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Posttranslational Acetylation of the Human Immunodeficiency Virus Type 1 Integrase Carboxyl-Terminal Domain Is Dispensable for Viral Replication▽

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A recent report sought to demonstrate that acetylation of specific lysines within integrase (IN) by the histone acetyltransferase (HAT) p300 regulates human immunodeficiency virus type 1 (HIV-1) integration and is essential for viral replication (A. Cereseto, L. Manganaro, M. I. Gutierrez, M. Terreni, A. Fittipaldi, M. Lusic, A. Marcello, and M. Giacca, EMBO J. 24:3070–3081, 2005). We can corroborate the efficient and specific acetylation of the IN carboxyl-terminal domain (CTD) (amino acids 212 to 288) by p300 using purified recombinant components. Although arginine substitution mutagenesis of the isolated CTD confirms that the majority of p300 acetylation occurs at lysine residues 264, 266, and 273, the pattern of acetylation is not uniform and a hierarchy of reactivity can be established. Several combinatorial mutations of the CTD lysines modified by p300 in vitro were reconstructed into an otherwise infectious proviral plasmid clone and examined for viral growth and frequency of productive chromosomal integration. In contrast to the findings of Cereseto and coworkers, who used epitope-tagged viruses for their experiments, we find that an untagged mutant virus, IN K(264/266/273)R, is fully replication competent. This discrepancy may be explained by the use of an acidic epitope tag placed at the extreme carboxyl terminus of integrase, near the target site for acetylation. Although the tagged, wild-type virus is viable, the combination of this epitope tag with the RRR substitution mutation results in a replication-defective phenotype. Although IN belongs to the very small set of nonhistone proteins modified by HAT-mediated activity, an obligate role for acetylation at the reactive CTD lysines in HIV-1 IN cannot be confirmed.

While considerable progress has been made in identifying viral and cellular components involved in human immunodeficiency virus type 1 (HIV-1) integration, a comprehensive understanding of the precise mechanistic details remains a distant goal. Nonetheless, retroviral integration is of basic interest and vital importance, and over the past few years the rational search for inhibitors of integrase function has expanded (15, 16, 25). Recently it has been shown that HIV-1 integrase (IN) is a substrate for histone acetyltransferase (HAT)-mediated p300 posttranslational acetylation both in vitro and in vivo (3). Interestingly, p300 modification targets lysine residues within the IN C-terminal domain (CTD), a domain known to possess intrinsic DNA binding activity in vitro (10, 11, 20, 21, 27, 40, 41). Since p300 acetylation affects the ability of a variety of DNA binding proteins to interact specifically with their respective target DNAs (12–14, 19, 30, 35, 42), these observations are of significant interest, especially in light of the related finding that posttranslational acetylation by p300 regulates HIV-1 integration (3).

To confirm and extend this important observation, we mutagenized an HXb2-derived HIV-1 proviral clone (R7/3) with three lysine-to-arginine substitutions at residues (K264, K266, and K273) that were previously identified as substrates for acetylation in integrase (3). The mutations were introduced by overlap PCR and the entire recombined fragment confirmed by DNA sequence analysis. Surprisingly, this IN mutant (RRR) replicated with kinetics similar to those of the wild-type (WT) virus. One possible explanation for the discrepancy between our finding and that of Cereseto et al. (3) is the use of two distinct genetic backgrounds for integrase. The earlier finding (3) was obtained in the context of Bru-FLAG, a proviral clone with a genetic pedigree convergent with that of R7/3 but encoding a FLAG tag epitope fused downstream and in frame with the C terminus of integrase (Fig. 1). This clone has been shown to be replication competent in tissue culture (24), but given the tag's proximity to the region of interest, we thought this might account for the discrepancy. To test this hypothesis, we cloned the Bru-FLAG-tagged integrase (wild type and RRR) into the R7/3 background. All viral stocks were prepared by transfecting plasmid DNA into 293T cells by using 8 μg/ml polyethylenimine. Viral supernatants (10 ng p24) were incubated overnight with CEM-SS cells (1.0×10^6). The cells were then spun down, washed once with phosphate-buffered saline, and resuspended in 8 ml fresh RPMI 1640 (containing 10% fetal bovine serum and penicillin-streptomycin). One hundred microliters of suspended cells was collected daily. Infected cultures were split 1:4 every 3 days. Viral titers were determined by use of an exogenous reverse transcriptase (RT) assay described previously (26). The radioactive products of the RT reactions were spotted on DE81 paper, detected by phosphorimaging, and quantified using ImageQuant 5.2 software. These data show that the presence or absence of the IN

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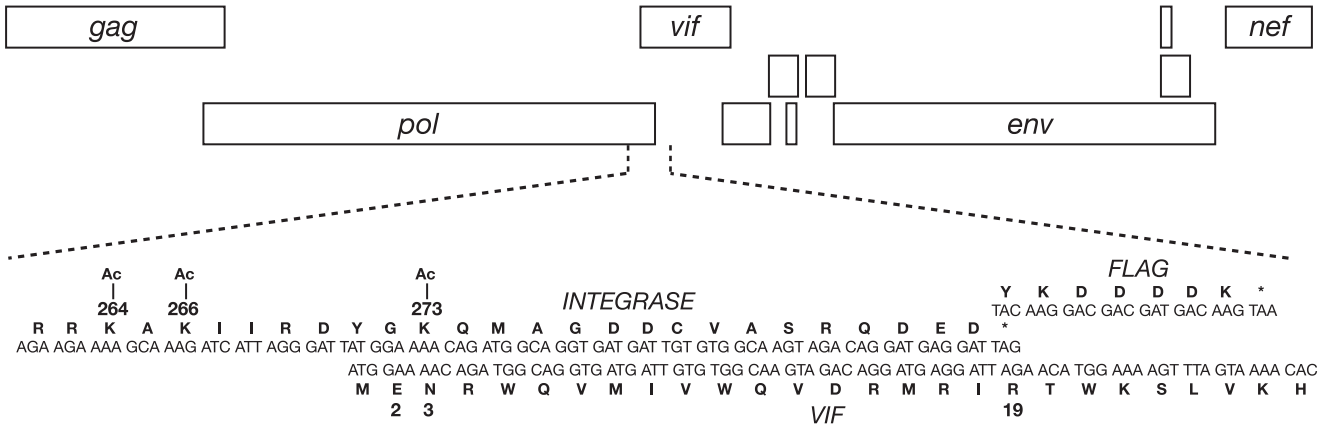

FIG. 1. Region of the IN CTD targeted for p300-mediated acetylation in vitro. The locations of the acetylated residues (Ac) are indicated. The position of the major residue targeted for acetylation in vitro, K273, is of particular interest since it is encoded in the region of the HIV-1 proviral genome where the *pol* and *vif* genes overlap. This implies that genetic constraints are likely imposed by the functional requirements of one molecule on the other, perhaps in both directions. Also shown is the sequence of the FLAG tag epitope positioned in frame with IN at its extreme carboxyl terminus.

FLAG tag is critical in the context of the mutations described (Fig. 2; Table 1). Thus, while the tagged and untagged WT viruses are replication competent, addition of the FLAG tag to the RRR substitution mutant results in a virus that is incapable of viral growth in culture. In contrast, the RRR mutant without a tag replicates well, as determined by tracking viral growth through culture (Fig. 2A) and in its ability to transduce a dominant selectable marker to recipient cells (see below and Table 1). Similar results were obtained during the infection of other sensitive cell lines (data not shown). These observations were further strengthened by comparing the relevant variables in all possible combinations (WT or RRR mutant, FLAG tagged or untagged, and IN R7/3 or IN Bru) for viral growth in culture (Fig. 2B). It is clear from the results that a replication defect occurs only when the epitope tag is present in combination with the closely spaced RRR mutation, regardless of the particular IN used, as the RRR mutation, by itself, does not preclude sustained viral replication in culture.

We are able to confirm that the integrase CTD is an efficient substrate for p300-catalyzed acetylation in vitro, with the lysine at position 273 being the primary target for modification (Fig. 3A). We constructed fusions between the maltose binding protein (MBP) in the pMAL-c2X vector (New England Biolabs) and the IN CTD (using purified protein extracts prepared as recommended by the manufacturer). Equivalent amounts of MBP or the MBP-IN CTD fusion protein were then incubated with recombinant glutathione *S*-transferase–p300 HAT domain (2) (purified over a glutathione *S*-transferase column) and ^{14}C-labeled acetyl-coenzyme A in buffer (2 mM dithiothreitol, 25% glycerol, 50 mM sodium butyrate, 145 mM Tris-HCl, pH 7.5). Reaction mixtures were incubated for 1 h at 30C. Samples were then heat denatured and electrophoresed on 4 to 12% bis-Tris polyacrylamide gels. Gels were stained with Coomassie brilliant blue to verify the normalization of the amount of protein reacted, impregnated with scintillant in drying solution (1 M sodium salicylate, 20% ethanol, 5% glycerol), dried, and exposed to film for 2 to 4 days at −80°C. It is clear that the IN CTD is a highly efficient substrate for acetylation by p300 in vitro. Indeed, on a molar basis, the IN CTD is as reactive as the positive control, a core histone preparation (Fig. 3A). Further analysis reveals that a hierarchy of reactivity can be established between the three modified residues, in which K273 ≫ K266 > K264. We also examined the specificity of reactivity of the IN CTD to two other classes of HAT proteins (30), MOZ (MYST superfamily) and PCAF (GNAT superfamily). Although these transferases efficiently acetylated the control core histone preparation, they were incapable of efficient acetylation of the IN CTD (Fig. 3B). Thus, while acetylation of the IN CTD in vitro appears to be specific for both the substrate and the class of HAT reacted, it is difficult to infer from these results their potential significance given the modest effect on viral kinetics that the untagged RRR mutant exhibits in vivo.

To determine the consequence of individual and combined substitutions, we constructed additional viral mutants and measured the accumulation of late RT products and two-long-terminal-repeat (2-LTR) circles, as well as integration frequency and syncytium formation (Table 1). None of the mutants, including RRR, exhibited significant RT defects. We can document a small but reproducible increase in 2-LTR circle formation. However, this increase is comparatively slight when contrasted with that of an integrase mutant (IN D116A) lacking all catalytic activity and associated with dramatic increases in the accumulation of 2-LTR circles (9, 38). The experiments were carefully controlled for the possibility of bacterial proviral plasmid contamination in the real-time PCRs by the use of the doubly substituted mutant proviral clone R7/3 RT$^-$ (RT D185A/D186A). The aspartic acid-to-alanine substitutions are within the highly conserved catalytic RT motif YMDD (33, 34), and the mutant is defective for reverse transcription (Table 1) but uncompromised for virion production or constituent Gag-Pol polyprotein processing (data not shown). Briefly, viral stocks were filtered (0.45 μm), aliquoted, analyzed for p24 concentration (enzyme-linked immunosorbent assay from Coulter), and stored at −80°C. Before infections, supernatants were treated with 50U/ml Turbo DNase (Ambion) at 37C for 60 min. One hundred nanograms of p24 was added to GHOST(3) X4/R5 cells (24-well plate, 90% confluent) and

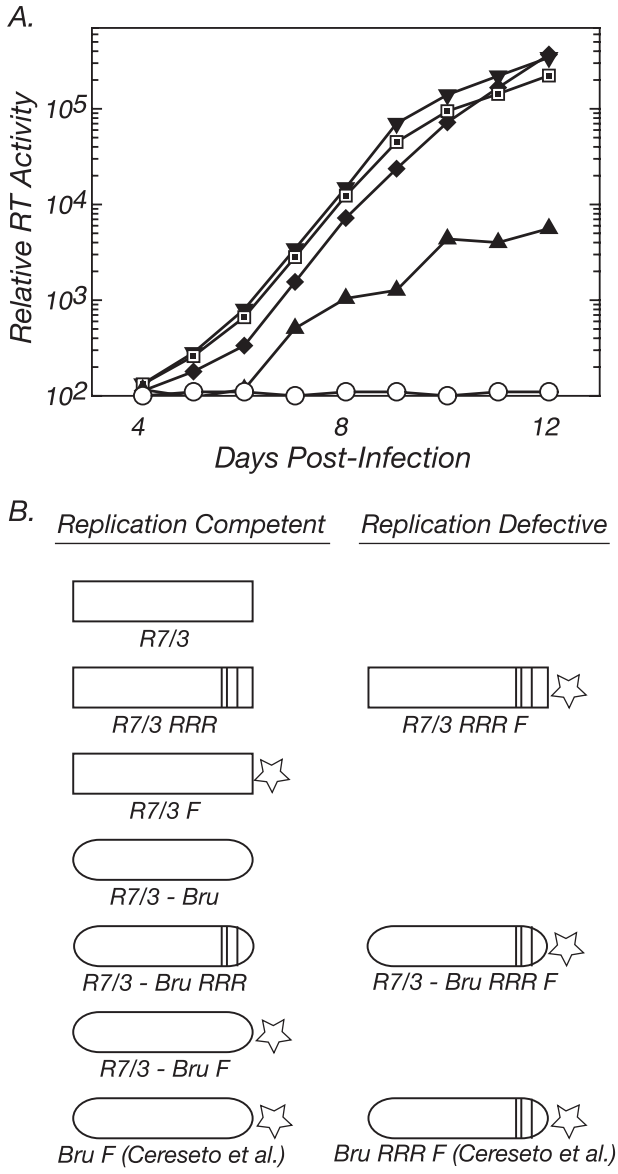

FIG. 2. Replication potential of the WT and mutant viruses in CEM-SS cells. (A) Growth curves of selected viral pairs. ▼, R7/3; ▣ R7/3 Bru F; ◆, R7/3 RRR; ▲, R7/3 Bru RRR F; ○, D116A (a catalytically inactive mutant included as a replication-defective control). (B) Summary of the replication statuses of all viruses at the end point. Viral infections were followed over 12 days and scored for replication by syncytium formation and p24 or RT production. The genotype for each is schematically indicated. The vertical lines represent the RRR substitution mutation. The presence of the FLAG tag (F) is denoted as a star. The rectangle is the integrase amino acid sequence from R7/3; the rounded rectangle, the integrase sequence from Bru (three amino acid differences exist between the two sequences). The experiments were performed using the specified integrase cassette (XbaI/SacII) in the otherwise isogenic R7/3 proviral background. Data generated using the complete Bru provirus were provided by the study of Cereseto et al. (3).

incubated in standard conditions for 24 h. Cells were washed, and total DNA was extracted using the Mini Blood kit (QIAGEN). The amount of purified DNA was determined by a PicoGreen assay (Invitrogen) and analyzed by quantitative PCR (reaction conditions and molecular beacon and primer sequences are available upon request). Data shown are the averages from duplicate infections and triplicate PCRs. At least three independent experiments were performed, and results never varied more than 10%. Viruses were also tested for their ability to productively infect CEM-SS cells by monitoring infected cultures for cytopathic effects and syncytium formation through several days in culture. These results closely paralleled those of the other assays. Finally, we employed a genetic assay to measure the respective integration frequency between the WT and mutant viruses. The WT and mutant proviral clones were recombined to replace *nef* with a dominant selectable marker, the blastocidin D gene (*bsd*), which also incorporated inactivating frameshift mutations in the genes *vpr* and *env*. Vesicular stomatitis virus G-pseudotyped viral stocks were prepared by cotransfecting R7/3 BSD expression vectors with pCI-VSV-G into 293T cells (detailed descriptions of all clones used are available upon request). HeLa P4R5 cells were plated in 24-well dishes at approximately 30% confluence and infected with 5 ng p24 in 0.5 ml for 8 h. The medium was changed, and cells were incubated for an additional 36 h before being trypsinized and serially diluted into medium supplemented with 5 μg/ml Blasticidin S HCl (Invitrogen). Cells were cultured for 1 week before staining with crystal violet. Colonies were counted to determine relative integration frequencies. Consistent with the results we have obtained using a similar

TABLE 1. Comparison of accumulation of viral DNA species, integration frequency, and sustained viral replication through culture for wild-type and integrase mutant viruses

R7/3 IN[a]	Late RT (%)[b,c]	2-LTR circles (%)[b,c]	Integration frequency (%)[c,d]	Sustained replication[e]
WT	100	100	100	+
RRR	91	411	61	+
KRR	ND[f]	ND	78	+
RKR	ND	ND	85	+
RRK	ND	ND	66	+
RKK	85	514	76	+
KRK	125	266	80	+
KKR	95	204	89	+
D116A	120	2231	<0.02	−
RTmut	<0.2	Undetected	ND	−
KKA	ND	ND	91	+
KKQ	ND	ND	82	+
KKE	ND	ND	18	−
IN Bru F	ND	ND	75	+
IN Bru RRR F	ND	ND	10	−

[a] To facilitate the cloning of the various integrase mutations, we modified R7/3 to specify unique XbaI and SacII restriction sites immediately flanking the integrase-coding sequence. The introduced XbaI and SacII sites are juxtaposed on either side of an approximately 900-base-pair integrase gene cassette maintaining wild-type code for both the *pol* and *vif* genes. The identity of the genotype of the wild-type and mutant genes cloned into R7/3 is shown.

[b] Kinetic PCR was used to precisely quantify this viral DNA species.

[c] Values are expressed as percentages relative to the value for the wild-type virus, which is set at 100, and are the averages from three independent experiments.

[d] A genetic integration assay based on the stable transduction of the dominant selectable marker Bsd.

[e] CEM-SS cells were monitored daily for the formation of multinucleated syncytia in culture. +, syncytia were apparent at 4 to 5 days postinfection; −, no syncytia were detected after 14 days of cell growth.

[f] ND, not determined.

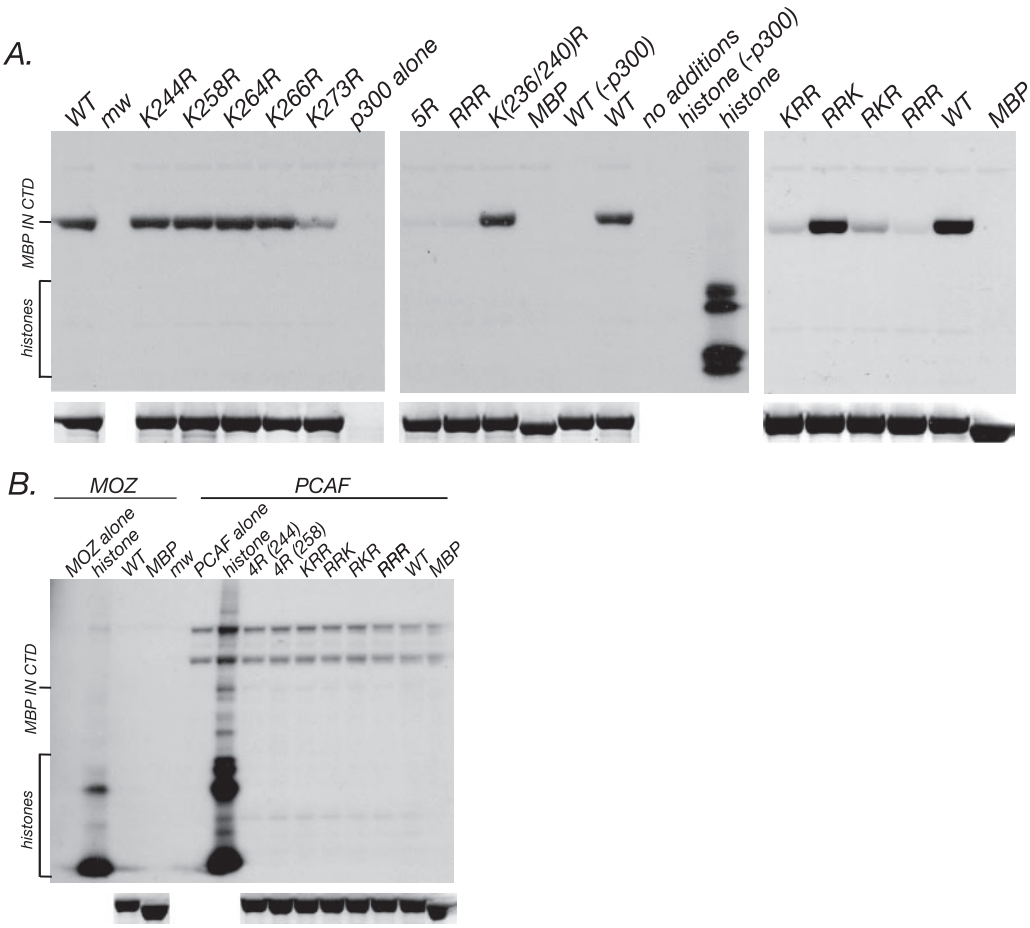

FIG. 3. The IN CTD is an efficient target for HAT-mediated acetylation by p300 in vitro. (A) Lysine residues K264, K266, and K273 are targeted for acetylation**.** The IN CTD contains nine lysine residues. Seven of the nine lysine residues (residues 215, 219, 236, 240, 244, 258, 264, 266, and 273) encoded within the IN CTD were mutagenized to specify arginine, but only three (residues 264, 266, and 273) were efficient targets of p300 modification in vitro. A hierarchy of reactivity to p300 modification exists, with K273 as the key residue targeted for acetylation. The specificity of acetylation by p300 for the IN-CTD is highlighted by its lack of any detectable reactivity with MBP alone (MBP has 36 lysines). 5R is K(244/258/264/266/273)R (B) Other HATs (MOZ and PCAF) efficiently acetylate a core histone preparation but not the IN CTD fusion protein. Recombinant MOZ and PCAF (Upstate, Lake Placid, NY) were reacted under the same conditions as p300 but failed to acetylate the MBP-IN-CTD fusion protein. Histone reactions are included as historic controls for the various recombinant HAT-dependent acetylation reactions. 4R (244) is K(244/264/266/273)R, 4R (258) is K(258/264/266/273)R. Shown below each panel is the band obtained for each of the corresponding MBP-IN CTD and MBP proteins stained with Coomassie blue G-250 before fluorography.

genetic assay (38, 39), integration frequencies of greater than 15 to 20% of that of the WT are required for sustained viral replication in culture. Here, the untagged R7/3 RRR virus integrated with a frequency of about 60% of that of the WT, whereas the FLAG-tagged mutant (R7/3 Bru RRR F) integrated with a frequency of about 12% compared to its tagged, WT comparator virus (R7/3 Bru F). This effect is not specific to the tagged Bru integrase sequence per se, since FLAG-tagged mutant R7/3 integrase (IN R7/3 RRR F) when contrasted to IN R7/3 F behaves in an identical fashion (data not shown). Taken together, these determinations cast doubt on the significance of these specific lysines as substrates for acetylation or other posttranslational modification in the cell types examined. In fact, Cereseto et al. (3) acknowledge a discrepancy between the marginal defects manifested by their mutant integrase in vitro and the dramatic-replication defective phenotype displayed by their FLAG-tagged mutant virus in culture. Here, we note that the modest effects reported by Cereseto et al. with N-terminally tagged mutant integrase in an assortment of assays in vitro are concordant with the results we have obtained in vivo using untagged mutant viruses. In this light, the slight integration defect we document for the R7/3 RRR mutant may be attributed to the cumulative effect of the three lysine-to-arginine substitution mutations. However, the diminution in integration frequency is not manifested in the replicative potential of this mutant compared to the WT during growth in culture (Fig. 2).

That the three lysines targeted for acetylation are highly conserved suggests that their preservation is important for some aspect of viral replication (3, 18). Despite this, all three residues can be conservatively replaced with arginine in single, double, or triple combination while remaining proficient for IN function and viral replication (Table 1). This suggests that, at least in the context of immortalized cell lines in culture, acetylation is not essential for IN function during infection. In fact, in addition to mutant K273R, mutants K273Q and K273A are

also replication competent in the CEM-SS cell line (Table 1). However, not all substitutions at these residues are tolerated in single point configuration, as K264E, K266A (18), and K273E (unpublished results and Table 1) are nonviable. Insight to explain K273 conservation is further complicated since this residue, the major site of p300-mediated acetylation in vitro (Fig. 3A), is encoded by a segment of proviral DNA that overlaps with the *vif* reading frame at codon positions 2 and 3 (Fig. 1). Therefore, it is likely that K273 conservation is dictated, in part, by constraints imposed by Vif protein function. The experiments conducted with CEM-SS cells do not reflect the potential for possible genetic pressure imposed by the *vif* sequence, since the activity of its gene product is irrelevant in the context of this cell line. Experiments are in progress to determine the replication status of K273 mutants under conditions where Vif activity is essential for viral passage (29, 31, 43). Interestingly, the region of *vif* that overlaps with *pol* encodes the amino terminus of Vif, a region noted for defining a specificity determinant for the interaction of this viral protein with APOBEC family members (28, 32, 36) and predictably conserved due to stringent functional pressure. In this light, it should be noted that with the exception of equine infectious anemia virus, which does not code for Vif (23), the overlap configured between the *pol* and *vif* reading frames has been preserved across all known lentiviral species.

In summary, we find that the isolated HIV-1 IN CTD is specifically acetylated by HAT activity associated with p300. However, when the lysine residues targeted for modification are conservatively replaced by arginine, an amino acid incapable of being acetylated, a mutant virus incorporating these changes (RRR) replicates well and integrates efficiently. Thus, we cannot confirm the original observation (3) that posttranslational acetylation by p300 is essential for viral integration and replication. The discrepancy between our work and that of Cereseto and coworkers (3) may be explained by the use of an acidic epitope tag placed at the extreme carboxyl terminus of integrase, near the target site for acetylation and mutation. Although tagging integrase in the WT virus did not preclude its efficient viral replication, additional alterations resulted in a low integration frequency and a defective virus. This effect was unrelated to either the tag or the substitution mutation in single combination; the phenotype was revealed only when the two alterations were together. These observations provide evidence for an unanticipated experimental caveat and highlight the need for caution when interpreting data generated from tagged viruses and their mutant derivatives.

The modest integration defect exhibited by the RRR mutant may be attributable to a lack of acetylation, but we believe that the lysine-to-arginine changes may degrade one of the functional activities of the integrase, perhaps by altering the ability of the IN CTD to bind either viral or target DNA (3, 4, 7, 8, 10, 11, 20, 21, 27, 40, 41). Although attempts to characterize integrase-DNA binding by computer modeling have been made (1, 5, 6, 17, 22, 37, 44), definitive structural details await description.

Although the importance of this region of integrase remains certain, a role for p300 and/or other posttranslational modifications at these residues is in doubt (at least in the context of viral replication in cultured cell lines). Cereseto et al. (3) have presented considerable evidence in support of an interaction between HIV-1 integrase and p300 in cells, but the significance of this observation remains undetermined. Progress towards uncovering any potential role that the modification of integrase by p300 has in the viral life cycle must involve studying the consequence of mutation of the targeted lysines during the infection of primary cells. To date this has not been possible for two reasons: (i) the major IN CTD residue modified by p300 (K273) is encoded within the *pol-vif* overlap region (Fig. 1), and (ii) mutation at its codon can simultaneously alter the amino acid sequences for both IN and Vif, confounding the interpretation of any mutant phenotype observable during infection. Progressive infection of both lymphocytes and macrophages requires the activity of the Vif protein. To circumvent these problems, we have constructed a replication-competent virus in which the *pol* and *vif* reading frames have been completely separated. This set of viruses will alleviate any potential for genetic interdependency between the two viral genes and allow us to study independently the effect of mutation within the C terminus of IN or the N terminus of Vif.

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REFERENCES

1. **Adesokan, A. A., V. A. Roberts, K. W. Lee, R. D. Lins, and J. M. Briggs.** 2004. Prediction of HIV-1 integrase/viral DNA interactions in the catalytic domain by fast molecular docking. J. Med. Chem. **47:**821–828.
2. **Bordoli, L., S. Husser, U. Luthi, M. Netsch, H. Osmani, and R. Eckner.** 2001. Functional analysis of the p300 acetyltransferase domain: the PHD finger of p300 but not of CBP is dispensable for enzymatic activity. Nucleic Acids Res. **29:**4462–4471.
3. **Cereseto, A., L. Manganaro, M. I. Gutierrez, M. Terreni, A. Fittipaldi, M. Lusic, A. Marcello, and M. Giacca.** 2005. Acetylation of HIV-1 integrase by p300 regulates viral integration. EMBO J. **24:**3070–3081.
4. **Chen, J. C., J. Krucinski, L. J. Miercke, J. S. Finer-Moore, A. H. Tang, A. D. Leavitt, and R. M. Stroud.** 2000. Crystal structure of the HIV-1 integrase catalytic core and C-terminal domains: a model for viral DNA binding. Proc. Natl. Acad. Sci. USA **97:**8233–8238.
5. **De Luca, L., A. Pedretti, G. Vistoli, M. L. Barreca, L. Villa, P. Monforte, and A. Chimirri.** 2003. Analysis of the full-length integrase-DNA complex by a modified approach for DNA docking. Biochem. Biophys. Res. Commun. **310:**1083–1088.
6. **De Luca, L., G. Vistoli, A. Pedretti, M. L. Barreca, and A. Chimirri.** 2005. Molecular dynamics studies of the full-length integrase-DNA complex. Biochem. Biophys. Res. Commun. **336:**1010–1016.
7. **Eijkelenboom, A. P., R. A. Lutzke, R. Boelens, R. H. Plasterk, R. Kaptein, and K. Hard.** 1995. The DNA-binding domain of HIV-1 integrase has an SH3-like fold. Nat. Struct. Biol **2:**807–810.
8. **Eijkelenboom, A. P., R. Sprangers, K. Hard, R. A. Puras Lutzke, R. H. Plasterk, R. Boelens, and R. Kaptein.** 1999. Refined solution structure of the C-terminal DNA-binding domain of human immunovirus-1 integrase. Proteins **36:**556–564.
9. **Engelman, A., G. Englund, J. M. Orenstein, M. A. Martin, and R. Craigie.** 1995. Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication. J. Virol. **69:**2729–2736.
10. **Engelman, A., A. B. Hickman, and R. Craigie.** 1994. The core and carboxyl-terminal domains of the integrase protein of human immunodeficiency virus type 1 each contribute to nonspecific DNA binding. J. Virol. **68:**5911–5917.
11. **Gao, K., S. L. Butler, and F. Bushman.** 2001. Human immunodeficiency virus type 1 integrase: arrangement of protein domains in active cDNA complexes. EMBO J. **20:**3565–3576.
12. **Goel, A., and R. Janknecht.** 2003. Acetylation-mediated transcriptional activation of the ETS protein ER81 by p300, P/CAF, and HER2/Neu. Mol. Cell. Biol. **23:**6243–6254.

13. **Gu, W., and R. G. Roeder.** 1997. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell **90:**595–606.
14. **Guidez, F., L. Howell, M. Isalan, M. Cebrat, R. M. Alani, S. Ivins, I. Hormaeche, M. J. McConnell, S. Pierce, P. A. Cole, J. Licht, and A. Zelent.** 2005. Histone acetyltransferase activity of p300 is required for transcriptional repression by the promyelocytic leukemia zinc finger protein. Mol. Cell. Biol. **25:**5552–5566.
15. **Hazuda, D. J., P. Felock, M. Witmer, A. Wolfe, K. Stillmock, J. A. Grobler, A. Espeseth, L. Gabryelski, W. Schleif, C. Blau, and M. D. Miller.** 2000. Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. Science **287:**646–650.
16. **Johnson, A. A., C. Marchand, and Y. Pommier.** 2004. HIV-1 integrase inhibitors: a decade of research and two drugs in clinical trial. Curr. Top. Med. Chem. **4:**1059–1077.
17. **Karki, R. G., Y. Tang, T. R. Burke, Jr., and M. C. Nicklaus.** 2004. Model of full-length HIV-1 integrase complexed with viral DNA as template for anti-HIV drug design. J. Comput. Aided Mol. Des **18:**739–760.
18. **Lu, R., H. Z. Ghory, and A. Engelman.** 2005. Genetic analyses of conserved residues in the carboxyl-terminal domain of human immunodeficiency virus type 1 integrase. J. Virol. **79:**10356–10368.
19. **Luo, J., M. Li, Y. Tang, M. Laszkowska, R. G. Roeder, and W. Gu.** 2004. Acetylation of p53 augments its site-specific DNA binding both in vitro and in vivo. Proc. Natl. Acad. Sci. USA **101:**2259–2264.
20. **Lutzke, R. A., and R. H. Plasterk.** 1998. Structure-based mutational analysis of the C-terminal DNA-binding domain of human immunodeficiency virus type 1 integrase: critical residues for protein oligomerization and DNA binding. J. Virol. **72:**4841–4848.
21. **Lutzke, R. A., C. Vink, and R. H. Plasterk.** 1994. Characterization of the minimal DNA-binding domain of the HIV integrase protein. Nucleic Acids Res. **22:**4125–4131.
22. **Ma, X. H., X. Y. Zhang, J. J. Tan, W. Z. Chen, and C. X. Wang.** 2004. Exploring binding mode for styrylquinoline HIV-1 integrase inhibitors using comparative molecular field analysis and docking studies. Acta Pharmacol. Sin. **25:**950–958.
23. **Navarro, F., and N. R. Landau.** 2004. Recent insights into HIV-1 Vif. Curr. Opin. Immunol. **16:**477–482.
24. **Petit, C., O. Schwartz, and F. Mammano.** 1999. Oligomerization within virions and subcellular localization of human immunodeficiency virus type 1 integrase. J. Virol. **73:**5079–5088.
25. **Pommier, Y., A. A. Johnson, and C. Marchand.** 2005. Integrase inhibitors to treat HIV/AIDS. Nat. Rev. Drug Discov. **4:**236–248.
26. **Potts, B.** 1990. "Mini" reverse transcriptase assay, p. 103–106. *In* A. Aldovini and B. Walker (ed.), Techniques in HIV research. Stockton Press, New York, NY.
27. **Ramcharan, J., D. M. Colleluori, G. Merkel, M. D. Andrake, and A. M. Skalka.** 2006. Mode of inhibition of HIV-1 integrase by a C-terminal domain-specific monoclonal antibody. Retrovirology **3:**34.
28. **Schrofelbauer, B., T. Senger, G. Manning, and N. R. Landau.** 2006. Mutational alteration of human immunodeficiency virus type 1 Vif allows for functional interaction with nonhuman primate APOBEC3G. J. Virol. **80:** 5984–5991.
29. **Sheehy, A. M., N. C. Gaddis, J. D. Choi, and M. H. Malim.** 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature **418:**646–650.
30. **Sterner, D. E., and S. L. Berger.** 2000. Acetylation of histones and transcription-related factors. Microbiol. Mol. Biol. Rev. **64:**435–459.
31. **Stopak, K., C. de Noronha, W. Yonemoto, and W. C. Greene.** 2003. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol. Cell **12:**591–601.
32. **Tian, C., X. Yu, W. Zhang, T. Wang, R. Xu, and X. F. Yu.** 2006. Differential requirement for conserved tryptophans in human immunodeficiency virus type 1 Vif for the selective suppression of APOBEC3G and APOBEC3F. J. Virol. **80:**3112–3115.
33. **Toh, H., H. Hayashida, and T. Miyata.** 1983. Sequence homology between retroviral reverse transcriptase and putative polymerases of hepatitis B virus and cauliflower mosaic virus. Nature **305:**827–829.
34. **Wakefield, J. K., S. A. Jablonski, and C. D. Morrow.** 1992. In vitro enzymatic activity of human immunodeficiency virus type 1 reverse transcriptase mutants in the highly conserved YMDD amino acid motif correlates with the infectious potential of the proviral genome. J. Virol. **66:**6806–6812.
35. **Wang, R., P. Cherukuri, and J. Luo.** 2005. Activation of Stat3 sequence-specific DNA binding and transcription by p300/CREB-binding protein-mediated acetylation. J. Biol. Chem. **280:**11528–11534.
36. **Wichroski, M. J., K. Ichiyama, and T. M. Rana.** 2005. Analysis of HIV-1 viral infectivity factor-mediated proteasome-dependent depletion of APOBEC3G: correlating function and subcellular localization. J. Biol. Chem. **280:**8387–8396.
37. **Wielens, J., I. T. Crosby, and D. K. Chalmers.** 2005. A three-dimensional model of the human immunodeficiency virus type 1 integration complex. J. Comput. Aided Mol. Des. **19:**301–317.
38. **Wiskerchen, M., and M. A. Muesing.** 1995. Human immunodeficiency virus type 1 integrase: effects of mutations on viral ability to integrate, direct viral gene expression from unintegrated viral DNA templates, and sustain viral propagation in primary cells. J. Virol. **69:**376–386.
39. **Wiskerchen, M., and M. A. Muesing.** 1995. Identification and characterization of a temperature-sensitive mutant of human immunodeficiency virus type 1 by alanine scanning mutagenesis of the integrase gene. J. Virol. **69:**597–601.
40. **Woerner, A. M., M. Klutch, J. G. Levin, and C. J. Marcus-Sekura.** 1992. Localization of DNA binding activity of HIV-1 integrase to the C-terminal half of the protein. AIDS Res. Hum. Retroviruses **8:**297–304.
41. **Woerner, A. M., and C. J. Marcus-Sekura.** 1993. Characterization of a DNA binding domain in the C-terminus of HIV-1 integrase by deletion mutagenesis. Nucleic Acids Res. **21:**3507–3511.
42. **Yao, Y. L., W. M. Yang, and E. Seto.** 2001. Regulation of transcription factor YY1 by acetylation and deacetylation. Mol. Cell. Biol. **21:**5979–5991.
43. **Zheng, Y. H., D. Irwin, T. Kurosu, K. Tokunaga, T. Sata, and B. M. Peterlin.** 2004. Human APOBEC3F is another host factor that blocks human immunodeficiency virus type 1 replication. J. Virol. **78:**6073–6076.
44. **Zhu, H. M., W. Z. Chen, and C. X. Wang.** 2005. Docking dinucleotides to HIV-1 integrase carboxyl-terminal domain to find possible DNA binding sites. Bioorg. Med. Chem. Lett. **15:**475–477.