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Mechanistic and pharmacological analyses of HIV-1 integration

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Abstract

Significant advances have taken place in the field of human immunodeficiency virus type 1 (HIV-1) integration in recent years. Considering its essential nature, integrase has long been a target of interest for antiviral drug development. The most significant advance was the approval of the Merck compound raltegravir, the first licensed integrase inhibitor, in October 2007. Another milestone was the identification and characterization of specific nucleoprotein complexes that mediate integrase 3' processing and DNA strand transfer activities in vitro. Genome-wide distribution analyses have furthermore revealed that different retroviruses differentially target distinctive regions of chromatin during integration. For examples, lentiviruses favor actively transcribed genes whereas γ -retroviruses such as Moloney murine leukemia virus prefer transcriptional start sites. Though the underlying mechanisms are unknown for most retroviruses, the lentiviral preference is in large part guided through the interaction with the integrase binding protein lens epithelium-derived growth factor (LEDGF)/p75. Experimental methods that formed the foundations for each of these advances are described in this issue of *Methods*.

1. Introduction

Retroviral replication proceeds through an obligate integration intermediate, the provirus. For all intents and purposes, integration converts a retrovirus from an exogenous infectious agent to a neat package of chromosomal genes. From there, the virus uses cellular transcriptional and translational machineries to make the raw materials for assembling new virions. The provirus also guarantees efficient segregation of the viral genetic material to daughter cells during mitosis.

The retroviral genome is plus-stranded RNA, so some significant genetic reorganization occurs during provirus formation. The RNA is first copied into linear double-stranded cDNA by the viral reverse transcriptase enzyme. cDNA integration occurs in a series of steps, which can be summarized as: (i) assembly of the viral integrase enzyme onto the newly formed cDNA ends, (ii) 3' processing of the cDNA ends by integrase, which in most cases hydrolyzes a dinucleotide from each end, (iii) transfer of the reactive 3'-hydroxyl groups to the 5'-phosphates of a double stranded staggered cut that is made by integrase in chromosomal DNA, and (iv) repair of the resulting gapped DNA recombination intermediate, which is likely mediated by cell enzymes. Integrase functions in the context of a large nucleoprotein complex, the preintegration complex (PIC), and PICs isolated from infected cells can integrate their viral cDNA into an exogenously added target DNA in vitro [1].

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Significant advances have taken place in the field of human immunodeficiency virus type 1 (HIV-1) integration in recent years. Considering its essential nature, integrase has long been a target of interest for antiviral drug development. The most significant advance was the approval of the Merck compound raltegravir, the first licensed integrase inhibitor, in October 2007 [2]. Another milestone was the identification and characterization of specific nucleoprotein complexes that mediate integrase 3' processing and DNA strand transfer activities in vitro [3, 4]. Genome-wide distribution analyses have furthermore revealed that different retroviruses differentially target distinctive regions of chromatin during integration. For examples, lentiviruses, like HIV-1, favor actively transcribed genes [5] whereas γ -retroviruses such as Moloney murine leukemia virus (MLV) prefer transcriptional start sites [6] (see reference [7] for review). Though the underlying mechanisms are unknown for most retroviruses, the lentiviral preference is in large part guided through the interaction with the integrase binding protein lens epithelium-derived growth factor (LEDGF)/p75 [8-10]. Experimental methods that formed the foundations for each of these advances are described in this issue of *Methods*.

2. In vitro analyses of HIV-1 integrase function

The general outline of the different steps in the integration pathway were determined by analyzing the end structures of viral cDNAs extracted from acutely-infected cells [11-15] as well as those present after the formation of PIC-mediated integration products in vitro [11, 12,15]. Parallel studies, pioneered primarily by the Craigie, Leis, and Skalka laboratories, established that recombinant integrase proteins possessed 3' processing and DNA strand transfer activities in vitro [16-23]. Many of these early assays employed radiolabeled, double-stranded oligonucleotides as the viral donor DNA substrate and the target DNA into which the processed donor end integrated. 3' Processing and DNA strand transfer activities were quantified following denaturing polyacrylamide gel electrophoresis. Such designs set the stage for a burst of information on integrase structure and function, including DNA substrate sequence requirements, essential amino acid residues, and reaction mechanism (reviewed in reference [24]). The identification of specific donor DNA–integrase complexes was by contrast difficult if not impossible, as such species formed large, intractable aggregates under reaction conditions [25]. Integrase also tended to perform only half of its job: the vast majority of HIV-1 DNA strand transfer reaction products resulted from the integration of one donor DNA into only one strand of target DNA [22], as compared to the in vivo situation whereby both viral DNA ends are integrated in concerted fashion into opposing strands of target DNA with defined spacing. Modifications that included longer donor DNA substrates, molecular crowding agents, as well as the conditions under which integrase was purified, led to significant increases in the yield of concerted HIV-1 integration products [26-29]. The article by Grandgenett et al. [30] in this issue details the procedures pioneered in this laboratory for the analysis of concerted HIV-1 integration.

The addition of a large excess of competitor DNA early during the reaction time course helped to identify functional integrase–donor DNA complexes [31,32]. A shortcoming of these early advances was the inability to directly visualize the reaction intermediate. Using conditions established to promote concerted DNA integration [28] together with electrophoresis modifications that reduced nonspecific interactions between integrase and the gel matrix, Li et al. identified the stable synaptic HIV-1 integrase–donor DNA complex as the functional intermediate en route to the strand transfer nucleoprotein complex [3]. The paper by Li and Craigie in this issue [33] details their methods for forming and analyzing the stable nucleoprotein complexes that mediate concerted HIV-1 DNA integration. These studies helped to solidify the contention that an integrase tetramer catalyzes concerted DNA integration in vitro [3,34-36], making it likely that the tetramer is also the active form in viral PICs.

DNA substrate modifications significantly helped to simplify and expand the utility of the oligonucleotide-based integration assay. For example, use of tractable dyes in place of radioactivity afforded time-resolved fluorescence anisotropy detection of HIV-1 integrase DNA binding and 3' processing activities [36-38]. Merkel et al. [39] not only detail here the use of fluorescence-labeled DNA substrates for real-time monitoring of these activities, they describe a novel DNA strand transfer assay that incorporates a second, biotinylated oligonucleotide as target DNA. These advances dispense the need for handling radioactivity, greatly increase the shelf life of labeled DNA substrates, and expand the number of conditions potentially analyzed per experiment, as individual reactions need not be analyzed on DNA sequencing gels.

Similar designs were previously formulated for high throughput approaches toward drug discovery [40-42]. Many of the early compounds selected via their ability to potently inhibit recombinant HIV-1 integrase failed to affect viral PIC activity in vitro, suggesting inhibition of step(s) particular to the purified integrase system [43]. One specific high throughput format, which immobilized oligonucleotide donor DNA to the plate well [41], turned out to be key. This provided significant flexibility to alter conditions after the formation of integrase-donor DNA complexes, revealing differential divalent metal co-factor requirements for complex assembly versus catalysis [44]. Reaction staging confirmed that previously identified inhibitors blocked the formation of integrase-DNA complexes in vitro [44], and was used to identify the first inhibitors that specifically blocked DNA strand transfer activity and HIV-1 infection at the integration step [45]. Grobler et al. [46] expand on this theme here by detailing scintillation proximity assays for inhibitor screening as well as characterization of integrase binding ligands in multi-well plate format.

3. Methods for analyzing integration during HIV-1 infection

Integration into the DNA of long-lived, quiescent cell types contributes a significant barrier to virus eradication (reviewed in reference [47]). Sensitive methods for quantifying HIV integration levels in human DNA are therefore important not only to understand integrase function, but also to monitor clinical disease progression. A number of early PCR-based assays employed inverse [48,49], linker-mediated ligation [50], or *Alu*-anchor [51-53] designs. Some used control samples isolated from cell lines known to harbor one or two unique proviruses, yet integration occurs throughout many genomic locations. In an initial real-time adaptation of the *Alu*-based method, Butler et al. incorporated the important concept of correlating integration signal to the known quantity of HIV-1 genomes in a DNA standard isolated from a typical polyclonal infection after propagating the cells for sufficient time to enable loss of unintegrated viral DNA forms [54]. Follow-up designs added a second nested PCR step [55, 56] and upped the number of repeat samples per experiment to increase the sensitivity limit of the *Alu*-based format [57]. Liszewski et al [58] detail here their repetitive-sampling PCR strategy for the quantification of HIV-1 integration in infected human cell DNA.

As mentioned, genome-wide integration site distribution analyses have defined features of host cell chromatin favored by different retroviral PICs during integration [7]. The technique has not only helped to understand important virus-host interactions that occur during integration, it has clinical utility for patients undergoing retroviral vector-mediated gene therapy [59]. Integration, which makes retroviral vectors attractive for therapeutic applications, has the potential side effect of insertional mutagenesis whereby genes that play important roles in cell growth control become deregulated. As an example, four of 10 children that received an MLV-based vector for the treatment of X-linked severe combined immunodeficiency syndrome developed T-cell leukemia linked to vector integration sites [59,60]. Integration site monitoring of patient samples will predictably remain an important clinical tool as vector use expands from γ -retroviruses to other viral genera. The paper by Ciuffi et al. [61] details molecular and

statistical methods developed in the Bushman laboratory for determining HIV-1 integration site distribution in cellular DNA.

One tactic for combating insertional mutagenesis is to override the natural tendency to integrate at many locations by engineering target sequence specificity, and an oft-used strategy fuses integrase to a site-specific DNA binding protein. Though such fusions could direct recombinant integrase to integrate nearby specific DNA sequences in vitro [62-64], initial transitions to the infected cell met with limited success. Integrase is expressed as part of a larger polyprotein precursor, which must be processed by the viral protease to form infectious virions, and the infectivities of viruses that harbored integrase fusion proteins in their genomes were marginal at best [64,65]. This detriment was subsequently sidestepped by supplying the integrase as a fusion to the HIV-1 accessory protein Vpr. Vpr is incorporated into virion particles at a relatively high copy number through an interaction with the p6 domain of the Gag precursor protein [66], and functional integrase was furthermore incorporated into virus particles in trans as a Vpr fusion protein [67,68]. Trans incorporated integrase proteins that harbored heterologous DNA binding elements catalyzed significant levels of HIV-1 integration [69, 70] and moreover imparted some target sequence specificity [70]. Su et al. [71] detail in this issue their virological and downstream PCR methods for quantifying site-specific HIV-1 integration in the human genome.

A number of mutations in integrase can confer resistance to inhibitors [45,72-75], including raltegravir [76-78]. These observations highlight the importance of developing second generation as well as new classes of integrase inhibitors (see reference [79]). An important aspect of preclinical drug evaluation is the selection of resistant viruses under ex vivo infection conditions, and then testing how the resulting changes in integrase impact viral fitness as well as cross resistance to other drug classes. Witmer and Danovich [80] describe here methods established in their laboratory for the selection and analysis of integrase inhibitor resistant viruses.

Analyses of PICs extracted from acutely infected cells remain a mainstay in HIV-1 integration research. For example, quantifying the level of integrase 3' processing activity that occurred during infection in the presence of a preclinical diketo acid inhibitor, as well as the in vitro integration activity of the resultant PICs, firmly established that this drug class specifically inhibited DNA strand transfer activity [45]. My colleagues and I have contributed an article on the methods we use for high multiplicity infection, extraction, partial PIC purification, and assessment of HIV-1 integrase 3' processing and DNA strand transfer activities [81].

4. Methods for integration co-factor analyses

A number of proteomic techniques have been used to identify cellular binding partners of interest. For HIV-1 integrase, these include yeast two-hybrid [82-85] and mass spectroscopic identification of co-immunoprecipitating proteins from human cells expressing recombinant integrase protein [86,87]. These results independently confirmed the identification of two now well-studied HIV-1 integrase binding proteins: integrase interactor 1 [82,84] and LEDGF/p75 [83,86,87]. Rain et al. detail the use of their yeast two-hybrid screen that identified these as well as other cellular binding partners of HIV-1 integrase [88].

Mixed results were obtained from initial studies that interrogated the role of LEDGF/p75 in HIV-1 infection using short-interfering (si) RNA [89-92]. Despite very efficient knockdowns as assessed by western blotting, some studies failed to detect an effect on HIV-1 infection [89,90] whereas others noted approximate two to five-fold defects [91,92]. Subsequent work using intensified lentiviral-mediated short-hairpin (sh) RNA expression vectors [93] and cells derived from LEDGF/p75 knockout mice [9,10] have suggested that a mere fraction of normal

protein levels can support a significant amount of viral integration. Initial differences were therefore likely due to siRNA transfection efficiency [89-91] or the use of macrophages [92], which express relatively low LEDGF/p75 levels [9]. The protein is chromatin-associated at steady-state, and a key analysis performed by Llano et al. [93] correlated residual chromatin levels to HIV-1 infectivity across a collection of cells transduced at high multiplicity with their shRNA silencing system. Cells that lacked detectable LEDGF/p75 protein supported only about 3% of residual HIV-1 infection [93]. Miest et al. [94] detail here the methods developed in the Poeschla laboratory for intensified knockdown of cell factors using lentiviral shRNA expression.

The integrase-LEDGF/p75 interaction has been studied using a variety of techniques including cellular co-localization [83,86,89,95,96], or in vitro pull-down [86,95,97-100] or co-crystallization [101,102] of purified recombinant proteins. The interaction has also been analyzed via protein-protein footprinting, a technique that ascertains surface amino acid residues of a target that are shielded from small molecule modification due to ligand binding [103]. This approach is quite flexible, as it has also been used to map protein-DNA interactions [104,105] and the binding sites of small molecules on HIV-1 integrase [106,107]. McKee et al. [108] detail herein the application of this versatile technique for the analysis of the LEDGF/p75-integrase interaction.

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References

1. Brown PO, Bowerman B, Varmus HE, Bishop JM. Cell 1987;49:347–356. [PubMed: 3032450]
2. Summa V, Petrocchi A, Bonelli F, Crescenzi B, Donghi M, Ferrara M, Fiore F, Gardelli C, Gonzalez Paz O, Hazuda DJ, Jones P, Kinzel O, Laufer R, Monteagudo E, Muraglia E, Nizi E, Orvieto F, Pace P, Pescatore G, Scarpelli R, Stillmock K, Witmer MV, Rowley M. J Med Chem 2008;51:5843–5855. [PubMed: 18763751]
3. Li M, Mizuuchi M, Burke TR Jr, Craigie R. EMBO J 2006;25:1295–1304. [PubMed: 16482214]
4. Pandey KK, Bera S, Zahm J, Vora A, Stillmock K, Hazuda D, Grandgenett DP. J Virol 2007;81:12189–12199. [PubMed: 17804497]
5. Schroder ARW, Shinn P, Chen H, Berry C, Ecker JR, Bushman F. Cell 2002;110:521–529. [PubMed: 12202041]
6. Wu X, Li Y, Crise B, Burgess SM. Science 2003;300:1749–1751. [PubMed: 12805549]
7. Bushman F, Lewinski M, Ciuffi A, Barr S, Leipzig J, Hannenhalli S, Hoffmann C. Nat Rev Microbiol 2005;3:848–858. [PubMed: 16175173]
8. Ciuffi A, Llano M, Poeschla E, Hoffmann C, Leipzig J, Shinn P, Ecker JR, Bushman F. Nat Med 2005;11:1287–1289. [PubMed: 16311605]
9. Marshall HM, Ronen K, Berry C, Llano M, Sutherland H, Saenz D, Bickmore W, Poeschla E, Bushman FD. PLoS ONE 2007;2:e1340. [PubMed: 18092005]
10. Shun MC, Raghavendra NK, Vandegraaff N, Daigle JE, Hughes S, Kellam P, Cherepanov P, Engelman A. Genes Dev 2007;21:1767–1778. [PubMed: 17639082]
11. Fujiwara T, Mizuuchi K. Cell 1988;54:497–504. [PubMed: 3401925]
12. Brown PO, Bowerman B, Varmus HE, Bishop JM. Proc Natl Acad Sci USA 1989;86:2525–2529. [PubMed: 2539592]
13. Roth MJ, Schwartzberg PL, Goff SP. Cell 1989;58:47–54. [PubMed: 2546673]
14. Pauza C. Virology 1990;179:886–889. [PubMed: 2238479]
15. Lee YM, Coffin JM. Mol Cell Biol 1991;11:1419–1430. [PubMed: 1847499]
16. Katzman M, Katz RA, Skalka AM, Leis J. J Virol 1989;63:5319–5327. [PubMed: 2555556]
17. Bushman FD, Fujiwara T, Craigie R. Science 1990;249:1555–1558. [PubMed: 2171144]

18. Craigie R, Fujiwara T, Bushman F. Cell 1990;62:829–837. [PubMed: 2167180]
19. Katz RA, Merkel G, Kulkosky J, Leis J, Skalka AM. Cell 1990;63:87–95. [PubMed: 2170022]
20. Sherman PA, Fyfe JA. Proc Natl Acad Sci USA 1990;87:5119–5123. [PubMed: 2164223]
21. Vora AC, Fitzgerald ML, Grandgenett DP. J Virol 1990;64:5656–5659. [PubMed: 2214031]
22. Bushman FD, Craigie R. Proc Natl Acad Sci USA 1991;88:1339–1343. [PubMed: 1847518]
23. LaFemina RL, Callahan PL, Cordingley MG. J Virol 1991;65:5624–5630. [PubMed: 1895409]
24. Engelman, A. Reverse transcription and integration. In: Kurth, R.; Bannert, N., editors. Retroviruses: Molecular Microbiology and Genomics. Horizon Scientific Press; Norwick, UK: 2009. in press
25. van Gent DC, Elgersma Y, Bolk MWJ, Vink C, Plasterk RHA. Nucleic Acids Res 1991;19:3821–3827. [PubMed: 1861975]
26. Goodarzi G, Im GJ, Brackmann K, Grandgenett D. J Virol 1995;69:6090–6097. [PubMed: 7666512]
27. Sinha S, Pursley MH, Grandgenett DP. J Virol 2002;76:3105–3113. [PubMed: 11884535]
28. Li M, Craigie R. J Biol Chem 2005;280:29334–29339. [PubMed: 15958388]
29. Sinha S, Grandgenett DP. J Virol 2005;79:8208–8216. [PubMed: 15956566]
30. Grandgenett DP, Bera S, Pandey KK, Vora AC, Sinha S. Methods 2009;48in press
31. Ellison V, Brown P. Proc Natl Acad Sci USA 1994;91:7316–7320. [PubMed: 8041787]
32. Vink C, Lutzke RA, Plasterk RH. Nucleic Acids Res 1994;22:4103–4110. [PubMed: 7937134]
33. Li M, Craigie R. Methods 2009;48in press
34. Bao KK, Wang H, Miller JK, Erie DA, Skalka AM, Wong I. J Biol Chem 2003;278:1323–1327. [PubMed: 12446721]
35. Faure A, Calmels C, Desjobert C, Castroviejo M, Caumont-Sarcos A, Tarrago-Litvak L, Litvak S, Parissi V. Nucleic Acids Res 2005;33:977–986. [PubMed: 15718297]
36. Guiot E, Carayon K, Delelis O, Simon F, Tauc P, Zubin E, Gottikh M, Mouscadet JF, Brochon JC, Deprez E. J Biol Chem 2006;281:22707–22719. [PubMed: 16774912]
37. Hawkins ME, Pfeiderer W, Mazumder A, Pommier YG, Balis FM. Nucleic Acids Res 1995;23:2872–2880. [PubMed: 7659509]
38. Deprez E, Tauc P, Leh H, Mouscadet JF, Auclair C, Hawkins ME, Brochon JC. Proc Natl Acad Sci USA 2001;98:10090–10095. [PubMed: 11504911]
39. Merkel G, Andrade MD, Ramcharan J, Skalka AM. Methods 2009;48in press
40. Craigie R, Mizuuchi K, Bushman FD, Engelman A. Nucleic Acids Res 1991;19:2729–2734. [PubMed: 2041748]
41. Hazuda DJ, Hastings JC, Wolfe AL, Emini EA. Nucleic Acids Res 1994;22:1121–1122. [PubMed: 8152918]
42. Vink C, Banks M, Bethell R, Plasterk RHA. Nucleic Acids Res 1994;22:2176–2177. [PubMed: 8029031]
43. Farnet CM, Wang B, Lipford JR, Bushman FD. Proc Natl Acad Sci USA 1996;93:9742–9747. [PubMed: 8790401]
44. Hazuda D, Felock P, Hastings J, Pramanik B, Wolfe A. J Virol 1997;71:7005–7011. [PubMed: 9261430]
45. Hazuda DJ, Felock P, Witmer M, Wolfe A, Stillmock K, Grobler JA, Espeseth A, Gabryelski L, Schleif W, Blau C, Miller MD. Science 2000;287:646–650. [PubMed: 10649997]
46. Grobler JA, Stillmock KA, Hazuda DJ. Methods 2009;48in press
47. Shen L, Siliciano RF. J Allergy Clin Immunol 2008;122:22–28. [PubMed: 18602567]
48. Lewis P, Hensel M, Emerman M. EMBO J 1992;11:3053–3058. [PubMed: 1322294]
49. Chun TW, Finzi D, Margolick J, Chadwick K, Schwartz D, Siliciano RF. Nat Med 1995;1:1284–1290. [PubMed: 7489410]
50. Vandegraaff N, Kumar R, Burrell CJ, Li P. J Virol 2001;75:11253–11260. [PubMed: 11602768]
51. Benkirane M, Corbeau P, Housset V, Devaux C. EMBO J 1993;12:4909–4921. [PubMed: 7505220]
52. Sonza S, Maerz A, Deacon N, Meanger J, Mills J, Crowe S. J Virol 1996;70:3863–3869. [PubMed: 8648722]

53. Chun TW, Stuyver L, Mizell SB, Ehler LA, Mican JA, Baseler M, Lloyd AL, Nowak MA, Fauci AS. *Proc Natl Acad Sci USA* 1997;94:13193–13197. [PubMed: 9371822]
54. Butler SL, Hansen MST, Bushman FD. *Nat Med* 2001;7:631–634. [PubMed: 11329067]
55. O'Doherty U, Swiggard WJ, Jeyakumar D, McGain D, Malim MH. *J Virol* 2002;76:10942–10950. [PubMed: 12368337]
56. Brussel A, Sonigo P. *J Virol* 2003;77:10119–10124. [PubMed: 12941923]
57. Agosto LM, Yu JJ, Dai J, Kaletsky R, Monie D, O'Doherty U. *Virology* 2007;368:60–72. [PubMed: 17631931]
58. Liszewski MK, Yu JJ, O'Doherty U. *Methods* 2009;48in press
59. Hacein-Bey-Abina S, Garrigue A, Wang GP, Soulier J, Lim A, Morillon E, Clappier E, Caccavelli L, Delabesse E, Beldjord K, Asnafi V, MacIntyre E, Dal Cortivo L, Radford I, Brousse N, Sigaux F, Moshous D, Hauer J, Borkhardt A, Belohradsky BH, Wintergerst U, Velez MC, Leiva L, Sorensen R, Wulffraat N, Blanche S, Bushman FD, Fischer A, Cavazzana-Calvo M. *J Clin Invest* 2008;118:3132–3142. [PubMed: 18688285]
60. Hacein-Bey-Abina S, Von Kalle C, Schmidt M, McCormack MP, Wulffraat N, Leboulch P, Lim A, Osborne CS, Pawliuk R, Morillon E, Sorensen R, Forster A, Fraser P, Cohen JI, de Saint Basile G, Alexander I, Wintergerst U, Frebourg T, Aurias A, Stoppa-Lyonnet D, Romana S, Radford-Weiss I, Gross F, Valensi F, Delabesse E, Macintyre E, Sigaux F, Soulier J, Leiva LE, Wissler M, Prinz C, Rabbitts TH, Le Deist F, Fischer A, Cavazzana-Calvo M. *Science* 2003;302:415–419. [PubMed: 14564000]
61. Ciuffi A, Ronen K, Brady T, Malani N, Wang G, Berry CC, Bushman FD. *Methods* 2009;48in press
62. Bushman FD. *Proc Natl Acad Sci USA* 1994;91:9233–9237. [PubMed: 7937746]
63. Goulaouic H, Chow SA. *J Virol* 1996;70:37–46. [PubMed: 8523550]
64. Katz RA, Merkel G, Skalka AM. *Virology* 1996;217:178–190. [PubMed: 8599202]
65. Bushman FD, Miller MD. *J Virol* 1997;71:458–464. [PubMed: 8985371]
66. Paxton W, Connor RI, Landau NR. *J Virol* 1993;67:7229–7237. [PubMed: 8230445]
67. Fletcher TM 3rd, Soares MA, McPhearson S, Hui H, Wiskerchen M, Muesing MA, Shaw GM, Leavitt AD, Boeke JD, Hahn BH. *EMBO J* 1997;16:5123–5138. [PubMed: 9305653]
68. Wu X, Liu H, Xiao H, Conway JA, Hunter E, Kappes JC. *EMBO J* 1997;16:5113–5122. [PubMed: 9305652]
69. Holmes-Son ML, Chow SA. *J Virol* 2000;74:11548–11556. [PubMed: 11090152]
70. Tan W, Dong Z, Wilkinson TA, Barbas CF III, Chow SA. *J Virol* 2006;80:1939–1948. [PubMed: 16439549]
71. Su K, Wang D, Ye J, Kim YC, Chow SA. *Methods* 2009;48in press
72. King PJ, Robinson WE Jr. *J Virol* 1998;72:8420–8424. [PubMed: 9733896]
73. Fikkert V, Van Maele B, Vercammen J, Hantson A, Van Remoortel B, Michiels M, Gurnari C, Pannecouque C, De Maeyer M, Engelborghs Y, De Clercq E, Debyser Z, Witvrouw M. *J Virol* 2003;77:11459–11470. [PubMed: 14557631]
74. Fikkert V, Hombrouck A, Van Remoortel B, De Maeyer M, Pannecouque C, De Clercq E, Debyser Z, Witvrouw M. *AIDS* 2004;18:2019–2028. [PubMed: 15577623]
75. Hazuda DJ, Anthony NJ, Gomez RP, Jolly SM, Wai JS, Zhuang L, Fisher TE, Embrey M, Guare JPI, Egbertson MS, Vacca JP, Huff JR, Felock PJ, Witmer MV, Stillmock KA, Danovich R, Grobler J, Miller MD, Espeseth AS, Jin L, Chen IW, Lin JH, Kassahun K, Ellis JD, Wong BK, Xu W, Pearson PG, Schleif WA, Cortese R, Emini E, Summa V, Holloway MK, Young SD. *Proc Natl Acad Sci USA* 2004;101:11233–11238. [PubMed: 15277684]
76. Charpentier C, Karmochkine M, Laureillard D, Tisserand P, Bélec L, Weiss L, Si-Mohamed A, Piketty C. *HIV Med* 2008;9:765–770. [PubMed: 18651855]
77. Kobayashi M, Nakahara K, Seki T, Miki S, Kawauchi S, Suyama A, Wakasa-Morimoto C, Kodama M, Endoh T, Oosugi E, Matsushita Y, Murai H, Fujishita T, Yoshinaga T, Garvey E, Foster S, Underwood M, Johns B, Sato A, Fujiwara T. *Antiviral Res* 2008;80:213–222. [PubMed: 18625269]
78. Malet I, Delelis O, Valantin MA, Montes B, Soulie C, Wirten M, Tchertanov L, Peytavin G, Reynes J, Mouscadet JF, Katlama C, Calvez V, Marcelin AG. *Antimicrob Agents Chemother* 2008;52:1351–1358. [PubMed: 18227187]

79. Kibanov OM. *Curr Opin Invest Drugs* 2009;10:190–200.
80. Witmer M, Danovich R. *Methods* 2009;48in press
81. Engelman A, Oztop I, Vandegraaff N, Raghavendra NK. *Methods* 2009;48in press
82. Kalpana GV, Marmon S, Wang W, Crabtree GR, Goff SP. *Science* 1994;266:2002–2006. [PubMed: 7801128]
83. Emiliani S, Mousnier A, Busschots K, Maroun M, Van Maele B, Tempe D, Vandekerckhove L, Moisant F, Ben-Slama L, Witvrouw M, Christ F, Rain JC, Dargemont C, Debyser Z, Benarous R. *J Biol Chem* 2005;280:25517–25523. [PubMed: 15855167]
84. Maroun M, Delelis O, Coadou G, Bader T, Segeral E, Mbemba G, Petit C, Sonigo P, Rain JC, Mouscadet JF, Benarous R, Emiliani S. *J Biol Chem* 2006;281:22736–22743. [PubMed: 16772295]
85. Christ F, Thys W, De Rijck J, Gijsbers R, Albanese A, Arosio D, Emiliani S, Rain JC, Benarous R, Cereseto A, Debyser Z. *Curr Biol* 2008;18:1192–1202. [PubMed: 18722123]
86. Cherepanov P, Maertens G, Proost P, Devreese B, Van Beeumen J, Engelborghs Y, De Clercq E, Debyser Z. *J Biol Chem* 2003;278:372–381. [PubMed: 12407101]
87. Turlure F, Devroe E, Silver PA, Engelman A. *Front Biosci* 2004;9:3187–3208. [PubMed: 15353349]
88. Rain JC, Cribier A, Gérard A, Emiliani S, Benarous R. *Methods* 2009;48in press
89. Llano M, Vanegas M, Fregoso O, Saenz D, Chung S, Peretz M, Poeschla EM. *J Virol* 2004;78:9524–9537. [PubMed: 15308744]
90. Vandegraaff N, Devroe E, Turlure F, Silver PA, Engelman A. *Virology* 2006;346:415–426. [PubMed: 16337983]
91. Vandekerckhove L, Christ F, Van Maele B, De Rijck J, Gijsbers R, Van den Haute C, Witvrouw M, Debyser Z. *J Virol* 2006;80:1886–1896. [PubMed: 16439544]
92. Zielske SP, Stevenson M. *J Virol* 2006;80:7275–7280. [PubMed: 16809334]
93. Llano M, Saenz DT, Meehan A, Wongthida P, Peretz M, Walker WH, Teo W, Poeschla EM. *Science* 2006;314:461–464. [PubMed: 16959972]
94. Miest T, Saenz D, Meehan A, Llano M, Poeschla EM. *Methods* 2009;48in press
95. Maertens G, Cherepanov P, Pluymers W, Busschots K, De Clercq E, Debyser Z, Engelborghs Y. *J Biol Chem* 2003;278:33528–33539. [PubMed: 12796494]
96. Vanegas M, Llano M, Delgado S, Thompson D, Peretz M, Poeschla E. *J Cell Sci* 2005;118:1733–1743. [PubMed: 15797927]
97. Cherepanov P, Devroe E, Silver PA, Engelman A. *J Biol Chem* 2004;279:48883–48892. [PubMed: 15371438]
98. Busschots K, Vercammen J, Emiliani S, Benarous R, Engelborghs Y, Christ F, Debyser Z. *J Biol Chem* 2005;280:17841–17847. [PubMed: 15749713]
99. Cherepanov P, Sun ZYJ, Rahman S, Maertens G, Wagner G, Engelman A. *Nat Struct Mol Biol* 2005;12:526–532. [PubMed: 15895093]
100. Cherepanov P. *Nucleic Acids Res* 2007;35:113–124. [PubMed: 17158150]
101. Cherepanov P, Ambrosio ALB, Rahman S, Ellenberger T, Engelman A. *Proc Natl Acad Sci USA* 2005;102:17308–17313. [PubMed: 16260736]
102. Hare S, Shun MC, Gupta SS, Valkov E, Engelman A, Cherepanov P. *PLoS Pathog* 2009;5:e1000259. [PubMed: 19132083]
103. McKee CJ, Kessl JJ, Shkriabai N, Dar MJ, Engelman A, Kvaratskhelia M. *J Biol Chem* 2008;283:31802–31812. [PubMed: 18801737]
104. Kvaratskhelia M, Miller JT, Budihas SR, Pannell LK, Le Grice SF. *Proc Natl Acad Sci USA* 2002;99:15988–15993. [PubMed: 12461175]
105. Zhao Z, McKee CJ, Kessl JJ, Santos WL, Daigle JE, Engelman A, Verdine G, Kvaratskhelia M. *J Biol Chem* 2008;283:5632–5641. [PubMed: 18093980]
106. Shkriabai N, Patil SS, Hess S, Budihas SR, Craigie R, Burke TR Jr, Le Grice SF, Kvaratskhelia M. *Proc Natl Acad Sci USA* 2004;101:6894–6899. [PubMed: 15118107]
107. Williams KL, Zhang Y, Shkriabai N, Karki RG, Nicklaus MC, Kotrikadze N, Hess S, Le Grice SFJ, Craigie R, Pathak VK, Kvaratskhelia M. *J Biol Chem* 2005;280:7949–7955. [PubMed: 15615720]
108. McKee CJ, Kessl JJ, Norris JO, Shkriabai N, Kvaratskhelia M. *Methods* 2009;48in press