HIV-1 Proviral DNA Excision Using an Evolved Recombinase

Indrani Sarkar, *et al.*

Science **316**, 1912 (2007);
DOI: 10.1126/science.1141453

The following resources related to this article are available online at www.sciencemag.org (this information is current as of March 7, 2008):

Updated information and services, including high-resolution figures, can be found in the online version of this article at:
http://www.sciencemag.org/cgi/content/full/316/5833/1912

Supporting Online Material can be found at:
http://www.sciencemag.org/cgi/content/full/316/5833/1912/DC1

A list of selected additional articles on the Science Web sites **related to this article** can be found at:
http://www.sciencemag.org/cgi/content/full/316/5833/1912#related-content

This article **cites 23 articles**, 8 of which can be accessed for free:
http://www.sciencemag.org/cgi/content/full/316/5833/1912#otherarticles

This article has been **cited by** 1 article(s) on the ISI Web of Science.

This article appears in the following **subject collections**:
Medicine, Diseases
http://www.sciencemag.org/cgi/collection/medicine

Information about obtaining **reprints** of this article or about obtaining **permission to reproduce this article** in whole or in part can be found at:
http://www.sciencemag.org/about/permissions.dtl

Downloaded from www.sciencemag.org on March 7, 2008

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. Copyright 2007 by the American Association for the Advancement of Science; all rights reserved. The title *Science* is a registered trademark of AAAS.

and Alix. The budding inhibition by Cep55 overexpression confirmed this functional link but did not necessarily imply that Cep55 was required for L-domain activity. To address this issue, we depleted endogenous Tsg101 from 293T cells by small interfering RNA (siRNA) and reintroduced it by transfecting siRNA-resistant plasmids with mutations in either the PTAP-binding site [Met^{95}→Ala^{95} (M95A)] (*26*) or in the Cep55-binding region (δ158-162). Depletion of Tsg101 results in a dramatic reduction of HIV-1 infectious particle release (Fig. 4A) (*10*), and the L-domain activity was rescued by transfecting an siRNA-resistant Tsg101 but not with Tsg101(M95A). Similar experiments with Tsg101(δ158-162) showed that the Cep55-binding region in Tsg101 was not required for HIV-1 L-domain activity (Fig. 4A). Additionally, depletion of Cep55 had no effect on either Tsg101- or Alix-dependent budding (fig. S4), indicating that Cep55 is not required for L-domain activity.

We next determined the role of the Cep55-Tsg101 interaction in cytokinesis by following a similar depletion-replacement approach in HeLa cells. The percentage of multinucleated cells in Tsg101-depleted cells was restored to normal levels when the siRNA-resistant Tsg101 was reintroduced (Fig. 4B), showing that the cytokinesis defect observed with the siRNA against Tsg101 was specific. An accumulation of cells arrested at the midbody stage was also observed in Tsg101-depleted cells (Fig. 4B), and midbody morphology was nearly identical to that in control cells, with formation of apparently normal Flemming bodies. Overall, the cytokinesis arrest observed in Tsg101-depleted cells is therefore consistent with defects in abscission. The replacement of Tsg101 mutants showed a partial cytokinesis defect in cells expressing Tsg101(δ158-162), and a similar partial phenotype was observed in Tsg101(M95A)-expressing cells (Fig. 4B), whereas a Tsg101 double mutant (M95A, δ158-162) recapitulated the phenotype of Tsg101-depleted cells (Fig. 4B). The effect of Tsg101(δ158-162) could be explained by the lack of binding to Cep55 and recruitment to the midbody, but Tsg101(M95A) was recruited to the Flemming body (Fig. 4C), suggesting that a downstream defect might explain its phenotype. Alternatively, efficient Tsg101 recruitment to the midbody might occur in a complex with Alix and Cep55. Alix binds to the ubiquitin E2 variant domain of Tsg101 through a PSAP motif in the PRR, and Tsg101(M95A) cannot bind Alix (*13*), although its binding to other components of the ESCRT machinery remained unchanged (fig. S3). Thus, the partial phenotype observed with Tsg101(M95A) may indicate a requirement for the Tsg101-Alix interaction to complete abscission, although more work is needed to prove this point unequivocally. An additional requirement for other components of ESCRT-I, specifically VPS28, was strongly suggested by the phenotype observed in cells expressing Tsg101(A3), which does not bind VPS28 (*25*). Tsg101(A3) was recruited to the central region of the midbody (Fig. 4C), and the percentage of multinucleated cells induced by the A3 mutation fully accounted for the phenotype of Tsg101-depleted cells (Fig. 4B), suggesting that the Tsg101-VPS28 interaction is required to complete abscission.

We found that Cep55, a key component of the cellular machinery that mediates abscission, interacts with two endosomal proteins that facilitate retroviral budding, namely Tsg101 and Alix. The cellular pathways that mediate retroviral L-domain activity and abscission are closely interconnected, which are consistent with a model whereby the ESCRT machinery mediates membrane fission events essential for efficient separation of the daughter cells in the last step of cell division. The role of ESCRT complexes in yeast cytokinesis is unclear, but mutations in the *Arabidopsis* homolog of Tsg101 induce cytokinesis defects (*27*), suggesting that the role of the ESCRT machinery in abscission might be conserved in multicellular organisms.

References and Notes

1. U. S. Eggert, T. J. Mitchison, C. M. Field, *Annu. Rev. Biochem.* **75**, 543 (2006).
2. M. Glotzer, *Annu. Rev. Cell Dev. Biol.* **17**, 351 (2001).
3. V. Jantsch-Plunger, M. Glotzer, *Curr. Biol.* **9**, 738 (1999).
4. R. Albertson, B. Riggs, W. Sullivan, *Trends Cell Biol.* **15**, 92 (2005).
5. A. R. Skop, H. Liu, J. Yates III, B. J. Meyer, R. Heald, *Science* **305**, 61 (2004).
6. A. Gromley *et al.*, *Cell* **123**, 75 (2005).
7. S. H. Low *et al.*, *Dev. Cell* **4**, 753 (2003).
8. P. D. Bieniasz, *Virology* **344**, 55 (2006).
9. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
10. J. E. Garrus *et al.*, *Cell* **107**, 55 (2001).
11. J. Martin-Serrano, T. Zang, P. D. Bieniasz, *Nat. Med.* **7**, 1313 (2001).
12. L. VerPlank *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 7724 (2001).
13. U. K. von Schwedler *et al.*, *Cell* **114**, 701 (2003).
14. B. Strack, A. Calistri, S. Craig, E. Popova, H. G. Gottlinger, *Cell* **114**, 689 (2003).
15. J. Martin-Serrano, A. Yarovoy, D. Perez-Caballero, P. D. Bieniasz, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12414 (2003).
16. J. H. Hurley, S. D. Emr, *Annu. Rev. Biophys. Biomol. Struct.* **35**, 277 (2006).
17. S. Welsch *et al.*, *Traffic* **7**, 1551 (2006).
18. W. Xie, L. Li, S. N. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 1595 (1998).
19. N. C. Shaner *et al.*, *Nat. Biotechnol.* **22**, 1567 (2004).
20. J. F. Rual *et al.*, *Nature* **437**, 1173 (2005).
21. W. M. Zhao, A. Seki, G. Fang, *Mol. Biol. Cell* **17**, 3881 (2006).
22. M. Fabbro *et al.*, *Dev. Cell* **9**, 477 (2005).
23. A. Krempler, M. D. Henry, A. A. Triplett, K. U. Wagner, *J. Biol. Chem.* **277**, 43216 (2002).
24. J. Ruland *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1859 (2001).
25. J. Martin-Serrano, T. Zang, P. D. Bieniasz, *J. Virol.* **77**, 4794 (2003).
26. O. Pornillos *et al.*, *EMBO J.* **21**, 2397 (2002).
27. C. Spitzer *et al.*, *Development* **133**, 4679 (2006).

28. We thank P. Bieniasz for stimulating discussions, M. Agromayor and M. Malim for critical reading of the manuscript, and W. Sundquist and M. Malim for reagents. This work was supported by Career Establishment Grant G0400207 from the Medical Research Council UK. J.G.C. is a Beit Memorial Research Fellow.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1143422/DC1
Materials and Methods
Figs. S1 to S4
References

4 April 2007; accepted 25 May 2007
Published online 7 June 2007;
10.1126/science.1143422
Include this information when citing this paper.

HIV-1 Proviral DNA Excision Using an Evolved Recombinase

Indrani Sarkar,[1]* Ilona Hauber,[2]* Joachim Hauber,[2]† Frank Buchholz[1]†

HIV-1 integrates into the host chromosome and persists as a provirus flanked by long terminal repeats (LTRs). To date, treatment regimens primarily target the virus enzymes or virus-cell fusion, but not the integrated provirus. We report here the substrate-linked protein evolution of a tailored recombinase that recognizes an asymmetric sequence within an HIV-1 LTR. This evolved recombinase efficiently excised integrated HIV proviral DNA from the genome of infected cells. Although a long way from use in the clinic, we speculate that this type of technology might be adapted in future antiretroviral therapies, among other possible uses.

Current highly active antiretroviral therapy (HAART) targeting the viral reverse transcriptase, protease, and virus-host fusion (*1*, *2*) has transformed HIV-1 infection into a chronic illness and curtailed the morbidity of infected individuals. Furthermore, new viral targets and novel inhibition strategies are being tested for improved control of HIV-1 (*3*–*7*). However, the current treatment strategies only suppress the viral life cycle without eradicating the infection, and new strains of HIV-1 are emerging that are resistant to suppressive treatments (*8*). An attractive alternative would be the specific eradication of the HIV-1 provirus.

[1]Max-Planck-Institute for Molecular Cell Biology and Genetics, Pfotenhauerstrasse 108, D-01307 Dresden, Germany. [2]Heinrich-Pette-Institute for Experimental Virology and Immunology, Martinistrasse 52, D-20251 Hamburg, Germany.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: joachim.hauber@hpi.uni-hamburg.de (J.H.); buchholz@mpi-cbg.de (F.B.)

Downloaded from www.sciencemag.org on March 7, 2008

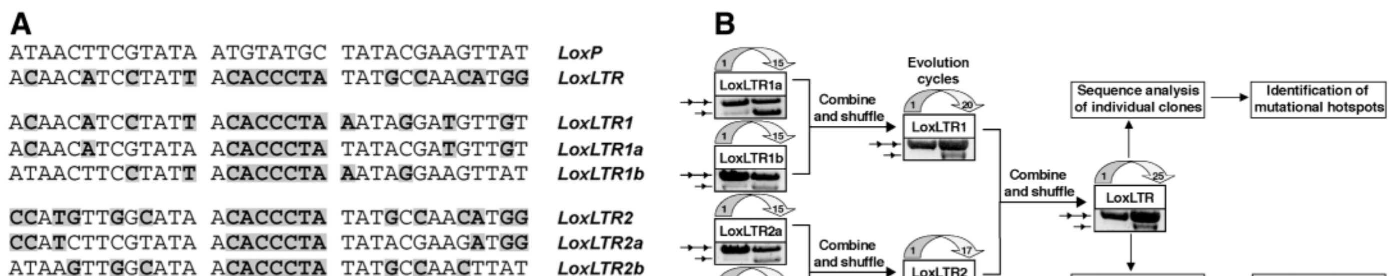

Fig. 1. Combinatorial directed-evolution strategy. (**A**) Recombinase target sites used during the evolution process are depicted. The bases highlighted in gray represent those different from loxP. (**B**) A summary of the 126 substrate-linked directed-evolution cycles is shown. The number of evolution cycles for each loxLTR subset is shown inside the arrows, with the final cycle number shown at the arrowhead. The recombinase library activity of the first and the last cycle of the target sites is shown underneath the respective targets. The unrecombined and the recombined bands are indicated as a line with two triangles or one triangle, respectively.

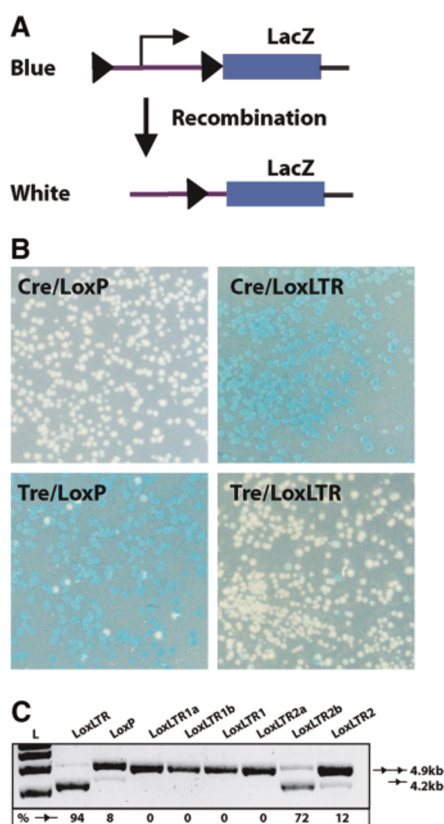

Fig. 2. Activity of Tre recombinase. (**A**) Schematic representation of the reporter assay. Site-specific recombination leads to the removal of the *E. coli* promoter, resulting in ablation of LacZ expression. (**B**) Recombination specificity of Tre illustrated using the indicated reporter plasmids. Cells were plated on X-galactosidase plates selecting for both reporter and recombinase plasmids. White colonies are produced as a result of the removal of the promoter driving lacZ expression after recombination. (**C**) Activity of Tre recombinase on indicated target sites. The lower recombined band is shown as a line with one triangle and the upper unrecombined band as a line with 2 triangles. The calculated percentage of recombined plasmid of each target is shown beneath the lanes.

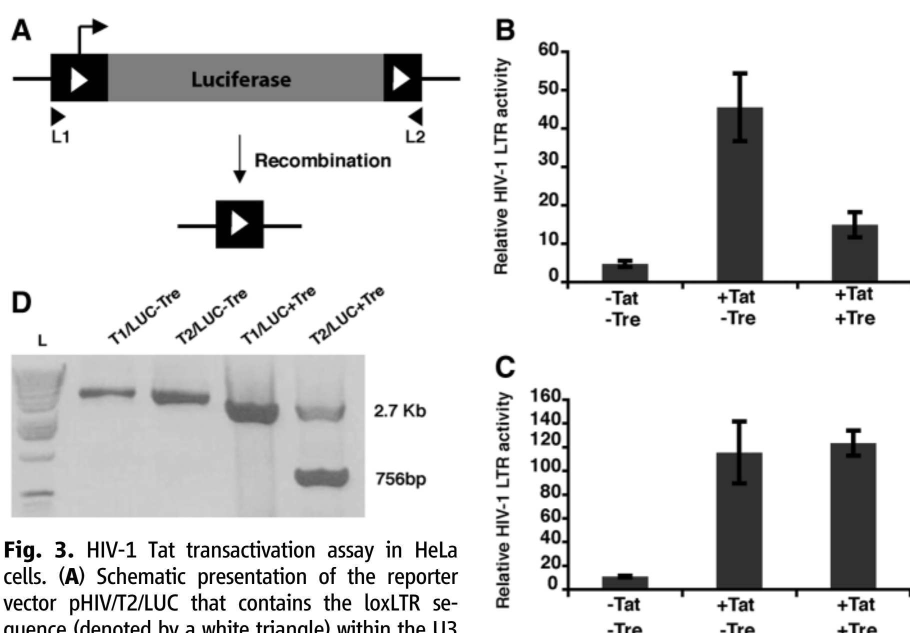

Fig. 3. HIV-1 Tat transactivation assay in HeLa cells. (**A**) Schematic presentation of the reporter vector pHIV/T2/LUC that contains the loxLTR sequence (denoted by a white triangle) within the U3 region of the HIV-1 LTR. Another copy of the loxLTR sequence, which is absent in the pHIV/T1/LUC control vector, is located 3′ of the luciferase gene. Tat transactivation is reflected by increased expression of firefly luciferase. L1 and L2 denote the primer pair used for PCR detection of recombination (black triangles). Recombination via the loxLTR sites will delete the luciferase coding region. (**B**) Relative HIV-1 LTR activity after transfection of HeLa cells with the pHIV/T2/LUC vector in presence and absence of Tre. (**C**) Relative HIV-1 LTR activity after cells were transfected with the control vector pHIV/T1/LUC in presence and absence of Tre. (**D**) PCR detection of Tre-mediated recombination in cells transiently cotransfected with Tat expression plasmid and the indicated vectors. The lower band represents the recombined fragment after loss of the luciferase gene and is only detectable in cells transfected with pHIV/T2/LUC and Tre expression vector.

Mutational and structural analyses have improved the understanding of the intricate enzymatic mechanism of site-specific recombinases and have permitted the identification of variants with altered properties [reviewed in (*9*) and (*10*)]. In particular, Cre recombinase, which has found widespread use in mouse genetics (*11*), has been intensively studied (*12*), and Cre target specificity can be altered to recognize moderately altered DNA target sites (*13*–*15*). These studies raise the possibility that new site-specific recombinases can be generated via directed evolution, which recombine more divergent target sites. More specifically, our aim was to evolve a recombinase that would recombine a sequence present within an HIV-1 LTR. Because Cre can efficiently remove genomic sequences that are flanked by two loxP sites (*16*), we and others have predicted that an evolved recombinase that would recombine a sequence present in the 5′-LTR and 3′-LTR of an integrated provirus could excise viral sequences from the genome (*13*, *17*–*19*).

To start the evolutionary process, we first scanned HIV-1 LTR sequences for a sequence with similarity to the canonical loxP site. The

Downloaded from www.sciencemag.org on March 7, 2008

chosen sequence belongs to the LTR of the primary HIV-1 strain TZB0003 (*20*) and is part of its modulatory U3 region. The selected loxLTR site is a 34-bp asymmetric sequence that has 50% sequence similarity to loxP, with four mismatches in the left element, six in the right element, and a completely different spacer (Fig. 1A). This sequence was examined in substrate-linked protein evolution in *Escherichia coli* (*13*). The loxLTR sequence was inserted into the evolution vector, and Cre and an archive of mutagenized Cre libraries (*13*) were tested for recombination activity (*21*). Recombination and subsequent polymerase chain reaction (PCR) would produce a 1.7-kb band reflecting recombination (fig. S1). However, Cre, as well as the library, failed to recombine the loxLTR sites, and no PCR product was obtained, which shows that the asymmetry and the mutations in loxLTR are too severe to result in recombination.

Because residual activity is required to start any directed evolution process (*22*), we split the original loxLTR target into two subsets. The palindromic target sites loxLTR1 and loxLTR2 were created based on the original asymmetric loxLTR sequence (Fig. 1A). However, when loxLTRs 1 and 2 were tested for recombination using either Cre or the library, no recombination was observed. Hence, the mutations in these sites were still too many for the starting library to display any activity, and this necessitated the further splitting of loxLTRs 1 and 2 by evenly dividing the half-site mutations to form four new subsets, termed loxLTRs 1a, 1b, 2a, and 2b (Fig. 1A). Splitting the mutations facilitated rec-

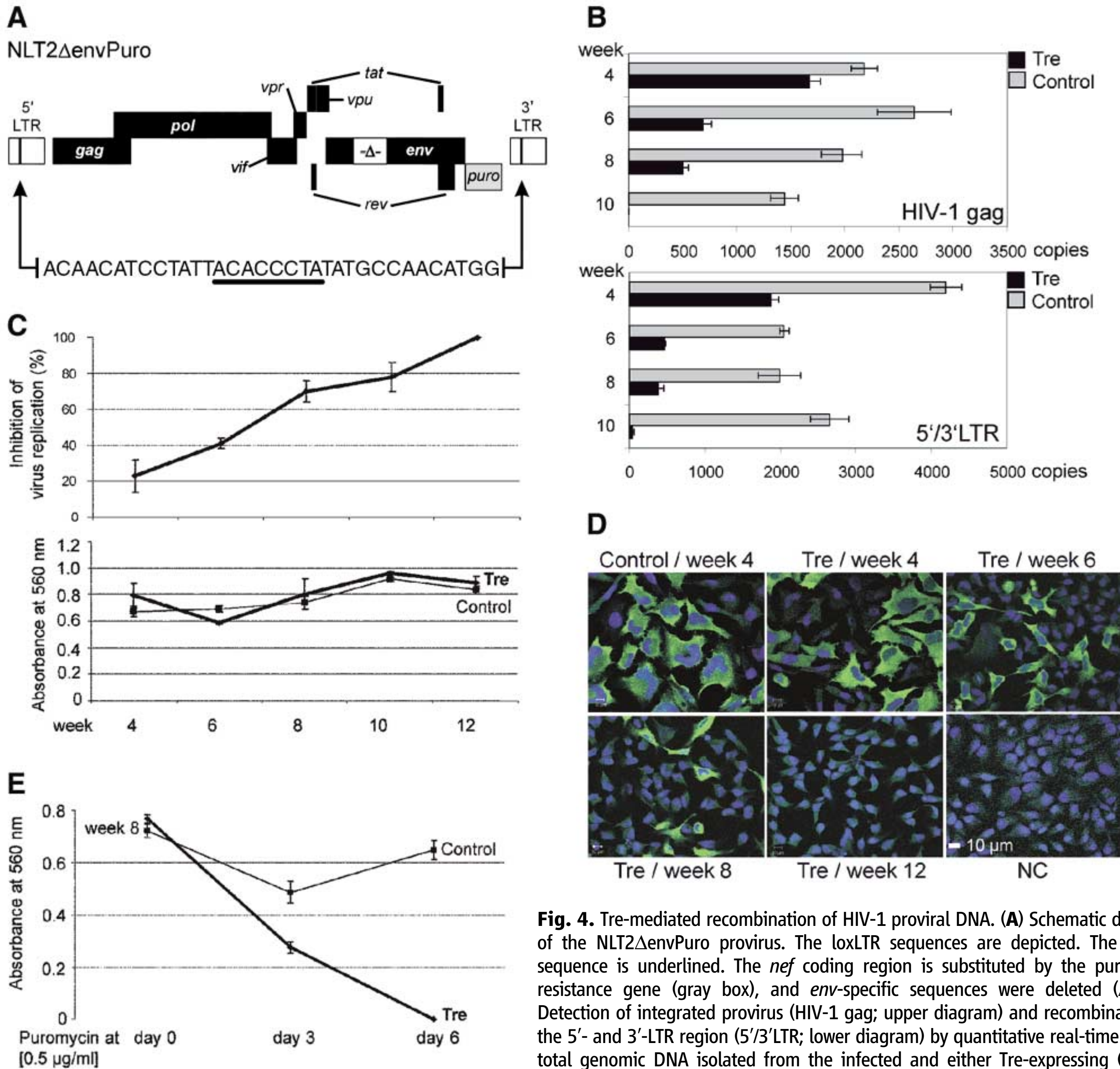

Fig. 4. Tre-mediated recombination of HIV-1 proviral DNA. (**A**) Schematic diagram of the NLT2ΔenvPuro provirus. The loxLTR sequences are depicted. The spacer sequence is underlined. The *nef* coding region is substituted by the puromycin resistance gene (gray box), and *env*-specific sequences were deleted (Δ). (**B**) Detection of integrated provirus (HIV-1 gag; upper diagram) and recombination of the 5′- and 3′-LTR region (5′/3′LTR; lower diagram) by quantitative real-time PCR of total genomic DNA isolated from the infected and either Tre-expressing (Tre) or Tre-deficient (Control) cells at indicated weeks after transfection. (**C**) Analysis of virus particle release and cell viabilities. Antigen $p24^{Gag}$ levels in the culture supernatants were determined by enzyme-linked immunosorbent assay (upper diagram) at the indicated weeks after transfection. The percentage of inhibition of viral replication within the Tre-expressing culture, compared with the control cells, is shown. Cell viabilities were simultaneously monitored (lower diagram). (**D**) Detection of Gag-expressing cells (green label) by indirect immunofluorescence in the control culture (Control) at week 4 or in the Tre-expressing culture at weeks 4, 6, 8, and 12 after transfection. Incubation of control cells with the secondary antibody alone served as negative staining control (NC). Nuclei were visualized by DRAQ5 staining (blue label). (**E**) Tre-expressing cells are sensitive to puromycin. Cells from the Tre-deficient (Control) or Tre-expressing (Tre) culture of week 8 were exposed to puromycin and monitored over time for cell viability.

Downloaded from www.sciencemag.org on March 7, 2008

ognition by recombinases in the library and hence served as a starting point for subsequent directed-evolution cycles. Reiterative directed-evolution cycles resulted in enrichment of the recombinase libraries with functional candidates (Fig. 1B). The number of evolution cycles required to obtain efficient recombinases for each loxLTR varied between the subsets, but eventually efficient recombination activity of the libraries was observed for all subsets.

To determine whether a combinatorial approach would now allow recombination of the next higher subsets, we pooled and shuffled the libraries 1a and 1b, and 2a and 2b, and cloned the products into the evolution vectors harboring loxLTR1 and loxLTR2, respectively. The combination of mutations from the different libraries resulted in synergistic effects and led to the generation of recombinases, which now recombined loxLTRs 1 and 2 (Fig. 1B), demonstrating that an evolutionary strategy traversing through intermediates can be used to achieve a desired activity.

Next, we tried to address the asymmetry of the loxLTR target site. A recombinase that recombines an asymmetric target site has to recognize half-sites of varying sequence. To determine whether this task can be accomplished through substrate-linked protein evolution, we pooled and shuffled libraries from loxLTR1 and loxLTR2 and assayed for recombination in the evolution vector harboring the loxLTR sequence. Very low recombination activity was detected in the first cycles that was enriched for functional candidates in later cycles (Fig. 1B), demonstrating that symmetry in the target site is not a prerequisite for the site-specific recombination reaction.

After a total of 126 evolution cycles, the evolution process was halted and individual loxLTR specific recombinases were examined for their recombination properties. Fifty individual recombinases were functionally analyzed in *E. coli*. The most active recombinase (termed Tre) showed efficient recombination of the loxLTR site with some residual activity for loxP (Fig. 2, A and B). To quantify the target specificity of Tre, we examined its recombination properties in the different loxLTR evolution vectors. As in the reporter assay, Tre efficiently recombined the loxLTR sequence and displayed residual activity on loxP. Tre also showed efficient recombination on loxLTR2b and residual activity on loxLTR2, but no recombination was observed on loxLTR1a, loxLTR1b, loxLTR1, and loxLTR2a (Fig. 2C). This is unexpected when taking into consideration that Tre evolved from these subsets (compare figs. S2 and S3). The reason for this target specificity is currently unknown. However, this observation confirms previous findings that target specificity is regained after initial relaxation in directed evolution over many generation cycles (*13*, *14*, *23*).

Evolved recombinases from all subsets were sequenced to monitor the evolution process. The sequences revealed clustering of mutations arising from the different subsets that were combined through the course of evolution and complemented by novel clusters in the higher subsets (fig. S2 and table S1). In total, Tre has 19 amino acid changes when compared with Cre, with many mutations originating from different subsets (fig. S3).

Next, we examined the recombination properties of Tre in mammalian cells. HeLa cells were cotransfected with recombinase expression and reporter plasmids, and recombinase activity was evaluated 48 hours after transfection. As in the *E. coli* assays, Cre efficiently recombined the loxP reporter but did not recombine loxLTR. Tre showed efficient recombination on the loxLTR reporter and some residual activity on loxP (fig. S4, A and B). To investigate whether Tre can recombine its target in a genomic context, a stable loxLTR reporter cell line was tested for recombination after transfection with a Tre expression plasmid. PCR assays and β-galactosidase activity measurements demonstrated that Tre recombines loxLTR sequences packaged in chromatin (fig. S4, C and D).

To address the question of whether recombination mediated by Tre is occurring within the context of an HIV-1 LTR, reporter constructs responsive to the HIV-1 Tat transcriptional regulator were generated and tested (*24*) (Fig. 3A). When HeLa cells were cotransfected with a Tre expression vector along with the Tat vector and pHIV/T2/LUC, luciferase activity decreased by a factor of three (Fig. 3B). In contrast, no decrease in luciferase expression was detected when the same experiment was performed using the pHIV/T1/LUC control, containing only one loxLTR site (Fig. 3C). We performed PCR analysis to prove that the observed decrease in luciferase expression was a result of recombination and not of blocking Tat-mediated transcription from the LTR promoter by the recombinase. This experiment demonstrated that the reduction of Tat activation was indeed due to Tre-mediated excision of the luciferase cassette (Fig. 3D). Gel extraction of the PCR fragments followed by sequencing confirmed the precise excision of the loxLTR-flanked sequence.

To examine whether Tre can excise the provirus from the genome of HIV-1–infected human cells, we produced loxLTR containing viral pseudotypes that were used to infect HeLa cells. A virus particle-releasing cell line was cloned and stably transfected, either with a plasmid expressing Tre or with the parental control vector. The respective cell pools were monitored with respect to recombinase activity and virus production. All assays performed demonstrated the efficient deletion of the provirus from the infected cells without obvious cytotoxic effects (Fig. 4, A to E).

These data reveal that it is possible to evolve a recombinase to specifically target an HIV-1 LTR and that this recombinase is capable of excising the respective provirus from its chromosomal integration site. Using substrate-linked protein evolution, we demonstrated that target recognition by Cre recombinase can be adapted to a target site that is asymmetric and very remote from its original site. Given the relative ease with which we have altered Cre specificity, it is likely that additional recombinases could be generated that target other sequences present in LTRs (fig. S5). We accept that this approach is unlikely to be of immediate therapeutic use and that considerable obstacles would need to be overcome before an engineered recombinase could be practically used in any clinical setting. The most important, and likely most difficult, among these is that the enzyme would need efficient and safe means of delivery and would have to be able to function without adverse side effects in relevant target cells. Nevertheless, the results we present offer an early proof of principle for this type of approach, which we speculate might form a useful basis for the development of future HIV therapies.

Downloaded from www.sciencemag.org on March 7, 2008

References and Notes

1. R. M. Gulick *et al.*, *N. Eng. J. Med.* **337**, 734 (1997).
2. J. P. Lalezari *et al.*, *N. Eng. J. Med.* **348**, 2175 (2003).
3. G. A. Donzella *et al.*, *Nat. Med.* **4**, 72 (1998).
4. H. Huthoff, M. H. Malim, *Virology* **334**, 147 (2005).
5. Y. L. Chiu *et al.*, *Nature* **435**, 108 (2005).
6. D. J. Hazuda *et al.*, *Science* **305**, 528 (2004).
7. I. Hauber *et al.*, *J. Clin. Invest.* **115**, 76 (2005).
8. S. J. Little *et al.*, *N. Eng. J. Med.* **347**, 385 (2002).
9. A. Akopian, W. Marshall Stark, *Adv. Genet.* **55**, 1 (2005).
10. N. D. Grindley, K. L. Whiteson, P. A. Rice, *Annu. Rev. Biochem.* **75**, 567 (2006).
11. S. Glaser, K. Anastassiadis, A. F. Stewart, *Nat. Genet.* **37**, 1187 (2005).
12. G. D. Van Duyne, *Annu. Rev. Biophys. Biomol. Struct.* **30**, 87 (2001).
13. F. Buchholz, A. F. Stewart, *Nat. Biotechnol.* **19**, 1047 (2001).
14. S. W. Santoro, P. G. Schultz, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 4185 (2002).
15. A. W. Rufer, B. Sauer, *Nucleic Acids Res.* **30**, 2764 (2002).
16. M. Lewandoski, *Nat. Rev. Genet.* **2**, 743 (2001).
17. C. C. Flowers, C. Woffendin, J. Petryniak, S. Yang, G. J. Nabel, *J. Virol.* **71**, 2685 (1997).
18. Y. Lee, J. Park, *Biochem. Biophys. Res. Commun.* **253**, 588 (1998).
19. T. Saraf-Levy *et al.*, *Bioorg. Med. Chem.* **14**, 3081 (2006).
20. J. T. Blackard *et al.*, *Virology* **254**, 220 (1999).
21. Materials and methods are available as supporting material on *Science* Online.
22. J. D. Bloom *et al.*, *Curr. Opin. Struct. Biol.* **15**, 447 (2005).
23. I. Matsumura, A. D. Ellington, *J. Mol. Biol.* **305**, 331 (2001).
24. M. Emerman, M. H. Malim, *Science* **280**, 1880 (1998).

25. We thank A. Bunk for technical assistance and A. F. Stewart for critical reading of the manuscript. The Max Planck Society supported this work. The Heinrich-Pette-Institute is a member of the Leibniz Gemeinschaft (WGL) and is supported by the Free and Hanseatic City of Hamburg and the Federal Ministry of Health.

Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5833/1912/DC1
Materials and Methods
SOM Text
Figs. S1 to S5
Table S1
References

20 February 2007; accepted 1 May 2007
10.1126/science.1141453