JOURNAL OF VIROLOGY, Aug. 1995, p. 4683–4692 Vol. 69, No. 8
0022-538X/95/$04.00+0

Substrate Specificity of Ty1 Integrase

SHARON P. MOORE,[1] MARILYN POWERS,[2] AND DAVID J. GARFINKEL[1]*

ABL-Basic Research Program[1] and SAIC Frederick,[2] NCI-Frederick Cancer Research and Development Center, Frederick, Maryland 21702-1201

Received 24 January 1995/Accepted 1 May 1995

Integration of the *Saccharomyces cerevisiae* retrotransposon Ty1 requires the element-encoded integrase (IN) protein, which is a component of cytoplasmic virus-like particles (VLPs). Using purified recombinant Ty1 IN and an oligonucleotide integration assay based on Ty1 long terminal repeat sequences, we have compared IN activity on substrates having either wild-type or altered donor ends. IN showed a marked preference for blunt-end substrates terminating in an A:T pair over substrates ending in a G:C pair or a 3′ dideoxyadenosine. VLP activity on representative substrates also showed preference for donor strands which have an adenosine terminus. Staggered-end substrates showed little activity when nucleotides were removed from the end of the wild-type donor strand, but removal of one nucleotide from the complementary strand did not significantly diminish activity. Removal of additional nucleotides from the complementary strand reduced activity to minimal detection levels. These results suggest that the sequence specificity of Ty1 IN is not stringent in vitro. The absence of Ty1 IN-mediated 3′ dinucleotide cleavage, a characteristic of retroviral integrases, was demonstrated by using selected substrates. In addition to the forward reaction, both recombinant IN and VLP-associated IN carry out the reverse disintegration reaction with long terminal repeat-based dumbbell substrates. Disintegration activity exhibits sequence preferences similar to those observed for the forward reaction.

The *Saccharomyces cerevisiae* genome contains approximately 25 copies of a transposable element, Ty1, which displays structural and functional similarities to retroviruses (for reviews of Ty elements, see references 2 and 19). Ty1 contains two partially overlapping genes, *TYA1* and *TYB1*, which are functionally equivalent to retroviral *gag* and *pol*, respectively (11, 12, 31). *TYA1* encodes a structural nucleocapsid protein, while *TYB1* gives rise to the catalytic proteins required for transposition: protease (PR), reverse transcriptase (RT)/RNase H (RH), and integrase (IN). The nucleocapsid protein, catalytic proteins, $tRNA^{Met}$ (8), and at least one mRNA copy of the genomic element are assembled into cytoplasmic virus-like particles (VLPs) (1, 20, 32). Reverse transcription takes place in the VLPs, which appear to be absolutely required for the transposition process. Although VLPs accumulate in the cytoplasm, it is likely that a protein-DNA complex returns to the nucleus, where integration takes place.

Ty elements are flanked by two long terminal repeats (LTRs) ending in the conserved inverted dinucleotide 5′TG. . . CA3′ (42). These terminal dinucleotides are absolutely required for transpositional integration of Ty1 in vivo (40). Although the precise mechanism of Ty integration has not been elucidated, analogous reactions mediated by Mu transposase (33) and human immunodeficiency virus type 1 (HIV-1) IN (18) have been shown to occur by a one-step transesterification. The observation that Ty1 transposition requires a 3′-OH (17) supports the hypothesis that Ty1 IN uses a similar mechanism.

In vitro studies using purified VLPs as a source of IN have yielded information regarding substrate preferences, optimal biochemical conditions, and crucial IN domains (3, 4, 14, 16, 17). To examine characteristics of IN outside the context of VLPs, we purified recombinant Ty1 IN to near homogeneity (35) and demonstrated its activity in a Ty-adapted oligonucleotide assay frequently used to measure retroviral IN activity (13, 27). Here, we extend our studies on Ty1 IN to include oligonucleotide substrates representing sequence or structural alterations of the wild-type Ty1 LTR ends. In addition, we show that Ty1 IN demonstrates a specific cleavage activity similar to the disintegration reaction of retroviral integrases (7, 9, 10, 15, 25, 26, 30, 39, 43, 44). This activity, monitored by the Ty1 IN-mediated excision of the LTR arm from a three-armed dumbbell substrate, exhibits substrate preferences that are similar to those of the forward reaction.

MATERIALS AND METHODS

Plasmids and strains. The construction of the galactose-inducible expression plasmid used for Ty1 IN production has been previously described (35). Briefly, the IN coding region of *TYB1* was amplified by PCR (36), and the product was cloned into the yeast expression vector pRDK249 (24). Sequences were added to provide a Met initiator codon and an N-terminal hexahistidine tag. Recombinant plasmids were introduced into *S. cerevisiae* RDKY 1293 (*MATα ura3-52 trp1 leu2-Δ1 his3-Δ200 pep4*::*HIS3 prb1-Δ11.6R can1*^{R} *GAL*) by lithium acetate transformation (22) to create the IN producer strain DG1377.

Purification of recombinant Ty1 IN. A method for purification of Ty1 IN using nickel-nitrilotriacetate chromatography has been published elsewhere (35). This method, with minor modifications, was used to purify IN for the experiments reported here. Strain DG1377 was grown and expression was induced essentially as described by Johnson and Kolodner (24). Following a 24-h induction, cells were harvested by centrifugation and washed three times in 20 mM Tris-HCl (pH 7.5)–150 mM NaCl. All subsequent steps were carried out at 4 °C. Cells were resuspended in 160 ml of buffer A (20 mM Tris-HCl [pH 7.5], 10% [vol/vol] glycerol, 10 mM 2-mercaptoethanol, 120 mM NaCl, 10 mM $NaHSO_3$, 2 mM benzamidine, 0.5 mM phenylmethylsulfonyl fluoride, 4 μM pepstatin A, 2 μM leupeptin). The cell suspension was transferred to a 320-ml polycarbonate chamber of an automated bead beater (Biospec). An equal volume of glass beads was added to the chamber, and cells were disrupted to approximately 70% lysis by 25 cycles (15 s on, 45 s off) glass bead blending. The suspension was centrifuged at 72,500 × *g*, and the pellet was washed once in buffer A, reextracted in buffer A–1.2 M NaCl–20 mM Pefabloc protease inhibitor (Boehringer Mannheim), and stirred overnight. The extract was centrifuged for 1 h at 105,000 × *g*, and the supernatant fraction was saved. A saturated solution of $(NH_4)_2SO_4$ was added to a final concentration of 45% (vol/vol) at a rate of 5 ml/min with constant stirring, allowed to stir for an additional 40 min, and centrifuged at 10,000 × *g*. The

* Corresponding author. Mailing address: Laboratory of Eukaryotic Gene Expression, ABL-Basic Research Program, NCI-Frederick Cancer Research and Development Center, P.O. Box B, Building 539, Frederick, MD 21702-1201. Phone: (301) 846-5604. Fax: (301) 846-6911. Electronic mail address: garfinke@fcrfvl.ncifcrf.gov.

TABLE 1. Blunt-end substrates[a]

Oligonucleotide	Sequence	Description	Activity
1	ACAATTATCTCAACATTCACCCATTTCTCA	Wild-type U5 end	
2	TGTTAATAGAGTTGTAAGTGGGTAAAGAGT		+++
3	----------GT	Terminal dinucleotides changed strands (flipped)	
4	----------CA		+++
5	----------AC	Terminal dinucleotides reversed	
6	----------TG		−
7	----------AG	Terminal dinucleotides flipped and reversed	
8	----------TC		−
9	----------AA	−1C→A	
10	----------TT	−1G→T	++
11	----------GCA	−3T→G	
12	----------CGT	−3A→C	+++
13	----------GTTTG-------	−8→−12 transversion; T→G, C→A	+++
14	----------CAAAC-------		
15	----------TT	2 T's added	
16	----------AA	2 A's added	++
17	----------AATAA	Last 5 bases changed, to all A's and T's	+
18	----------TTATT		
19	----------GGCGG	Last 5 bases changed, to all G's and C's	−
20	----------CCGCC		
21	----------GAGAC	Transversion of last 5 bases; T→G, C→A	−
22	----------CTCTG		
1	----------A	Terminal A-A mismatch	++
23	----------A		
5	----------C	Terminal C-C mismatch	−
8	----------C		

[a] Sequences are presented from 5′ to 3′; the first oligonucleotide of each pair represents the positive strand, and the second oligonucleotide represents the negative strand. Only those sequences different from the wild-type sequence are shown; all others are represented by a dash. Activity was estimated as described in Materials and Methods. These substrates are shown in Fig. 1.

resulting pellet was resuspended in buffer B (20 mM $NaPO_4$ [pH 7.5], 10% glycerol, 0.5 M NaCl) and batch bound overnight to 12 ml of nickel-nitrilotriacetate resin (InVitrogen). Following two rinses with buffer B, the resin was poured into a C16/20 column (Pharmacia), washed extensively with buffer B containing 60 mM imidazole, and eluted with buffer B containing 240 mM imidazole. Peak fractions were concentrated, and the buffer was exchanged by Centricon 30 filtration (Amicon). The fractions were stored in buffer C (20 mM Tris-HCl [pH 7.5], 1 mM dithiothreitol, 0.1 mM EDTA, 5% [vol/vol] glycerol, 300 mM KCl) at −70 °C. Ty1 VLPs were isolated from strain GRY458 (21) by using established methods (16).

Oligonucleotide integration assay. The oligonucleotide integration assay widely used to assay retroviral IN activity (5, 6, 7, 10, 13, 22a, 26, 27, 27a, 27b, 29, 37, 39, 41, 44, 45) was used in our studies to assess the activity of Ty1 IN on substrates representing the wild-type Ty1-H3 U5 LTR as well as sequence or structural variants (see Tables 1 and 2 for sequences). Oligonucleotides were synthesized by using an Applied Biosystems 380B or 394 DNA synthesizer and purified by electrophoresis through a 16% polyacrylamide–7 M urea gel. T4 polynucleotide kinase (U.S. Biochemicals) was used to 5′ end label 200 ng of donor oligonucleotide with [γ-^{32}P]ATP (6,000 Ci/mmol; 1 Ci = 37 GBq; Amersham) prior to annealing with a complementary oligonucleotide. Following addition of threefold-excess complementary oligonucleotide, the reaction mixture was heated to 85 °C and allowed to cool slowly to room temperature overnight. The unincorporated label and unannealed single strands were removed by a Sephadex G-25 spin column (Boehringer Mannheim). Specific activity of these substrates was approximately 2×10^8 cpm/μg of DNA.

Standard assay conditions consisted of reaction in 20 μl of a mixture containing 10 mM Tris-HCl (pH 7.4), 3 mM $MnCl_2$, 0.1 mM dithiothreitol, 50 mM KCl, 0.8% (vol/vol) glycerol, 5% (wt/vol) polyethylene glycol 8000, 3.3 μl of purified Ty1 IN, and 1.5 pmol of labeled double-strand substrate (see Tables 1 and 2 for substrate sequences) followed by incubation for 1 h at 30 °C. The reaction products were boiled in sequencing gel loading buffer for 3 min and visualized by autoradiography after electrophoresis through a 20% polyacrylamide–8 M urea gel. An estimate of the efficiency of each substrate relative to the wild type (oligonucleotide pair 1+2) is given as follows: +++, comparable to wild type in band intensities, although certain bands may be absent; ++, band intensities reduced but easily visible; +, band intensities greatly reduced; +/−, minimal band intensities; −, no activity observed. These estimations were made because of variable band intensities within a given reaction. Buffer C was substituted for IN in control reactions.

Assay for 3′ dinucleotide cleavage. Oligonucleotides were labeled as described to a specific activity of approximately 5×10^7 cpm. Reaction conditions for Ty1 IN are as described above except that incubation at 30 °C was terminated after 20 min. Assay buffer for HIV-1 IN consisted of 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.5), 10 mM $MnCl_2$, 1 mM dithiothreitol, 0.05% (vol/vol) Nonidet P-40, 2 μl of purified HIV-1 IN at a concentration of 45 mg/ml (generously provided by P. Clark, E. Barsov, and S. Hughes), and 1.5 pmol of labeled double-strand substrate in a final reaction volume of 20 μl. HIV-1 IN reaction mixtures were incubated at 37 °C for 20 min. Reaction products were resolved by electrophoresis and visualized by autoradiography as described above.

Assay for disintegration. The assay for disintegration monitored the specific cleavage of a portion of an oligonucleotide representing an LTR sequence, or variants, annealed in a dumbbell formation to mimic the structure of a half-site integration event (9, 15, 43, 44) (see Table 3 for sequences). When the molecule was annealed correctly, two *Msp*I sites were formed that could be used to check for folding in the three-armed conformation. The substrates were labeled as described above except that nonradioactive rATP was added to the labeling reaction mixture to a final concentration of 26 μM and no additional oligonu-

TABLE 2. Staggered-end substrates[a]

Oligonucleotide	Sequence	Description (strand)	Activity
1	ACAATTATCTCAACATTCACCCATTTCTCA	Wild-type U5 end	+++
2	TGTTAATAGAGTTGTAAGTGGGTAAAGAGT		
24	ACAATTATCTCAACATTCACCCATTTCTC	−1 base (+)	−
2	TGTTAATAGAGTTGTAAGTGGGTAAAGAGT		
25	ACAATTATCTCAACATTCACCCATTTCT	−2 bases (+)	+
2	TGTTAATAGAGTTGTAAGTGGGTAAAGAGT		
26	ACAATTATCTCAACATTCACCCATTTC	−3 bases (+)	−
2	TGTTAATAGAGTTGTAAGTGGGTAAAGAGT		
27	ACAATTATCTCAACATTCACCCATTT	−4 bases (+)	+
2	TGTTAATAGAGTTGTAAGTGGGTAAAGAGT		
1	ACAATTATCTCAACATTCACCCATTTCTCA		
28	TGTTAATAGAGTTGTAAGTGGGTAAAGAG	−1 base (−)	+++
1	ACAATTATCTCAACATTCACCCATTTCTCA		
29	TGTTAATAGAGTTGTAAGTGGGTAAAGA	−2 bases (−)	+/−
1	ACAATTATCTCAACATTCACCCATTTCTCA		
30	TGTTAATAGAGTTGTAAGTGGGTAAAG	−3 bases (−)	+
1	ACAATTATCCTCAACATTCACCCATTTCTCA		
31	TGTTAATAGAGTTGTAAGTGGGTAAA	−4 bases (−)	+/−
Additional bases beyond wild-type ends			
1	ACAATTATCTCAACATTCACCCATTTCTCA		
16	TGTTAATAGAGTTGTAAGTGGGTAAAGAGTAA	2 extra A's (−)	+++
15	ACAATTATCTCAACATTCACCCATTTCTCATT	2 extra T's (+)	+++
2	TGTTAATAGAGTTGTAAGTGGGTAAAGAGT		
24	ACAATTATCTCAACATTCACCCATTTCTC	−1 base (+)	
16	TGTTAATAGAGTTGTAAGTGGGTAAAGAGTAA	+2 bases (−)	−
32	ACAATTATCTCAACATTCACCCATTTCTCAT	+1 base (+)	+++
2	TGTTAATAGAGTTGTAAGTGGGTAAAGAGT		

[a] Sequences are presented as in Table 1. These substrates are shown in Fig. 3. Activity was estimated as described in Materials and Methods.

cleotides were added before annealing. Unincorporated label was removed as described above. The specific activity of these substrates was approximately 6.6×10^6 cpm/μg of DNA, and 1.1 pmol was used for each reaction. Otherwise, the assay conditions were identical to those listed for the oligonucleotide integration reaction.

Construction of a substrate with a 3 -terminal dideoxyadenosine. Radioactively labeled oligonucleotide 24 was annealed with oligonucleotide 2 (Table 2) as previously described, resulting in a one-base recess in the donor strand. With the complementary strand used as a template, the recessed end was filled in with either dATP or ddATP by T7 DNA polymerase. Identical polymerization reactions were performed with oligonucleotide pair 1+2 (Table 1) to ensure that steps required for the construction of the substrate did not subsequently alter IN activity. Unincorporated nucleotides were removed by a Sephadex G-25 spin column (Boehringer Mannheim).

TABLE 3. Disintegration substrates[a]

Oligonucleotide	Sequence	Description	Activity	Corresponding forward substrate
	LTR LTR			
D1	TGTTGG TTT CCAACA—target	Wild-type U3	+++	NA
D2	TGAGAA TTT TTCTCA—target	Wild-type U5	+++	1+2
D3	AGAGAA TTT TTCTCT—target	U5 last base flipped	+++	3+4
D4	TTTTTA TTT TAAAAA—target	U5 all A's and T's	+++	17+18
D5	GTAGAA TTT TTCTAC—target	U5 reversed end	−	5+6
D6	TTAGAA TTT TTCTAA—target	−1C→A, −1G→T	+++	9+10
D7	TGCGAA TTT TTCGCA—target	−3T→G, −3A→C	+++	11+12

[a] Complementary sequences comprising the LTR arms of the substrates are designated by brackets. The three non-LTR thymine residues are included to form the hairpin structure. Target sequences are the same as shown in Fig. 6. Activity was estimated as described in Materials and Methods. NA, not applicable.

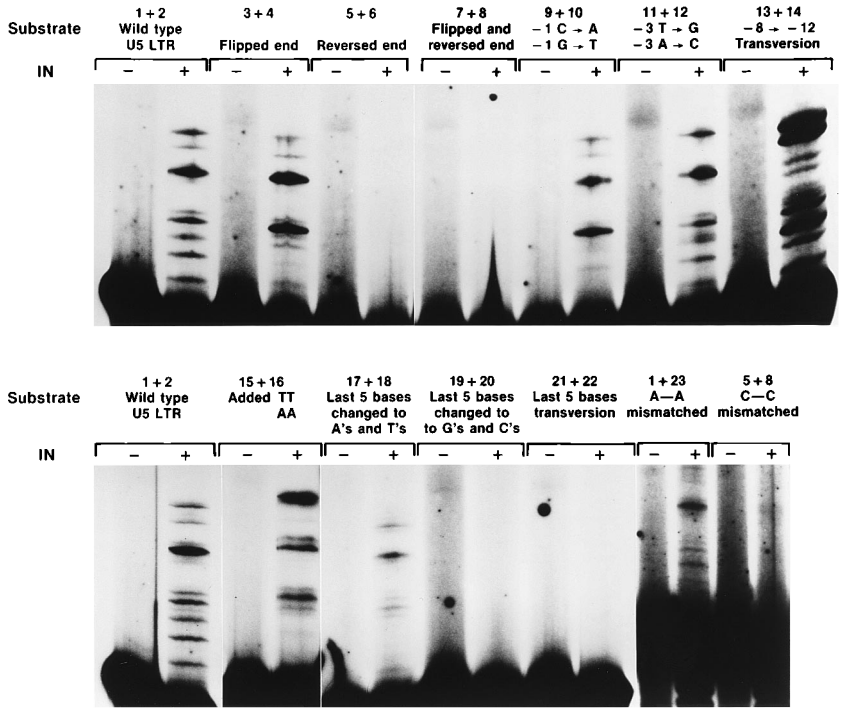

FIG. 1. IN activity with blunt-end substrates that contain different sequences. The first lane of each pair shows the reaction with the designated substrate in which buffer C was substituted for IN. Sequences of the substrates used are listed in Table 1. Donor strand (top; positive), complementary strand (bottom; negative), and orientation are designated for oligonucleotide pair 1+2 in Table 1. Oligonucleotide pairs 1+23 and 5+8 were assayed in a parallel experiment. Oligonucleotide pair 1+2 was included to normalize activity (34).

RESULTS

IN activity with blunt-end substrates. Ty1 IN substrate preferences were determined in an oligonucleotide integration assay. This assay monitors the insertion of a radioactively labeled strand of a short DNA duplex into either strand of a separate but identical duplex. Activity is defined as the formation of products longer than the original substrate as visualized by autoradiography. In this assay, the putative donor strand is designated the positive strand, while the complementary strand is designated as the negative strand. Although the negative strand may also theoretically act as a donor, it is unlabeled; consequently, long products formed in this manner would not be detected. Purified recombinant Ty1 IN and VLP-associated IN gave identical sequence-specific products with both wild-type U3 and U5 oligonucleotide substrates in this assay (35) (see below). However, VLPs catalyzed the formation of less product than did purified Ty1 IN, presumably because there are fewer active IN molecules in VLPs.

The oligonucleotide integration assay showed that recombinant Ty1 IN formed strand-joining products with several non-wild-type substrates containing sequence changes at the 3′ end of the positive strand (Fig. 1; Table 1). For example, a transversion of the terminal 2 bp with the reciprocal strand (oligonucleotide pair 3+4) produced a substrate that was approximately as effective as the wild-type sequence. Other terminal sequence changes that resulted in usable substrates included the addition of two base pairs (T:A pairs) to the wild-type sequence (oligonucleotide pair 15+16), conversion of the last five bases to all A's and T's (oligonucleotide pair 17+18), and a terminal A-A mismatch (oligonucleotide pair 1+23). All of these substrates contained a terminal A or T on the positive strand. In contrast, none of the oligonucleotides containing a terminal G or C were effective IN substrates (oligonucleotide pairs 5+6, 7+8, 19+20, 21+22, and 5+8). Two substrates with terminal mismatches, A-A (oligonucleotide pair 1+23) and C-C (oligonucleotide pair 5+8), were included to determine whether stronger G:C pairing bonds inhibited strand joining. If these bonds precluded utilization of a G:C terminus, then a C-C mismatch should have been as effective as an A-A mismatch substrate. However, the A-A mismatch showed nearly the wild-type level of strand joining, while the C-C mismatch showed no product. This result suggests that terminal base pairing is not a determining factor in substrate suitability.

We also tested two oligonucleotide pairs containing single subterminal mutations (Fig. 1; Table 1, oligonucleotide pairs 9+10 and 11+12) and found little, if any, effect on activity. This finding is in contrast to the results of Sharon *et al*. (40), who have shown that a Ty1 element containing the alteration represented by oligonucleotide pair 9+10 is incapable of transpositional integration in vivo. The most drastic internal mutation involved a transversion in which all wild-type T's were converted to G's, all wild-type C's were converted to A's, and vice versa at positions −8 to −12 (oligonucleotide pair 13+14). This internal change did not diminish activity, but the integration banding pattern was different from that obtained with wild-type oligonucleotides or with oligonucleotides containing terminal sequence changes. The transversion may have resulted in altered target site preferences. A similar effect has also been observed with HIV-1 IN (22a, 41).

We tested three oligonucleotides containing multiple sequence variations within the terminal 5 bp (Fig. 1; Table 1, oligonucleotide pairs 17+18, 19+20, and 21+22). Two of these involved indiscriminately changing the last 5 bp to either all A:T pairs (oligonucleotide pair 17+18) or all G:C pairs (oligonucleotide pair 19+20). Activity was observed with terminal A:T pairs but at greatly reduced levels compared with either

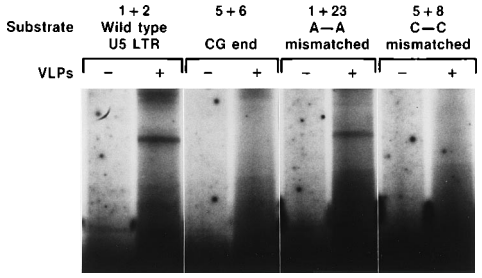

FIG. 2. VLP-associated IN activity with blunt-end substrates containing either a wild-type end (oligonucleotide pair 1+2) or variant ends C:G (oligonucleotide pair 5+6), A-A mismatch (oligonucleotide pair 1+23), and C-C mismatch (oligonucleotide pair 5+8). Refer to Table 1 for sequences of these substrates. The first lane of each pair shows the reaction in which buffer was substituted for Ty1 VLPs; the second lane contains 1.5 μl of Ty1 VLPs. The major product band obtained with wild-type and A-A mismatched substrates corresponds to the major product in Fig. 1, wild-type U5 LTR (oligonucleotide pair 1+2) (34).

the wild-type sequence or single subterminal mutations, suggesting that optimal activity requires multiple internal wild-type sequences. We have not yet determined whether there is a subterminal position more crucial than −1 or −3 which, when mutated, might reduce the level of activity to that observed with oligonucleotide pair 17+18. No activity was observed with terminal G:C pairs (oligonucleotide pair 19+20) or with terminal transversion (oligonucleotide pair 21+22); however, both of these oligonucleotides ended in a G:C or C:G pair, respectively, which was an ineffective substrate in simpler contexts.

To determine if the bias against C:G-ended substrates is a characteristic of recombinant Ty1 IN but not of Ty1 VLPs, we used VLPs as a source of IN in an assay with U5 oligonucleotides having the wild-type A:T end (Fig. 2, oligonucleotide pair 1+2) or variant ends, including a C:G end (oligonucleotide pair 5+6), an A-A mismatch (oligonucleotide pair 1+23), or a C-C mismatch (oligonucleotide pair 5+8). As with recombinant Ty1 IN, product was observed only with the wild-type substrate and the A-A mismatch.

IN activity with staggered-end substrates. Deletion of nucleotides from the wild-type U5 substrate generally reduced or abolished substrate utilization (Fig. 3; Table 2). The exception is that a deletion of the terminal T on the negative bottom strand (5′ end), causing an unpaired A on the donor positive top strand, showed activity at approximately wild-type levels. Progressive deletion of the four 3′ nucleotides on the positive strand yielded alternating terminal C:G or A:T pairs with additional 5′ unpaired bases on the negative strand (oligonucleotide pairs 24+2, 25+2, 26+2, and 27+2). Of these substrates, those in which the terminal paired bases are T:A (oligonucleotide pairs 25+2 and 27+2) show detectable levels of activity, while those in which the terminal paired bases are C:G (oligonucleotide pairs 24+2 and 26+2) did not. However, the level of IN activity did not strictly follow the progression of deletion of terminal nucleotides. For example, deletion of one nucleotide (oligonucleotide pair 24+2) from the positive strand completely abolished strand joining, but deletion of two bases (oligonucleotide pair 25+2) allowed low levels of product formation. Likewise, deletion of three bases (oligonucleotide pair 26+2) yielded no product, but deletion of four bases (oligonucleotide pair 27+2) showed a minimally detectable level of product. In contrast, substrates having 5′ deletions from the negative strand (oligonucleotide pairs 1+28, 1+29, 1+30, and 1+31) did not follow a sequence-related trend. As mentioned above, an overhanging, unpaired 3′ adenosine (oligonucleotide pair 1+28) yielded substantial product even though the terminal paired bases were C:G. Additional dele-

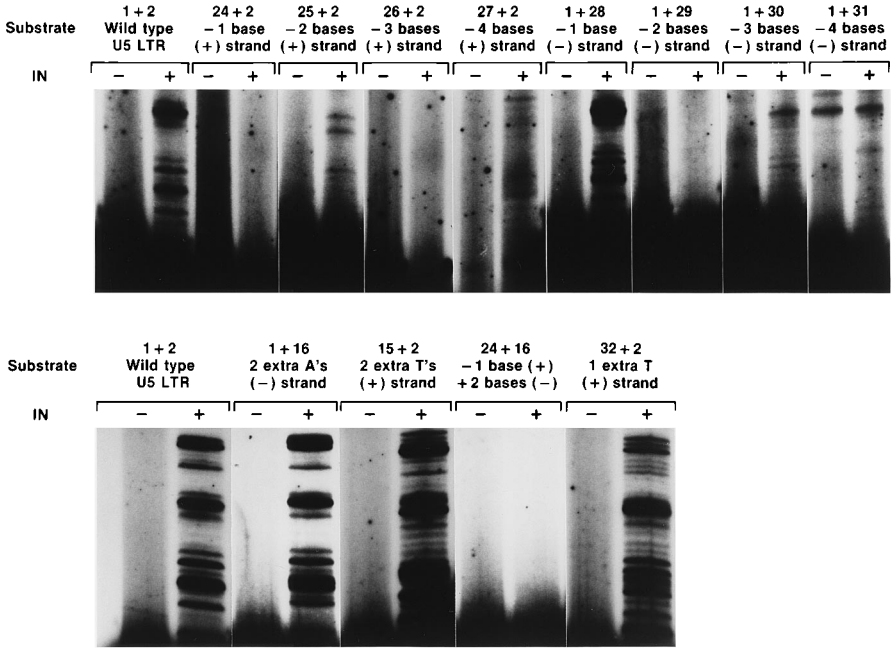

FIG. 3. IN activity with substrates that contain staggered ends. The first lane of each pair shows the reaction with the designated substrate in which buffer C was substituted for IN. Sequences of the substrates used are listed in Table 2. Donor strand (top; positive), complementary strand (bottom; negative), and orientation are designated for oligonucleotide pair 1+2 in Table 2. The top row shows the wild-type blunt-end substrate (oligonucleotide pair 1+2) followed by substrates having up to four bases deleted from either the positive [(+)] or negative [(−)] strand. The bottom row shows substrates which represent extra bases added to the wild-type U5 end and were assayed in a separate but parallel experiment.

tions (oligonucleotide pairs 1+29, 1+30, and 1+31) showed drastic reductions in activity, regardless of the terminal paired sequence. This result suggests that although an unpaired base can serve as a donor (oligonucleotide pair 1+23), deletion of more than one base from the negative strand severely inhibits activity.

If the wild-type end was preserved but nucleotides were added to either strand (oligonucleotide pairs 1+16, 15+2, and 32+2), product formation was comparable to that observed with the blunt-end wild-type substrate. However, the addition of two extra adenosines on the complementary strand could not compensate for the deletion of the terminal adenosine on the donor strand (oligonucleotide pair 24+16).

Comparison of substrates with 3′ deoxyadenosine or dideoxyadenosine. The absence of strict sequence specificity gave rise to the possibility that at least some of the product observed could have resulted from a contaminating activity. Although the precise mechanism of Ty IN-mediated strand joining is not known, similar joining reactions mediated by HIV-1 IN (18) or Mu transposase (33) proceed by a one-step transesterification. Since transesterification requires a 3′-OH, a substrate lacking this terminus should be unable to integrate. Eichinger and Boeke (17) have shown that a similar substrate does not undergo transposition when VLPs are used as a source of IN. However, a terminal dideoxyadenosine should not affect internal sequences if an endonuclease gives rise to a 3′-OH, which could then be available for subsequent ligation or strand transfer by IN. To test this hypothesis, we constructed donor substrates that ended in either a 3′-OH or a 3′-H by adding either dATP or ddATP, respectively, to oligonucleotide pair 24+2 with T7 DNA polymerase, using the complementary oligonucleotide as a template (Fig. 4A). The results of this experiment are shown in Fig. 4B. Controls consisted of oligonucleotide pair 1+2, which should have been unaffected by treatment with either dATP or ddATP, since this substrate had no unpaired nucleotide. All substrates having a 3′-OH end showed similar levels of product formation, but no product was observed when the substrate contained a terminal 3′-H. Figure 4C shows the substrates separated through a 20% sequencing gel to monitor conversion from a 29-mer to a 30-mer by addition of the final nucleotide. Although conversion was incomplete with ddATP, approximately 60% of the substrate was shifted to the higher-molecular-weight species. Given the level of product observed with all other substrates in this experiment, a level of 60% efficiency is well above the threshold of detection. The complete absence of activity with the terminal dideoxyadenosine substrate indicated that a terminal 3′-H was not a usable donor and that no other usable ends arising by other mechanisms were available. Donzella *et al*. (15) have shown that Moloney murine leukemia virus IN also does not carry out an end-joining reaction when a terminal dideoxyadenosine is incorporated into the splice site of a Y substrate.

Detection of Ty1 IN-mediated 3′ dinucleotide cleavage. A specific endonucleolytic activity resulting in the removal of the 3′-terminal dinucleotide of the donor strand has been observed with retroviral integrases (5–7, 13, 18, 22, 22a, 27a, 27b, 29, 39, 41, 45). Sequence analysis of Ty1-target junctions indicates that the conserved TG. . .CA inverted repeat which delimits the Ty1 element is preserved after integration (3, 4, 16, 17). In addition, there are no extra nucleotides present between the conserved dinucleotides at the U3 and U5 borders and the priming sites for Ty1 minus- and plus-strand reverse transcription (2, 19). These results suggest that Ty1 IN does not carry out a dinucleotide cleavage step prior to integration. However, since interpretation of the results in this study relies on the integrity of the terminal sequence prior to strand transfer, it

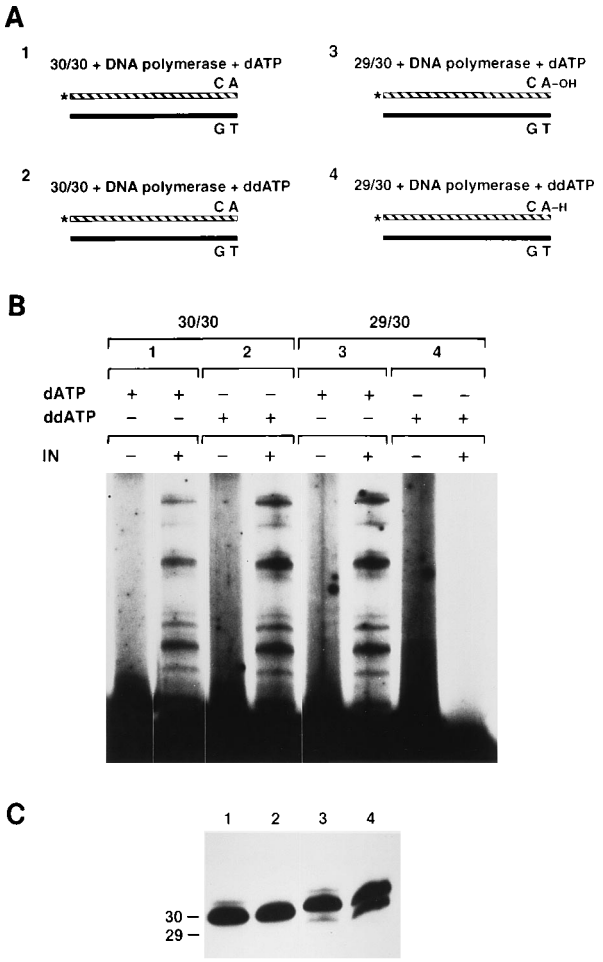

FIG. 4. IN activity with U5 substrates that contain either dATP or ddATP at the 3′ donor end. (A) Diagram of the substrates. Oligonucleotide 30/30 represents wild-type U5 end (oligonucleotide pair 1+2) in which a 30-mer is annealed to a complementary 30-mer. Oligonucleotide 29/30 represents the U5 end with the terminal adenosine residue deleted from the positive strand (oligonucleotide pair 24+2; Fig. 2 and Table 2). (B) IN activity with the substrates shown in panel A. The first lane of each pair represents the reaction in which buffer C was substituted for IN. (C) Substrates 1 to 4 were loaded at reduced counts per minute to determine the efficiency of filling in the terminal nucleotide with either dATP or ddATP and with DNA polymerase I (refer to Materials and Methods).

was necessary to ascertain whether Ty1 IN exhibits a functional 3′ trimming activity in vitro in the presence of retrovirus-like substrates in which two nucleotides 3′ of the U5 CA end are present.

The experiment was designed to determine whether incubation of a labeled oligonucleotide substrate with recombinant Ty1 IN gives rise to a specific product two bases shorter than the starting substrate (Fig. 5). The substrates, diagrammed in Fig. 5A, were labeled at the 5′ end of the donor strand with ^{32}P. Oligonucleotide pairs 25+2 (Fig. 5B, lane 3) and 1+16 (lane 7) and HIV-1 precleaved (lane 11), which were two nucleotides shorter than the experimental substrate, were also included. The shorter substrates were incubated under the same conditions as the experimental substrate, but without IN, and served as markers for the dinucleotide cleavage product. When the Ty1 wild-type substrate (oligonucleotide pair 25+2) was incubated without IN (lane 1) or with Ty1 IN (lane 2), negligible dinucleotide cleavage was observed. These results show that Ty1 IN does not remove a dinucleotide from the

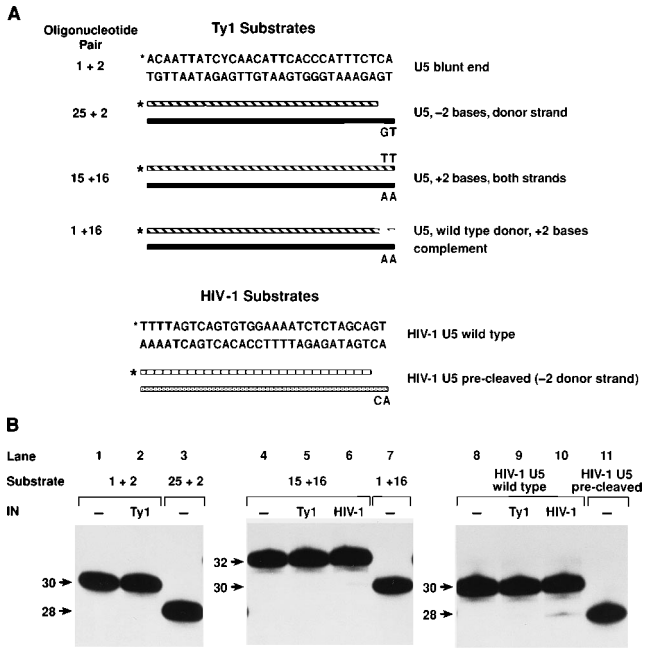

FIG. 5. Assay for IN-mediated 3′-terminal dinucleotide cleavage of Ty1 and HIV-1 oligonucleotide substrates. (A) Diagram of Ty1 and HIV-1 substrates. Oligonucleotide pair numbers correspond to those in Tables 1 and 2. Electrophoretic mobility of cleavage products was monitored by comparison of untreated oligonucleotides that were two nucleotides shorter (oligonucleotide pairs 25+2 and 1+16 and HIV-1 precleaved) than the test substrates with substrates that were full length (oligonucleotide pair 1+2 and HIV-1 wild type) or two nucleotides longer than the wild type (oligonucleotide pair 15+16). (B) Dinucleotide cleavage activity in the absence (lanes 1, 3, 4, 7, 8, and 11) or presence of Ty1 IN (lanes 2, 5, and 9) or HIV-1 IN (lanes 6 and 10). Numbers to the left of each set of substrates indicate sizes in nucleotides. Size markers, in nucleotides (lanes 3, 7, and 11), were run alongside each of the dinucleotide cleavage reactions.

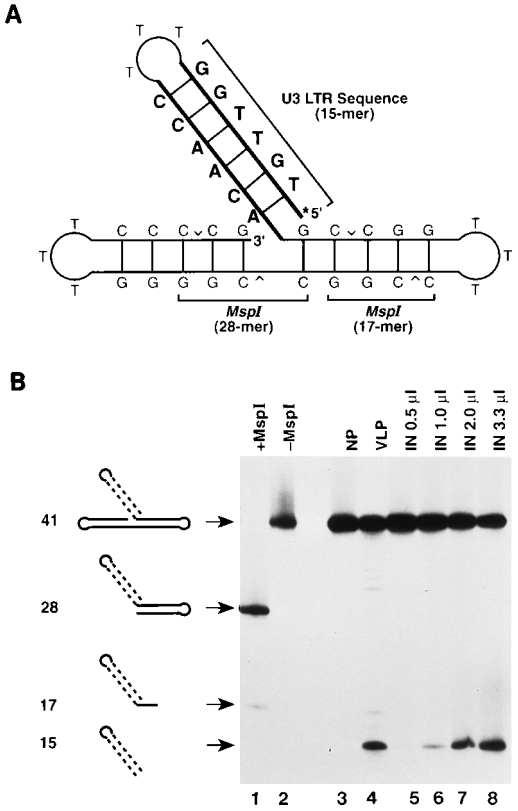

FIG. 6. Disintegration activity of Ty1 IN. (A) Diagram of the Ty1 U3-based disintegration dumbbell substrate. (B) Polyacrylamide gel (20% [wt/vol]) showing susceptibility of the substrate in panel A to *Msp*I digestion (lanes 1 and 2) and cleavage product of the LTR arm with either VLP-associated IN (lane 4) or 0.5, 1.0, 2.0, and 3.3 μl of purified IN (lanes 5 to 8, respectively). Numbers and structures to the left indicate predicted size and structure of the unreacted substrate and disintegration products. No-protein (NP) control is shown in lane 3.

wild-type U5 end (compare lanes 1, 2, and 3). When a substrate with an additional dinucleotide pair (TT:AA) 3′ of the wild-type Ty1 positive terminus (oligonucleotide pair 15+16), which is the same as the terminal dinucleotide pair of Moloney murine leukemia virus, was incubated without IN (lane 4), with Ty1 IN (lane 5), or with HIV-1 IN (lane 6), negligible dinucleotide cleavage was also observed. Our results indicate that neither Ty1 IN nor HIV-1 IN produces a significant dinucleotide cleavage product with this substrate, which, if present, would be expected to migrate with the marker oligonucleotide (lane 7). When wild-type HIV-1 U5 substrate was incubated without IN (lane 8), with Ty1 IN (lane 9), or with HIV-1 IN (lane 10), dinucleotide cleavage was not observed with Ty1 IN but was observed with HIV-1 IN (compare lanes 8, 9, 10, and 11). Strand transfer products were observed with Ty1 IN (lanes 2, 5, and 9) and with HIV-1 IN (lane 10) after longer autoradiography (34). Taken together, our results indicate that Ty1 IN carries out strand transfer without dinucleotide cleavage and strongly suggest that the terminal sequences of the Ty1 substrates used in this study are those directly involved in strand transfer.

Disintegration activity. Several retroviral integrases catalyze a reverse integration reaction (7, 9, 10, 15, 25, 26, 30, 39, 43, 44) termed disintegration. We found that purified recombinant Ty1 IN and VLP-associated IN also carried out disintegration (Fig. 6 and 7). The substrate used for this reaction was a three-armed dumbbell (43) modified such that one arm represented the terminal 6 bp of Ty1 LTR and the remainder of the molecule consisted of nucleotides representing target sequences (Fig. 6A). We monitored correct annealing by the formation of two *Msp*I sites in the target portion of the substrate (Fig. 6). Disintegration activity was indicated by specific cleavage of the LTR arm from the substrate, giving rise to a discrete 15-base product. These results show that the substrate could be completely digested by *Msp*I, indicating proper an-

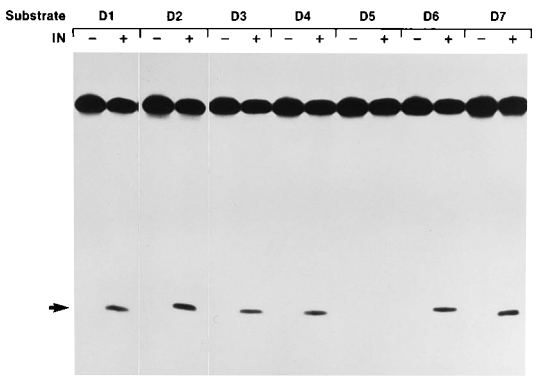

FIG. 7. Disintegration activity of U3 wild type with that of U5 wild type or substrates representing selected U5 variants. The arrow designates the cleavage product. The sequences of the U5 wild-type substrate and variants are listed in Table 3. Only the LTR arm sequence was varied in these substrates.

nealing and that a discrete product of the expected size resulted from incubating the substrate either with VLPs or with purified recombinant IN. Furthermore, the amount of product increases with increasing IN concentrations.

Substrate specificity of disintegration. To determine whether the disintegration activity of recombinant Ty1 IN displays a substrate preference similar to that of the oligonucleotide integration reactions, we assayed several disintegration substrates in which the LTR arm represented either the wild-type U5 sequence or sequence variations corresponding to some of the variant integration substrates. These substrates are shown in Table 3, where the target is identical in sequence to the non-LTR arms in Fig. 6A. The results of this experiment (Fig. 7) are that the disintegration activity of Ty1 IN exhibits sequence preferences similar to those of the forward reaction. All molecules in which an adenosine (oligonucleotides D1, D2, D4, D6, and D7) or thymidine (oligonucleotide D3) is the incorporating LTR nucleotide showed levels of disintegration similar to that of the wild-type U5 substrate, but a substrate having a cytidine in the incorporating position (oligonucleotide D5) was not susceptible to disintegration. However, unlike the case for the forward reaction, the levels of activity were nearly equal among all effective substrates. This result is in contrast to that reported by Vincent *et al*. (43) for HIV-1 IN. Using a splicing-disintegration assay, they have shown that a CA→TC substitution in the LTR arm is a viable, albeit less efficient, disintegration substrate. Although we show a short exposure of the autoradiograph in Fig. 7 to illustrate the minimal quantitative differences in product formation, we observed no product with substrate D5 after longer exposures (34).

DISCUSSION

We have determined whether oligonucleotides representing sequence or structural variations of Ty1 LTR could serve as substrates for purified recombinant IN in an oligonucleotide integration assay. Blunt-end oligonucleotide pairs contained either sequence alterations of the conserved terminal two nucleotides or the terminal five nucleotides, subterminal sequence variations, or terminal 1-bp mismatches (Table 1). Of these substrates, IN showed a marked preference for sequences having a terminal A:T pair. When the sequence variation was confined to the terminal dinucleotide, a thymine terminus on the donor strand was as effective as the wild-type adenine terminus. No IN activity was observed with any of the substrates having a G:C terminus. These results indicate that Ty1 IN utilizes oligonucleotide pairs having A:T ends on either strand and is unable to use any substrate that ends in a G:C pair. This sequence stringency does not appear to be related to simple base pairing, since a terminal A-A mismatch was an efficient substrate whereas a terminal C-C mismatch was not. These results differ somewhat from those reported by Braiterman and Boeke (4). In an assay designed to monitor two-ended integration of a molecule containing 12 bp of Ty1 LTR sequence on each end, or sequence variants thereof, and VLPs as a source of Ty1 IN, several altered molecules, including certain substrates with G:C termini, are effective donor substrates. Conversely, not all substrates with A:T termini are competent in the VLP/two-ended donor assay. An analysis of effective and ineffective substrates supports the conclusion that the number of adenosine residues within the last 4 bp of each terminus is positively correlated with integration activity. Although we have not tested oligonucleotide substrates representing sequences identical to the termini tested or analyzed by Braiterman and Boeke (4), our observation that adenosine residues are crucial for substrate efficiency is consistent with their results. Whether an oligonucleotide substrate having a terminal G:C pair could be rendered more effective by the concomitant alteration of the two adjacent subterminal nucleotides to adenosine residues, which represents the only effective G:C terminal substrate reported by Braiterman and Boeke (4), is not known. However, substrates having G:C terminal preceded by a single A:T pair, regardless of the strand orientation, are ineffective substrates in both studies.

A comparison of Ty data with those derived from retroviral IN studies is not straightforward, since retroviral IN does not carry out strand joining of blunt-end substrates. However, the 3′ dinucleotide cleavage of retroviral LTR substrates gives rise to a 3′ adenosine as the incorporating nucleotide. Studies with IN from different retroviruses have indicated that replacing the −3 A:T pair with a C:G pair severely impairs or abolishes strand-joining activity (6, 13, 29, 39, 41) as well as 3′ cleavage. Leavitt *et al*. (29) have shown that the reduction in strand joining is not a direct result of impaired strand cleavage by using a 3′ recessed substrate containing a terminal G:C pair with two unpaired negative strand nucleotides. This substrate does not exhibit IN-mediated strand joining.

It is not yet known why Ty1 IN and retroviral IN proteins are unable to use oligonucleotide substrates with G:C ends. Results with oligonucleotide pair 5+6, in which the pyrimidine cytosine is in the terminal donor position, along with those for oligonucleotide pair 7+8, having the purine guanine in this position, suggest that the gross structure of the terminal nucleotide probably does not account for this difference. Furthermore, reduced activity obtained with multiple sequence changes indicates that some effect is contributed by subterminal positions. A similar subterminal position effect has been reported for several prokaryotic transposases (23, 28, 38, 46).

Although certain mutations in the blunt-end substrates retain wild-type levels of strand-joining efficiency, removal of nucleotides to produce a staggered-end substrate generally reduces Ty1 IN activity. However, this reduction appears to be related more to the removal of crucial nucleotides than to the introduction of a recessed or overhanging end, since the addition of nucleotides to either strand of a wild-type substrate does not limit strand joining. The ability of Ty1 IN to utilize staggered-end substrates has allowed us to determine whether Ty1 IN reacts with 3′-recessed as well as blunt-end LTR substrates derived from a variety of retroviruses, including avian sarcoma virus, HIV-1, Moloney murine leukemia virus, and feline immunodeficiency virus. All of these substrates yielded strand-joining products when incubated with Ty1 IN in the assay conditions normally used for Ty1 substrates (34). However, all of the 3′-recessed retroviral substrates end in the permissive CA dinucleotide, while the blunt-end substrates end in either a permissive TT or GT dinucleotide.

The absence of strand-joining products with a substrate lacking a 3′-OH supports and extends the observation of Eichinger and Boeke (17) that a substrate with a dideoxyadenosine terminus is not competent for transposition when VLPs are used as a source of IN. Together, these results support the idea that Ty1 integration also proceeds by a transesterification process similar to that reported for HIV-1 IN (18). The results presented here, along with our previously reported observation that purified recombinant Ty1 IN produces strand-joining patterns that, although sequence specific, are identical to those obtained with purified Ty1 VLPs (35) (Fig. 2), minimize the likelihood that the observed products arise from contaminating activities.

Ty1 IN carries out a disintegration reaction yielding products similar to those obtained with retroviral IN proteins with the three-armed substrate. However, unlike HIV-1 IN, which

shows relaxed substrate stringency for disintegration compared with the forward reaction, Ty1 IN exhibits similar substrate sequence requirements for both the joining reaction and the disintegration reaction. This specificity is illustrated by the inability of Ty1 IN to cleave the LTR arm of substrate D5 (Table 3; Fig. 7) in which a C is in the incorporating position. Vincent *et al*. (43) and Chow and Brown (9) have shown that an analogous substitution in a Y disintegration substrate was cleaved by HIV-1 IN, although at reduced efficiency. Bushman and Wang (7) suggested that for HIV-1 IN, disintegration activity and 3′ dinucleotide cleavage activity may be related. A similar relationship between the two activities is not supported for Ty1 IN, since Ty1 IN does not catalyze a 3′ dinucleotide cleavage (Fig. 5).

In summary, the results reported here, obtained by using oligonucleotide substrates for Ty1 IN, differ somewhat from those obtained in a different joining assay (4) as well as data obtained from in vivo transposition with aberrant substrates (40). These differences may represent, at least in part, differences in the integration assays used. The oligonucleotide assay probably represents the simplest form of IN-mediated strand transfer, while a two-ended assay may have additional sequence requirements or conditions. Furthermore, Ty1 transposition in vivo may rely on host cell factors to provide substrate specificity and structural stringency.

ACKNOWLEDGMENTS

We thank Ellie Scott and Colleen Umbenhouer for oligonucleotide production, Joan Hopkins for expert assistance in preparation of the manuscript, Anne Arthur for editorial suggestions, and Conrad Lichtenstein, Charlotte Paquin, Dwight Nissley, and Lori Rinckel for critical review of the manuscript.

This research was supported by the National Cancer Institute under contract N01-CO-46000 with ABL.

REFERENCES

1. **Adams, S. E., J. Mellor, K. Gull, R. B. Sim, M. F. Tuite, S. M. Kingsman, and A. J. Kingsman.** 1987. The functions and relationships of Ty-VLP proteins in yeast reflect those of mammalian retroviral proteins. Cell **49:**111–119.
2. **Boeke, J. D., and S. B. Sandmeyer.** 1992. Yeast transposable elements, p. 193–261. *In* J. R. Broach, J. Pringle, and E. Jones (ed.), The molecular and cellular biology of the yeast *Saccharomyces*: genome dynamics, protein synthesis and energetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
3. **Braiterman, L. T., and J. D. Boeke.** 1994. In vitro integration of retrotransposon Ty1: a direct physical assay. Mol. Cell. Biol. **14:**5719–5730.
4. **Braiterman, L. T., and J. D. Boeke.** 1994. Ty1 in vitro integration: effects of mutations in *cis* and in *trans*. Mol. Cell. Biol. **14:**5731–5740.
5. **Bushman, F. D., and R. Craigie.** 1991. Activities of human immunodeficiency virus (HIV) integration protein in vitro: specific cleavage and integration of HIV DNA. Proc. Natl. Acad. Sci. USA **88:**1339–1343.
6. **Bushman, F. D., and R. Craigie.** 1992. Integration of human immunodeficiency virus DNA: adduct interference analysis of required DNA sites. Proc. Natl. Acad. Sci. USA **89:**3458–3462.
7. **Bushman, F. D., and B. Wang.** 1994. Rous sarcoma virus integrase protein: mapping functions for catalysis and substrate binding. J. Virol. **68:**2215–2223.
8. **Chapman, K. B., A. S. Bystrom, and J. D. Boeke.** 1992. Initiator methionine tRNA is essential for Ty1 transposition in yeast. Proc. Natl. Acad. Sci. USA **89:**3236–3240.
9. **Chow, S. A., and P. O. Brown.** 1994. Substrate features important for recognition and catalysis by human immunodeficiency virus type 1 integrase identified by using novel DNA substrates. J. Virol. **68:**3896–3907.
10. **Chow, S. A., K. A. Vincent, V. Ellison, and P. O. Brown.** 1992. Reversal of integration and DNA splicing mediated by integrase of human immunodeficiency virus. Science **255:**723–726.
11. **Clare, J., and P. Farabaugh.** 1985. Nucleotide sequence of a yeast Ty element: evidence for an unusual mechanism of gene expression. Proc. Natl. Acad. Sci. USA **82:**2829–2833.
12. **Clare, J. J., M. Belcourt, and P. J. Farabaugh.** 1988. Efficient translation frameshifting occurs within a conserved sequence of the overlap between the two genes of a yeast Ty1 transposon. Proc. Natl. Acad. Sci. USA **85:**6816–6820.
13. **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.
14. **Devine, S. E., and J. D. Boeke.** 1994. Efficient integration of artificial transposons into plasmid targets in vitro: a useful tool for DNA mapping, sequencing and genetic analysis. Nucleic Acids Res. **22:**3765–3772.
15. **Donzella, G. A., C. B. Jonsson, and M. J. Roth.** 1993. Influence of substrate structure on disintegration activity of Moloney murine leukemia virus integrase. J. Virol. **67:**7077–7087.
16. **Eichinger, D. J., and J. D. Boeke.** 1988. The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: cell-free Ty1 transposition. Cell **54:**955–966.
17. **Eichinger, D. J., and J. D. Boeke.** 1990. A specific terminal structure is required for Ty1 transposition. Genes Dev. **4:**324–330.
18. **Engelman, A., K. Mizuuchi, and R. Craigie.** 1991. HIV-1 DNA integration: mechanism of viral DNA cleavage and DNA strand transfer. Cell **67:**1211–1221.
19. **Garfinkel, D. J.** 1992. Retroelements in microorganisms, p. 107–157. *In* J. A. Levy (ed.), The retroviridae. Plenum Press, New York.
20. **Garfinkel, D. J., J. D. Boeke, and G. R. Fink.** 1985. Ty element transposition: reverse transcriptase and virus-like particles. Cell **42:**507–517.
21. **Garfinkel, D. J., A.-M. Hedge, S. D. Youngren, and T. D. Copeland.** 1991. Proteolytic processing of *pol-TYB* proteins from the yeast retrotransposon Ty1. J. Virol. **65:**4573–4581.
22. **Gietz, D., A. St. Jean, R. A. Woods, and R. H. Schiestl.** 1992. Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:**1425.

22a. **Hong, T., E. Murphy, J. Groarke, and K. Drlica.** 1993. Human immunodeficiency virus type 1 DNA integration: fine structure target analysis using synthetic oligonucleotides. J. Virol. **67:**1127–1131.

23. **Ichikawa, H., K. Ikeda, J. Amemura, and E. Ohtsubo.** 1990. Two domains in the terminal inverted repeat sequence of transposon Tn2. Gene **86:**11–17.
24. **Johnson, A. W., and R. D. Kolodner.** 1991. Strand exchange protein 1 from *Saccharomyces cerevisiae*. J. Biol. Chem. **266:**14046–14054.
25. **Jonsson, C. B., G. A. Donzella, and M. J. Roth.** 1993. Characterization of the forward and reverse integration reactions of the Moloney murine leukemia virus integrase protein purified from *Escherichia coli*. J. Biol. Chem. **268:**1462–1469.
26. **Jonsson, C. B., and M. J. Roth.** 1993. Role of the His-Cys finger of Moloney murine leukemia virus integrase protein in integration and disintegration. J. Virol. **67:**5562–5571.
27. **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.

27a. **Katzman, M., R. Katz, A. M. Skalka, and J. Leis.** 1989. The avian retroviral integration protein cleaves the terminal sequence of linear viral DNA at the in vivo site of integration. J. Virol. **63:**5319–5327.

27b. **LaFemina, R., P. Callahan, and M. Cordingley.** 1991. Substrate specificity of recombinant human immunodeficiency virus integrase protein. J. Virol. **65:**5624–5630.

28. **Lavoie, B. D., B. S. Chan, R. G. Allison, and G. Chaconas.** 1991. Structural aspects of a higher ordered nucleoprotein complex: induction of an altered DNA structure at the Mu-host junction of the Mu type I transposon. EMBO J. **10:**3051–3059.
29. **Leavitt, A. D., R. B. Rose, and H. E. Varmus.** 1992. Both substrate and target oligonucleotide sequences affect in vitro integration mediated by human immunodeficiency virus type 1 integrase protein produced in *Saccharomyces cerevisiae*. J. Virol. **66:**2359–2368.
30. **Mazumder, A., A. Engelman, R. Craigie, M. Fesen, and Y. Pommier.** 1994. Intermolecular disintegration and intramolecular strand transfer activities of wild-type and mutant HIV-1 integrase. Nucleic Acids Res. **22:**1037–1043.
31. **Mellor, J., S. M. Fulton, M. J. Dobson, W. Wilson, S. M. Kingsman, and A. J. Kingsman.** 1985. A retrovirus-like strategy for expression of a fusion protein encoded by yeast transposon Ty1. Nature (London) **313:**243–246.
32. **Mellor, J., M. H. Malim, K. Gull, M. F. Tuite, S. McCready, T. Dibbayawan, S. M. Kingsman, and A. J. Kingsman.** 1985. Reverse transcriptase activity and Ty RNA are associated with virus-like particles in yeast. Nature (London) **318:**583–586.
33. **Mizuuchi, K., and K. Adzuma.** 1991. Inversion of the phosphate chirality at the target site of Mu DNA strand transfer: evidence for a one-step transesterification mechanism. Cell **66:**129–140.
34. **Moore, S. P., and D. J. Garfinkel.** 1995. Unpublished results.
35. **Moore, S. P., and D. J. Garfinkel.** 1994. Expression and partial purification of enzymatically active recombinant Ty1 integrase in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **91:**1843–1847.
36. **Mullis, K., F. Faloona, S. Scharf, R. Saiki, G. Horn, and H. Erlich.** 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. Cold Spring Harbor Symp. Quant. Biol. **51:**263–273.
37. **Murphy, J. E. and S. P. Goff.** 1992. A mutation at one end of Moloney murine leukemia virus DNA blocks cleavage of both ends by the viral integrase in vivo. J. Virol. **66:**5092–5095.
38. **Nissley, D. V., F. Lindhand, and M. A. Fennewald.** 1991. Mutations in the inverted repeats of Tn*3* affect binding of transposase and transposition

immunity. J. Mol. Biol. **218:**335–347.
39. **Pahl, A., and R. M. Flugel.** 1993. Endonucleolytic cleavages and DNA-joining activities of the integration protein of human foamy virus. J. Virol. **67:**5426–5434.
40. **Sharon, G., T. J. Burkett, and D. J. Garfinkel.** 1994. Efficient homologous recombination of Ty1 element cDNA when integration is blocked. Mol. Cell. Biol. **14:**6540–6551.
41. **Sherman, P. A., M. L. Dickson, and J. A. Fyfe.** 1992. Human immunodeficiency virus type 1 integration protein: DNA sequence requirements for cleaving and joining reactions. J. Virol. **66:**3593–3601.
42. **Temin, H. M.** 1980. Origin of retroviruses from cellular moveable genetic elements. Cell **21:**599–600.
43. **Vincent, K. A., V. Ellison, S. A. Chow, and P. O. Brown.** 1993. Characterization of human immunodeficiency virus type 1 integrase expressed in *Escherichia coli* and analysis of variants with amino-terminal mutations. J. Virol. **67:**425–437.
44. **Vink, C., K. H. van der Linden, and R. H. A. Plasterk.** 1994. Activities of the feline immunodeficiency virus integrase protein produced in *Escherichia coli*. J. Virol. **68:**1468–1474.
45. **Vink, C., D. C. vanGent, Y. Elgersma, and R. H. A. Plasterk.** 1991. Human immunodeficiency virus integrase protein requires a subterminal position of its viral DNA recognition sequence for efficient cleavage. J. Virol. **65:**4636–4644.
46. **Wieter, L. A., and N. D. F. Grindley.** 1991. γδ transposase: purification and analysis of its interaction with a transposon end. J. Biol. Chem. **266:**1841–1849.