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HMG Protein Family Members Stimulate Human Immunodeficiency Virus Type 1 and Avian Sarcoma Virus Concerted DNA Integration In Vitro

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We have reconstituted concerted human immunodeficiency virus type 1 (HIV-1) integration in vitro with specially designed mini-donor HIV-1 DNA, a supercoiled plasmid acceptor, purified bacterium-derived HIV-1 integrase (IN), and host HMG protein family members. This system is comparable to one previously described for avian sarcoma virus (ASV) (A. Aiyar et al., J. Virol. 70:3571–3580, 1996) that was stimulated by the presence of HMG-1. Sequence analyses of individual HIV-1 integrants showed loss of 2 bp from the ends of the donor DNA and almost exclusive 5-bp duplications of the acceptor DNA at the site of integration. All of the integrants sequenced were inserted into different sites in the acceptor. These are the features associated with integration of viral DNA in vivo. We have used the ASV and HIV-1 reconstituted systems to compare the mechanism of concerted DNA integration and examine the role of different HMG proteins in the reaction. Of the three HMG proteins examined, HMG-1, HMG-2, and HMG-I(Y), the products formed in the presence of HMG-I(Y) for both systems most closely match those observed in vivo. Further analysis of HMG-I(Y) mutants demonstrates that the stimulation of integration requires an HMG-I(Y) domain involved in DNA binding. While complexes containing HMG-I(Y), ASV IN, and donor DNA can be detected in gel shift experiments, coprecipitation experiments failed to demonstrate stable interactions between HMG-I(Y) and ASV IN or between HMG-I(Y) and HIV-1 IN.

Integration of retroviral DNA into the host chromosome is catalyzed by the virally encoded enzyme integrase (IN) and requires short DNA sequences at the ends of the long terminal repeats (LTRs). Model systems utilizing short duplex oligodeoxynucleotides that represent single ends of viral DNA have shown that IN is both necessary and sufficient to cleave and join viral to an acceptor DNA. However, the efficiency of the reaction is low, and there is no synergism between oligonucleotides representing U3 and U5 termini when mixed together. Interaction between the two termini is characteristic of concerted integration in vivo (18). Previously, Fitzgerald et al. (10) and Vora et al. (30) described a reconstituted integration system which used purified avian myeloblastosis virus (AMV) IN, lambda as an acceptor DNA, and a donor DNA, 3,400 or 487 bp in length, containing 30 bp from the ends of the LTRs. The donor DNAs were formed by digestion of tandemly linked AMV U5 and U3 sequences with NdeI so that they contain preprocessed 5′ AT overhangs rather than TT/AA blunt ends. A small percentage of the integrants isolated from these reactions exhibited concerted integration and displayed properties expected of avian sarcoma virus (ASV) DNA integration in vivo. However, the remainder arose through nonconcerted integration events, which produced deletions in the acceptor DNA. Further analysis of the concerted integration products showed that they resulted from two one-ended integration events by different donor DNAs into the same acceptor rather than both ends being provided by the same donor.

More recently, we developed an in vitro reconstituted ASV IN-dependent system that utilizes a small linear blunt-ended donor DNA substrate containing 15 bp derived from the U3 and U5 ends of the LTRs flanking a *supF* gene, purified ASV IN, and a host protein from the high-mobility-group (HMG) family (1). DNA integration with this system is concerted, using the two ends of the same DNA donor molecule for integration. The products of the reaction exhibit all of the properties of integration in vivo, including dependence on both LTRs, the loss of 2 bp from the LTR ends, and the duplication of the cell DNA at the site of integration. Integration occurs randomly in the acceptor DNA, although a low percentage of the integrants were detected at specific hot spots. The efficiency of the ASV IN reconstituted system was increased upon addition of an HMG-1 (1). A subsequent report presented evidence that preintegration complexes isolated from human immunodeficiency virus type 1 (HIV-1)-infected cells contained another HMG family member, HMG-I(Y) (9). Depletion of the preintegration complex of HMG-I(Y) prevented integration, while addition of purified HMG-I(Y) protein restored integration.

HMG proteins are members of a class of eukaryotic nonhistone DNA-binding proteins that modulate chromatin structure and function (5, 6, 12). Because of their many functional similarities, two subgroups of HMG proteins, the HMG-1/-2 and HMG-I(Y) families, in particular, have common functional features that include (i) binding to the minor groove of double-stranded DNA, (ii) recognizing DNA structure rather than sequence, (iii) preferentially interacting with bent, supercoiled, or distorted DNA structures, (iv) binding to non-B-form DNA structures such as four-way junctions and *cis*-platin adducts, (v) unwinding, bending, and supercoiling DNA substrates, in the

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absence of ATP hydrolysis, and (vi) selectively interacting with other sequence-specific transcription factors as part of gene transcription regulatory complexes.

In this report, we describe a reconstituted minidonor DNA system for HIV-1 IN, in which the integration products show all of the properties associated with HIV-1 DNA integration in vivo. The HIV-1 system differs from that described by Goodarzi et al. (11) in that it uses blunt-ended DNA donors and HMG proteins and the observed concerted DNA integration products arise from using two ends of the same DNA donor molecule. We have used this system to compare the mechanism of integration to that of ASV and to study the role of HMG proteins, particularly HMG-I(Y), in the reaction.

MATERIALS AND METHODS

Bacterial strains, growth conditions, plasmids, and reagents. ASV IN was prepared as described by Jones et al. (15). HMG-1 was purified as described by Chow et al. (7). HMG-I(Y) and HMG-2 were prepared and purified as described by Nissen et al. (19). All other reagents, plasmids, bacterial strains, and growth conditions were as previously described (1). Oligodeoxynucleotides were synthesized by Genosys Biotechnologies Inc. (The Woodlands, Tex.) or Midland Certified Reagent Company (Midland, Tex.) and purified by denaturing polyacrylamide gel electrophoresis (PAGE) followed by reverse-phase chromatography as previously described (1). The supercoiled acceptor DNA is pBCSK+ (Stratagene, San Diego, Calif.). The oligodeoxynucleotides used in this study were ASV U5 (WT) (5′AATGAAGCCTTCTGCTGGGCGGAGCCTATG3′, ASV U3 (WT) (5′AATGTAGTCTTATGCGTTGCCCGGATCCGG3′), ΔU3 (5′AG CAATGGCAACAACGTTGCCCGGATCCGG3′), ΔU5 (5′AGCACTCGCAA CAACTGGGCGGAGCCTATG3′), ASV/HIV-1 U5seq (5′TTCAAAAGTCCG AAA3′), ASV/HIV-1 U3seq (5′AGAATTCGGCGTTGC3′), HIV U5 (5′ACT GCTAGAGATTTTCCACACTGGGCGGAGCCTATG3′), and HIV U3 (5′A CTGGAAGGGCTAATTCACTCGTTGCCCGGATCCGG3′.

The ASV U5 (WT) and ASV U3 (WT) oligodeoxynucleotides were used to prepare the wild-type ASV donor DNA substrate, and the HIV U5 and HIV U3 oligodeoxynucleotides were used to prepare the wild-type HIV-1 donor DNA substrate. The ΔU5 and ΔU3 oligodeoxynucleotides were used to prepare donor substrates with random terminal sequences at their ends. They have also been used to construct a donor DNA with both ends changed (ΔΔ donor DNA). The U5seq and U3seq oligodeoxynucleotides were used as sequencing primers. The U3seq primer is complementary to plasmid πvx nucleotides 326 to 312, and the U5seq primer is complementary to plasmid πvx nucleotides 116 to 130.

HMG-I(Y) mutants. The cDNA coding for the full-length human HMG-I(Y) protein (11.7 kDa) consists of an open reading frame with 107 amino acid residues. Standard in vitro mutagenesis techniques were used to introduce four amino acid substitutions (P57A, P61A, P83A, and P87A) to produce a cDNA encoding the dominant-negative mutant protein designated HMG-I(Y) (II, III). Construction and use of the N- and C-terminally truncated mutant protein designated HMG-I(Y) Δ50-91 has been described previously (13). The 41-amino-acid-residue HMG-I(Y) Δ50-91 protein consists of the last two AT-hook DNA-binding domains of the protein together with the intervening peptide backbone.

PCR amplification and purification of integration donor DNA. PCR amplification and purification of integration donors, as well as integration reaction conditions using the ASV IN, were as previously described (1).

HIV-1 integration assay. The HIV-1 integration reaction conditions were similar to those described by Engelman and Craigie (8). For the Mg^{2+}-dependent reaction, 4.6 μmol of wild-type HIV-1 IN was incubated at 37°C for 2 h in a volume of 10 μl with final concentrations of 25 mM morpholinepropanesulfonic acid (MOPS; pH 7.2), 23 mM NaCl, 10 mM dithiothreitol, 5% polyethyleneglycol 8000, 10% dimethyl sulfoxide, 0.05% Nonidet P-40, 1.6 mM HEPES (pH 8.0), 3.3 μM EDTA, 1 mM $MgCl_2$, 1% glycerol, supercoiled plasmid acceptor DNA (0.02 pmol), and ^{32}P-labeled HIV-1 donor DNA (544,000 cpm/pmol, 0.3 pmol). In the Mn^{2+}-dependent reaction, 18.4 μmol of wild-type HIV-1 IN was incubated as described above except with 10 mM 2-mercaptoethanol, 11% glycerol, 0.1 mg of bovine serum albumin per ml, and 1.0 mM $MnCl_2$. The reactions were stopped by increasing the volume to 160 μl with the addition of EDTA (final concentration of 4.25 mM), sodium dodecyl sulfate (SDS; final concentration of 0.44%), and proteinase K (final concentration of 0.06 mg/ml). After digestion for 60 min at 37°C, the reaction mixtures were extracted with phenol followed by phenol-chloroform-isoamyl alcohol (25:24:1 mixture); 17 μl of 3 M sodium acetate (pH 5.2) was added along with 1 μl of glycogen (10-mg/ml stock solution). The reaction products were precipitated by the addition of 400 μl of 100% ethanol and washed four times with 70% ethanol prior to electrophoresis and autoradiography. The products were separated on a 1% agarose gel run in 0.5× Tris-borate-EDTA at 10 V/cm for 2 h. Gels were stained with ethidium bromide, and bands were detected by exposure to UV light. Following electrophoresis, gels were submerged in 5% trichloroacetic acid (TCA) for 20 min or until the bromophenol blue dye turned bright yellow. The gels were dried and exposed to autoradiographic film overnight at −80°C in a film cassette with GAFMEDTA-3 midspeed screens and quantified as described in reference 1.

ASV integration assay. The conditions for reconstitution of integration in vitro with the ASV IN were as described by Aiyar et al. (1). Briefly, 6 pmol of ASV IN was incubated at 37°C for 90 min in a volume of 30 μl with final concentrations of 20 mM Tris-HCl (pH 7.5), 166 mM NaCl, 5 mM dithiothreitol, 10% dimethyl sulfoxide, 0.05% Nonidet P-40, 1% glycerol, 1.6 mM HEPES (pH 8.0), 3.3 μM EDTA, and 6.7 mM $MgCl_2$. The reaction was stopped by increasing the volume to 160 μl with final concentrations of 4.25 mM EDTA, 0.44% SDS, and 0.06 mg of proteinase K per ml.

Cloning and sequencing of integrants. Cloning and sequencing of integrants were done as previously described (1).

Gel shift assay. Assays contained 0.02 pmol of labeled donor DNA, 4.0 pmol of IN, and 4.0 pmol of HMG-1 or HMG-I(Y) protein. Labeled donor was incubated with ASV IN, HMG-1 protein, HMG-I(Y) protein, or both ASV IN and HMG proteins for 25 min in the presence or absence of antisera prepared against either ASV IN or HMG-I(Y). The reactions were carried out in a 10% glycerol buffer (0.5 M NaCl, 1 mM dithiothreitol, 0.1 mM EDTA, 50 mM HEPES [pH 8.5]). The formed complexes were separated on a 1.5% agarose gel (50% GTG SeaKem, 50% SeaPlaque agarose; FMC, Rockland, Maine]) run in 0.5× Tris-borate-EDTA at 10 V/cm for 2 h at 4°C. Following electrophoresis, gels were submerged in 5% TCA for 20 min or until the bromophenol blue dye turned bright yellow. After being washed with water, the gels were dried on DE-81 paper (Whatman) in a Bio-Rad slab gel dryer for approximately 2 h under vacuum. The dried gels were exposed to autoradiographic film for 45 min at 25°C.

Coprecipitation analysis of complexes of IN with HMG proteins in the presence and absence of donor DNA. For analysis of complexes containing ASV IN, we used proteins with glutathione *S*-transferase (GST) that were produced and purified as previously described (2). IN fusion proteins (85 pmol) were preincubated on ice for more than 5 h with HMG proteins (40 pmol) either in the presence or in the absence of cognate donor DNA (1 pmol). Buffer conditions were then adjusted to those used for concerted integration assays in a total volume of 30 μl, and complexes were incubated for 1.5 h at 25°C. A slurry of glutathione-agarose was then added, and the mixture was incubated for 30 min at 25°C with gentle mixing. The reaction was divided in half, and GST-IN-containing complexes were pelleted; one pellet was washed with 20 mM Tris-HCl (pH 7.4)–50 mM NaCl, and the other pellet was not washed. SDS sample buffer was added to supernatants, and pellets and proteins were analyzed by SDS-PAGE followed by either silver staining (Bio-Rad silver stain plus) or Western blotting by standard procedures (4). Western blots used rabbit polyclonal antisera against HMG-I(Y) at a 1:2,000 dilution and a secondary antibody of goat anti-rabbit–horseradish peroxidase conjugate (1:2,000) for chemiluminescence detection (Pierce).

For analysis of complexes containing HIV-1 IN, a soluble six-His-tagged HIV-1 IN (F185K, C280S) protein was used; the polyhistidine sequence was used to pellet complexes with Ni^{2+}-charged chelating Sepharose (Pharmacia). The six-His-tagged IN was incubated on ice with HMG proteins either in the presence or in the absence of cognate donor DNA for greater than 1 h in buffer (0.1 M HEPES [pH 8.1], 100 mM NaCl, 8% glycerol [components from protein storage buffers]) in a total volume of 40 μl. $CaCl_2$ at 10 mM was included in one experiment as noted in the text. Wash buffer (10 mM HEPES [pH 8.1], 100 mM NaCl) was added, followed by 20 μl of a 1:1 slurry of Ni^{2+}-charged chelating Sepharose, and the mixture was incubated with occasional mixing on ice. Complexes were pelleted by brief centrifugation, and the supernatant was removed to a labeled tube. Chelating Sepharose pellets were washed with 100 μl of wash buffer and repelleted, and the wash supernatant was removed to a labeled tube. Complexes were subsequently eluted with 100 μl of elution buffer consisting of 15 mM Tris-HCl (pH 7.5), 0.4 M NaCl, 7 mM 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO), 80 mM EDTA, and 330 mM imidazole. The elution was done at 25°C, and buffer was removed to a labeled tube. Each of these three fractions was TCA precipitated (10%) for 30 min on ice, and all proteins were pelleted for 30 min at 15,000 × *g* at 4°C. The protein pellets were washed with acetone, dried, suspended in SDS protein sample buffer, and analyzed by SDS-PAGE. The remaining chelating Sepharose beads were also boiled in SDS protein sample buffer and analyzed by SDS-PAGE followed by silver staining or Western blotting as described above.

RESULTS

Reconstitution of HIV-1 concerted DNA integration in vitro. An HIV-1 IN-independent integration system using a mini-HIV DNA substrate was reconstituted in vitro by a method patterned after a previously described system for ASV IN (1). The HIV-1 substrate DNA differs from that of ASV in that it contains 20 bp representing the LTRs of HIV-1 DNA flanking the *supF* gene (Fig. 1A). Possible replicative form II (RF II) integration intermediates from the reconstituted reactions are presented diagrammatically in Fig. 1B. They include integrants derived from concerted integration of a single donor by using

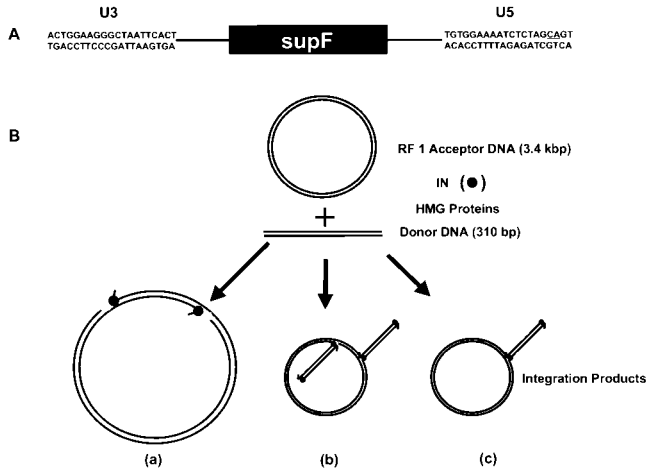

FIG. 1. Reconstitution of integration in vitro. (A) Diagrammatic representation of a mini-HIV-1 donor DNA substrate of 310 bp in length. The 20 bp shown are from the U3 and U5 HIV-1 LTRs. The underlined nucleotides denote the highly conserved CA dinucleotide found near the ends of the LTR sequences. The solid box represents an expression cassette for the *supF* suppressor tRNA. (B) Diagrammatic representation of reconstituted integration with an acceptor DNA, purified IN, HMG proteins, and mini-donor DNA. Possible products include those that result from concerted donor integration (majority product) (a) and from nonconcerted integration by two (b) or one (c) donor DNA(s) via one-ended insertion events.

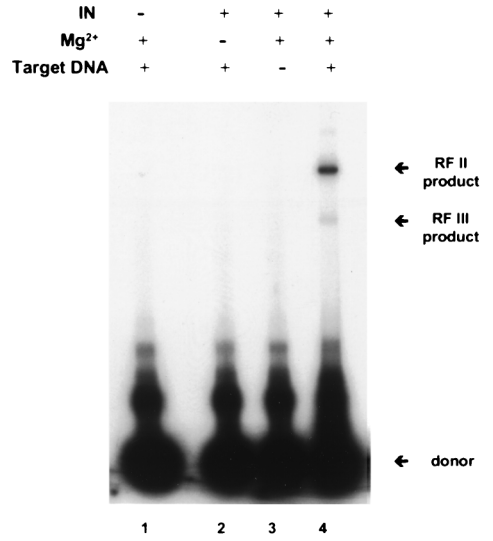

FIG. 2. Requirements for reconstitution of HIV-1 IN-dependent integration in vitro. Integration reactions with wild-type HIV-1 donor DNA (0.3 pmol), acceptor DNA (0.02 pmol), and bacterially expressed HIV-1 IN (6.4 μmol) were incubated and analyzed as described in Materials and Methods. The arrows indicate the labeled donor, RF II, and RF III integration products. Lane 1, IN omitted; lane 2, Mg^{2+} omitted; lane 3, acceptor DNA omitted; lane 4, complete reaction with HMG-I(Y) present. Migration on the gel is from top to bottom.

both LTR ends (product a) and from nonconcerted integration of one or two donors by using a single LTR end (products b and c). Not shown are linear RF III intermediates that could arise by two donors integrating at the same insertion site. The incubation conditions of the reconstituted HIV-1 integration system differ from those used for ASV. They include Mn^{2+} or Mg^{2+} as the divalent cation, lower ionic strengths, and different buffers (see Materials and Methods). The majority of the integration products are detected as a slowly migrating band of nicked circular DNA (RF II product), with an estimated size of 3.7 kb, at the top of the gel marked by the arrow (Fig. 2, lane 4). The product band migrating faster than the RF II product represents a linear form of the same integration product (RF III; Fig. 2, lane 4). The product migrating slower than the RF II product (unmarked) may represent an acceptor DNA with multiple donors inserted. Appearance of each of these integration products was dependent on the addition of IN, divalent cations, and acceptor DNA (Fig. 2, lanes 1 to 3). If the buffer conditions described for ASV IN were substituted for those used with HIV-1 IN, no detectable integration products were observed (data not shown). The HIV-1 assay conditions also used nanomole rather than picomole amounts of HIV IN to compensate for the lower specific activity of the HIV enzyme compared to that of ASV IN.

The ability of different HMG proteins to stimulate HIV-1 IN-independent integration is shown in Fig. 3. Incubation of the HIV donor, HIV IN, and acceptor DNA with either divalent cation produced limited but detectable amounts of RF II integration products (Fig. 3A, lanes 1 and 5). The addition of HMG-I(Y) stimulated integration in vitro with either divalent cation (Fig. 3A, lanes 2 and 6). The extent of stimulation varied, depending on the concentration of HMG protein used or the divalent cation included in the reaction. For example, the titration of HMG-I(Y) in $MgCl_2$-dependent reactions is shown in Fig. 3B. The optimum amount of HMG-I(Y) was found to be 2 pmol, or about a sixfold molar excess over donor DNA. Addition of more HMG-I(Y) to the reaction (Fig. 3B, lanes 1 to 3) resulted in detection of less integration products. This behavior is similar to the effect of addition of other HMG proteins (data not shown). In the presence of $MnCl_2$, 20 pmol of HMG-I(Y) (Fig. 3A, lane 2) gave the maximum stimulation of integration. This concentration of HMG-I(Y) is 10 times higher than that required with the $MgCl_2$-dependent reactions.

In the presence of $MnCl_2$, neither HMG-1 nor HMG-2 protein reproducibly stimulated integration above that observed with HIV-1 IN alone (Fig. 3A, lane 3 or 4, respectively). However, if IN was first preincubated with Mn^{2+}, as described by Asante-Appiah and Skalka (3), HMG-1 was found to stimulate the integration reaction about threefold (data not shown). In contrast to these results, HMG-2 (10 pmol) stimulated integration in the presence of $MgCl_2$ more than 10-fold (Fig. 3A, lane 8) without preincubation. This level of stimulation was greater than that obtained with the optimum concentration of HMG-I(Y) under these same conditions (Fig. 3A, lane 6). HMG-1 did not stimulate integration significantly in the presence of $MgCl_2$ (Fig. 3A, lane 7). While we noted the differences between Mg^{2+}- and Mn^{2+}-dependent reactions (Fig. 3A), further study will be required to understand their significance.

HMG-I(Y) stimulates concerted DNA integration in vitro. To determine if the HIV-1 integration products were the result of concerted DNA integration events, HIV-1 donor substrates substituting random DNA (Δ3 and Δ5; see Materials and Methods) for either the U5 or the U3 LTR were used in the assay. When the ΔU3 donor substrate was used in HMG-2- or HMG-I(Y)-stimulated reactions, there was a significant decrease in the amount of integration products detected, consistent with the results obtained with the reconstituted ASV IN system (data not shown). However, a smaller decrease in integration products was observed when the ΔU5 donor was used, suggesting that a significant portion of the products detected resulted from nonconcerted one-ended U3 LTR donor DNA-driven integration events. With this reconstituted system, individual integrants can be cloned and isolated from

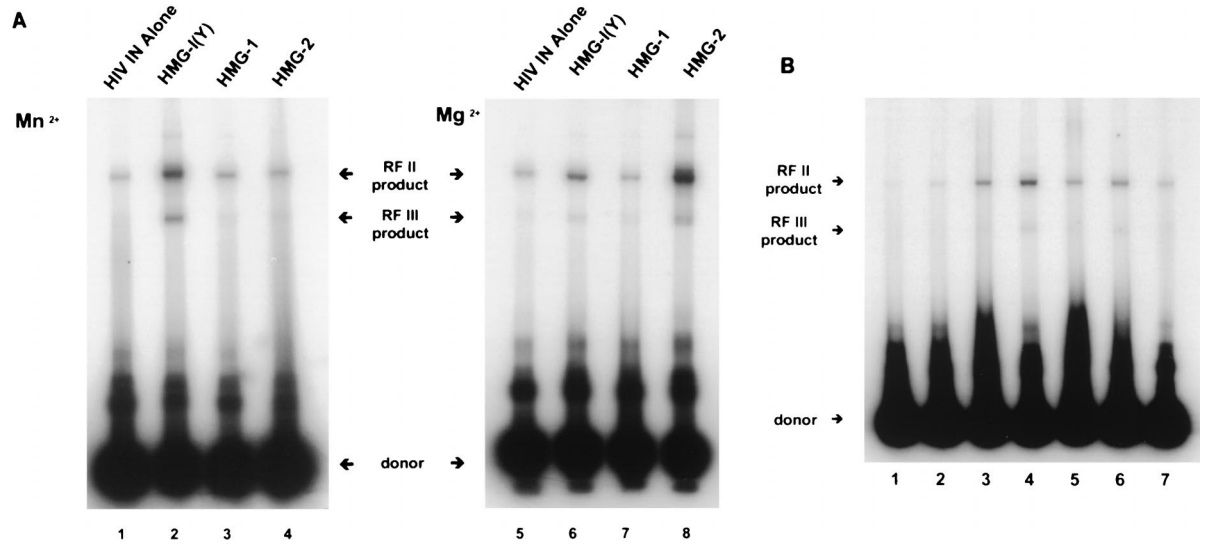

FIG. 3. (A) HMG protein family members stimulate reconstituted DNA integration catalyzed by HIV-1 IN. Reaction conditions were as described in Materials and Methods. Wild-type donor DNA was incubated in the presence of $MnCl_2$ (lanes 1 to 4) or $MgCl_2$ (lanes 5 to 8) with HMG protein family members as follows: lanes 1 and 4, no HMG protein; lanes 2 and 6, 20 and 4 pmol of HMG-I(Y), respectively; lanes 3 and 7, 20 pmol of HMG-1; lanes 4 and 8, 20 and 10 pmol of HMG-2, respectively. (B) Effect of varying the concentration of HMG-I(Y) on HIV-1 IN-dependent integration in vitro. HIV-1 IN-dependent integration reactions were reconstituted with $MgCl_2$ in the presence of increasing concentrations of HMG-I(Y), and the products were analyzed by gel electrophoresis as described in Materials and Methods. HMG-I(Y) was added as follows: lane 1, 20 pmol; lane 2, 10 pmol; lane 3, 4 pmol; lane 4, 2 pmol; lane 5, 1 pmol; lane 6, 0.4 pmol. Lane 7, no added HMG-I(Y). All other notations are as in legend to Fig. 2.

bacteria after *supF* selection and then sequenced. To estimate the percentage of the integration products that arose by concerted integration, individual HIV-1 integrants were cloned and sequenced. Concerted DNA integration events produce short duplications of the acceptor DNA at the junctions with viral DNA, whereas nonconcerted events result in deletions of the acceptor DNA at the sites of integration. Such deletions may arise either from recombination between two donors that have inserted at different sites on the acceptor or from one donor DNA in which the ends have inserted at distal sites.

Summaries of junction sequences for HIV-1 integrants obtained from HMG-2- and HMG-I(Y)-stimulated reactions in the presence of $MgCl_2$ are presented in Tables 1 to 4. Of the 13 HIV-1 integrants sequenced from HMG-2-stimulated reactions, 6 were derived from concerted (Table 1) and 7 were derived from nonconcerted (Table 2) integration events. In each case, the concerted integration events (Table 1) produced a 5-bp duplication of the acceptor DNA and the loss of 2 bp from the LTR ends. Integration occurred in the f1 origin and *lacZ* multiple cloning sequences of the acceptor, as summarized in Fig. 4. The seven nonconcerted DNA integration events showed small deletions of the acceptor DNA without duplications (Table 2). We also examined nine integrants obtained in the presence of $MgCl_2$ from HMG-I(Y)-stimulated reactions (Tables 3 and 4). In this instance, seven of nine integrations were concerted. All but one resulted in 5-bp duplications of the acceptor, and integration occurred again in the f1 and *lacZ* regions of the acceptor DNA (Fig. 4). The two events that were nonconcerted both had 18-bp deletions (Table 4). From these results, it appears that while HMG-2-stimulated integration in the presence of $MgCl_2$ to a greater extent than HMG-I(Y), it did so by increasing multiple one-ended donor insertions that result in nonconcerted integration. Thus, HIV-1 IN-dependent reactions stimulated by HMG-I(Y) more closely resemble integration in vivo.

HMG family members stimulate ASV IN-dependent integration in vitro. Reconstitution of ASV IN-catalyzed concerted DNA integration in vitro was previously described (1) and

TABLE 1. Sites of concerted integration of HIV-1 into acceptor DNA stimulated by HMG 2

Sequence of donor-acceptor junction[a]	Size (bp) of duplication of plasmid DNA	Plasmid position of integration
caaccACGAT.....AAGGTcaacc	5	486–490
gtgagACGAT.....AAGGTgtgag	5	874–878
cggaaACGAT.....AAGGTcggaa	5	992–996
ttttcACGAT.....AAGGTttttc	5	1001–1005
ttccgACGAT.....AAGGTttccg	5	1003–1007
gcaaaACGAT.....AAGGTgcaaa	5	1037–1041

[a] Deoxynucleotide sequence junction of the donor DNA integrated into the acceptor DNA. The sequence of only one strand of the duplex is shown. Lowercase letters denote acceptor DNA; uppercase letters indicate the processed viral DNA sequences, which have lost 2 bp from each end.

TABLE 2. Sites of nonconcerted integration of HIV-1 into acceptor DNA stimulated by HMG 2

Sequence of donor-acceptor junction[a]	Plasmid position of integration	
	U5	U3
aaatcACGAT.....AAGGTaacac	291	690
cttgcACGAT.....AAGGTttctt	351	339
tttggACGAT.....AAGGTCAtagtc	579	2152
aaaggACGAT.....AAGGTgacac	973	956
tcttaACGAT.....AAGGTcgtcg	1370	1387
gtttgACGAT.....AAGGTaacaa	1559	1586
tcggcACGAT.....AAGGTtatcc	2051	901

[a] Deoxynucleotide sequence junction of the donor DNA integrated into the acceptor DNA. The sequence of only one strand of the duplex is shown. Lowercase letters denote acceptor DNA; uppercase letters indicate the processed viral DNA sequences, which have lost 2 bp from each end.

TABLE 3. Sites of concerted integration of HIV-1 into acceptor DNA stimulated by HMG-I(Y)

Sequence of donor-acceptor junction[a]	Size (bp) of duplication of plasmid DNA	Plasmid position of integration
CaaggACGAT.....AAGGTcaagg	5	286–290
CcgACGAT.....AAGGTccg	3	374–376
CgataACGAT.....AAGGTcgata	5	517–521
CcattACGAT.....AAGGTccatt	5	568–572
CttcgACGAT.....AAGGTcttcg	5	866–870
GaccgACGAT.....AAGGTgaccg	5	1033–1037
GaaggACGAT.....AAGGTgaagg	5	1520–1524

[a] Deoxynucleotide sequence junction of the donor DNA integrated into the acceptor DNA. The sequence of only one strand of the duplex is shown. Lowercase letters denote acceptor DNA; uppercase letters indicate the processed viral DNA sequences, which have lost 2 bp from each end.

TABLE 4. Sites of nonconcerted integration of HIV-1 into acceptor DNA stimulated by HMG-I(Y)

Sequence of donor-acceptor junction[a]	Plasmid position of integration	
	U5	U3
aagaaACGAT.....AAGGTgcaag	356	339
aagtgACGAT.....AAGGTggtac	657	640

[a] Deoxynucleotide sequence junction of the donor DNA integrated into the acceptor DNA. The sequence of only one strand of the duplex is shown. Lowercase letters denote acceptor DNA; uppercase letters indicate the processed viral DNA sequences, which have lost 2 bp from each end.

shown to be stimulated four- to fivefold by the inclusion of purified rat HMG-1 protein. Since the different HMG proteins stimulated HIV-1 IN-dependent integration to different extents, we used our ASV reconstituted system to test whether other HMG proteins [HMG-2 and HMG-I(Y)] would stimulate integration in vitro. The products from the reconstituted integration reactions are shown in Fig. 5. In each case, the optimum stimulatory concentration of the HMG protein determined by a titration experiment was used in the reaction. The addition of a greater than optimum amount HMG protein resulted in a decrease in the rate of formation of integration products for all HMG proteins tested. As reported previously, the addition of 4 pmol of rat HMG-1 stimulated concerted integration (Fig. 5, lane 3) compared to reactions with ASV IN alone (lane 1). A similar result was obtained if a comparable amount of purified calf thymus HMG-1 was substituted for the rat protein (data not shown). The addition of calf thymus HMG-2 (4 pmol) to the reaction stimulated the integration of the donor into the acceptor plasmid DNA to the same extent as observed with HMG-1 protein (Fig. 5, lane 4). In contrast, when human HMG-I(Y) (2 pmol) was added to the reaction, there was a more than 10-fold stimulation of the integration reaction (lane 2).

All of the ASV HMG-1-stimulated concerted integration events that were previously sequenced showed the hallmarks of retroviral DNA integration in vivo, including the loss of 2 bp from the ends of the viral DNA and small duplications of acceptor DNA at the site of integration (1). Because HMG-I (Y) was more effective for stimulating ASV DNA integration in vitro, individual integrants from this reaction were isolated and sequenced (Table 5). All eight integrants examined arose from a concerted mechanism. Each had lost 2 bp from the ends of the LTRs, and there was short duplications of 5 or 6 bp of the acceptor DNA at the site of insertion. Sites of integration in the acceptor DNA and the size of duplication of the acceptor DNA are shown in Fig. 4 and compared to the integration data previously derived from HMG-1-stimulated reactions (1). All of the integration events in the presence of HMG-I(Y) sequenced were at different sites in the acceptor than found for reactions in the presence of HMG-1. However, the insertions were in the same general region where most HMG-1-stimulated integration events were mapped. Note also that these sites of integration are in the same region as detected for HIV-1-dependent integration events. If the HMG-I(Y)-stimulated reaction was repeated with a donor DNA that substituted a random sequence for either a U5 or a U3 LTR (Δ3 and Δ5; see Materials and Methods), there was an 80 to 90% reduction in the amount of radioactive donor inserted into the acceptor plasmid for each substrate (data not shown). This result further demonstrates that these reactions are mostly concerted and dependent on the presence of both LTRs, as previously described for reactions containing HMG-1 (1). From these results we conclude that at least three HMG protein family members stimulate ASV IN concerted integration in vitro, although HMG-I(Y) is the most effective.

Several altered HMG-I(Y) proteins have been engineered, and their DNA-binding properties have been investigated (13, 20, 25, 27). One such mutant, HMG-I(Y) Δ50-91, has N- and C-terminal truncations and consequently contains only the last two DNA-binding motifs. It nevertheless binds to DNA substrates with the same specificity and with nearly the same affinity as the full-length native protein (13) but loses the ability to interact with other known wild-type HMG-I(Y) protein partners (reference 13 and unpublished data). Another mutant protein is HMG-I(Y) (II, III), which contains specific amino acid substitutions which replace critical proline residues in the last two DNA-binding regions of the HMG-I(Y) protein that are essential for formation of the AT-hook structural motifs necessary for substrate binding (13). HMG-I(Y) (II, III) does not bind either to AT-rich B-form DNA or to synthetic four-way junction DNA, both of which are preferred binding substrates for the wild-type HMG-I(Y) protein (data not shown). The HMG-I(Y) (II, III) protein does, however, retain the ability to specifically interact with other known protein partners and to act as a dominant-negative competitor protein when expressed as a transgene in cells (unpublished observations). Both of these purified HMG-I(Y) proteins were tested for stimulation of integration in vitro. As shown in Fig. 5, the HMG-I(Y) Δ50-91 protein, which retains the ability to bind to DNA, stimulates integration in vitro (lane 7), whereas the HMG-I(Y) (II, III) protein, which has lost the ability to bind to DNA, does not (lane 8). This finding suggests that HMG-I(Y) DNA-binding capability is important for stimulating ASV IN-catalyzed integration in vitro.

Analysis of complexes of HMG proteins, ASV IN, and donor DNA. To examine interactions between HMG proteins, ASV IN, and donor DNA, the donor was internally labeled with [α-^{32}P]dCTP and mixed with the appropriate proteins, and the resultant complexes were subjected to agarose gel electrophoresis as described in Materials and Methods. The relative positions of complexes were noted by preparing an autoradiogram of the dried gel. In the absence of added protein, labeled DNA (0.02 pmol of ends) migrated to the bottom of the gel (Fig. 6A, lane 1). The addition of a 200-fold molar excess of ASV IN (4 pmol) to the reaction resulted in some donor DNA being retarded in the gel as a broad, slowly migrating smear or at the top of the gel in the wells (lane 2). In contrast, addition of HMG-I(Y) to a concentration of 200-fold in excess to donor (lane 4) shifted all of the DNA as a series of bands. The

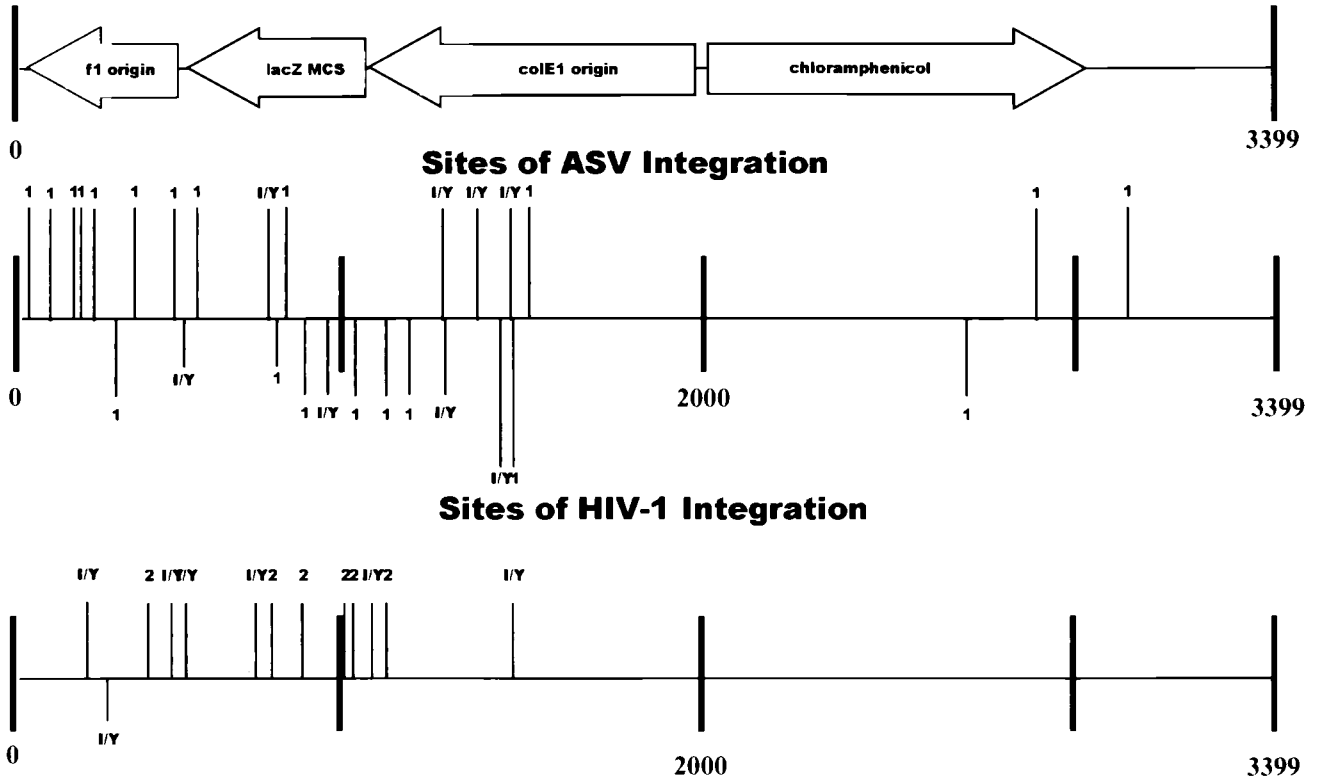

FIG. 4. Sites of concerted integration of donor ASV or HIV-1 DNA. The locations and lengths of flanking duplications of cell DNA for integrants using wild-type ASV or HIV-1 donor DNA as described in the tables are presented. Data for wild-type ASV from Aiyar et al. (1) are also included. The plasmid acceptor data is drawn in a linear representation to scale. The genes of the plasmid and origin of replication are indicated by the open arrows. MCS, multiple cloning site. The thick vertical lines represent numbers of nucleotide in the plasmid DNA. Each thin vertical line represents a separate sequenced integration event. The length of the thin vertical line above or below each bar represents either 4-, 5-, 6-, or 7-bp duplication of the acceptor DNA. The thin lines above the bar are in vitro duplications of acceptor DNA that match duplications observed in vivo, six for ASV and five for HIV-1. The thin lines below the bar represents base pair duplications of the acceptor DNA different from duplications observed in vivo. The presence of HMG-I(Y), HMG-1, or HMG-2 in the reaction is indicated for ASV or HIV-1 integrants above or below the thin vertical lines.

combination of equimolar amounts of ASV IN (4 pmol) and HMG-I(Y) (4 pmol) resulted in the shift of the donor DNA to a slower-migrating band (Fig. 6A, lane 8). This shift was not observed with either protein alone at the protein concentration used (compare lane 8 to lane 2 or 4), although addition of twice as much HMG-I(Y) protein by itself resulted in a band that migrated similarly to the one observed in lane 8 (lane 5). To establish that both ASV IN and HMG-I(Y) form stable complexes with donor DNA, antibodies directed at either protein were added to the reaction and then analyzed by gel electrophoresis. As shown in lane 3, the addition of the anti-IN antiserum supershifted donor DNA to slower-migrating complexes. Similarly, the addition of anti-HMG-I(Y) sera shifted the donor DNA to several slower-migrating bands (lanes 6 or 7). Reactions containing both ASV IN and HMG-I(Y) proteins were also supershifted in the presence of anti-IN (lanes 10 and 11) or in the presence of anti-HMG-I(Y) (lanes 12 or 13). The extent of donor DNA supershifted by the various antibodies was similar to that observed when IN or HMG-I(Y) was added alone (lanes 3 and 6). As a control, no supershift in DNA bands was detected if the anti-IN serum was added to a reaction containing only DNA and HMG-I(Y) or if the anti-HMG-I(Y) serum was added to a reaction containing only DNA and ASV IN (data not shown).

Substitution of the Δ50-91 HMG-I(Y) (Fig. 6B, lanes 7 and 8) but not the HMG-I(Y) (II, III) mutant protein (Fig. 6B, lanes 11 and 12) resulted in a similar gel shift pattern. This result confirms that formation of the complex of ASV IN, HMG-I (Y), and donor DNA was dependent on the ability of HMG-I (Y) protein to bind to DNA. In contrast to these results, we could not demonstrate an altered gel shift pattern when HMG-1 was substituted for HMG-I(Y), even though HMG-1 stimulates integration in vitro (data not shown). To determine whether the gel shifts observed with ASV IN, HMG-I(Y), and donor DNA were dependent on recognition of the 15 terminal bases of U3 or U5 IN recognition sequences in our donor DNA molecule, a donor DNA of similar size but with random sequences at both termini was substituted for the wild type. The results from these gel shift experiments (Fig. 6B, lanes 1 to 4) parallel those obtained with the wild-type donor DNA (Fig. 6A). From these observations, we conclude that HMG-I(Y) does not specifically recognize LTR sequences.

An alternative method to examine interactions between ASV IN, HMG proteins, and donor DNAs is to use affinity chromatography and coprecipitation to isolate and analyze complexes. GST-IN fusion protein was allowed to interact with HMG proteins in the presence or absence of cognate donor DNAs, and complexes were pelleted with glutathione-coated beads. Bound proteins in the complex were washed and analyzed by SDS-PAGE. The results show that more HMG-1 is bound to the beads with ASV IN in the absence of DNA than in its presence (Fig. 7A; compare lanes 4 and 5 with lanes 1 and 2). In the presence of DNA, most of the HMG-1 is found in the supernatant fraction (lane 3). Because the GST-ASV IN fusion

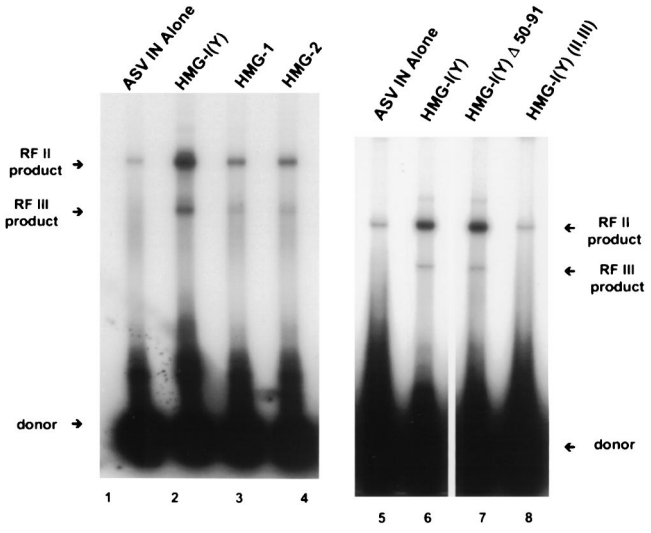

FIG. 5. Reconstituted avian IN-dependent concerted DNA integration is stimulated by several HMG protein family members. Integration reactions with the wild-type ASV ^{32}P-labeled donor DNA (0.3 pmol), acceptor DNA (0.02 pmol), and bacterially expressed IN (6 pmol) were incubated as described in Materials and Methods and analyzed by agarose gel electrophoresis. The arrows indicate the labeled donor, RF II, and RF III integration products. Different HMG protein family members or altered HMG proteins were added to the reaction as follows: lanes 1 and 5, no HMG protein added; lanes 2 and 6, wild-type HMG-I(Y) (4 pmol); lane 3, HMG-1 (8 pmol); lane 4, HMG-2 (8 pmol); lane 7, HMG-I(Y) (Δ50-91) derivative with an N-terminal truncation (4 pmol); lane 8, HMG-I(Y) (II, III) with amino acid substitutions in the A-T hook regions of the protein (4 pmol).

protein can pull down wild-type ASV IN (nonfused) (lanes 7 and 8), we conclude that protein-protein interactions between ASV IN monomers are maintained under these conditions. The same fusion protein was used to test binding to HMG-I (Y). In this case no binding could be detected (even with Western blot analysis) with or without donor DNA present (data not shown). As a control, we did not detect binding of HMG-1 to the glutathione beads in the absence of IN or DNA (data not shown).

To test the binding of HIV-1 IN to HMG proteins, a His-tagged IN (F185K, C280S) protein was used to form complexes with HMG proteins in the absence or presence of cognate donor DNA. Complexes were immobilized on Ni^{2+}-charged chelating Sepharose, pelleted, washed, and then eluted prior to analysis by SDS-PAGE. In contrast to results with ASV IN, some HMG-1 protein is coprecipitated with HIV-1 IN when donor DNA is present (Fig. 7B; compare lanes 3 to 6). Note that there is a low amount of nonspecific HMG-1 binding to the chelating Sepharose (seen in lane 9). While coprecipitation of HMG-1 in the absence of DNA is comparable to this background, coprecipitation of HMG-1 in the presence of DNA is at least threefold higher than the background. These results indicate that interactions between HIV-1 IN and HMG-1 require binding to a common DNA donor. The same His-tagged HIV-1 IN was used to test interactions with HMG-I(Y). Under these conditions, no complexes between these two proteins were detected either in the presence or in the absence of donor DNA. In both instances, HMG-I(Y) was found only in the supernatant and wash fractions (Fig. 7C, lanes 1, 2, 5, and 6). Complex formation with these proteins was also tested in the presence of 10 mM Ca^{2+}, with the same result (data not shown). We conclude that interactions between IN and HMG protein vary with the protein tested. Some HIV-1 IN complex formation with HMG-1 can be detected in the presence of donor DNA, while DNA is not required for interactions between ASV IN and HMG-1. However, neither IN protein can be detected in complex with HMG-I(Y) under these same assay conditions.

TABLE 5. Sites of concerted integration of ASV into acceptor DNA stimulated by HMG-I(Y)

Sequence of donor-acceptor junction[a]	Size (bp) of duplication of plasmid DNA	Plasmid position of integration
ctttTGTAG...CTTCActtt	4	536–539
taagtTGTAG...CTTCAtaagt	5	1373–1377
cgcctcTGTAG...CTTCAcgcctc	6	842–847
gtttgccTGTAG...CTTCAgttgcc	7	1552–1558
catgtTGTAG...CTTCAcatgt	5	981–985
cagcggTGTAG...CTTCAcagcgg	6	1565–1570
gtggtgTGTAG...CTTCAgtggtg	6	1452–1457
cgtgtcTGTAG...CTTCAcgtgtc	6	1367–1372

[a] Deoxynucleotide sequence junction of the donor DNA integrated into the acceptor DNA. The sequence of only one strand of the duplex is shown. Lowercase letters denote acceptor DNA; uppercase letters indicate the processed viral DNA sequences, which have lost 2 bp from each end.

DISCUSSION

The development of ASV and HIV-1 reconstituted concerted integration systems has permitted a direct comparison of the two reactions as well as a vehicle to study the role of host proteins belonging to the HMG family. All of the ASV integration products sequenced that were stimulated by HMG-1 or HMG-I(Y) and used a wild-type donor DNA arose from a concerted mechanism. This is in contrast to the HIV-1 IN-dependent reaction in the presence of Mg^{2+}, in which less than half of the HMG-2 and approximately 75% of the HMG-I(Y) integrants examined resulted from a concerted reaction. One may speculate that the higher percentage of nonconcerted integration events catalyzed by HIV-1 than by ASV IN is an indication that complexes between this IN and donor DNA ends are not as stable as complexes with ASV IN. Alternatively, this difference could reflect the lower specific activity of the HIV-1 IN or some difference in the multimer structure of the two enzyme preparations.

In the case of ASV IN, the percentage of nonconcerted integration events can be increased by introducing base changes into the LTR sequences (unpublished observations), which presumably alters the binding affinity of IN for the LTR recognition sequences. While HIV-1 IN appears to be less efficient in catalyzing concerted integration, the size of the duplication of the acceptor DNA at the site of integration is more homogeneous. All but one of the HIV-1 concerted integrants showed a 5-bp duplication of the acceptor DNA, characteristic of in vivo integration. In contrast, about 60% of the ASV-dependent integrants exhibited the expected 6-bp duplication (Fig. 4). This could reflect differences in stability of protein-protein interactions between ASV or HIV-1 IN protomers with the ends of the donor and with the acceptor DNA. The configuration of the IN protomers could change the spacing of staggered breaks introduced into the acceptor at the site of integration and thereby alter the size of the duplications. The fact that both ASV and HIV-1 IN-dependent in vitro systems produce a high percentage of concerted DNA integration products suggest that they will be very useful for the discovery of new drugs to treat AIDS.

The results from these in vitro studies indicate that multiple HMG proteins can stimulate integration. However, our obser-

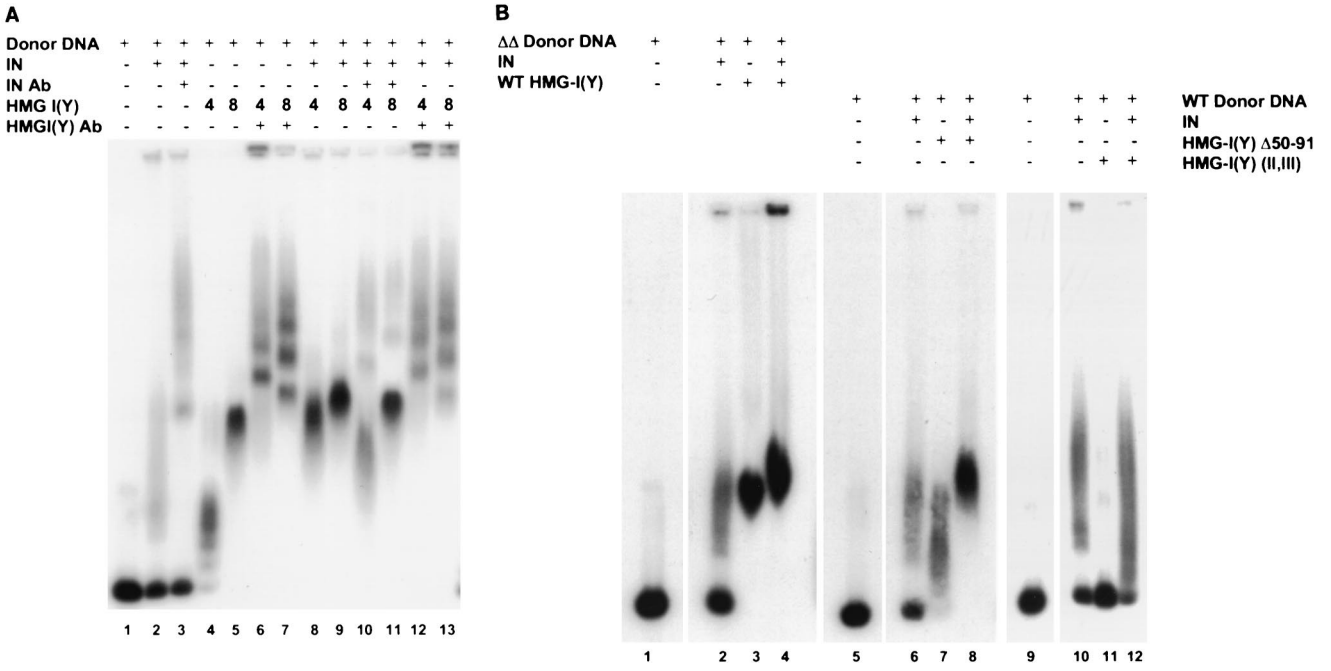

FIG. 6. Complexes of donor DNA, ASV IN, and HMG-I(Y) detected by gel shift assays. (A) ^{32}P-labeled donor DNA was incubated with ASV IN and/or HMG-I(Y) protein in the presence or absence of antibodies (Ab) to IN or HMG-I(Y) as described in Materials and Methods. The complexes formed were analyzed by agarose gel electrophoresis. Lane 1, labeled donor DNA alone; lane 2, donor DNA and 4 pmole ASV IN; lanes 3, donor DNA, 4 pmol ASV IN and anti-IN serum; lane 4, donor DNA and 4 pmol of wild-type HMG-I(Y); lane 5, donor DNA and 8 pmol of HMG-I(Y); lane 6, donor DNA, 4 pmol of HMG-I(Y), and anti-HMG-I(Y) serum; lane 7, donor DNA, 8 pmol of HMG-I(Y) and anti-HMG-I(Y); lane 8, donor DNA, 4 pmol each of ASV IN and HMG-I(Y); lane 9, donor DNA, 4 pmol of ASV IN, and 8 pmol of HMG-I(Y); lane 10, donor DNA, 4 pmol of ASV IN, 4 pmol HMG-I(Y), and anti-IN serum; lane 11, donor DNA, 4 pmol of ASV IN, 8 pmol of HMG-I (Y), and anti-IN serum; lane 12, donor DNA, 4 pmol of ASV IN, 4 pmol HMG-I(Y), and anti-HMG-I(Y) serum; lane 13, donor DNA, 4 pmol of ASV IN, 8 pmol HMG-I(Y), and anti-HMG-I(Y) serum. (B) Gel shifts using wild-type ASV donor DNA or donor DNA with random sequences at each end (ΔΔ), ASV IN, and HMG-I (Y) protein or mutants HMG-I(Y) (II, III) and HMG-I(Y) Δ50-91. Lanes 1 to 4 use the ΔΔ donor DNA. Lane 1, no IN or HMG protein; lane 2, 1 pmol of ASV IN; lane 3, 2 pmol of HMG-I(Y); lane 4, 1 pmol of ASV IN and 2 pmol of HMG-I(Y). Lanes 5 to 12 use the wild-type ASV donor DNA and HMG-I(Y) Δ50-91 where indicated. Lane 5, no IN or mutant HMG protein; lane 6, 1 pmol of ASV IN; lane 7, 2 pmol of HMG-I(Y) Δ50-91; lane 8, 1 pmol of ASV IN and 2 pmol of HMG-I (Y) Δ50-91. Lanes 9-12 use the wild-type ASV donor DNA and 1 pmol of HMG-I(Y) (II, III). Lane 9, no IN or mutant HMG protein; lane 10, 1 pmol of ASV IN; lane 11, 2 pmol of HMG-I(Y) (II, III); lane 12, 1 pmol of ASV IN and 2 pmol of HMG-I(Y) (II, III).

vation that HMG-I(Y) is the most effective HMG protein to stimulate concerted DNA integration by ASV and HIV-1 IN supports the notion that HMG-I(Y) facilitates integration in vivo. Recently, HMG-I(Y) has been detected in HIV-1 preintegration complexes isolated from infected cells (9). Moreover, the activity of these complexes was reported to be dependent on the HMG-I(Y) protein. In contrast to the results with the purified system presented here, these investigators could not demonstrate stimulation of preintegration complexes by HMG-2. The reason for this difference is unclear.

The results of coprecipitation and gel shift experiments show that direct protein-protein interactions between IN and HMG proteins vary with the different proteins tested and are probably not required for stimulation. In addition, HMG proteins from several different species, including calf thymus and rat HMG-1, human HMG-I(Y), and human HMG-2, all stimulate integration of a given IN protein. These observations argue that the stimulatory action of HMG proteins does not require species specific protein-protein interactions. However, both stimulation of enzymatic activity and complex formation require HMG proteins that are competent to bind DNA. The truncated HMG-I(Y) protein (Δ50-91) that lacks the N-terminal-most DNA-binding domain motif (A-T hook region) and its acidic C-terminal tail region is as capable of stimulating integration as wild-type HMG-I(Y). The truncated protein also assembles a ternary complex detected by gel shift. In contrast, another mutant protein, HMG-I(Y) (II, III), which has a number of single amino acid substitutions in the last two A-T hooks that prevent protein-DNA interactions while retaining the capacity for specific protein-protein interaction, did not stimulate integration in vitro or gel shift the donor DNA into a ternary complex in the presence of IN. These results clearly indicate the HMG-I(Y) protein needs to associate with the donor DNA both to form a ternary complex with IN and to stimulate integration. In addition, they indicate that only the last two A-T hook DNA-binding regions and the intervening peptide backbone of HMG-I(Y) are necessary for this stimulation.

The DNA-binding domains of the HMG-1/-2 and HMG-I (Y) proteins have markedly different three-dimensional structures and somewhat different DNA-binding properties (6). This raises the question as to the mechanism(s) by which apparently different proteins stimulate the same integration reaction. For example, the HMG-1/-2 proteins interact in a sequence-independent manner with the minor groove of DNA. The interaction occurs through two DNA-binding domains known as HMG-1 boxes (17) which are a conserved set of amino acids folded into three alpha helices forming an L-shaped structure (22, 31). The HMG-I(Y) proteins also bind preferentially to the minor groove of A-T-rich regions of B-form DNA but through DNA binding domains known as A-T hooks (23). When bound to DNA, these domains assume an extended planar crescent-shaped structure similar to the A-T-minor-groove-binding drugs netropsin and distamycin (13). While these two classes of HMG proteins possess different folds, they both insert segments directly into the minor groove and can exert similar effects on the DNA to which they bind.

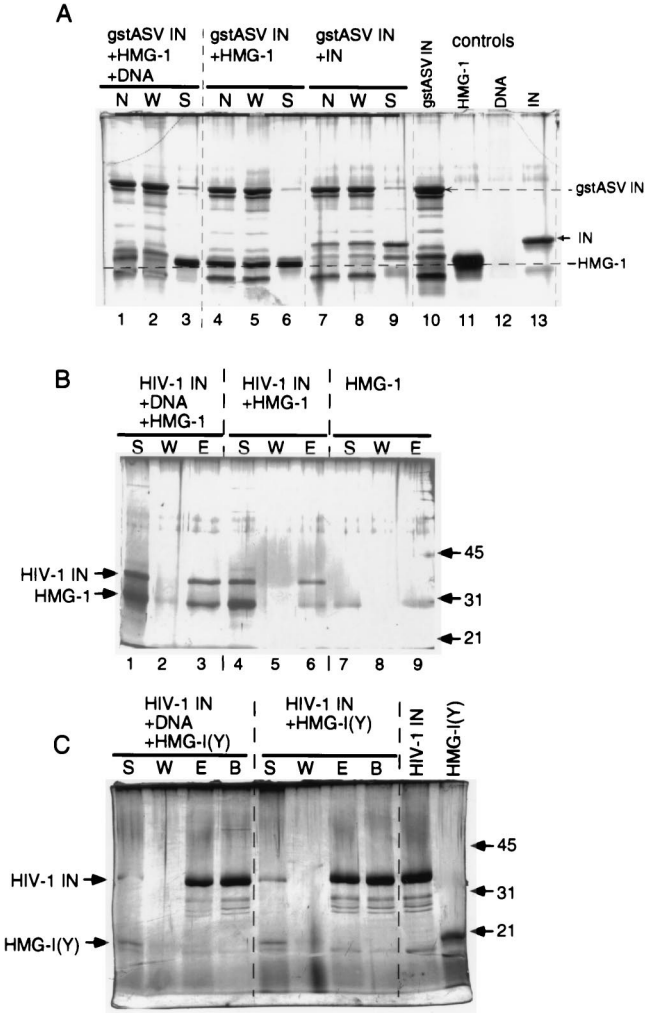

FIG. 7. Analysis of complexes of HMG proteins and IN in the presence or absence of DNA. (A) GST-ASV IN was incubated with HMG-1 in the presence (lanes 1 to 3) or absence (lanes 4 to 6) of donor DNA as described in Materials and Methods. As a positive control for protein-protein interactions, GST-ASV IN was incubated with wild-type ASV IN and these complexes were treated in a identical fashion (lanes 7 to 9). Complexes were pelleted with glutathione-agarose, and supernatants (lanes S) were removed. Half of the pelleted complexes were washed (lanes W), and the other half were directly analyzed (lanes N) by SDS-PAGE followed by silver staining. Lanes 10 to 13 show starting amounts of each component loaded directly on the gel in separate lanes; arrows and dashed lines show the migration positions of each component. (B) His-tagged HIV-1 IN was incubated with HMG-1 in the presence (lanes 1 to 3) or absence (lanes 4 to 6) of donor DNA as described in Materials and Methods. Complexes were pelleted by interaction with Ni^{2+}-charged chelating Sepharose, and the unbound supernatant fraction was removed (S). Pellets were washed, and this fraction (W) was removed and subjected to elution with buffer containing Imidazole and EDTA (E). Each of these three fractions (S, W, and E) were TCA precipitated and analyzed by SDS-PAGE followed by silver staining. Lanes 7 to 9 show the partitioning of HMG-1 alone with no His-tagged IN or DNA present and show a minor level nonspecific binding of HMG-1 to the Sepharose beads (lane 9). Positions of proteins are marked at the left, and positions of molecular weight markers are shown in kilodaltons on the right. (C) His-tagged HIV-1 IN was incubated with HMG-I(Y) in the presence (lanes 1 to 4) or absence (lanes 5 to 8) of donor DNA and analyzed as described for panel B. Any protein remaining with the beads following removal of the elution fraction was released by boiling in SDS sample buffer (fraction B) and also analyzed by SDS-PAGE. Lanes 9 and 10 show starting amounts of each protein loaded directly on the gel in separate lanes.

This may account for the functional similarities observed with proteins from each class. As noted previously, among these common functional characteristics is the ability to bend and unwind DNA in vitro. For example, DNA ligase-mediated ring closure (i.e., cyclization) assays have demonstrated that both HMG-1 (21) and HMG-I(Y) (24a) are capable of bending short, rigid pieces of DNA (i.e., below the persistence length) into closed circles. Therefore, the simple notion has been advanced that both the HMG-1 (1) and HMG-I(Y) (9) proteins might stimulate concerted integration by bending the donor DNA to bring the ends into close proximity. This would facilitate concerted recognition of the termini by IN and accelerate the efficiency and/or rate of the in vitro integration reaction.

Alternatively, because HMG-1 (14) and HMG-I(Y) (20, 24) proteins are capable of unwinding DNA substrates in vitro, it is possible that they function by modulating the helical twist at the ends of the donor DNA. This could improve the efficiency of nucleophilic attack on the acceptor DNA. Recent experiments (15a) indicate that ASV IN unwinds and distorts DNA ends. This distortion appears to be required for viral DNA end processing, but the enzyme will also unwind and distort DNA ends that lack viral sequences. Others (26) have shown that HIV-1 IN preferentially processes frayed DNA ends. Thus, it seems possible that the unwinding activity of HMG proteins could facilitate binding of IN proteins to DNA ends and their subsequent distortion. In earlier experiments (1), we observed that HMG protein maximally facilitated concerted joining when preincubated with the donor but not the acceptor DNA. Preferential activity of HMG on DNA ends is consistent with the fact that the donor but not the acceptor DNA molecules possess free ends.

It is interesting that there is a striking similarity in the biochemical mechanisms determined for retroviral integration and the initial steps in immunoglobin gene V(D)J recombination, catalyzed by the cellular enzymes RAG1 and RAG2 (28). Moreover, like retroviral integration, V(D)J recombination is stimulated by HMG protein family members (29).

ACKNOWLEDGMENTS

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