JOURNAL OF VIROLOGY, Apr. 1991, p. 1910–1915
0022-538X/91/041910-06$02.00/0

Vol. 65, No. 4

Determination of Viral Proteins Present in the Human Immunodeficiency Virus Type 1 Preintegration Complex

CHRIS M. FARNET AND WILLIAM A. HASELTINE*

Division of Human Retrovirology, Dana-Farber Cancer Institute, Department of Pathology and Committee on Virology, Harvard Medical School, 44 Binney Street, Boston, Massachusetts 02115

Received 10 October 1990/Accepted 9 January 1991

Cytoplasmic extracts prepared from cells infected with metabolically radiolabeled virions of human immunodeficiency virus type 1 contain viral DNA in association with labeled viral proteins. Viral DNA can be purified from these extracts by gel filtration chromatography and sucrose gradient sedimentation as a part of a nucleoprotein complex containing integrase as the only viral protein detectable by immunoprecipitation and gel electrophoretic analysis. The purified complex contains no detectable *gag* gene products, including p17, p24, p7, or p6, and contains no additional *pol* gene products, including the p10 protease, p66 and p51 polymerase, or the p15 RNase H. Nearly all of the purified nucleoprotein complexes are capable of integrating into heterologous DNA targets in vitro. These observations demonstrate that integrase is a component of the human immunodeficiency virus type 1 preintegration complex and suggest that integrase may be the only viral protein necessary for the integration of retroviral DNA.

The retroviral replication cycle begins when the fusion of the viral envelope and cell membrane occurs and the virion core containing the viral RNA genome is released into the cytoplasm of the infected cell. The viral core contains all of the functions necessary for the conversion of the single-stranded RNA genome into a linear, double-stranded DNA molecule (15). Following its synthesis in the cytoplasm, the viral DNA intermediate migrates to the nucleus, where integration into the cellular genome establishes a proviral template for the synthesis of viral RNA. Presumably, some or all of the viral core proteins remain associated with the linear viral DNA and mediate the functions, such as nuclear localization and integration, necessary to establish the provirus.

Linear viral DNA can be isolated from the cytoplasm of infected cells as a discrete nucleoprotein complex (1, 6). When separated from the bulk of cellular proteins, the nucleoprotein complex is capable of mediating the integration of viral DNA into heterologous DNA targets in vitro, suggesting that these complexes may contain all of the functions necessary for viral integration (2, 6). Little is known about the structure or composition of the cytoplasmic preintegration complex. Here, the viral proteins associated with fully integration-competent human immunodeficiency type 1 (HIV-1) DNA purified from the cytoplasm of newly infected cells are characterized.

MATERIALS AND METHODS

Cells and viruses. The chronically HIV-1-infected Molt IIIB human T-cell line (HTLV-IIIB strain) was the source of virus for cell-free infections. Virus production from the cells was stimulated approximately 10-fold by incubation with phorbol esters. To prepare ^{3}H-labeled virions, 10^8 Molt IIIB cells were cultured for 20 h in arginine-free RPMI 1640 medium containing 20% fetal calf serum, 10 ng of phorbol 12-myristate 13-acetate per ml, and 200 μCi of [2,3-^{3}H]arginine per ml. Supernatant from stimulated cells was collected and filtered through a 0.2-μm (pore size) cellulose acetate filter. ^{3}H-labeled virus was pelleted by centrifugation of filtered supernatants for 1 h at 25,000 × *g* at 4°C. The virus pellet was gently washed with RPMI 1640 medium containing 10% fetal calf serum, resuspended in a minimal volume of the same, and used to infect cells of the SupT1 human T-cell line.

Preparation of cell extracts. SupT1 cells (50×10^8 cells) were infected with concentrated, ^{3}H-labeled virus (300 to 500 ng of p24 viral core protein per ml) in 25 ml of RPMI 1640 medium containing 10% fetal calf serum. Cytoplasmic extracts (1.2-ml volume) were prepared from cells 4 to 5 h postinfection as previously described (6) and treated with RNase A (20 μg/ml) for 30 min at room temperature. Triton X-100 (Sigma) was added to a final concentration of 0.5% to extracts before fractionation by gel filtration. Cell extracts from uninfected SupT1 cells were prepared in the same manner, except that they were not treated with RNase A prior to the addition of detergent.

Sephacryl S400 chromatography. Cytoplasmic extracts (0.8 ml) were loaded onto a 22-ml Sephacryl S400 (Pharmacia) column, 28 cm in height, equilibrated in buffer K (20 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid], pH 7.4, 150 mM KCl, 5 mM $MgCl_2$, 1 mM dithiothreitol, 25 mM aprotinin) containing 0.5% Triton X-100 at 4°C. Fractions (0.5 ml) were collected at a flow rate of approximately 0.2 ml/min.

Sucrose gradient sedimentation. S400 column fractions containing viral DNA were pooled, and 1.5 ml was layered onto 12-ml gradients of 15 to 30% sucrose in buffer K–0.5% Triton X-100 and centrifuged at 35,000 rpm for 3 h at 4°C in a Beckman SW41 rotor. Fractions (1 ml) were collected from the bottom of the gradient.

Analysis of viral proteins. Proteins were analyzed by immunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). For immunoprecipitations, cytoplasmic extracts, S400 column fractions, and sucrose gradient fractions were adjusted to 1% Triton X-100, and sodium deoxycholate and SDS were added to final concentrations of 1 and 0.1%, respectively. Antiserum (5 to 10 μl) was added and incubated for 12 to 16 h at 4°C. Immune complexes were precipitated by incubation with protein

* Corresponding author.

A-Sepharose CL4B (Pharmacia) for 1 h at 4°C. Precipitated immune complexes were washed four times in a buffer containing 10 mM Tris HCl, pH 7.4, 150 mM NaCl, 1% Triton X-100, and 0.1% SDS and once in the same buffer minus detergents. Proteins were analyzed by electrophoresis on 10 to 18% polyacrylamide gradient gels containing SDS followed by fluorography.

Immunoprecipitation of viral DNA. S400 column fractions containing the peak of viral DNA were pooled, and 0.75 ml was incubated with 10 μl of antiserum for 1 h at 4°C with occasional gentle mixing. The Sepharose beads were allowed to settle at unit gravity, the supernatant was collected for the analysis of viral DNA (see below), and the beads were washed three times in a buffer K–0.5% Triton X-100 at 4°C in the same manner. Immunoprecipitated viral DNA was recovered by suspending the Sepharose beads in buffer K and extracting the DNA as described below.

Analysis of viral DNA. Viral DNA was recovered from cytoplasmic extracts, S400 column fractions, sucrose gradient fractions, and immunoprecipitation supernatants and pellets by SDS-proteinase K treatment and phenol extraction as previously described (6). Agarose gel electrophoresis and Southern blotting and hybridization analyses of viral DNA were performed as previously described (6).

Preparation of rabbit antiserum against *trpE*-integrase fusion protein. A fusion protein of the HIV-1 integrase and the *Escherichia coli trpE* protein was expressed in bacteria by using a plasmid vector provided by Steven Goff. Growth of bacteria, induction of the *trp* operon, and preparation of bacterial extracts were as described previously (14). Fusion protein was recovered from polyacrylamide gel slices following SDS-PAGE of cell extracts. A New Zealand White female rabbit was injected intranodally with 100 to 200 μg of protein in 50% complete Freund adjuvant (GIBCO) and boosted 2 weeks later with an intraperitoneal injection of 100 μg of protein in incomplete Freund adjuvant. Serum was collected 10 days after the booster immunization. A preimmune serum sample was collected prior to the initial immunization.

Other antisera. The following sera were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases; sheep antiserum to HIV-1 p17 (catalog no. 286, M. Phelan); rabbit antiserum to HIV-1 p24 (no. 384, K. Steimer); rabbit antiserum to HIV-1 protease C-terminal peptide (no. 226, B. Korant); and human polyclonal immunoglobulin G to HIV-1 reverse transcriptase (no. 187, J. Laurence). Sheep antiserum to HIV-1 $p15^{gag}$ was obtained from Seromed, Berlin, Federal Republic of Germany. An HIV-1-positive human serum sample was provided by Bruce Walker.

RESULTS

The synthesis of linear viral DNA occurs synchronously in the cytoplasm of SupT1 cells infected with concentrated HIV-1 (6). To determine the viral proteins associated with the newly synthesized viral DNA, cells were infected with ^{3}H-arginine labeled virions. High concentrations of labeled virus were prepared by treatment of chronically infected Molt IIIB cells with phorbol esters in medium containing ^{3}H-arginine. The SupT1 human T-cell line was used as a target for infection, as this cell line expresses a high concentration of surface CD4 receptors and is easily infected.

Cytoplasmic extracts of cells infected with the labeled virus were fractionated by gel filtration chromatography. The methods used for preparation of the cell extracts and chromatography were those previously demonstrated to yield functional preintegration complexes (6). The cytoplasmic extracts were fractioned on a Sephacryl S400 column. The column fractions were analyzed for the presence of viral DNA by Southern blot analysis using an HIV-1-specific probe. The Southern blot analysis showed that DNA eluted near the void volume, well separated from the peak of cellular proteins (Fig. 1B and data not shown).

Viral proteins cofractionating with the linear viral DNA were analyzed by immunoprecipitation and SDS-PAGE analysis of column fractions by using an HIV-1-positive human serum. This serum recognized all of the viral *gag* and *pol* gene products (Fig. 1A, lane 1). The identities of the immunoreactive viral proteins were confirmed by using polyclonal antisera raised to purified individual HIV-1 proteins (see below). Three viral proteins, p17, p24, and the integrase p31, eluted in or near the void volume of the column (Fig. 1A, fractions 16 to 20). However, only the viral integrase protein eluted in the column fractions containing viral DNA. The void volume fractions containing capsid proteins p17 and p24 were devoid of viral DNA (Figure 1A and B, fractions 16 and 17). The amounts of p17 and p24 proteins eluting in the void volume fractions were variable and at most represented only a small fraction of the total amount of these proteins eluted from the column. The bulk of the capsid proteins eluted in the included volume fractions, well separated from fractions containing viral DNA (Fig. 1A, fractions 27 to 32). It is likely that the p17 and p24 proteins which were present in the void volume represented high-molecular-weight aggregates of these proteins. Alternatively, these proteins may be part of viral core structures derived from infecting virions that failed to synthesize viral DNA.

Column fractions containing the peak of viral DNA were immunoprecipitated with antisera specific for HIV-1 *pol* gene products to confirm the identity of the single protein species cofractionating with viral DNA. A polyclonal antiserum to HIV-1 integrase precipitated a 31-kDa protein from the column fractions containing viral DNA, identifying this protein as the viral integrase (Fig. 2, lanes 2 to 5). The antiserum used for this experiment was raised to a *trpE*-integrase fusion protein which contains a portion of the carboxy-terminal region of the viral RNase H protein. For this reason, this antiserum also detected the p66 form of reverse transcriptase and the 15-kDa RNase H protein when used to precipitate the unfractionated cytoplasmic extracts (Fig. 2, lane 1). Neither of these cross-reacting viral proteins is present in the column fractions containing viral DNA (Fig. 2, lanes 2 to 5). The p51 form of the viral polymerase is not recognized by this antiserum.

Human polyclonal immunoglobulin G reactive with HIV-1 reverse transcriptase recognized the envelope glycoprotein gp120, the p66 and p51 viral DNA polymerase, and the p31 integrase in the unfractionated extracts, consistent with previous reports (8) (Fig. 2, lane 6). An additional protein, with a molecular weight identical to that of the small protein recognized by the integrase antiserum, is detected by this antiserum. This protein is probably the viral RNase H. Only the 31-kDa integrase protein was precipitated by this antiserum from column fractions containing viral DNA (Fig. 2, lanes 7 to 10). Viral p17 and p24 and the 10-kDa protease were not precipitated from column fractions containing viral DNA with polyclonal antisera for these proteins (Fig. 3, lanes 2, 4, and 8). Each of these proteins was readily detectable in immunoprecipitates of fractions eluting with

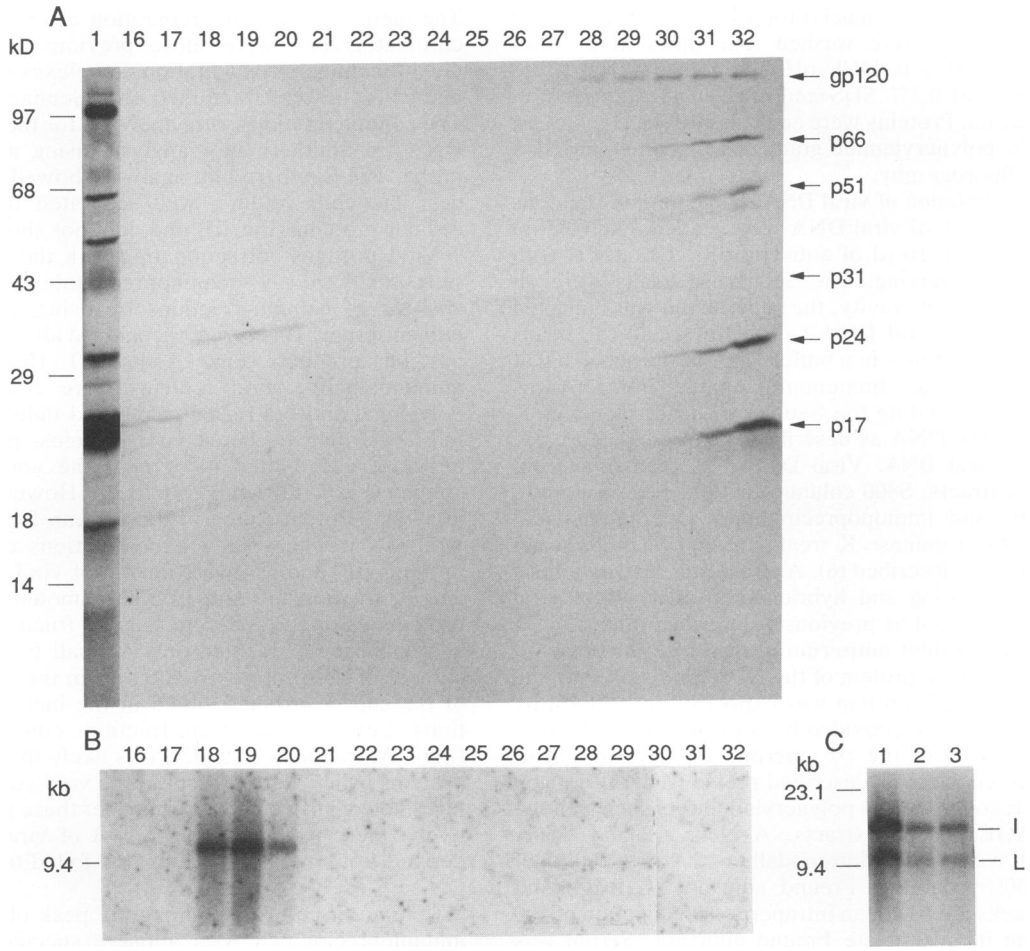

FIG. 1. Sephacryl S400 chromatography of extracts from cells infected with ^{3}H-labeled virus. (A) Lane 1, 0.2 ml of unfractionated cytoplasmic extract immunoprecipitated with HIV-1-positive human serum; lanes 16 to 32, 0.5-ml fractions from S400 column immunoprecipitated with the same serum. The migration positions of molecular mass standards are indicated on the left. The identities of viral protein bands are indicated on the right. (B) Southern blot analysis of viral DNA in column fractions. Supernatants from immunoprecipitations of viral proteins in column fractions (see above) were analyzed for viral DNA. Numbers above the lanes correspond to column fractions. (C) Southern blot analysis of the in vitro integration products of viral DNA in unfractionated extracts (lane 1) and in S400 column fractions (lanes 2 and 3). Lane 1, Linear ϕX174 DNA target (10 μg/ml) was added to 0.2 ml of unfractionated extract and incubated at 37°C for 1 h; lane 2, fractions 19 and 20 from the S400 column were pooled, linear ϕX174 DNA target (10 μg/ml) was added, and the mixture was incubated at 37°C for 1 h; lane 3, same as lane 2, except an equal volume of uninfected SupT1 cytoplasmic extract was added prior to incubation at 37°C. L, Linear viral DNA; I, integration product.

the included volume of the column (lanes 1, 3, and 7). Likewise, a polyclonal antiserum to the uncleaved precursor of p7 and p6, the p15^{gag} protein, recognized small proteins having molecular weights expected of the viral nucleocapsid proteins from unfractionated extracts but failed to detect these proteins in the column fractions containing viral DNA (lanes 5 and 6).

Cytoplasmic extracts were treated with RNase A prior to gel filtration in order to ensure that the void volume fractions did not contain viral RNA in association with viral proteins derived from the infecting virions. Cytoplasmic extracts were also prepared from cells infected with ^{3}H-labeled virus 30 min postinfection, at a time when linear viral DNA is not detectable (6). The 30-min extract was treated with RNase A and fractionated by Sephacryl S400 chromatography. Void volume fractions were analyzed for viral DNA and protein. Neither viral DNA nor integrase protein was detectable in the void volume fractions, even though labeled viral proteins were readily detectable in the included volume fractions of the column (data not shown).

The ability of the linear viral DNA eluted from the column to integrate into target DNA molecules in vitro was determined. The assay used to detect the integration activity was that previously described, integration of viral DNA into a linearized ϕX174 DNA target (6). The junction formed between viral DNA and target sequences in vitro is identical to that observed in infected cells. Joining of viral DNA occurs at the highly conserved 5′-TG/CA-3′ sequences present at the ends of the linear viral DNA, and a 5-bp direct repeat of target DNA sequences flanks the integrated provirus (9). In the physical assay, integration is detected by the shift of linear viral DNA on Southern blots into a higher-molecular-weight form (6). Figure 1C, lane 2, shows the 15-kDa DNA fragment produced upon incubation of the column fractions containing viral DNA with the target DNA. Densitometric scanning of the blots indicates that 90 to 98%

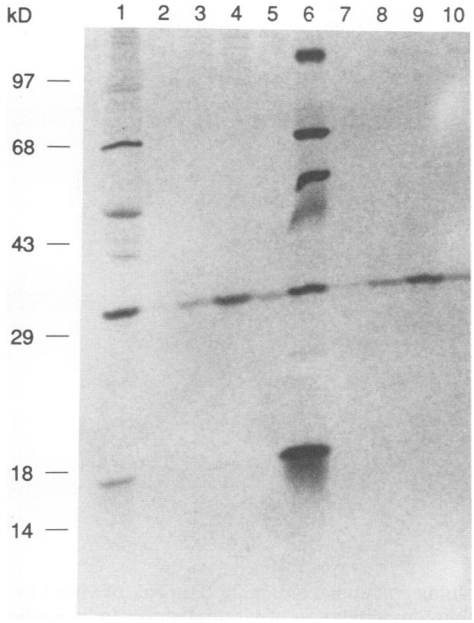

FIG. 2. Immunoprecipitation of S400 column fractions with antiserum specific for HIV-1 integrase (lanes 1 to 5) and human polyclonal immunoglobulin G to HIV-1 reverse transcriptase (see text for a description of this antiserum) (lanes 6 to 10). Lanes 1 and 6, Immunoprecipitated proteins from 0.3 ml of unfractionated cytoplasmic extract; lanes 2 to 5 and 7 to 10, immunoprecipitation of S400 column fractions 17 to 20, respectively.

of the viral DNA molecules have integrated into target after a 1-h incubation. The integration efficiency of the viral DNA in column fractions was indistinguishable from that of viral DNA in the unfractionated extracts (Fig. 1C, lane 1). Addition of a cytoplasmic extract from uninfected SupT1 cells to the column fractions containing viral DNA has no effect on the extent or rate of integration of viral DNA (Fig. 1C, lane 3).

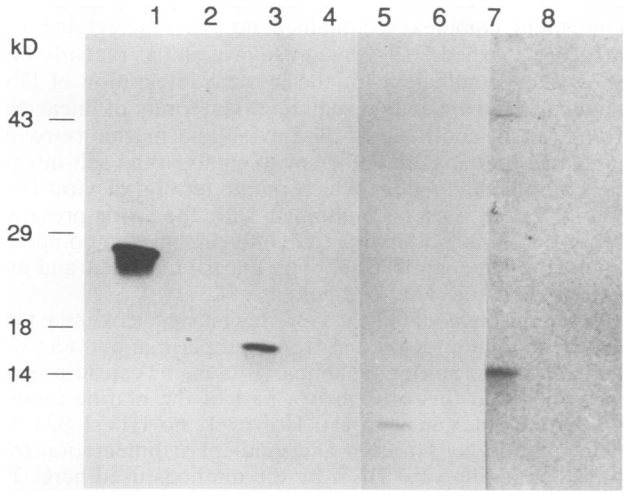

FIG. 3. Immunoprecipitation of unfractionated cell extracts (lanes 1, 3, 5, and 7) and S400 column fractions 19 and 20 (lanes 2, 4, 6, and 8) with antisera specific for HIV-1 p24 (lanes 1 and 2), p17 (lanes 3 and 4), $p15^{gag}$ (lanes 5 and 6), or protease C-terminal peptide (lanes 7 and 8).

Sucrose gradient sedimentation. To verify that integrase protein which eluted in the void volume of the column was associated with viral DNA, fractions containing the peak of viral DNA and integrase were analyzed by sucrose gradient sedimentation. Figure 4C shows the Southern blot detection of viral DNA in fractions collected from a 15 to 30% sucrose gradient. Viral DNA eluted from the Sephacryl S400 column migrated with a sedimentation profile similar to that of viral DNA present in unfractionated cell extracts. The gradient fractions containing viral DNA also contained integrase protein as detected by immunoprecipitation and SDS-PAGE analysis (Fig. 4A and B, lane 3), indicating an association between these two viral components. Gradient fractions containing the peak of viral DNA and integrase contained no p17 or p24 detectable by immunoprecipitation with polyclonal sera to p17 and p24 proteins (Fig. 4B, lanes 1 and 2). The viral DNA present in the sucrose gradient fractions was fully competent for integration in vitro (Fig. 4C, lanes 5′ and 6′), indicating that no component necessary for the in vitro integration reaction was lost by this additional step of purification.

Immunoprecipitation of viral DNA with antiserum to integrase. If the 31-kDa integrase protein is tightly bound to the proviral DNA, as suggested by coelution from the Sephacryl column and by cosedimentation on the sucrose gradient, it should be possible to precipitate viral DNA with antiserum to the 31-kDa integrase protein. To examine this possibility, several antisera to HIV-1 proteins were tested for the ability to immunoprecipitate viral DNA from column fractions. Viral DNA was found only in the supernatants of immunoprecipitation reactions when antiserum to capsid protein p17 or p24 was used (Fig. 5, lanes 5 and 7). However, full-length linear proviral DNA was detected in the precipitated fractions when antiserum to the 31-kDa integrase protein was used (Fig. 5, lanes 1 and 2). Deproteinated viral DNA was not precipitated by antiserum to integrase (Fig. 5, lanes 11 and 12).

DISCUSSION

The viral integrase protein was shown to be a major component of the integration-competent nucleoprotein complex isolated from the cytoplasm of cells newly infected with HIV-1. Furthermore, integrase was the only viral protein that remained associated with the viral DNA through successive purification by gel filtration and gradient sedimentation. All other virion-associated proteins carried into the cell during infection were separated from the viral preintegration complex without affecting its ability to integrate into DNA targets in vitro. The purified complex contained no detectable *gag* proteins, including p17, p24, p7, and p6. The preintegration complex was similarly devoid of other *pol* gene products, including the p66 and p51 viral DNA polymerase proteins, the 15-kDa RNase H, and the 10-kDa viral protease. This is the first report that defines the viral proteins present in a purified, fully functional retroviral preintegration complex.

Integrase eluted in the void volume fractions of a Sephacryl S400 column by virtue of its association with newly synthesized viral DNA. This association was demonstrated by the ability of a polyclonal antiserum to HIV-1 integrase to immunoprecipitate the viral DNA from column fractions and by the comigration of integrase and viral DNA in column fractions on sucrose density gradients. The preintegration

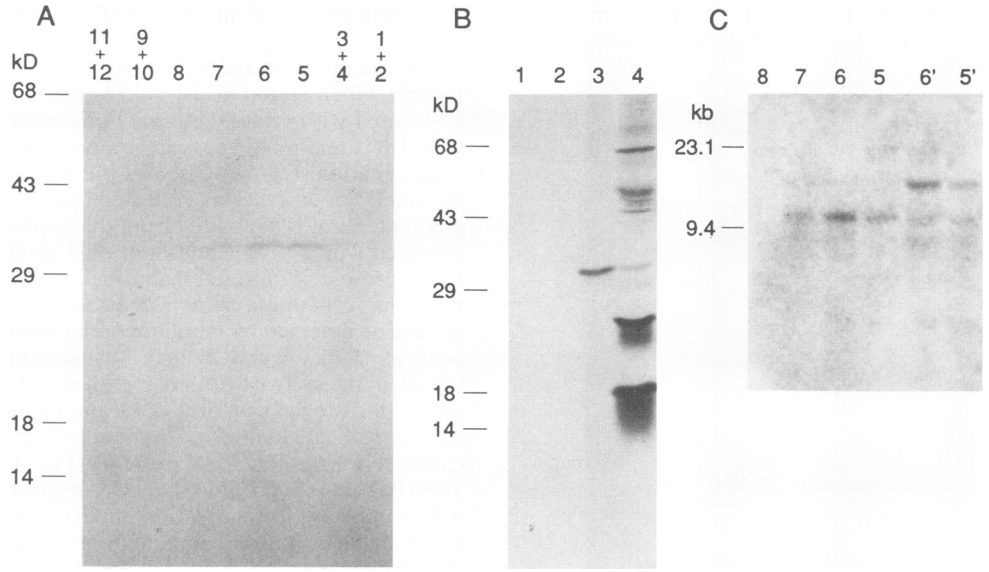

FIG. 4. Sucrose gradient sedimentation of preintegration complexes. S400 column fractions containing the peak of viral DNA (fractions 19 and 20) were pooled and sedimented on a 15 to 30% sucrose gradient. (A) Immunoprecipitation of viral integrase from gradient fractions with HIV-1-positive human serum. Numbers above the lanes correspond to gradient fractions. Fraction 1 is the bottom of the gradient, and fraction 12 is the top. (B) Sucrose gradient fractions 5 to 8 were pooled and immunoprecipitated with antiserum to p17 (lane 1), p24 (lane 2), or integrase (lane 3). Lane 4, Volume fractions of S400 column (fractions 30 to 35) immunoprecipitated with HIV-1-positive human serum. (C) Southern blot analysis of viral DNA in sucrose gradient fractions (lanes 5 to 8) and in vitro integration products of viral DNA in gradient fractions (lanes 5′ and 6′). Numbers above the lanes correspond to gradient fractions. Lanes 5′ and 6′, Linear φX174 DNA (10 μg/ml) was added to gradient fractions 5 and 6 and incubated at 37°C for 1 h. L, Linear viral DNA; I, integration product.

complex present in unfractionated cytoplasmic extracts and the complex purified by gel filtration had similar sucrose gradient sedimentation profiles (Fig. 4 [6]), suggesting that gel filtration did not significantly alter the overall composition or structure of the complex. As much as 98% of the viral DNA present in column fractions and gradient fractions was able to integrate into target DNA molecules in vitro, further evidence that the fractionation procedures used did not disrupt any structural or functional features of the complex needed for integration.

The presence of integrase in a retroviral preintegration complex is not surprising, given the genetic evidence that this protein is required for the establishment of proviral DNA in the infected cell (5, 10, 11, 13). Integrase has also been shown to be responsible for the generation of the proper terminal structure of the linear viral DNA required for integration in vivo (12). These findings suggest an association between integrase and viral DNA in vivo; this report presents the first direct evidence for such an association.

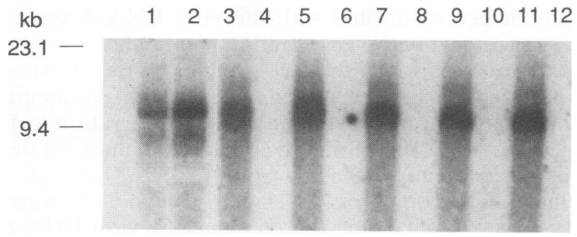

FIG. 5. Southern blot analysis of viral DNA in immunoprecipitation supernatants and pellets. Lanes 1, 3, 5, 7, 9, and 11, Viral DNA in supernatants following immunoprecipitation; lanes 2, 4, 6, 8, 10, and 12, viral DNA in pellets following immunoprecipitation. S400 column fractions containing the peak of viral DNA (fractions 18 to 20) were pooled and immunoprecipitated with antiserum to integrase (lanes 1 and 2), p17 (lanes 5 and 6), p24 (lanes 7 and 8), or reverse transcriptase (lanes 9 and 10). Lanes 3 and 4, Immunoprecipitation with preimmune serum from rabbit used to raise the integrase antiserum, lanes 11 and 12, immunoprecipitation of deproteinated linear viral DNA with the rabbit polyclonal antiserum to HIV-1 integrase used in lanes 1 and 2.

The role of other viral proteins in the integration reaction remains unknown. Work presented here indicates that integrase may be the only viral protein needed for integration after viral DNA synthesis has occurred, as fully integration-competent complexes contained no other detectable viral proteins. Purified HIV-1 integrase protein has recently been shown to be sufficient for the in vitro integration of DNA molecules having ends resembling the termini of linear viral DNA (3). In addition, purified avian and murine retroviral integrase proteins are sufficient to catalyze the self-integration of oligonucleotides which mimic termini of viral DNA (4, 7). These reports, combined with the work presented here, raise the possibility that fully integration-competent nucleoprotein complexes contain linear viral DNA and integrase as the sole viral components.

Nucleoprotein complexes of the murine leukemia virus can be immunoprecipitated from cytoplasmic extracts with antisera to the murine leukemia virus major capsid protein, indicating that this protein is a part of the murine preintegration complex in vivo (1). However, no HIV-1 p24 was detectable in gel filtration and gradient sedimentation fractions containing viral DNA by the methods used here. The presence of a small amount of viral p24 in the nucleoprotein complex undetectable by the methods used here may explain the discrepancy in the two findings. Alternatively, these findings may indicate that the structures of the preintegration complexes of these two retroviruses differ.

The role of cellular factors in the in vitro integration reaction also remains unknown. Addition of the cytoplasmic extracts from uninfected cells to preintegration complexes purified by gel filtration had no effect on the integration reaction. This result does not preclude the involvement of host factors in the integration reaction, as cellular proteins may cofractionate with the preintegration complex and may indeed contribute to the structure of the complex. The presence of host proteins in the preintegration complex is currently under investigation.

ACKNOWLEDGMENTS

We thank Dag Helland for help throughout the course of this work and for helpful comments on the manuscript, Amy Emmert for assistance with the graphics, and Jan Welch for work on the manuscript.

This work was supported by grant AI 24845 from the National Institutes of Health National Cooperative Drug Development Group.

REFERENCES

1. **Bowerman, B., P. O. Brown, J. M. Bishop, and H. E. Varmus.** 1989. A nucleoprotein complex mediates the integration of retroviral DNA. Genes Dev. **3**:469–478.
2. **Brown, P. O., B. Bowerman, H. E. Varmus, and J. M. Bishop.** 1987. Correct integration of retroviral DNA in vitro. Cell **49**:347–356.
3. **Bushman, F. D., T. Fujiwara, and R. Craigie.** 1990. Retroviral DNA integration directed by HIV integration protein in vitro. Science **249**:1555–1558.
4. **Craigie, R., T. Fujiwara, and F. Bushman.** 1990. The IN protein of Moloney murine leukemia virus processes the viral DNA ends and accomplishes their integration *in vitro*. Cell **62**:829–837.
5. **Donehower, L. A., and H. E. Varmus.** 1984. A mutant murine leukemia virus with a single missense codon in pol is defective in a function affecting integration. Proc. Natl. Acad. Sci. USA **81**:6461–6465.
6. **Farnet, C. M., and W. A. Haseltine.** 1990. Integration of human immunodeficiency virus type 1 DNA in vitro. Proc. Natl. Acad. Sci. USA **87**:4164–4168.
7. **Katz, R. A., G. Merkel, J. Kulkosky, J. Leis, and A. M. Skalka.** 1990. The avian retroviral IN protein is both necessary and sufficient for integrative recombination in vitro. Cell **63**:87–95.
8. **Laurence, J., A. Saunders, and J. Kulkosky.** 1987. Characterization and clinical association of antibody inhibitory to HIV reverse transcriptase activity. Science **235**:1501–1504.
9. **Myrick, K., C. Farnet, and W. Haseltine.** Submitted for publication.
10. **Panganiban, A. T., and H. M. Temin.** 1984. The retrovirus pol gene encodes a product required for DNA integration: identification of a retroviral int locus. Proc. Natl. Acad. Sci. USA **81**:7885–7889.
11. **Quinn, T. P., and D. P. Grandgenett.** 1988. Genetic evidence that the avian retrovirus DNA endonuclease domain of *pol* is necessary for viral integration. J. Virol. **62**:2307–2312.
12. **Roth, M. J., P. L. Schwartzberg, and S. P. Goff.** 1989. Structure of the termini of DNA intermediates in the integration of retroviral DNA: dependence on IN function and terminal DNA sequence. Cell **58**:47–54.
13. **Schwartzberg, P. L., J. Colicelli, and S. P. Goff.** 1984. Construction and analysis of deletion mutations in the pol gene of Moloney murine leukemia virus: a new viral function required for productive infection. Cell **37**:1043–1052.
14. **Tanese, N., M. J. Roth, and S. P. Goff.** 1986. Analysis of retroviral *pol* gene products with antisera raised against fusion proteins induced in *Escherichia coli*. J. Virol. **59**:328–340.
15. **Varmus, H. E., and P. O. Brown.** 1989. Retroviruses, p. 53–108. *In* M. Howe and D. Berg (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.