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Characterization of Cre–*lox*P Interaction in the Major Groove: Hint for Structural Distortion of Mutant Cre and Possible Strategy for HIV-1 Therapy

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Abstract Although the crystal structure of Cre recombinase complexed with DNA, named *lox*A, was elucidated a couple of years ago, it has not yet been determined which amino acids of the protein are involved in the specific Cre–*lox*P interaction. Arg259 and Gln90 interact with DNA substrate in the major groove from which the specificity of protein–DNA interaction comes. In this study, we substituted these residues for other amino acids. Also, two mutated DNA substrates were constructed. In each mutant, one of the bases that interact with Arg259 or Gln90 was changed into another base. In vitro binding assays and recombination assays of variant *lox* sites with wild-type and mutant-type Cre revealed that Arg259 plays a key role in Cre–*lox*P binding but Gln90 does not. However, the recombination activity still remained intact, although the binding between Cre and DNA substrate was not ensured. J. Cell. Biochem. 80:321–327, 2001. © 2001 Wiley-Liss, Inc.

Key words: Cre–*lox*P recombination; site-directed mutagenesis; protein–DNA interaction; major groove; HIV-1 therapy

The 38 kDa Cre recombinase catalyzes site-specific recombination in vitro [Abremski et al., 1983] and in vivo [Sternberg and Hamilton, 1981]. DNA substrate at two *lox* sites are cleaved, exchanged, and rejoined to new partners in a well-ordered way without any synthesis or degradation of the DNA [Stark et al., 1992]. The Cre–*lox*P recombination system does not require any accessory factors other than 38 kDa Cre and 34 bp *lox*P [Abremski et al., 1983]. The enzyme can mediate recombination efficiently at the *lox* sites in various procaryotic and eucaryotic cells [Dale and Ow, 1990; Odell et al., 1990; Sauer, 1987; Sauer and Henderson, 1988]. Consequently, it has been used in general target gene manipulation and antiviral strategies [Flowers et al., 1997; Russ et al., 1996].

Although the structure of Cre recombinase complexed with DNA, named *lox*A, was elucidated a couple of years ago [Guo et al., 1997], it has not yet been determined which residues of the protein are involved in the specific Cre–*lox*P interaction since the result only gives us knowledge of the recombination mechanism and intermediate structure. From NUCPLOT [Luscombe et al., 1997] analysis of Cre–*lox* co-crystal structure coordination (1crx.pdb), it has been established that the major grooves of the thymine at position 1 and the guanine at position 6 of *lox*P interact with Gln90 and Arg259, respectively. In the current study, only interactions that involve atoms in the DNA major groove were included, so we constructed Cre mutants (R259Q, R259N, R259D and R259L; Q90R, Q90N, Q90E, Q90D and Q90L) and two engineered *lox* sequences, designated *lox*IL2 and *lox*IL3, which have a corresponding sequence of *lox*LTR1 at the 6th or 1st positions of inverted repeat sequences, respectively, based on the structure of *lox*IL1. The recognition of *lox*IL2 and *lox*IL3 by Cre protein seems to be a critical step in the recognition process of

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*lox*LTR1 since their binding affinity depends primarily on major groove interaction in many cases. By estimating the degree of interaction which should be proper in order to mediate efficient catalysis, we were able to capture information about the possibility of HIV-1 gene therapy. In this paper, we report the reduced binding affinity of the mutants and the characterization of their involvement in recombination events.

METHODS

Preparation of DNA Substrate

*Lox*IL2 and *lox*IL3, asymmetric *lox*P mutants, were designed based on *lox*LTR-1 and *lox*IL1 (Table I). To construct *lox*IL2 and *lox*IL3-ligated plasmids, pYLIL2 and pYLIL3 respectively, *lox*IL2 and *lox*IL3 were made flanked by *Bam*HI and *Xho*I sites (Fig. 1). After annealing of both strands, the cohesive ends of *Eco*RI and *Hind*III were formed. The annealed DNA was substituted for the *lox*P in pRH43 [Abremski et al., 1983] as previously described [Lee and Park, 1988; Lee and Saito, 1998]. Plasmid pRH43 was kindly presented from Dr. R. Hoess (DuPont Merck Pharmaceutical Co.).

Construction of Wild- and Mutant-Type Cre Expression Vector

The *cre* gene was cloned into between *Nde*I–*Xho*I site at pET-22b(+) vector (Novagen). A fragment of Cre coding sequence was synthesized by PCR using pRH200 as a template and *Pfu* DNA polymerase (Stratagene). Plasmid pRH200 was also the gift from Dr. R. Hoess. The 5′-, N-terminal primer contains an *Nde*I site, and has the sequence of 5′ TAGGGCATCATATGTCCAATTTACTGACCGTACAC 3′ [Shaikh and Sadowski, 1997]. The 3′-, C-terminal primer contains a *Xho*I site, and has the sequence of 5′ AGGATCTCGAGCTAATCGCCATCTTCCAGC 3′. All site-directed mutagenesis of Cre were performed by 'Megaprimer' methods [Sarkar and Sommer, 1990] with improved modification [Datta, 1997]. Three PCR primers were used to perform the two rounds of PCR. The 5′- and 3′-primers are described above, and the sequences of oligonucleotides used for mutagenesis are indicated in Table II. The first round PCR using the mutagenic primer and the 3′-primer was performed to produce megaprimer (325 bp) for each mutation. The megaprimer was used as a 3′-primer for the second round PCR. The second PCR product bearing nucleotide changes was also cloned into the *Nde*I–*Xho*I sites of pET-22b(+) expression vector. The DNA sequences of the mutants were confirmed by DNA sequencing.

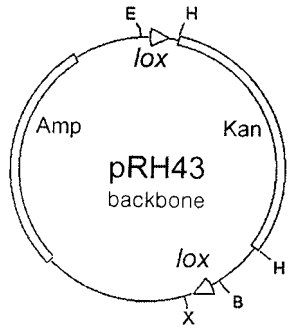

Fig. 1. Schematic diagram of variant *lox*-ligated plasmids (pYLSL, pYLIL1, pYLIL2, pYLIL3). (E, *Eco*RI; H, *Hind*III; B, *Bam*HI; and X, *Xho*I. Amp, Ampicillin resistant gene; and Kan, Kanamycin resistant gene.)

Purification of Cre Protein

The recombinant Cre expression vectors were transformed into *Escherichia coli* strain BL21(DE3) (Novagen). Wild- and mutant-type Cre were purified essentially as described by Abremski and Hoess [Abremski and Hoess, 1984] with slight modification [Lee and Park, 1998; Lee and Saito, 1998]. Wild-type Cre and most Cre mutants were eluted with $0.6 \times$ TSEG ($X \times$ TSEG = 20 mM Tris–HCl, pH 7.5, 1 mM EDTA, 10% (v/v) glycerol, and X is the molar concentration of NaCl [Abremski et al., 1983]) from phosphocellulose column (Whatman). However, R259Q and R259D were eluted with $0.4 \times$ TSEG buffer. Protein concentration was determined by the method of Peterson [Peterson, 1977]. Each of the protein was checked by SDS-PAGE [Laemmli, 1970] to confirm purity and relative protein concentration. Both wild- and mutant-type Cre represented about 90% homogeneity.

In Vitro Recombination Assay

The *lox*IL2 and *lox*IL3 recombination was determined by the restriction enzyme analysis [Abremski et al., 1983] with slight modification [Lee and Park, 1998; Lee and Saito, 1998]. Briefly, recombination reactions were carried out in a 50 μl containing 50 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 30 mM NaCl, 0.5 mg/ml bovine serum albumin, 0.5 μg purified wild-type Cre, and 2 μg *lox*P, *lox*IL1, *lox*IL2 or

TABLE I. Design of Engineered *lox* Sequence Containing Part of Long Terminal Repeat of HIV-1; Comparison of Variant Lox Sequences[a]

	Variant *Lox* Sequence
*lox*P	5′ ATAACTTCGTATAATGTATGCTATACGAAGTTAT 3′
loxSL	5′ ATAACTTCGTATA**AGTTGAAC**TATACGAAGTTAT 3′
loxIL1	5′ **TC**AACTTCGTATA**AGTTGAAC**TATACGAAGTT**GA** 3′
loxIL2	5′ **TC**AACTT**A**GTATA**AGTTGAAC**TATAC**C**AAGTT**GA** 3′
loxIL3	5′ **TC**AACTTCGTAT**CAGTTGAACC**ATACGAAGTA**GA** 3′
loxLTR-1	5′ **TC**AA**G**TT**A**GTA**CC****AGTTGAACC**A**G**A**G**C**A**AGT**AGA** 3′

[a]Spacer regions are underlined and in bold-faced, and engineered sites are only in bold-faced.

TABLE II. Sequences of Oligonucleotides Used for Site-Directed Mutagenesis of Gln90 or Arg259[a]

	Sequence of oligonucleotides (5′——→3′)	
Glu90	GCCCAAATGTTGCTGGATAGTTTTTACTGCCAG	wild-type
Q90R	GCCCAAATGACGCTGGATAGTTTTTACTGCCAG	(CGT: Arg)
Q90N	GCCCAAATGGTTCTGGATAGTTTTTACTGCCAG	(AAC: Asn)
Q90E	GCCCAAATGTTCCTGGATAGTTTTTACTGCCAG	(GAA: Glu)
Q90D	GCCCAAATGGTCCTGGATAGTTTTTACTGCCAG	(GAC: Asp)
Q90L	GCCCAAATGCAGCTGGATAGTTTTTACTTGCCAG	(CTG: Leu)
Arg259	CTATCTTCTCGCGCCCTGGAAGGGATTTTTGAAGC	wild-type
R259Q	CTATCTTCTCAGGCCCTGGAAGGGATTTTTGAAGC	(CAG: Gln)
R259N	CTATCTTCTAACGCCCTGGAAGGGATTTTTGAAGC	(AAC: Asn)
R259D	CTATCTTCTGACGCCCTGGAAGGGATTTTTGAAGC	(GAC: Asp)
R259L	CTATCTTCTCTGGCCCTGGAAGGGATTTTTGAAGC	(CTG: Leu)

[a]Mutated regions for coding different amino acid are underlined and corresponding codons and amino acids are listed in parentheses.

*lox*IL3-ligated plasmid DNA (pRH43, pYLIL1, pYLIL2, or pYLIL3, respectively). Reaction mixtures were incubated at 37°C for 30 min, and then the reactions were terminated by phenol/chloroform extraction followed by ethanol precipitation. The recovered DNA was treated with 20 units of *Eco*RI for the restriction enzyme analysis and DNA fragments were detected by ethidium bromide staining after electrophoresis on 1% agarose gel in 1 × TBE (1 × TBE contains 90 mM Tris-borate and 2 mM EDTA).

Electrophoretic Mobility Shift Assay (EMSA)

EMSA was used under the condition described by Wierzbick et al. [Wierzbicki et al., 1987]. *Lox*P probe was obtained from 50-bp *Bam*HI–*Xho*I fragment of pRH43. *Lox*IL2 and *lox*IL3 probes were from 52-bp *Eco*RI–*Hind*III fragment of pYLIL2 and pYLIL3, respectively. Each fragment was labeled with (γ-^{32}P)ATP (NEN) and T4 polynucleotide kinase (TaKaRa). Binding reactions were carried out in 30 μl of reaction volume containing 50 mM Tris-HCl, pH 7.5, 30 mM NaCl, 32 μg/ml bovine serum albumin, 10 mM 1,4-dithiothreitol, 10 mM $MgCl_2$, 5% (v/v) glycerol, 10,000 cpm of ^{32}P-labeled probe, and various amounts of wild-type Cre. Twenty narograms of each of wild- or mutant-type Cre (CreK244R and CreK244L) were used in EMSA. The mixtures were incubated at 30°C for 15 min followed by the addition of 0.1 μg of heparin (Sigma) as a nonspecific competitor or 1 μg of poly (dI-dC) as a weaker one to each sample. After 1 min, samples were loaded onto a 5% native polyacrylamide gel in the presence of 0.25 × TBE. The gel was dried and autoradiographed with Agfa CP-BU film at −70°C for 12 h. The percentage of retarded probe was quantified by image analysis program, SigmaGel (SPSS Inc.).

RESULTS

Characterization of Arg259 and Gln90 Residues of Cre

In order to determine the role of the two residues in Cre–*lox*P interaction, we performed site-directed mutagenesis in which Arg259 and Gln90 were changed into four and

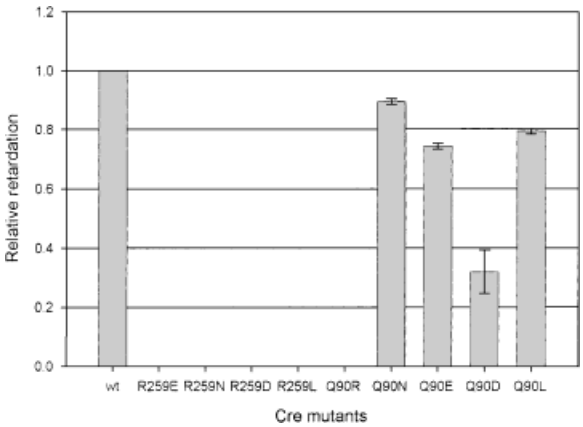

Fig. 2. Relative retardation of *lox*P by Cre mutants, five Gln90 mutants and four Arg259 mutants, compared with that by wild-type Cre. Twenty narograms of protein was used in this EMSA. The retardation of each probe by wild-type Cre with respect to complex II was regarded as 1 ×. Each value shows the mean of three independent experiments. Error bar indicates the standard deviation.

five different amino acids, respectively (R259Q, R259N, R259D and R259L; Q90R, Q90N, Q90E, Q90D and Q90L). They were preferred to other mutants in that they have proper chemical properties in order to interact efficiently with their DNA substrates. We carried out EMSA with ^{32}P-labeled *lox*P and the Cre mutants.

Figure 2 shows that Arg259, which interacts with DNA substrate by strong hydrogen bonding using its guanidinium group at the end of the side chain, is one of the most important residues in Cre–*lox*P interaction. Therefore, its mutants completely lose their binding affinity. Several single amino acid-substituted Cre mutants were tested before by Hartung and all of them had at least 5% retardation. It has been known that Cre protein have a few and scattered binding domains. This is a novel finding since Arg259 make more strong interaction than any other domain. This result also shows that pivotal interaction between protein and DNA is achieved in the major groove as we expected.

In the case of Gln90, which has a polar side chain forming a hydrogen bond with DNA at physiological pH, the binding affinity of the mutants was more retained compared with that of wild-type Cre. However, Q90R did not restore its binding affinity at all because the side chain of the mutant is too bulky to interact with DNA in the proper position and orientation. It is noticeable that Gln90 is not likely to bind to DNA strongly by hydrogen bonding because Q90L, which is likely to bind mostly to DNA by hydrophobic interaction by leucine at the residue, shows about 80% retardation compared to wild-type Cre. The slightly decreased binding affinities of Q90N and Q90E may be due to the shortened length of the carbon chain, and a negatively charged side chain that produces electrostatic repulsion against the phosphate group in DNA, respectively. These two factors regarding Q90N and Q90E can also be applied to the case of Q90D, so it shows about 30% retardation compared to wild-type Cre.

In vitro recombination assays using pRH43 as a substrate were carried out with two kinds of Cre mutants. Figure 3 reveals that all mutants except Q90R were able to recombinase the substrate efficiently. As mentioned above, Q90R has a very bulky side chain which effectively prevents its interaction with DNA. DNA (2.5 kbp) linearized by *Eco*R1 and 1.8 kbp supercoiled DNA were identified as expected

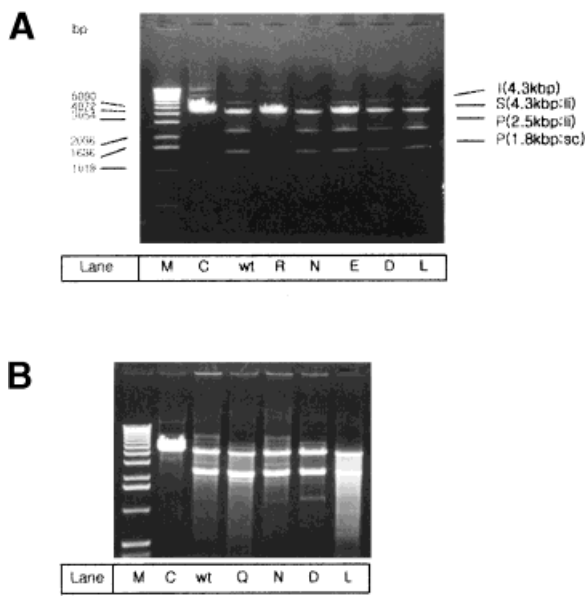

Fig. 3. In vitro recombination assay using pRH43 as a substrate. Two micrograms of substrate DNA and 500 ng of Cre were used. (**A**) Lane M, DNA size marker (DNA Molecular Weight Maker X, Boehringer Mannheim); C, without Cre; wt, wild-type Cre; R, Q90R; N, Q90N; E, Q90E; D, Q90D; L, Q90L. S, P, and I are substrate, product, and intermediate, respectively. (li, linear; and sc, supercoiled DNA.) (**B**) Lane M, the same DNA size marker used in (A); C, without Cre; wt, wild-type Cre; Q, R259Q; N, R259N; D, R259D; L, R259L. The same pattern as (A) is observed.

products [Lee and Park, 1998; Lee and Saito, 1998]. This result indicates that it is not the case that strong binding affinity is needed to operate efficient catalysis.

Charaterization of 1- and 6- Positions in Inverted Repeat Sequences of *lox*LIL1

The construction of the *lox*P mutants only at position 1 or 6 in inverted repeat sequences was already accomplished by Hartung et al. [Hartung and Kisters-Woike, 1998]. However, we performed the *lox*P mutation based on the structure of *lox*IL1, which has the corresponding sequence of *lox*LTR-1 at the spacer region and the last two bases in inverted repeat sequences. Figure 4 indicates that the 12th and 13th bases of the *lox*IL1 were not crucial for Cre–*lox*P interaction so we could use it as an intermediate to effect further modification of the DNA.

In order to test the effect of variation of the DNA in the major groove, we constructed two asymmetric engineered *lox* fragments, designated *lox*IL2 and *lox*IL3. From Figure 4, we could not detect any Cre molecules that bind to *lox*IL2 or *lox*IL3. This might easily be expected from the fact that even a single mutation in the inverted repeat sequences of *lox*P can cause a significant decrease in its binding affinity to Cre [Hartung and Kisters-Woike, 1998]. No trace of retardation band was identified with 50 ng of Cre.

To assay the recombination efficiency of *lox*IL2 and *lox*IL3, in vitro recombination assays were carried out with pRH43, pYLSL, pYLIL1, pYLIL2 and pYLIL3. Figure 5 reveals that the efficiency of recombination of the pYLIL2 and pYLIL3 was as clear as pRH43, that is, it was extremely efficient. This implies that pYLIL2 and pYLIL3 are still excellent DNA substrates in spite of their negligible binding affinity to Cre. It is likely that there was a difference in experiment results between in vivo and in vitro. Hartung described that even a single symmetric substitution at position 2 or 6 in inverted repeat sequences can abolish catalysis mediated by wild-type Cre in in vivo recombination assay. However, according to the results we obtained, asymmetric mutations at three positions including position 6 did not inhibit in vitro recombination events. We performed another experiments with Cre mutants of Arg259 or Gln90, and obtained the same results as shown in Figure 3A and B in vitro recombination assay as expected (data not shown). We performed another EMSA using a weaker non-specific competitor of DNA, poly (dI-dC). In EMSA using 1 μg of poly (dI-dC), Q90R forms the most retarded complex with *lox*IL3 among five mutants (data not shown). This indicates that bulky arginine in this residue causes steric hindrance with vicinal amino acid, so the 3D structure of Cre molecule was severely distorted and interaction with

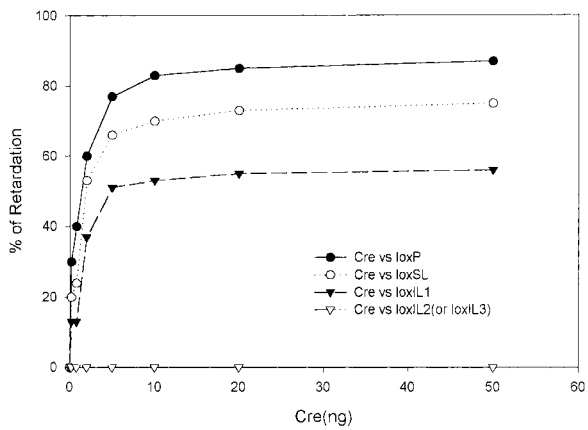

Fig. 4. Retardation of *lox*P or its variants, *lox*SL, *lox*IL1, *lox*IL2 and *lox*IL3 with various amount of wild-type Cre. Retardation values were determined in average of three independent experiments by quantification of SigmaGel after autoradiography. The percentage of retardation is calculated by the following equation: (Retarded probe/(Retarded probe+Unbound free probe)) × 100 (%).

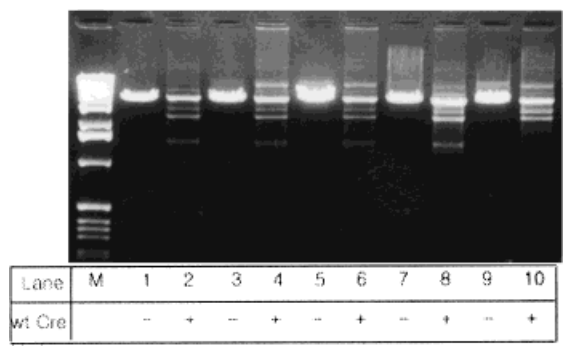

Fig. 5. In vitro recombination assay using variant *lox*-ligated plasmids (pRH43, pYLSL, pYLIL1, pYLIL2, pYLIL3) as substrates. Three micrograms of each substrate DNA and wild-type Cre were used. Lane M, DNA size marker (DNA Molecular Weight Maker X, Boehringer Mannheim); 1 and 2, pRH43; 3 and 4, pYLSL; 5 and 6, pYLIL1; 7 and 8, pYLIL2; 9 and 10, pYLIL3 (Odd numbers, without Cre; Even numbers, with Cre). The pattern of recombination activity is optically identical throughout all five different DNA substrates.

*lox*P was hampered. However, the binding affinity by *lox*IL3 was somewhat increased without catalytic activity. The result indicated that in Cre–*lox* system single binding between Cre and DNA substrate is not sufficient to undergo efficient recombination and vice versa.

DISCUSSION

In this report, we have described the Cre–*lox*P interaction in the major groove where Gln90 and Arg259 of Cre interact with the corresponding bases at the 1st and 6th positions of inverted repeat sequences, respectively. Four Arg259 mutants and five Glu90 mutants were made and *lox*IL2 and *lox*IL3 were also presented as substrates. From EMSA, we concluded that Arg259 is the most important residue in the Cre–*lox*P interaction among the residues that have been tested so far and Gln90 is not. Also, arginine itself is believed to be one of the important residues and has a critical role in protein–DNA interaction. This was inferred from the following two facts. First, Arginine has a bulky side chain that could disturb protein–DNA interaction unless the position and orientation are proper. On the other hand, the guanidinium group at the end of the side chain can be utilized as an excellent hydrogen bonding donor. So only wild-type Cre fully retained its binding affinity and the binding of Q90R was completely disrupted only among the five Gln90 mutants. It is noticeable that Q90L has only about 20% decreased binding affinity compared with wild-type Cre, which means that the interaction between Gln90 and DNA might not be maintained principally by hydrogen bonding. In summary, Arg259 and Gln90 must absolutely belong to the binding domain because mutation of the residues was solely responsible for the abrupt decrease in binding affinity but did not change their recombination activity. Figures 4 and 5 also show that the decreased binding affinity caused by the mutation of the base of DNA had no effect whatsoever on Cre–*lox* recombination activity at all. So *lox*IL2 and *lox*IL3 are still excellent substrates for Cre mediated recombination. This means there is no strict condition of base identity in inverted repeat sequences for catalysis. *Lox*IL2 and *lox*IL3 have about 79 and 82% total sequence identities with *lox*LTR-1 as depicted in Table II. In addition, the result that Q90R forms the most retarded complex with *lox*IL3 among the five mutants for the case in which 1 μg of poly (dI-dC) is used implied that Q90R allowed better partitioning into the *lox*IL3 than other mutants, even though it has no recombination activity at all (data not shown). This indicates that strong binding is not required to operate Cre–*lox* recombination and vice versa. Unfortunately, among the single amino acid-substituted Cre mutants we made, we could not find any that could mediate recombination between two *lox*LTR-1 sites (data not shown). In spite of this result, since we found that Arg259 is the most important residue in Cre–DNA interaction and many parts of the protein are involved in binding to its substrate, we could make a Cre protein that could still mediate recombination on DNA with two *lox*LTR-1 sites by substitution of some of the residues into proper ones. This is one of the most promising candidate methods for HIV-1 gene therapy. Therefore, it is possible to utilize Cre–*lox*P system as a common tool in a variety of gene manipulations including antiviral strategies.

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REFERENCES

Abremski K, Hoess R. 1984. Bacteriophage P1 site-specific recombination. J Biol Chem 259:1509–1514.

Abremski K, Hoess R, Sternberg N. 1983. Studies on the properties of P1 site-specific recombination: evidence for topologically unlinked products following recombination. Cell 32:1301–1311.

Dale EC, Ow DW. 1990. Intra- and intermolecular site-specific recombination in plant cells mediated by bacteriophage P1 recombinase. Gene 91:79–85.

Datta AK. 1997. Efficient amplification using 'megaprimer' by asymmetric polymerase chain reaction. Nucleic Acids Res 23:4530–4531.

Flowers CC, Woffendin C, Petryniak J, Yang S, Nabel GJ. 1997. Inhibition of recombinant human immunodefficiency virus type 1 replication by a site-specific recombinase. J Virol 71:2685–2692.

Guo F, Gopaul DN, van Duyne GD. 1997. Structure of Cre recombinase complexed with DNA in a site-specific recombination synapse. Nature 389:40–46.

Hartung M, Kisters-Woike B. 1998. Cre mutants with altered DNA binding properties. J Biol Chem 273: 22884–22891.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685.

Lee Y, Park J. 1998. A novel mutant *lox*P containing part of long terminal repeat of HIV-1 in space region: presentation of possible target site for antiviral strategy using site-specific recombinase. Biochem Biophys Res Commun 253:588–593.

Lee G, Saito I. 1998. Role of nucleotide sequences of *lox*P spacer region in Cre-mediated recombination. Gene 216:55–65.

Luscombe NM, Laskowski RA, Thornton JM. 1997. NUCPLOT: a program to generate schematic diagrams of protein–nucleic acid interactions. Nucleic Acids Res 25:4940–4945.

Odell J, Caimi P, Sauer B, Russell S. 1990. Site-directed recombination in the genome of transgenic tobacco. Mol Gen Genet 223:369–378.

Peterson GL. 1977. A simplification of the protein assay method of Lowry et al. which is more generally applicable. Anal Biochem 83:346–356.

Russ AP, Friedel C, Grez M, von Melchner H. 1996. Self-deleting retrovirus vectors for gene therapy. J Virol 70:4927–4932.

Sarkar G, Sommer SS. 1990. The Megaprimer method of site-directed mutagenesis. Biotechniques 8:404–407.

Sauer B. 1987. Functional expression of the Cre-*lox* site-specific recombination system in the yeast *Saccharomyces cerevisiae*. Mol Cell Biol 7:2087–2096.

Sauer B, Henderson N. 1988. Site-specific DNA recombination in mammalian cells by the Cre recombinase of bacteriophage P1. Proc Natl Acad Sci USA 85: 5166–5170.

Shaikh AC, Sadowski PD. 1997. The Cre recombinase cleaves the *lox* site in Trans. J Biol Chem 272:5695–5702.

Stark WM, Boocock MR, Sherratt DJ. 1992. Catalysis by site-specific recombinase. Trends Genet 8:432–439.

Sternberg N, Hamilton D. 1981. Bacteriophage P1 site-specific recombination. J Mol Biol 150:467–486.

Wierzbicki A, Kendall M, Abremski K, Hoess R. 1987. A mutational analysis of the bacteriophage P1 recombinase Cre. J Mol Biol 195:785–794.