*Proc. Natl. Acad. Sci. USA*
Vol. 91, pp. 5913–5917, June 1994
Biochemistry

# Human immunodeficiency virus integrase directs integration to sites of severe DNA distortion within the nucleosome core

(DNA structure/chromatin/kinks)

DMITRY PRUSS*, FREDERIC D. BUSHMAN†, AND ALAN P. WOLFFE*

*Laboratory of Molecular Embryology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892; and †Infectious Disease Laboratory, Salk Institute, La Jolla, CA 92014

*Communicated by Howard A. Nash, March 3, 1994 (received for review January 13, 1994)*

**ABSTRACT** **We have examined the consequences of DNA distortion and specific histone–DNA contacts within the nucleosome for integration mediated by the human immunodeficiency virus (HIV)-encoded integrase enzyme. We find that sites of high-frequency integration cluster in the most severely deformed, kinked DNA regions within the nucleosome core. This may reflect either a preference for a wide major groove for association of the integrase or a requirement for target DNA distortion in the DNA strand transfer mechanism. Both the distortion and folding of the target DNA through packaging into nucleosomes may influence the selection of HIV integration sites within the chromosome.**

During their life cycle, retroviruses must integrate a DNA copy of their genome into the chromosomal DNA of the host cell (1). Although some integration systems show considerable target-site specificity (2–4), the exact mechanisms determining where retroviral integration takes place within the chromosome are not well understood (5, 6). Chromosome structure has been proposed as one influence on target selection, since sites that are preferentially accessible to DNase I appear to be favored for integration (7–10). These DNase I-hypersensitive sites represent points of disruption of the chromatin fiber at which trans-acting factors are associated with regulatory elements. However, DNase I-hypersensitive sites are not free of nucleosomes (11). Both the integrity of the chromatin fiber and the organization of DNA within the nucleosome might influence target selection. Although the murine leukemia virus and human immunodeficiency virus (HIV) integrase proteins show only modest primary sequence specificity on naked DNA, Varmus and colleagues found that nucleosome assembly can enhance integration at particular sites, so that integration occurs more often at sites in nucleosomal DNA than at the corresponding sites in free DNA (12–14). This important observation was extended to demonstrate that chromatin structure influences target site selection *in vivo* and *in vitro* (12–14). These studies did not examine whether DNA distortion or specific histone–DNA contacts within the nucleosome determine the exact sites of integration. This is because minichromosomes were used as substrates for integration, in which the nucleosomes contain defined DNA sequences. The exact organization of DNA and the location of histone–DNA contacts in these nucleosomes is not known. We have extended the work of Varmus and colleagues (12–14) by using nucleosome core particles in which these parameters have been defined. In this way we determine whether the precise structure of DNA and its placement within the nucleosome core influences target site selection by HIV integrase.

The nucleosome core particle isolated after limited digestion of chicken erythrocyte chromatin by micrococcal nuclease consists of 146 base pairs (bp) of DNA wrapped around the globular domains of the core histones (15). The organization of DNA in this structure is well defined: DNA is wrapped around the histones in a left-handed superhelix with about 80 bp per turn of the superhelix. A striking feature of the path of DNA around the histones is that there are sharp bends in DNA at four symmetrically located positions (±1.5 and ±3.5 turns away from the dyad axis) (15). Importantly, these core particles contain a mixture of all DNA sequences found in nucleosomes within the nucleus; consequently, our results on integration selectivity will reflect the effects of nucleosomal structure *per se* on integration. In nucleosomes containing defined DNA sequences (12–14), any primary sequence preference shown by the integrase might predominate over the influence of DNA structure or histone–DNA interaction. Isolated core particles also avoid any influence on integration due to the potential folding of nucleosomal arrays within minichromosomes.

In agreement with previous work (12–14), we find that the HIV integrase effectively utilizes a nucleosomal template for integration; however, we find that not all DNA within the nucleosome core is utilized equivalently. DNA that is more severely distorted and that has a wider major groove within the nucleosome is a preferential target for integration. Our results also demonstrate that it is the wrapping of DNA around the globular domains of the core histones that determines the pattern of integration and not interaction of the integrase with the N-terminal domains of the histones that lie on the outside of the nucleosome.

## MATERIALS AND METHODS

**Partial Purification of Soluble HIV Integrase.** Overexpression and partial purification of insoluble HIV integrase has been described (16, 17). The HIV integrase-containing insoluble fraction from two 150-cm$^2$ tissue culture flasks was solubilized in a buffer containing 4 M urea, fractionated on a Superose 12 column, and dialyzed to remove the urea (18). HIV integrase protein was the predominant species after electrophoresis on SDS/PAGE gels and staining with Coomassie blue.

**The Integration Reaction.** The synthetic HIV long terminal repeat (LTR) substrate used for the integration reaction corresponded to a 3′ processed intermediate of integration of the viral *U3* end (17). It was made by annealing the oligonucleotides RB67 [30-nucleotide (nt)-labeled integrating strand ($^{32}$P-5′-end-labeled CGATAGGATCCGAGTGAATTAGCCCTTCCA)] and RB50 [32-nucleotide nonintegrating strand (5′-ACTGGAAGGGCTAATTCACTCGGATCCTATCG)], followed by gel filtration and acetone precipitation. These annealed oligonucleotides are shown in Fig. 1*A* undergoing a self-integration reaction in which the synthetic HIV LTR is

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV, human immunodeficiency virus; LTR, long terminal repeat

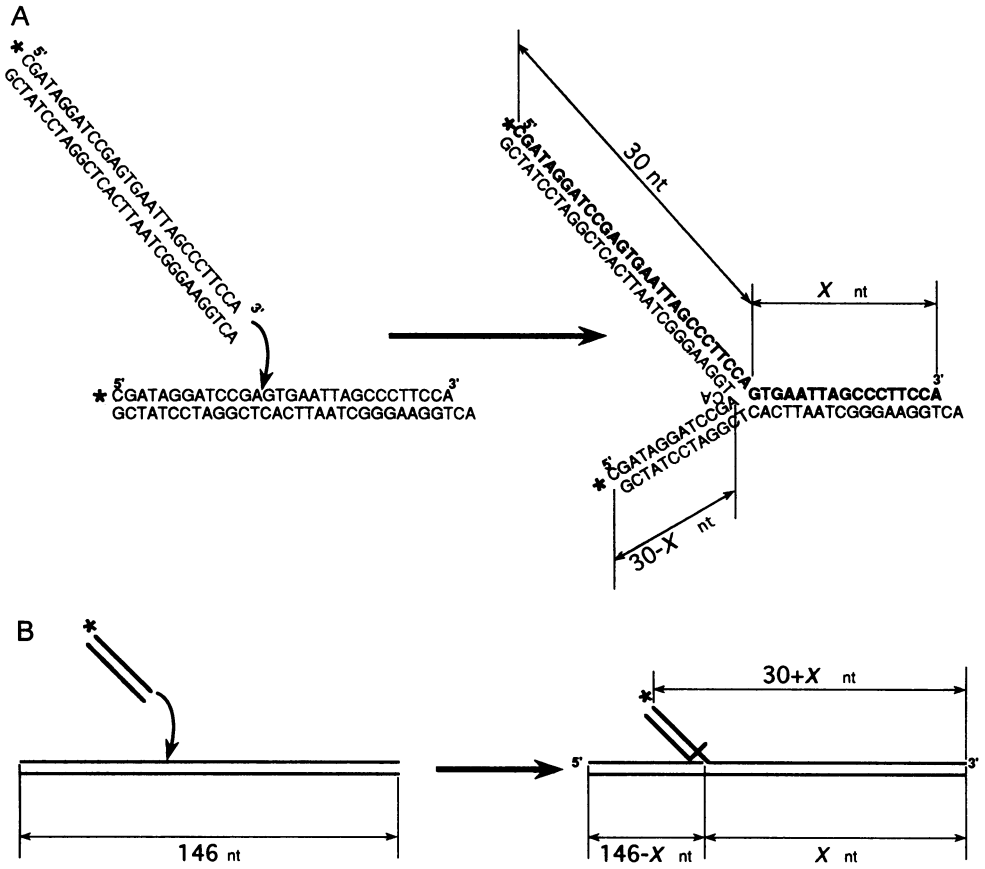


FIG. 1. The integration reaction. (*A*) The synthetic HIV LTR is shown undergoing a self-integration reaction. The 5′ and 3′ ends of the 30-nt-labeled integrating strand are shown, together with the radiolabeled 5′ end (asterisks). *X* indicates the variable number of nucleotides from the site of integration to the 3′ end of the target oligonucleotide. When the synthetic HIV LTR functions as both substrate and target for integration, the radiolabeled oligonucleotides are (30 + *X*) and (30 − *X*) nucleotides long. (*B*) In this diagram, the experimental condition used to examine HIV LTR integration into nucleosomal cores or deproteinized core DNA (146 bp long) is shown. Here only the synthetic HIV LTR substrate is radiolabeled (asterisks) as in *A*; the target nucleosomal cores or core DNA are unlabeled. *X* indicates the number of nucleotides from the site of integration to the 3′ end of the target DNA. The single-stranded DNA fragment whose length is measured consists of the 30-nt-labeled integrating strand plus *X* nucleotides. Because the nucleosome is dyad symmetric (see Fig. 4), integration sites in the two equivalent DNA strands can be mapped in a single experiment.

both substrate and target for the reaction. The reaction mixture for integration contained 0.5–1 μg of the synthetic HIV LTR substrate per ml, 0.1–0.5 μg of the soluble HIV integrase, and 40 ng of naked DNA or nucleoprotein target (146 bp long; shown in Fig. 1*B*) in a buffer containing 7.5 mM $MnCl_2$, 20 mM Hepes·NaOH (pH 7.3), 10 mM 2-mercaptoethanol, 10% (vol/vol) glycerol, and 0.75 mM CHAPS within a final reaction volume of 24 μl. The HIV integrase was solubilized at 0.2–1 μg/μl in 1 M NaCl/20 mM Hepes·NaOH, pH 7.3/0.1 mM EDTA/1 mM dithiothreitol/20% glycerol. The addition of enzyme solution or buffer solution alone led to an increase of ionic strength equivalent to 20 mM NaCl. The target was always the last component to be added to the reaction mixture. The reaction was allowed to proceed for 2 hr at 37°C, before it was stopped through addition of 10 mM EDTA. Proteins were removed by digestion with Proteinase K (0.04 μg/μl) in the presence of 0.1% SDS at 37°C for 1 hour. This was followed by phenol/chloroform extraction and precipitation of DNA with ethanol.

Integration products were resolved on denaturing 8% polyacrylamide sequencing gels containing 7 M urea to identify the sites of integration or on 4% nondenaturing acrylamide gels to estimate the overall yield of integrated products (19, 20). Quantitation was by laser scanning of autoradiograms with a Molecular Dynamics densitometer. The actual distance between the integration site and the DNA 3′ terminus was calculated as the integrated product size minus 30 nt, and the distance between the integration site and the 5′ terminus of core particle DNA was calculated as the length of the core particle DNA (146 bp) plus the length of the radiolabeled integrating strand (30 nt), minus the length of the integration product (Fig. 1*B*).

**Preparation of Intact and Trypsinized Core Particles.** Nucleosome core particles (0.2 mg of DNA per ml) in 35 mM NaCl/10 mM Tris·HCl (pH 8.0)/1 mM EDTA/1 mM dithiothreitol were prepared as described (20), and portions were treated with trypsin from bovine pancreas (L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated; Sigma) at 6 μg/ml for 7 min at 25°C to remove the core histone tails. The reaction was stopped by adding trypsin inhibitor from hen egg white (Boehringer Mannheim) to 60 μg/μl and cooled on ice. The integrity of the histones before and after trypsin digestion and HIV integrase treatment was analyzed in SDS/18% polyacrylamide gels.

DNase I digestion products from chicken erythrocyte nuclei were used as markers. These were prepared by incubating the DNA fragments after deproteinization for 37°C for 30 min with 50 pmol of [$\gamma^{32}$P]ATP and 5 units of bacteriophage T4 polynucleotide kinase (BRL) in 10 mM Tris·HCl, pH 8.0/2.5 mM $MgCl_2$ in a 20-μl volume. The reaction was terminated and processed for electrophoresis as described above for the integration reaction.

## RESULTS

**DNA Conformation Within the Nucleosome Influences the Site of Integration.** We probed integration into mixed se-

quence nucleosomes, using purified HIV-1 integrase and model viral DNA substrates. A duplex oligonucleotide matching in sequence one end of the unintegrated linear viral DNA was labeled on one strand with $^{32}P$ and incubated with nucleosomal target DNA in the presence of HIV integrase. Reaction products were analyzed after denaturation by electrophoresis on a DNA sequencing-type gel. The labeled oligonucleotide DNA can itself be used as a target DNA, so a ladder of low molecular weight product bands is seen in all lanes, including that with no added target (Fig. 2*A*, lanes 2–4). Because the nucleosome is dyad symmetric (ref. 15, see Fig. 4), integration sites in the two equivalent DNA strands can be mapped in a single experiment. The DNA present within nucleosome cores did not itself provide any preferential sites for integrase action, since deproteinized target DNA showed even reactivity across its length (Fig. 2*A*, compare lane 2 with lane 4). However, nucleosomal DNA displayed strikingly uneven reactivity (Fig. 2*A*, lane 2), indicating that contact with the histones provides the basis for preferential site utilization.

The average position (*i*) of maximal cleavage for reagents that preferentially interact with DNA across the minor groove, such as hydroxyl radical or DNase I (20), and (*ii*) of maximal integration were out of phase by half a helical turn, indicating that integration took place on the major groove side. Our results therefore confirm previous work (12) in demonstrating that the HIV integrase prefers to integrate into DNA within the nucleosome core where the major groove is oriented toward solution (away from the surface of the histone octamer) (Figs. 2 and 3).

**Integration Occurs Preferentially at Sites of Severe DNA Distortion.** The frequency of integration into nucleosomes where the major groove is exposed varied dramatically. At the dyad axis of the nucleosome core, the minor groove was directed toward solution (Fig. 3, fat arrow) (15). Integration was very inefficient in this region for 1.5 turns of DNA to either side of the dyad axis, including two sites at which the major groove was exposed (Fig. 2, bar). These three turns of DNA were unusual in their configuration within the nucleosome core. DNA in this region is only slightly bent, lying on the surface of histones H3 and H4 (15, 22). The helical periodicity of the three turns of DNA (32 bp) at the dyad axis was 10.7 bp as opposed to 10.0 bp in the remainder of the nucleosome core (19, 20). In fact, the distance between the preferred sites of integration 1.5 turns to either side of the dyad axis (+60 and +92) was exactly 32 bp (Fig. 3), whereas outside of this region integration occurred every 10 bp. DNA at the dyad axis was also relatively exposed (see Fig. 4 *Inset*), so the adjacent turns of DNA were not expected to impede access to the double helix at this site. Despite this exposure, integration frequency remained low in this region. This is also probably true for defined sequence nucleosomes (12–14) (although in these instances the dyad axis of the nucleosome has not been unambiguously determined). However, occasionally integration events in the region around the dyad axis have been seen (12–14); presumably these are due to sequence specific influences on integration efficiency. Integration over the three turns of DNA at the dyad axis may be impeded by histone–DNA interactions or by the unusual DNA helical periodicity. Alternatively, the integration system may not favor target DNA that is relatively undeformed.

At about 1.5 and 3.5 turns of DNA from the dyad axis, there were highly preferred sites of integration (Figs. 2 and 3, +39, +60, +92, and +112). These sites overlap the regions of most severe DNA distortion seen in the structure of the nucleosome core (15). DNA in these regions has been proposed to be kinked such that base stacking changes, opening up base planes toward the major groove (24, 25). The regions 1.5 turns from the dyad axis (+60 and +92) are hyperreactive to cleavage by singlet oxygen (24). These regions represent the sites within the nucleosome with the widest major grooves (see Fig. 4). Integration is also efficient at a site 5.5 turns from the center of dyad symmetry. The path of DNA at this site is not clearly resolved within the 7-Å nucleosome structure (15), and thus the basis for efficient integration at this site is unclear. The origin of the 20-nt period for integration events at the periphery of the nucleosome core (Fig. 1*A*, lane 2) thus appears related to a 20-nt modulation in the path of DNA around the histone octamer; however, whether this reflects any comparable modulation in histone DNA contacts has not yet been resolved.

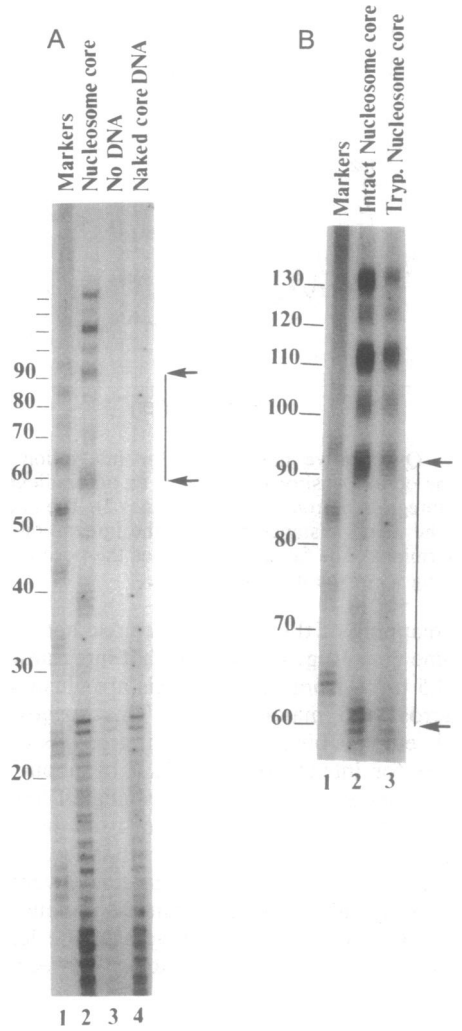


FIG. 2. Integration sites within the nucleosome. Integration products made with or without a heterologous target were resolved in a sequencing gel. (*A*) Nucleosome cores show preferred sites of integration compared with naked DNA. Lanes: 1, markers of DNA fragments end-labeled with [$\gamma$-$^{32}P$]ATP and T4 polynucleotide kinase after DNase I digestion of chicken erythrocyte chromatin; 2, integration into nucleosome cores; 3, self-integration products in the absence of target DNA; 4, integration into naked DNA extracted from nucleosome cores. The numbers represent the actual distance in nucleotides between the integration site and the DNA 3′ terminus (i.e., the integrated products sizes minus 30 nt, the length of the integrated oligonucleotide). Note the self-integration products of the oligonucleotide DNA below position 26. The arrows indicate the sites of preferred integration 1.5 turns to either side of the dyad axis of the nucleosome core at which integration is favored; the vertical line between them indicates the 32 bp of DNA that includes the dyad axis at which integration occurs less frequently. (*B*) The core histone tails do not influence integration site selection. Lanes: 1, markers as in *A*; 2, integration into nucleosome cores as in *A*; 3, integration into nucleosome cores from which the core histone tails have been removed with trypsin (20, 21). Markers are as in *A*.

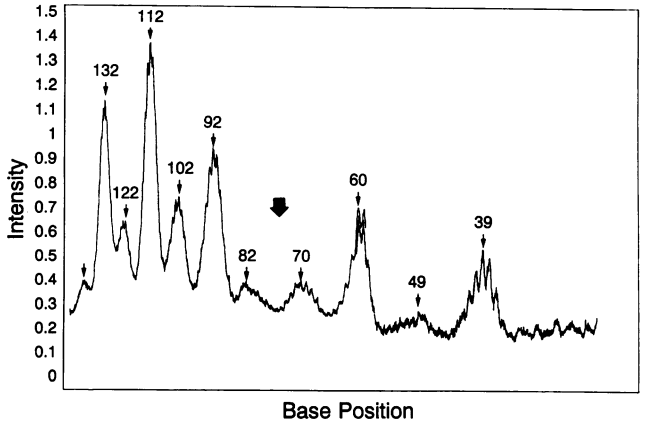


FIG. 3. Quantitative mapping of the integration sites in the nucleosome core. Densitometer scan of an autoradiograph of a gel resolving integration products (Fig. 2, lanes 2). The numbers correspond to the actual distance between the integration site and the DNA 3′ terminus. The fat arrow indicates the position of the dyad axis (0 in Fig. 4) of the nucleosome core.

Exact mapping of the sites of integrase action within the nucleosome core (Fig. 4) revealed that integration events at 1.5 and 3.5 turns from the dyad axis are clustered on each strand across the major groove. This suggests that the multimeric enzyme binds in the major groove so that the two active sites for integration are offset by about 5 bp. It is possible to deduce the *in vivo* spacing of the points of attachment of the two viral DNA ends from the length of the short duplication of target sequences that accompanies integration. Because the target DNA unpairs between the points of integration, and the single-stranded regions are subsequently filled in by a DNA polymerase, the length of the target duplication reflects the spacing between the initial points of attachment of each viral DNA end (1). For HIV, the target site duplication was found to be 5 bp, a value consistent with the observed spacing between points of attachment on each strand at the ±1.5 and ±3.5 hotspots (Fig. 4).

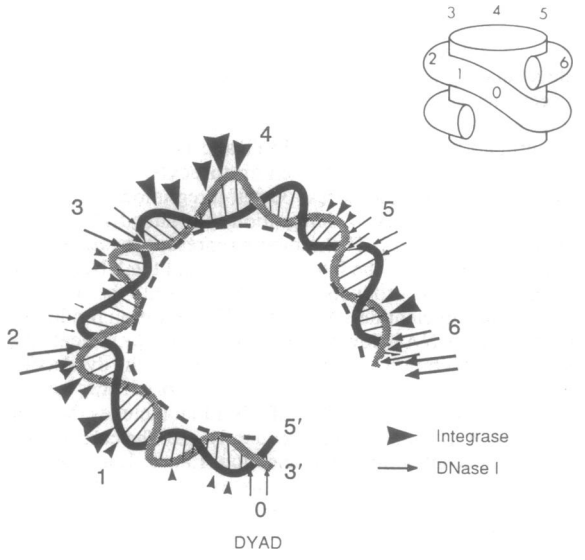


FIG. 4. Integration events are clustered in the distorted major grooves of the nucleosomal DNA. The DNA backbone shape within the nucleosome core is given (15), and the numbers indicate turns of DNA away from the dyad axis (19, 20). The dyad axis is indicated by 0. The broken line represents the surface of the histone octamer. Arrows indicate the major DNase I cleavage sites (refs. 20 and 23), and the arrowheads indicate the major integration sites (the sign size corresponds to the integration efficiency). (*Inset*) Illustration of the nucleosome core, showing the positions of the turns of DNA represented in the larger portion of the figure. DNA is the tube, wrapped around the cylinder made up of the globular domains of the core histones.

**The Core Histone N-terminal Tails Do Not Influence the Sites of Integration.** We next examined whether the preferred reactivity of a few intranucleosomal sites may reflect not only a differential deformation of DNA within the nucleosome cores but also differential steric hindrance by the histones. The histones have two domains, a central globular domain around which DNA is wrapped and a positively charged N-terminal tail domain that lies on the outside of the nucleosome core (26). The tail domains can be removed from the nucleosome by using trypsin without disturbing the wrapping of DNA around the globular domains of the histones (20, 21). Removal of the histone tails can facilitate the access of transacting factors to DNA (27). We found that integrase acts at the same sites in the nucleosome core in the presence or absence of the tail domains (Fig. 2*B*, compare lanes 2 and 3). Differences between the favored and disfavored sites for integration appeared to be less pronounced in the trypsinized cores; however, it is clear that the histone tails did not provide a major steric impediment to the integrase–substrate complex. Thus, the wrapping of DNA around the globular domains of the core histones is the major determinant of integration selectivity.

**Wrapping DNA Around the Core Histones Enhances Integration.** Finally, we quantitated the frequency of integration events, using different concentrations of nucleosome cores or naked DNA isolated from nucleosome cores as the substrate and a fixed concentration of integrase. This point is important for our interpretation, since the observation of selective target site utilization on nucleosomes could be due to either suppression of integration at the disfavored sites or stimulation of integration at the favored sites. After subtracting the background signal obtained from self integration events of the labeled primer DNA (e.g., Fig. 2*A*, lane 3), we found that wrapping DNA around the core histones provided an overall 2- to 3-fold enhancement of integration (Fig. 5). Thus, DNA distortion in the nucleosome core apparently promotes the integration reaction and explains why DNA wrapped in a nucleosome is a more efficient target. The possibility that excess naked target DNA might reduce the level of integration by potentially competing with the viral substrate for integrase (12) was examined by reducing target DNA concentrations below those optimal for detection of integration.

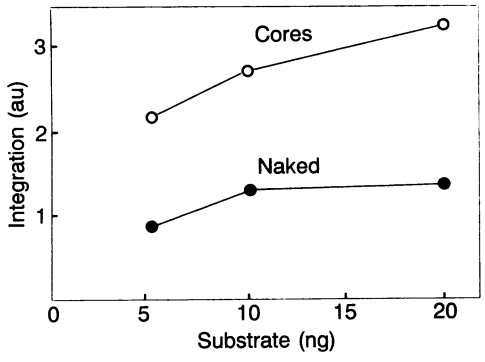


FIG. 5. HIV integration occurs more effectively in nucleosome cores. The integration yield curves at different target inputs for the nucleosome cores (○) or the naked core DNA (●) were plotted by resolving the reaction products and scanning and integrating the target-specific bands. Self-integration products were subtracted (see Fig. 2, lane 3). The mass of integrase (0.25 μg) was kept constant, while the amount of substrate was varied as indicated; other reaction conditions were as described in text. au, arbitrary units.

The apparent preference for nucleosomal cores over naked DNA was retained (Fig. 5; also data not shown).

## DISCUSSION

Our experiments with HIV integrase and nucleosome cores containing random DNA sequences provide new information about the selectivity of the integration reaction for chromatin templates and extend our previous understanding about the structure of DNA in the nucleosome. Previous work has established that DNA has a nonuniform structure in the nucleosome core. Three turns of DNA at the dyad axis are relatively straight with a measured helical periodicity of 10.7 bp per turn (15, 19, 20). Outside of this region DNA is bent around the core histones with a helical period of 10.0 bp per turn (15, 19, 20). The junctions between these regions of distinct helical period lie 1.5 turns to either side of the dyad axis of the nucleosome, where DNA is severely deformed or kinked (15, 24). DNA is also sharply bent ±3.5 turns to either side of the dyad axis (15). We find that HIV integrase detects these structural features of DNA in the nucleosome core. For the three turns of DNA including the dyad axis of the nucleosome core, integration is inefficient. However ±1.5 and ±3.5 turns from the dyad axis, the integration reaction readily occurs. The peaks of integration efficiency at these sites ±1.5 turns from the dyad axis are separated by 32 bp (Figs. 2 and 3); outside of this region, peaks of integration are separated by multiples of 10 bp. These results are consistent with the observations that an altered helical periodicity for DNA exists at the dyad axis of the nucleosome (19, 20) and that a distinct DNA structure exists ±1.5 and ±3.5 turns from the dyad axis of the nucleosome (15, 24). The retroviral integration complex approaches and faces DNA where the major groove is facing directly out from the nucleosome core (ref. 12; Fig. 4). Should the integrase interact directly with the major groove, this would be different from the most commonly utilized probes of DNA structure within nucleoprotein structures, such as DNase I and hydroxyl radical, which interact with the minor groove (19, 20). Our results therefore offer the potential of insight into the local deformation of the major groove within the nucleosome core in solution, which may confirm and extend existing information on the organization of DNA derived from probes of minor groove configuration (19, 20).

The preference of HIV integrase for nucleosomal DNA (Fig. 5) may reflect either a preference for interaction with DNA segments having a wide major groove (Fig. 4) or the utilization of the preexisting deformation of DNA within the nucleosome core to facilitate the reaction mechanism itself. Wrapping of DNA around the histones causes a periodic widening of the major groove (15) that might allow the HIV integrase to gain more ready access to these sites. Alternatively, any DNA deformation that promotes base pair unstacking (24, 25) might facilitate the integrase reaction directly.

Chromatin structure serves two general functions: (*i*) it compacts DNA while still enabling metabolic processes involving DNA to occur (26), and (*ii*) it serves to restrict the accessibility of certain trans-acting factors to regulatory elements (27, 28). We provide an example of a third potential role for the histone proteins—i.e., to deform DNA in a manner that can increase its utilization by an enzyme complex. Both the accessibility and distortion of DNA as it is folded and compacted within nucleoprotein complexes will probably have an important role in determining integration sites within the chromosome.

We thank Drs. Harold Varmus and Hans-Peter Muller for their encouragement of this work. We are also grateful to Drs. Muller and Varmus for a critical reading of the manuscript. We thank Ms. Thuy Vo for manuscript preparation. F.D.B. is a Special Fellow of the Leukemia Society of America and is supported by National Institutes of Health Grant RO1 AI 34786.

1. Varmus, H. E. & Brown, P. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington, DC), pp. 53–108.
2. Chalker, D. L. & Sandemeyer, S. B. (1992) *Genes Dev.* **6**, 117–128.
3. Shih, C. C., Stoye, J. P. & Coffin, J. M. (1988) *Cell* **53**, 531–537.
4. Ji, H., Moore, D. P., Blumkey, M. A., Braiterman, T., Voytas, D. F., Natsoulis, G. & Boeke, J. D. (1993) *Cell* **73**, 1007–1018.
5. Bushman, F. (1993) *Curr. Biol.* **3**, 533–535.
6. Craigie, R. (1992) *Trends Genet.* **8**, 187–190.
7. Rohdewohld, H., Weiher, H., Reik, W., Jaenisch, R. & Breindl, M. (1987) *J. Virol.* **61**, 336–343.
8. Scherdin, U., Rhodes, K. & Breindl, M. (1990) *J. Virol.* **64**, 907–912.
9. Mooslehner, K., Karls, U. & Harbers, K. (1990) *J. Virol.* **64**, 3056–3058.
10. Vijaya, S., Stefan, D. L. & Robinson, H. L. (1986) *J. Virol.* **60**, 683–692.
11. Elgin, S. C. R. (1988) *J. Biol. Chem.* **263**, 19259–19264.
12. Pryciak, P. M. & Varmus, H. E. (1992) *Cell* **69**, 769–780.
13. Pryciak, P. M., Sil, A. & Varmus, H. E. (1992) *EMBO J.* **11**, 291–303.
14. Pryciak, P. M., Muller, H. P. & Varmus, H. E. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9237–9241.
15. Richmond, T. J., Finch, J. T., Rushton, B., Rhodes, D. & Klug, A. (1984) *Nature (London)* **311**, 532–537.
16. Bushman, F. D., Fujiwara, T. & Craigie, R. (1990) *Science* **249**, 1555–1558.
17. Bushman, F. D. & Craigie, R. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1339–1343.
18. Craigie, R., Fujiwara, T. & Bushman, F. D. (1990) *Cell* **62**, 829–837.
19. Hayes, J. J., Tullius, T. D. & Wolffe, A. P. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 7405–7409.
20. Hayes, J. J., Clark, D. J. & Wolffe, A. P. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6829–6833.
21. Ausio, J., Dong, F. & van Holde, K. E. (1989) *J. Mol. Biol.* **206**, 451–463.
22. Arents, G., Burlingame, R. W., Wang, B. W., Love, W. E. & Moudrianakis, E. N. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10148–10152.
23. Lutter, L. C. (1978) *J. Mol. Biol.* **124**, 391–420.
24. Hogan, M. E., Rooney, T. F. & Austin, R. H. (1987) *Nature (London)* **328**, 554–557.
25. Crick, F. H. C. & Klug, A. (1975) *Nature (London)* **255**, 530–533.
26. van Holde, K. E. (1989) *Chromatin* (Springer, Berlin).
27. Lee, D. Y., Hayes, J. J., Pruss, D. & Wolffe, A. P. (1993) *Cell* **72**, 73–84.
28. Wolffe, A. P. (1992) *FASEB J.* **6**, 3354–3361.