24.24 ± 0.95% ($P < 0.05$). However, this increased rate of recombination was not seen in cells co-transfected with a human non-silencing duplex small RNA (82.25 ± 1.05% of the control, $P > 0.09$, Figure 3 column D). These results indicated that the endogenous DAXX can slightly inhibit the integration efficiency of ΦC31 in HEK293 cells (Figure 3).

## DISCUSSION

DAXX is known for its distribution in nucleus domain 10 (ND10) and it relocates to the cytoplasm through nuclear export in response to certain signals (16–18). Consistent with this, we found that punctate DAXX-DsRed fluorescence was mainly localized in the nucleus. Meanwhile, the GFP-ΦC31 fusion protein was predominantly located in the cytoplasm. The GFP fusion protein possesses the same subcellular distribution as ΦC31 itself, and was localized with an antibody against ΦC31 protein (to be published elsewhere). The predominant cytoplasmic localization of ΦC31 was also consistent with an earlier observation that adding a nuclear localization signal could increase the integration

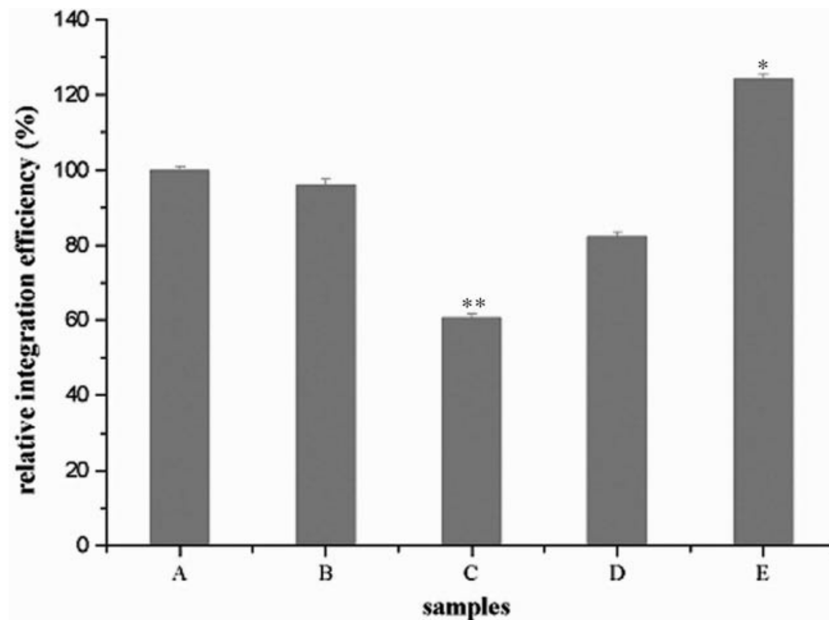


**Figure 3.** Effects of DAXX overexpression and siRNA knock down on efficiency of ΦC31-integrase-mediated recombination in the ΦC31 activity reporter cells PB[EGFP]. The efficiency of recombination was first calibrated using the transfection efficiency in each culture and then expressed as percentages of the cultures transfected with pCMVInt only. PB[EGFP] reporter cells were transfected with A, pCMVInt, B, pCMVInt/pDsRed1, C, pCMVInt/pDAXX-DsRed1, D, pCMVInt/non-silencing siRNA, and E, pCMVInt/siDAXX. $*P < 0.05$, $**P < 0.01$.

efficiency in mammalian cells (19). The lack of apparent co-localization between DAXX and ΦC31 integrase with fluorescent microscopy in contrast to the high affinity between the two proteins demonstrated by yeast-two-hybrid screening and co-immunoprecipitation may be attributed to the insufficient sensitivity of fluorescence microscopy and/or low amounts of the co-localized proteins. Strong red fluorescence of the DAXX-DsRed fusion protein in the nucleus may mask the green fluorescence of EGFP-ΦC31 fusion protein due to the relatively small amount of intranuclear-localized ΦC31 and vice versa for the cytoplasmic-localized DAXX-DsRed. Indeed, low levels of EGFP fluorescence seemed present in the nuclei of a few cells under microscope although the fluorescence failed to show in microphotographs. This argument is supported by the fact that, although little EGFP-ΦC31 green fluorescence was seen in the nuclei, ΦC31-mediated site-specific integration, which only takes place in the nucleus, was found in a significant percentage of ΦC31 activity reporter cells. Furthermore, a functional interaction between the two proteins was also demonstrated by both overexpression and knockdown DAXX in HEK293 cells. It is especially noteworthy that the ΦC31-mediated integration rate was increased by 24% in DAXX siRNA-treated cells because this suggests the existence of an endogenous interaction between the two proteins. The relatively mild increase in the ΦC31 integration efficiency may be attributed to the extreme abundance of DAXX in the nucleus causing incomplete DAXX knock down by the siRNA. However, it is important to determine whether ΦC31 predominantly localized in the cytoplasm can interfere with the function of DAXX when the latter is translocated to the cytoplasm upon activation by other cellular signals.

The mutational analysis of ΦC31 protein showed that the DAXX binds to 451RFGK454 in the ΦC31. The integrase activity assay further indicated that the tetramer is essential for integrase activity and therefore, binding to this region by DAXX may inhibit the integrase. It is worth pointing out that previous studies have indicated that the catalytic activity of ΦC31 was located between residues 1 and 120 at the N-terminus of the protein, and that the $Ser^{12}$ is the putative catalytic serine while the putative DNA binding domain is possibly at the C-terminus (20,21). We showed here that 451RFGK454 is crucial for the integration activity of ΦC31. However, the exact role of this region remains to be investigated.

Our yeast-mating test showed that only the construct containing the DAXX 627–740 fragment, but not DAXX 573–627, interacted with ΦC31, suggesting that this 627–740 amino acid region is crucial for the DAXX-ΦC31 interaction. Interestingly, the same domain is also well known for its interaction with other cellular proteins including Fas. To date, more than 20 proteins have been reported to interact with DAXX resulting in a broad range of effects including apoptotic regulation, transcriptional regulation, chromatin remodeling, involvement in signal pathways and regulation of the interaction between virus and host cells (22,23). DAXX was also reported to bind to the integrase of Avian Sarcoma Virus (ASV) (24). Interestingly, we found that DAXX binds to ΦC31 through the same domain as that for binding to ASV integrase. More importantly, the interaction between DAXX and ASV integrase causes suppression of viral replication, which was also reported in human cytomegalovirus-infected cells where DAXX had also been found to interact with a viral protein, pp71, causing inhibition of viral replication (25–27), suggesting that DAXX may play a role in cellular defense against viral infection.

The present study was the first to reveal that a phage integrase, ΦC31, interacts with an important cellular protein,

DAXX. The strong binding of ΦC31 to DAXX indicates the importance of understanding potential interactions between the integrases and cellular proteins in mammalian cells since the information gained would aid in developing a gene therapy strategy for site-specific genomic insertion. On the other hand, the inhibitory effect of DAXX on viral proteins across a wide range of species (from phage, avian to human viruses) suggests that DAXX might play a role in maintaining the stability of the genome through preventing extrinsic DNA integration.

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*Conflict of interest statement*. None declared.

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