THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 276, No. 6, Issue of February 9, pp. 4373–4381, 2001

Printed in U.S.A.

The Mu Enhancer Is Functionally Asymmetric Both in *cis* and in *trans*

TOPOLOGICAL SELECTIVITY OF Mu TRANSPOSITION IS ENHANCER-INDEPENDENT*

Received for publication, September 18, 2000, and in revised form, November 9, 2000
Published, JBC Papers in Press, November 17, 2000, DOI 10.1074/jbc.M008523200

Hong Jiang and Rasika M. Harshey‡

From the Section of Molecular Genetics and Microbiology and Institute of Cellular and Molecular Biology, University of Texas, Austin, Texas 78712

Mu DNA transposition from a negatively supercoiled DNA substrate requires interaction of an enhancer element with the left (attL) and right (attR) ends of Mu. The orientation of the L and R ends with respect to each other (inverted) and with respect to the enhancer is normally inviolate. We show that when the enhancer is provided in *trans* as a linear fragment, the head to head orientation of the L/R ends is still required. Each functional half of the linear enhancer maintains the same "cross-wise" interaction with the subsites L1 and R1, when present in *cis* or in *trans*. In reactions catalyzed by an enhancer-independent variant of the Mu transposase, the need for negative supercoiling of the substrate and the inverted orientation of L and R ends is not relaxed. These results show that the orientation specificity of the enhancer is not determined by its topological linkage to the Mu ends. There is a functional asymmetry inherent to the enhancer. Furthermore, the enhancer does not directly impose topological constraints on the transposition reaction or specify the reactive orientation of the Mu ends.

Site-specific recombination systems in both prokaryotes and eukaryotes require interaction of specific DNA sequences. Although some systems can carry out recombination with the interacting recombination sites in any orientation (*e.g.* phage λ integration/excision, phage P1 Cre/lox recombination, yeast 2-μm plasmid Flp/FRT recombination), other systems have a strict requirement for just one orientation (*e.g.* Mu transposition, Tn3/γδ resolvase reactions, Hin/Gin inversion reactions) (see Ref. 1). A hallmark of the latter systems is the employment of enhancers (or accessory DNA sites) and the requirement for negative DNA supercoiling. Either one or both of these elements (enhancers and DNA supercoiling) could contribute to the orientation specificity of the recombination sites in these systems. For the Mu transposition system, the role of supercoiling was explored by Craigie and Mizuuchi (2) prior to the discovery of the enhancer. Transposition of the L and R ends of Mu present in two different orientations on catenated or knotted supercoiled DNA substrates was monitored. The pattern of reactivity of Mu ends on these novel substrates led the authors to suggest that the normally inverted L and R ends might juxtapose most easily in an "intertwined" parallel configuration on negatively supercoiled DNA; for directly oriented sites an energy barrier must be overcome to bring them together in this parallel configuration. Thus, DNA topology was responsible for sensing the relative orientation of the Mu ends. Experiments with Tn3 resolvase were consistent with the Mu results in suggesting the existence of a "topological filter" (defined as a combined effect of constraints derived from both the circularity of the DNA substrate and the requirement for a highly specific interwrapping of the recombining sites; see Ref. 1) that limits productive interaction to a particular orientation of sites (3). Recent experiments with Tn3 resolvase have implicated interwrapping at the enhancer in determining the topological selectivity of the reaction (4); enhancer-independent resolvase mutants simultaneously lost their normal specificity for directly oriented sites and for supercoiled substrates. Similar results have been reported with the Gin invertase family (5, 6). Since the Mu experiments implicating DNA topology in determining the orientation specificity of the ends (2) were done before the discovery of the enhancer, one objective of the present study was to dissect the contribution of the enhancer, if any, to the topological filter. These studies were facilitated by the existence of an enhancer-independent variant of the Mu transposase (7).

A second objective of this study was to understand the basis of the orientation specificity of the transposition enhancer itself (8–10). Since the Mu ends are inverted with respect to each other, the transposase synapse (in which the enhancer interacts with the L and R ends to form an LER complex; see Ref. 11; see Fig. 1*A*) is expected to be most closely related to the synapse made by the Hin/Gin invertases, whose enhancers do not display orientation specificity (13, 14). Does the orientation specificity of the Mu enhancer arise as a result of an inherent asymmetry in its sequence and/or from being topologically linked to the L and R ends? If the enhancer were free from the constraints of DNA topology, might it acquire the freedom to be active in either orientation? Two developments facilitated this investigation. The first was the demonstration that the enhancer is functional "in *trans*" on a linear DNA fragment (15). The second was the availability of a convenient assay for testing the orientation specificity of the enhancer by exploiting similarities and differences between the transposition systems of Mu and the Mu-related phage D108; these two systems share the same att site specificity but have different enhancer specificities (16). The latter studies found a highly specific "crosswise" interaction between the left O1 site of the enhancer and the right R1 att site and between the right O2 site of the enhancer and the left L1 att site (Fig. 1*B*). We have used these two assays to explore the orientation specificity of both the enhancer and the L/R ends in this study.

* This work was supported by National Institutes of Health Grant GM33247 and in part by Robert F. Welch Foundation Grant F-1531. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 512-471-6881; Fax: 512-471-7088; E-mail: rasika@uts.cc.utexas.edu.

DNA supercoiling plays a critical role in recombination systems that employ accessory enhancer sites to organize the recombination synapse (*e.g.* Mu transposition, Hin/Gin inversion, Tn3/γδ resolution; see Refs. 12, 17, and 18). Varied roles for supercoiling have been deduced in these systems (see "Discussion"). In the Mu transposition system supercoiling is not essential at the enhancer, which can function in *trans* on a linear DNA fragment when the L/R ends are present on supercoiled DNA (15). These results do not rule out a role for supercoiling at the enhancer when it is present in *cis*. It is clear that a *cis* location increases the effective concentration of the enhancer, since a 50-fold molar excess is required for function when the enhancer is supplied in *trans* in the presence of *Escherichia coli* protein IHF (15). A requirement for the DNA bending protein IHF (which binds between the O1 and O2 sites of the enhancer; see Ref. 19) on linear enhancer DNA fragments, or on circular DNA with low superhelical densities (10), suggests that supercoiling may also favor DNA bending at the enhancer. In addition, supercoiling has been shown to be important for binding HU protein at the L end (20) as well as for a critical post-synaptic event (21, 22).

The enhancer interacts with the L and R ends early in the Mu transposition reaction to form an unstable nucleoprotein complex LER (see Fig. 1*A*; not shown are three sets of individual transposase-binding sites L1–L3, O1–O3, and R1–R3 within L, enhancer, and R, respectively). Interactions within LER lead to formation of a stable type 0 complex in which the Mu transposase (MuA protein) assumes its active tetrameric form, catalyzing the cleavage (type I complex) and joining (type II complex) reactions of transposition (12). Only two subunits within the tetramer, those located on L1 and R1, have thus far been implicated in catalysis on supercoiled substrates (Fig. 1*B*; see Ref. 23). Similar results have been reported using R1–R2 oligonucleotide substrates under dimethyl sulfoxide (Me_2SO) reaction conditions (24). The catalytic "DDE" residues of these active subunits work in *trans* (25, 26), *i.e.* DDE^+ subunit at L1 is responsible for cleavage and strand transfer of the opposite R end whereas the DDE^+ subunit at R1 is responsible for these reactions at the L end (23). The specific function of the other two subunits is not known (although they also interact with the enhancer).

We report that Mu ends maintain their orientation specificity as well as their requirement for a supercoiled substrate in reactions with a topologically unlinked enhancer as well as in those not dependent on the enhancer. We compare the role of the enhancer in imposing topological selectivity (defined as the specificity for a particular orientation of topologically linked reactive sites) in other recombination systems.

MATERIALS AND METHODS

Transposition Substrates—Plasmids pJMM and pJDD have been described (16). pJHN (enhancer$^-$) was created by deleting a *Sal*I-*Eco*RI fragment encoding the enhancer from pJMM. pJH25 (enhancer$^-$, inverted attL) was created by inverting a 233-bp[1] *Xba*I-*Bgl*II fragment encompassing attL in pJHN. pJH26 (enhancer$^+$, inverted attL) was constructed in a manner similar to pJH25, except that pJMM was used as the substrate. pJH27 (O2$^-$) was constructed by first incorporating an *Eco*RI site at nt 964 (between IHF and O2 sites) in a PCR primer used to amplify the O1-IHF region on pJMM. A *Bgl*II-*Eco*RI digest of the PCR product was exchanged into similar sites on pJMM, resulting in deletion of O2. pJH28 (O1$^-$) was constructed in a manner similar to pJH27, except by introducing a *Sal*I site at nt 938 (between O1 and IHF sites) into the PCR primer used for amplifying the IHF-O2 region, followed by exchange of the *Sal*I-*Eco*RI-digested PCR product into these sites on pJMM. pJH29 (IHF$^-$ O2$^-$) was constructed similarly, by introducing the *Eco*RI site at nt 943 between O1 and IHF sites.

[1] The abbreviations used are: bp, base pair; nt, nucleotide; PCR, polymerase chain reaction; OC, open circle.

TABLE I
Linear enhancer fragments derived by PCR amplification

Enhancer	Description	Template	Size	Nuc on Mu/D108 genome
			bp	
MM	O1-O2 Mu	pJMM	171	872–1042
DD	O1-O2 D108	pJDD	140	831–970
MD	O1 Mu-O2 D108	pJMD	140	872, 970
DM	O1 D108-O2 Mu	pJDM	167	831, 1042
O1L	O1 Mu	pJH27	171	872–1042
O1	O1 Mu	pJMM	103	872–974
O2	O2 Mu	pJMM	89	954–1042

Linear Enhancer Fragments—These were prepared by PCR amplification of template DNA with appropriate primers. They are shown in Table I. O1L (long) was designed to be the same size as MM and contains nonenhancer DNA in place of O2. The numbers in the last column are the starting and ending nucleotide coordinates of the enhancer fragments encompassing the Mu and/or D108 genome.

Proteins—Purification of MuA, MuA(E392A), MuAΔ84, D108A, D108(E392A), and HU proteins has been described (7). IHF was a generous gift from Steve Goodman, University of Southern California.

In Vitro Assays for Mu DNA Cleavage—Strand cleavage (type I complex) assays were done in 20-μl reaction volumes in 20 mM HEPES-KOH (pH 7.6), 140 mM NaCl, 10 mM $MgCl_2$. Final Me_2SO concentrations were 15% when included. In reactions employing linear enhancer DNA fragments the molar ratio of the donor plasmid DNA to enhancer DNA was 1:50 and that of enhancer DNA to IHF protein was 1:1.6 (15). Besides the amounts of transposase proteins (0.1–0.4 μg), the reaction contained 0.8 μg of donor mini-Mu DNA and 0.2 μg of *E. coli* HU protein. Reaction mixtures were incubated at 30 °C for 20 min and analyzed by agarose gel electrophoresis.

DNA bands were excised from ethidium bromide-stained agarose gels as described (16) and digested with either *Bam*HI plus *Xba*I or with *Bam*HI plus *Aat*II. The DNA fragments were labeled with [α-^{32}P]cordycepin phosphate using terminal nucleotidyltransferase, electrophoresed on 6% denaturing polyacrylamide gels, and detected by autoradiography as described (16).

RESULTS

The "cleavage-in-*trans*" rule (*i.e.* the action of a catalytically active MuA monomer away from the end to which it is bound) has provided the rationale for mapping the enhancer-att interactions, whose functional relationships were deduced by assaying how enhancers of Mu-D108 hybrid specificity respond to mixtures of MuA and D108A proteins and their catalytically inactive DDE^- variants (see Ref. 16; these two transposases bind to each other's att sites but are highly specific for their cognate enhancers). We have now employed the same system for testing the orientation specificity of an enhancer when supplied on a linear fragment (in *trans*) to attL and attR ends present on supercoiled DNA. These studies will be presented first, followed by those employing a variant transposase, which functions in the absence of the enhancer.

Linear Enhancer Substrates—Both Mu and D108 enhancers are composed of three transposase binding regions (O1-O3; see Ref. 16). The polarity of these sites with respect to each other is not known (see "Discussion"). Although the O3 site participates in optimal enhancer function, the O1-O2 sites are sufficient for activity (10, 16). We have therefore used O1-O2 as the enhancer in the present study. Linear DNA fragments encoding wild-type enhancers from Mu and D108 (MM and DD, respectively) as well as hybrid enhancers (MD and DM) were generated by PCR amplification as described under "Materials and Methods." The first and second letters in the enhancer name denote the source of O1 and O2, respectively: M for Mu and D for D108. The logic for the design of the hybrid enhancer substrates has been described (16). The *E. coli* IHF protein is required for function of linear Mu enhancer fragments (15).

Strand Cleavage in the Presence of D108-Mu Hybrid Enhancer (DM) in Trans—When the enhancer and att sites are all

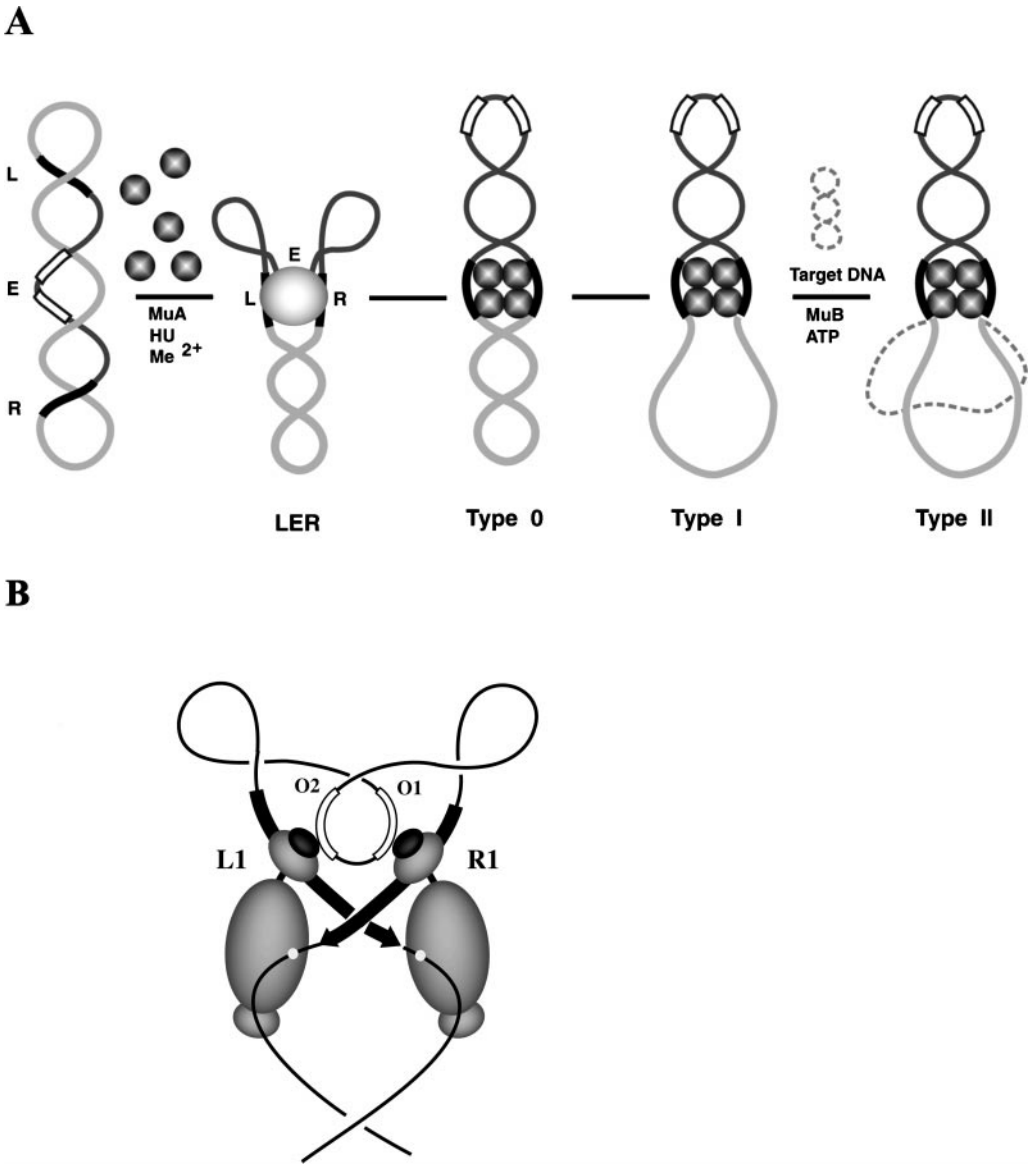

FIG. 1. **Nucleoprotein complexes in Mu transposition.** *A,* monomeric MuA protein binds to the two Mu ends L and R (each composed of three att sites), as well as interacts with an enhancer element E on a negatively supercoiled plasmid, to promote rapid formation of the LER complex in the presence of divalent metal ions and *E. coli* HU protein. Ca^{2+} ions support conversion of LER to type 0, in which MuA has tetramerized and the enhancer is no longer associated with the ends. Mg^{2+} or Mn^{2+} ions also support formation of type 0, promoting cleavage of the synapsed Mu ends to produce the type I complex. MuB protein modulates the activity of MuA at each stage of the reaction and captures target DNA in the presence of ATP to generate the type II strand transfer complex (12). *B,* a model for the arrangement of catalytic MuA subunits within LER. Subunits bound through their DNA-binding domains to L1 and R1 interact cross-wise with O1 and O2 regions of the enhancer. The central domain (containing catalytic DDE residues) of each subunit acts in *trans* to cleave (*white dot*) and subsequently strand transfer (not shown) specific phosphodiester bonds at the two Mu ends. There are two other MuA subunits in the tetramer, not shown because specific structural/catalytic functions have not yet been assigned to them.

present on the same plasmid, the O1 site specifies the occupancy of the transposase monomer at R1, and the O2 site promotes placement of its cognate transposase at L1 (Fig. 1*B*). The O1-R1 rule is rigid; the O2-L1 rule is less so (16). These conclusions were reached from strand cleavage assays using negatively supercoiled plasmid substrates containing the hybrid enhancers DM and MD in *cis* (16).

Reactions of an enhancer-less plasmid pJHN with the DM enhancer provided in *trans* are shown in Fig. 2. Except where indicated, reactions contained the IHF protein. Wild-type MuA did not yield the cleaved type I complex (*lane b*), and the catalytically inactive variant MuA(E392A) did not produce the uncleaved type 0 complex (*lane d*). We know that MuA and its variants are not active on a DM substrate in *cis* unless D108A is present (16). Wild-type D108A was only weakly active (*lane c*; the type I band is barely visible). Interestingly, this activity was elevated when IHF was omitted from the reaction (*lane a**). D108A(E392A) gave type 0 complex (*lane h*) and, as expected, no type I product. When wild-type D108A was paired with MuA(E392A), the type I product and, more prominently, the nicked circular product were formed (*lanes e–g*). From previous work (16), we know that single cleavage at the left end of Mu results in an unstable form of the type I complex. This product migrates as the open circle (OC) during electrophoresis due to relaxation of a substrate domain that is normally held supercoiled in the stable complex. The diffused migration of the OC band suggests dissociation of MuA from the DNA, perhaps during electrophoresis. Further characterization of the cleavage product is described below. In reactions that paired MuA with D108A(E392A), stable type I complex was formed (*lanes i–k*). The strand cleavage position was mapped in this product as well. Unlike left end cleavage, single cleavage at the right

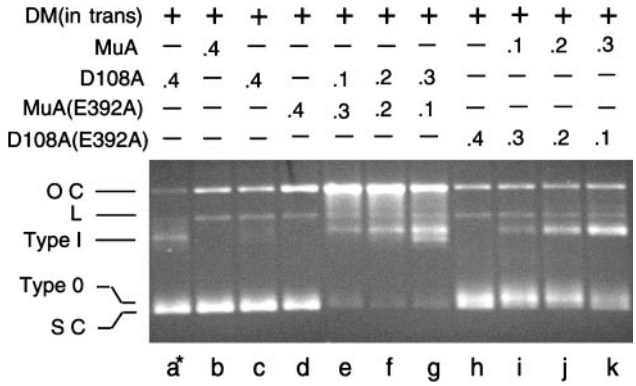

FIG. 2. **Reaction of MuA, D108A, and their variants with enhancer-less plasmid pJHN and the DM hybrid enhancer supplied in *trans*.** The DM substrate was incubated with mixtures of indicated amounts (in μg) of wild-type MuA or D108A proteins and their DDE$^-$ variants and assayed for type I or type 0 complex formation. Except for *lane a** all reactions included IHF. The supercoiled (*SC*), open circular (*OC*), and linear (*L*) forms of the plasmid are indicated. Intramolecular strand transfer complexes migrate slightly ahead of the type I complex (see *lane g*).

end does not destabilize type I (16). Note that these reactions also yielded the type 0 complex, its formation being favored at higher molar ratios of D108A(E392A).

The reason for the higher activity of wild-type D108A in the absence of IHF, rather than its presence, is not known (Fig. 2, compare *lanes a* and *c*). All the other reactions in Fig. 2, including those containing D108A as one of the binary protein partners, were strictly dependent on IHF (data not shown). In our previous work (16), we observed that D108A acted efficiently on a negatively supercoiled substrate containing the DM enhancer in *cis* under reaction conditions that did not require IHF. The hybrid enhancer may not have retained all the structural features of the native enhancer, causing the odd behavior of D108A noted here. For example, an intrinsic bend or flexibility within the DM enhancer could be unfavorably modulated by IHF. Subtle differences between wild-type and mutant proteins in their binding affinities for the enhancer and/or att sites and in their intersubunit cooperativities could also come into play. However, these factors do not diminish the significance of the clear-cut selectivity in strand cleavage observed with specific protein pairs (see below).

Orientation Specificity of the DM Enhancer Is the Same in Cis and in Trans—The scheme for mapping the cleavage positions in the type I and OC products formed in Fig. 2 is diagrammed in Fig. 3*A,* and the results are shown in Fig. 3*B*. The DNA samples extracted from the excised gel bands were digested with *Bam*HI and *Xba*I in one case and with *Bam*HI and *Aat*II in the other. The 3′-hydroxyl ends generated from the digestion as well as those produced by cleavage of Mu ends were radioactively labeled with ^{32}P and fractionated by electrophoresis in denaturing polyacrylamide gels. The diagnostic bands for left end cleavage are LC1 and LC2; those for right end cleavage are RC1 and RC2 (see Ref. 16 for more details).

The results of the cleavage analysis from reactions in Fig. 2, *lanes f* (containing equimolar D108A and MuA(E392A)) and *lane j* (containing equimolar MuA and D108A(E392A)), are displayed in Fig. 3*B*. The OC product from the f reaction was cleaved almost exclusively at the left end (LC1 and LC2 in *lane 2* and undetectable RC1 and RC2 in *lane 6*). The type I product from the f reaction also showed predominant cleavage at the left end (LC1 and LC2 in *lane 3* and faint bands of RC1 and RC2 in *lane 7*). By contrast, the type I product from the j reaction shown in Fig. 2 (MuA/D108A(E392A)) showed nearly exclusive cleavage at the right end (RC1 with undetectable LC1 and LC2 in *lane 4*; RC1 and RC2 in *lane 8*).

The conclusions from the data in Fig. 2 and Fig. 3*B* are summarized in Fig. 3*C*. The placement of the wild-type or mutant MuA or D108A monomers is dictated by the *trans* rule for DDE donation during MuA active site assembly; left end and right end cleavages require the DDE-containing monomer to be positioned at the R1 and L1 sites, respectively (23). The DM enhancer strictly specifies the occupancy of D108A at R1, either wild-type (Fig. 3*C, left*) or the DDE mutant (Fig. 3*C, right*). Left end cleavage is promoted in the former case, whereas it is blocked in the latter. The DM enhancer also promotes the occupancy of MuA (over D108A) at the L1 end, but the specificity is less rigorous. L1 is primarily occupied by MuA(E392A) when paired with D108A (Fig. 3*B*; the preponderance of cleavage at the left over the right end in *lanes 2* and *3* and *6* and *7* corresponding to reaction f in Fig. 2) and incorporates wild-type MuA when paired with D108A(E392A) (right end cleavage in *lanes 4* and *8* in Fig. 3*B* corresponding to reaction j in Fig. 2). However, the presence of D108A(E392A) at L1 in a fraction of the molecules is denoted by the formation of the type 0 complex (for example, reaction j in Fig. 2). Similarly, D108A is not totally excluded from L1, as indicated by the faint cleavage observed at the right end (*lane 7* in Fig. 3*B* corresponding to reaction f in Fig. 2).

In summary, our results with the DM enhancer in *trans* are essentially identical to those obtained by Jiang and co-workers (16) for the same enhancer in *cis*. Together, these findings ascertain that the distribution of the transposase subunits at the Mu ends is determined by their cross-wise specificities for the enhancer elements. The left O1 region of the enhancer specifies the right R1 site; and to a lesser degree, the right O2 region specifies L1. This rule for the distribution of transposase molecules is the same regardless of whether the enhancer is present in *cis* or in *trans*.

We have carried out assays similar to those in Fig. 2 with the same substrate plasmid pJHN and the MD enhancer supplied in *trans*. The efficiency of the MD reactions was very poor.

Orientation Specificity of Natural Enhancers (MM and DD) Is Also the Same in Cis and in Trans—The action of the native enhancers MM or DD in *trans* on the plasmid pJHN was assayed in presence of IHF, and the points of strand cleavage were mapped (Fig. 4). A control set of reactions was done with plasmid pJMM containing MM in *cis* (Fig. 4*A*) and plasmid pJDD containing DD in *cis* (data not shown). The *cis* reactions used standard conditions for supercoiled substrates that did not include IHF.

Wild-type MuA was active and D108A was inactive in the type I reaction with the MM enhancer in *cis* (Fig. 4*A, lanes a* and *b*) or in *trans* (*lanes d* and *e*). An equimolar mixture of D108A and MuA(E392A) yielded both type I and type 0 products (*lanes c* and *f*). The type 0 complex would be expected to contain MuA(E392A) monomers at R1 and L1. The results were similar for the DD enhancer in *trans* (*lanes g–i*). Type I reaction was obtained with D108A (*lane g*) but not MuA (*lane h*), and both type I and type 0 were obtained with a mixture of MuA and D108A(E392A) (*lane i*). The reaction profile was the same for the plasmid containing DD in *cis* (not shown).

The type I complex from the wild-type reactions (Fig. 4*B, lanes a, d,* and *g*) contained both left and right end cleavages as expected (LC1, LC2, and RC1 in *lanes 2, 5,* and *8*; RC1, RC2, and LC1 in *lanes 11, 14,* and *17*). On the other hand, type I from the mixed reactions (*lanes c, f,* and *i*) harbored only right end cleavages (RC1 alone in *lanes 3, 6,* and *9*; RC1 and RC2 alone in *lanes 12, 15,* and *18*). These results are in accordance with the strict O1-R1 rule and the less stringent O2-L1 rule. With the MM enhancer, MuA(E392A) would occupy R1, thereby negating left end cleavage. When MuA(E392A) occupies L1 as

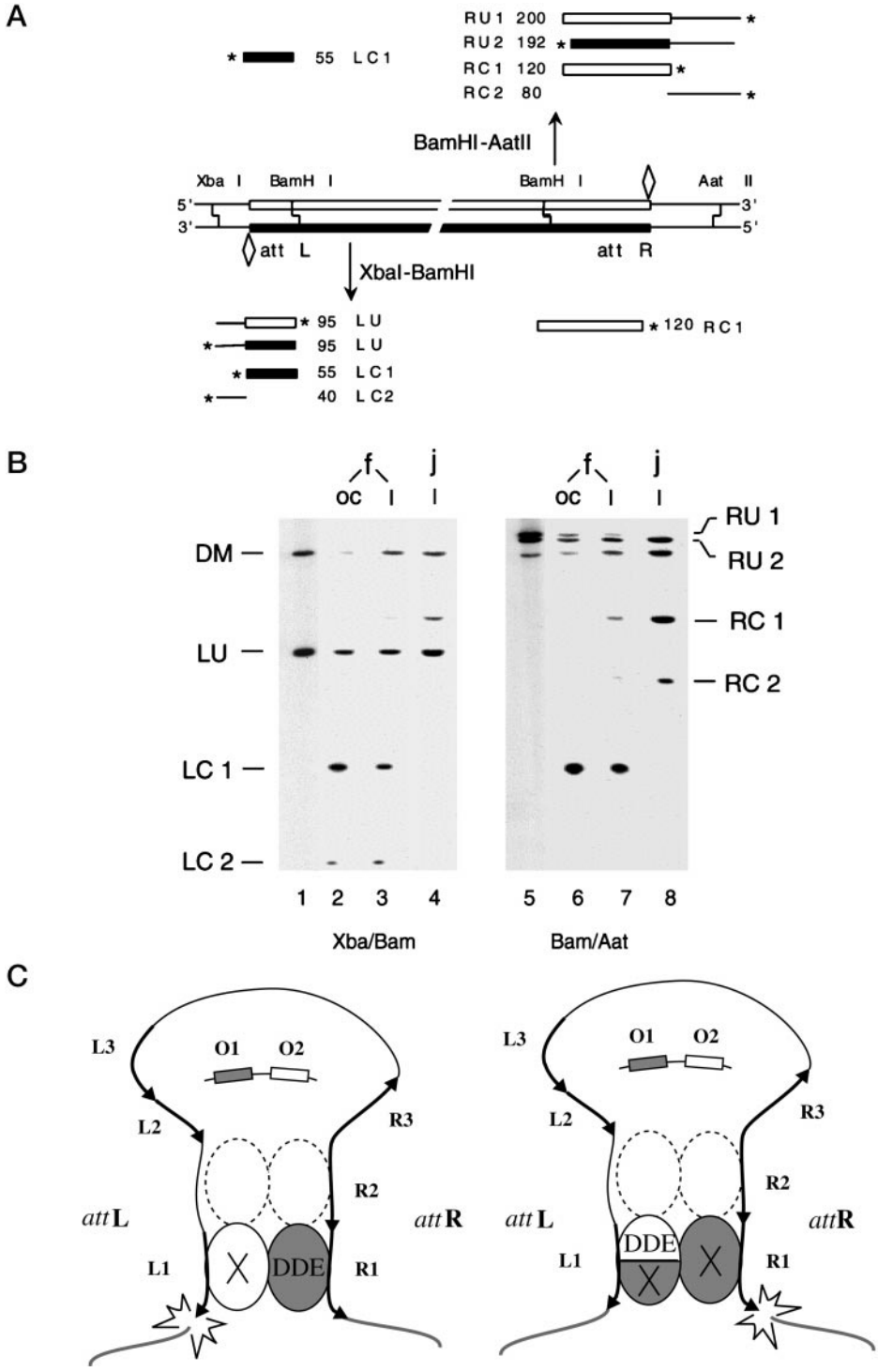

FIG. 3. **Analysis of cleaved ends in pJHN when DM is supplied in *trans*.** *A,* cleavage at Mu ends yield free 3′-hydroxyl groups, hence a 3′ end labeling strategy (described by Jiang and co-workers (16)) was used to assay left or right end cleavages in the type I complex. The top and bottom strands of the Mu genome are represented by *unfilled* and *filled bars*, respectively. The *diamonds* indicate the strand cleavage positions. Double digestion with *Bam*HI-*Xba*I or *Bam*HI-*Aat*II, followed by 3′ end labeling (indicated by the *asterisk*) would give rise to the indicated radioactive products. They can be revealed by electrophoresis in denaturing polyacrylamide gels (see *B*). Uncleaved attL generates an LU doublet consisting of a 95-nt fragment from the bottom strand, which has the same length as the fragment from the top strand; uncleaved attR generates RU1 from the top strand, which differs in length by 8 nt from the corresponding bottom strand fragment RU2. The products specific to left and right end cleavages are denoted by LC and RC, respectively. (*U* and *C* indicate uncleaved and cleaved fragments, respectively.) *B,* the reactions in *lanes f* and *j* of Fig. 2 were analyzed by the strategy outlined in *A*. *Lanes 1* and *5* represent the substrate DNA that was not treated with MuA or D108A proteins. *Xba*I-*Bam*HI and *Bam*HI-*Aat*II restriction digestions are indicated. The symbols *OC* and *I* correspond to the isolated open circular product and the type I complex, respectively. *DM* denotes the contaminating linear enhancer fragment that is found distributed throughout the lanes in Fig. 2 likely due to its high concentration. *C,* deduced position of transposase subunits at L1 and R1. DDE = DDE^+ subunit; *X* = DDE^- subunit. *Gray oval* = D108A or its variant; *white oval* = MuA or its variant. The disposition of all six att sites (L1–L3 and R1–R3) is shown. The occupancy of L1 by either of the two transposases is represented by the *half-white/half-gray oval.* The two subunits in the tetramer whose enhancer specificity could not be addressed in these experiments are indicated as *dotted ovals*. *Gray* and *white* O1 and O2 sites represent D and M enhancers, respectively.

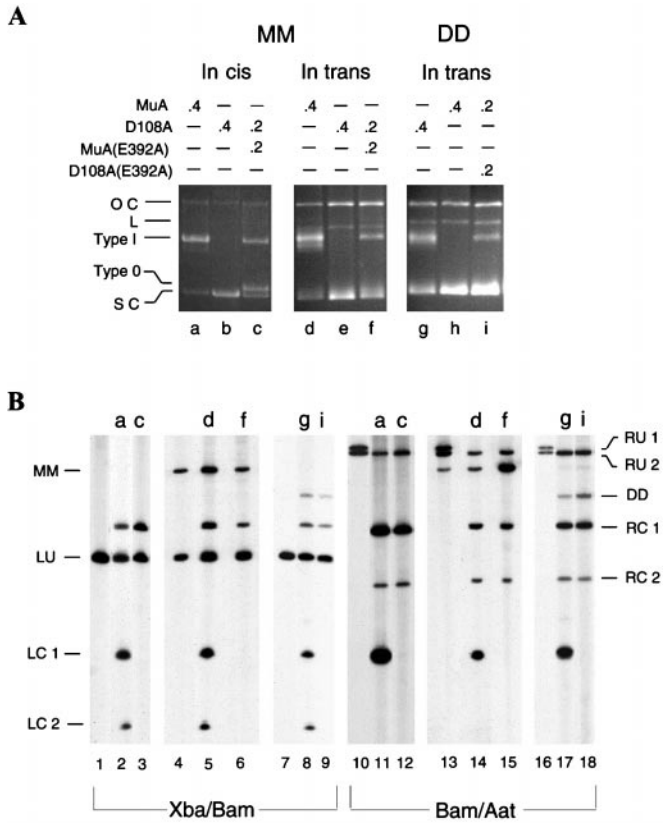

FIG. 4. **Reactions of the natural enhancer substrates MM and DD with MuA, D108A, and their variants both in *cis* and in *trans*.** *A* and *B*, as in Fig. 2 and Fig. 3*B*, respectively, except with the MM and DD substrates. Position of contaminating linear enhancer fragments from lanes in *A* is indicated as MM and DD in *B*.

well, neither end can be cleaved, yielding the type 0 complex. When D108A occupies L1, right end cleavage can occur. These arguments apply to the DD enhancer as well.

Table II summarizes the activities of various *cis* and *trans* arrangements of the Mu enhancer (see "Materials and Methods: for details). Some of these experiments have been previously reported (10, 15), but we present them here for the sake of completeness. The negatively supercoiled pJMM plasmid (harboring MM in *cis*) was active in the type I reaction with or without added IHF and provides a reference for the other reactions. The enhancer-less pJHN could be complemented in *trans* by MM in presence of IHF. No complementation was observed with O1 alone, O2 alone, O1 plus O2 supplied as separate DNA fragments and by O1L (containing O1 + IHF-binding site + nonspecific DNA) even at a 50-fold molar excess over the att sites (see also Ref. 15). An inactive derivative of pJMM lacking O1 (pJH28) could be partially rescued by O1-O2 (MM) in *trans* but not by O1 alone or by O1L. In a 30-min period, a plasmid deleted for O2 alone (pJH27) retained ~60% of wild-type pJMM activity, which saturates more rapidly (see also Ref. 10). By comparison, the corresponding relative activity at 5′ was only 20%. The pJHN reactions with the MM enhancer in *trans* also displayed similar slow kinetics (data not shown; see Ref. 15). Addition of MM in *trans* in the presence or absence of IHF did not improve the reactivity of pJH27. Deletion of the IHF-binding site in addition to O2 (but leaving O1 intact) did not change the activity of the resulting substrate (pJH29).

We noticed that addition of IHF (in amounts used for the enhancer in *trans* experiments) inhibits the intrinsic activity of plasmid pJH27 that lacks O2 (but retains O1 and the adjacent IHF site) (Table II). The inhibition was relieved with added

TABLE II
Activity of cis and trans configurations of complete and partial enhancers

Plasmid	Enhancer in *trans*	IHF	Reaction efficiency[a]
pJMM (W.T.)	–	–	+++
	–	+	+++
pJHN (enhancer–)	–	–	–
	MM	+	+++
	O1	+	–
	O2	+	–
	O1 + O2	+	–
	O1L	+	–
pJH28 (O1–)	–	–	–
	MM	+	+
	O1	+	–
	O1L	+	–
pJH27 (O2–)	–	–	++
	–	+	–
	MM	+	++
	MM	–	++
pJH29 (O2–, IHF–)	–	–	++

[a] Reaction efficiency (type I complex/input donor substrate): +++, 80–100%; ++, 50–70%; +, ~30%.

MM, probably by titration of IHF. Note that IHF had no inhibitory effect on pJMM that contains both O1 and O2. Some of the unexpected results with IHF might be due to its architectural effects on DNA in a context-dependent manner. Recall that IHF also inhibited the activity of the DM enhancer in *trans* in the type I reaction with pJHN (Fig. 2*A, lane a versus c*).

The sum of the data in Figs. 2–4 and Table II corroborate and extend the rules for enhancer-att interactions previously deduced (16). Our current results demonstrate that both hybrid and natural enhancers obey these rules in *cis* and in *trans* reactions. Consistent with its relaxed specificity, O2 is not essential for activity in *cis*. In pJH27 and pJH29 lacking O2, the normal L1 preference for MuA over D108A in mixed reactions is lost, and the placement of protein monomers on L1 become completely randomized (results not shown).

A Normal Orientation of Mu Ends as Well as DNA Supercoiling Is Required When the Enhancer Is Topologically Unlinked or Is Completely Bypassed—Since the organization of the transposition structure requires at least a transient three-site complex (LER), their precise alignment in a circular supercoiled molecule would be subject to large entropic constraints. We have tested whether the topological and geometric requirements of the reaction may be relaxed by unlinking the enhancer from the att sites in two ways. First, we provided the enhancer in *trans* to plasmid substrates containing the attL and attR sites in direct and indirect orientation. We note that whereas Surette and Chaconas (15) have reported that the enhancer does not function in *trans* when the ends are inverted, their experiments were conducted on the relaxed and linear form but not the supercoiled form of the inverted substrate; we now know that DNA supercoiling is essential for both pre- and post-synaptic events in transposition under normal reaction conditions (20–22). Second, we assayed the activity of an enhancer-independent MuA variant (MuAΔ84; Ref. 7) on these plasmids. The results are shown in Fig. 5. Among the three test plasmids, pJHN is the control in which the att sites are in their correct orientation. In pJH25 and pJH26, the sites are in a head to tail arrangement. Only pJH26 contains the enhancer in *cis*.

Type I complex was formed from pJHN with MuA and the MM enhancer in *trans* (Fig. 5, *lane 1*) and with MuAΔ84 in the absence of MM (*lane 2*). When the pJHN substrate was nicked

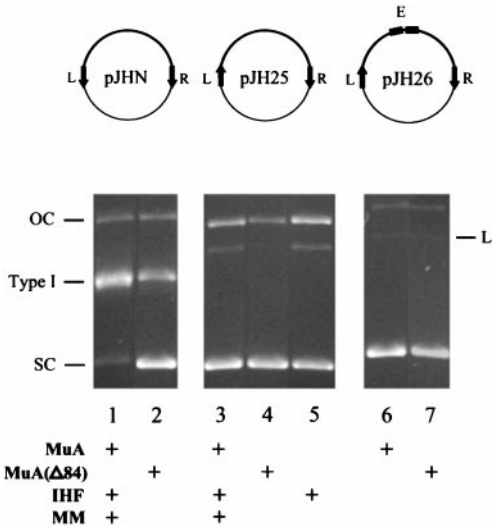

FIG. 5. **Contribution of the enhancer to topological selectivity of the Mu ends.** Disposition of att (*L* and *R*) and enhancer (*E*) sites on plasmid substrates is indicated on the *top panel*. Reactivity of these plasmids in the presence of wild-type MuA with MM enhancer in *trans* (*lanes 1* and *3*) or in *cis* (*lane 6*) or MuAΔ84 in the absence of added enhancer (*lanes 2* and *4*) or presence of enhancer in *cis* (*lane 7*) is shown in the *bottom panel*. *Lane 5* is a control with IHF alone. Other symbols as in Fig. 2.

or linearized, no cleavage at the left or right Mu end was detected in the MuA plus MM (also reported in Ref. 15) or MuAΔ84 reactions (data not shown). Neither pJH25 nor pJH26 was reactive under any of the conditions tested (*lanes 3–7*). Thus, the correct orientation of Mu ends and DNA supercoiling are essential even when the enhancer is unlinked from the att sites or when the reaction is enhancer-independent.

Loss of Topological Specificity with Me_2SO Cannot Be Due to Enhancer Independence Alone—It has long been known that addition of 15% Me_2SO to the transposition reaction results in enhancer independence and abrogates the need for circularity or supercoiling of the substrates (2, 9). The plasmids pJHN, pJH25, and pJH26 (see Fig. 5) were reacted with either MuA or MuAΔ84 under Me_2SO conditions and were analyzed directly (Fig. 6, −*SDS*) or after deproteinizing (Fig. 6, +*SDS*). All three plasmids, including the relaxed form of pJHN, were reactive with both MuA and MuAΔ84. Under these conditions, the type I reaction proceeded nearly quantitatively to type II, resulting in multiple intramolecular strand transfer product bands. An exception was the MuAΔ84 reaction with pJH26. In this case, the predominant product was the cleaved type I complex, and the type II product bands were weak (*lane 9*). After protein removal (+*SDS, lanes 13–20*), the type II strand transfer products from pJHN migrated below the supercoiled plasmid band (*lanes 13 and 14*; *ST*), whereas those from pJH25, pJH26, and relaxed pJHN migrated close to the open circular form (*lanes 15–20; ST*). This difference in topology is expected. In pJHN, with the correct orientation of att sites, only the vector domain will be relaxed following cleavage of Mu ends. The supercoils present within the Mu domain of the plasmid will therefore be trapped in the strand transfer products. In pJH25 and pJH26, with incorrectly oriented att sites, the strand nicks will be located in both plasmid domains. The plasmid would thus be relaxed fully prior to strand transfer.

We note that cleaved inverted end complexes are unstable in the absence of the enhancer, giving rise to material at the OC position (*pJH25*, *lanes 5* and *6*). The absence of OC products in the presence of the enhancer in complexes formed with MuA but not MuAΔ84 (*pJH26, lanes 8* and *9*) suggests that the enhancer stabilizes inverted end complexes in Me_2SO conditions, allowing them to mature into strand transfer products.

In attempts to mimic the Me_2SO effect under the standard Me_2SO-free reaction condition, we carried out MuA/pJH26 and MuAΔ84/pJH25 reactions under combinations of different ionic strengths (100–200 mM NaCl or KCl; 4–16 mM Mg^{2+}) and varying superhelical plasmid densities (σ of −0.015 to −0.075). Under none of these conditions did we observe any activity (data not shown). In summary, comparison of data presented in Figs. 5 and 6 shows that the breakdown of topological and geometric restrictions in the presence of Me_2SO cannot be due to enhancer independence alone.

DISCUSSION

In this study, we have examined whether the topological selectivity of Mu transposition might arise from the restrictive nature of aligning three DNA sites (attL, attR, and the enhancer) for productive synapsis on supercoiled DNA. We have found that unlinking the enhancer from the att sites or carrying out the reaction in an enhancer-independent manner fails to override the requirement for supercoiling or for the inverted orientation of the att sites. We have also found that the crosswise interactions observed between the enhancer and att sites on supercoiled DNA are maintained when the enhancer is provided in *trans*. We discuss these results in detail below.

Functional Asymmetry of the Mu Enhancer Is Independent of Its Linkage to the Att Sites

In a supercoiled plasmid, the enhancer cannot be transferred outside the Mu domain or reversed in orientation inside it without markedly affecting transposition, signifying a defined order and direction of linkage among attL, attR, and the enhancer (8–10). An important outcome from our present study is the demonstration that the O1-R1 and O2-L1 relations are maintained in both the *cis* and *trans* configurations of the enhancer (Figs. 2 and 3), arguing for an inherent functional asymmetry of the enhancer, independent of its topological linkage to Mu ends.

What is the significance of the functional asymmetry of the enhancer? The enhancer DNA segment is relatively large. The O1-O2 region is ~150 bp in Mu and 100 bp in D108 (see Ref. 16). Consensus sequences of 14 and 8 bp have been identified as potential transposase-binding sites in the Mu and D108 enhancer regions, respectively (19, 27). Since these sequences appear repeatedly and in both orientations, the organization of the enhancer *per se* does not suggest an obvious functional polarity to it. A single IHF-binding site present between O1 and O2 could, in principle, introduce asymmetry when bound by IHF. However, IHF is not an obligatory transposition factor and is required only when the supercoiling density of the substrate is well below −0.06 (10). Our results suggest that the functional asymmetry of the enhancer is a reflection of its physical asymmetry and that the specific interacting elements within the large O1-O2 regions must be positioned to match the asymmetry of the ends with which they interact. It is known that two left ends or two right ends are inefficient in transposition compared with the normal attL/attR configuration (9). We have also determined that substitution of a single att site L1 for R1 does not alter the O1-right/O2-left interactions.[2] Thus, not only are interactions with the enhancer unique at each end, but they also involve more than one att site, consistent with the network of end-enhancer interactions deduced from genetic studies (28) and from the observation that efficient transpososome assembly requires that all four subunits in the MuA tetramer carry the enhancer-binding domain (29).

[2] H. Jiang and R. M. Harshey, unpublished results.

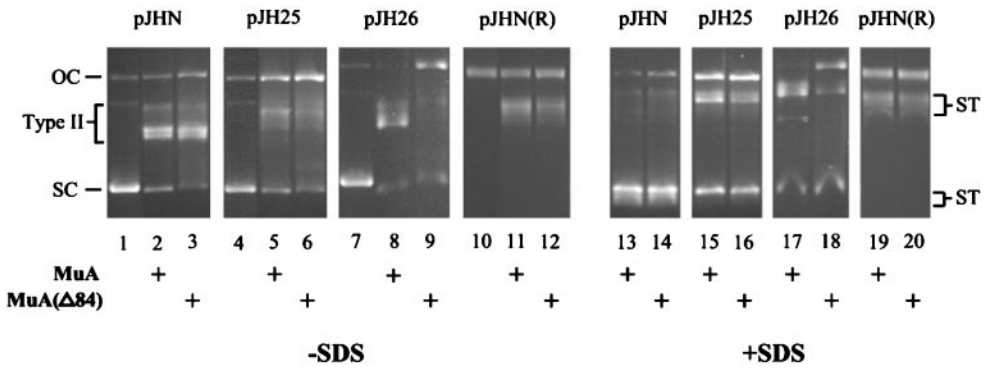

FIG. 6. **Loss of topological selectivity in Me_2SO.** Reactivity of plasmids shown in Fig. 5 to wild-type MuA or to MuAΔ84 under Me_2SO reaction conditions (see "Materials and Methods"). *Lanes 1, 4, 7,* and *10* are controls with no transposase protein added. The relaxed form (*R*) of pJHN was generated by topoisomerase I treatment. Samples were electrophoresed in the absence (−) or presence (+) of SDS. Type II strand transfer complexes or ST strand transfer products are indicated. Other symbols as in Fig. 2.

The unique end-enhancer interactions are likely designed to build the MuA tetramer along specific protein interfaces, thus precisely regulating assembly of the active transpososome.

Mu Enhancer Function Requires O1 to Be in Cis with O2

We have shown in this study that O1 is indispensable in *cis* or in *trans* (Table II). O2 can be substituted with nonspecific DNA, at least in supercoiled substrates, with retention of greater than half the normal activity (see also Ref. 10). However, lack of O2 results in slower kinetics of the type I reaction and a loss of the O2-specified preferential placement of transposase subunits at L1. Although the enhancer can be unlinked from the att sites as the O1-O2 entity, attempts to unlink O1 and O2 have not been successful. The functional relevance of the physical coupling between O1 and O2 is supported by a number of observations (see Table II). It is significant that a plasmid lacking the Mu enhancer (pJHN) is restored to near normal activity by MM supplied in *trans*, whereas MM in *trans* has only a weak effect on a plasmid lacking O1 but retaining O2 (pJH28). So, the modest activity of a plasmid missing O2 but containing O1 is not improved by MM in *trans*. Thus, the presence of either half of the functional enhancer in *cis* effectively blocks the effects of the full enhancer in *trans*. These results are indicative of strong cooperativity between O1 and O2, disruption of which results in loss of enhancer function.

Orientation Specificity of the Mu Ends Is Independent of the Enhancer, A Common Role for Enhancers in Site-specific Recombination?

Our finding that the requirement for an inverted orientation of Mu ends is not alleviated by unlinking the enhancer or by eliminating it entirely through use of an enhancer-independent variant (Fig. 5, *MuAΔ84*) suggests that the enhancer is not the primary determinant of the orientation specificity of Mu ends on supercoiled substrates. This finding is somewhat surprising in view of the fact that systems requiring only two recombination target sites enjoy greater topological freedom than those that require a third site (30–33). This is because a three-site synapse in a circular, supercoiled substrate is thought to occur by DNA slithering or branching, rather than by random collision (34–37), and can be highly restrictive with respect to the permissible orientation of the interacting sites. Although our results suggest that the orientation specificity of the Mu system is a property of the two ends themselves, they do not directly address its mechanism. The asymmetric orientation of the multiple att sites at the two ends, combined with the trans activity of the transposase, are likely to favor stable transpososome assembly only within a unique synapse on negatively supercoiled DNA, an idea compatible with the original proposal by Craigie and Mizuuchi (2) of a special "plectosome" structure in which two double-stranded DNA segments are associated with a specific superhelical geometry.

We discuss below the plausible contribution of the Mu enhancer to plectosome assembly by considering the properties of enhancer-independent reactions in other site-specific DNA recombination systems.

The Hin/Gin System—In contrast to the Mu enhancer, the enhancers in the Hin/Gin invertase systems function in either orientation. This is likely due to the 2-fold symmetry present in the enhancers (bound by Fis) as well as in their recombination target sites hix/gix (see Ref. 17). However, as with Mu, supercoiling in the Gin system is essential only at the gix site and not at the Fis site (38). Point mutations that confer enhancer independence relieve topological constraints, permitting the mutant recombinases to carry out the normally prohibited deletion reaction and intermolecular transposition (5, 6). Although wild-type Gin (and Hin) can form synaptic complexes with both orientation of sites (14, 39), the mutant reaction allows processive recombination and hence loss of the normal discrimination that disallows joining at directly oriented sites after 180° rotation due to the mispaired core sequence. Unlike the wild-type Gin reaction, which occurs exclusively from a synapse with two negative supercoil nodes, the mutant reaction occurs from a broad spectrum of synapses yielding topologically complex products (40). The ability of an enhancer-independent Gin to induce local DNA unwinding and effect strand cleavage at a gix site in the absence of Fis, DNA supercoiling, or synapsis with a second gix site, suggests that the role of the enhancer is to normally promote Gin-Gin contacts to effect DNA unwinding at the gix sites (41). Thus, the role of the enhancer in these systems is to organize a synapse with a unique topology, promoting protein-protein interactions that facilitate DNA unwinding at the recombination sites, assisted by the energetics of DNA supercoiling.

The Resolvase System—The complex organization of the Mu att sites and the functional asymmetry of the Mu enhancer are perhaps more similar to the Tn3/γδ resolvase system whose recombination target site "res" is a composite of three sites, I, II, and III, all of which bind resolvase dimers (18). Only the dimer at site I is catalytic and acts as the recombinase. Those bound at sites II and III mediate the enhancer effect and promote correct alignment of sites I (42, 43). As in the Mu and invertase systems, supercoiling has two distinct roles here, both pre- and post-synaptic. Either supercoiling or catenation of res-containing circles in the absence of supercoiling supports synapsis. Supercoiling is also required after synapsis since relaxed synaptic complexes or relaxed catenanes cannot proceed through recombination (44).

Point mutations of Tn3 resolvase have been obtained that allow recombination between sites I, independent of sites II and III (4). Like in the Gin system, these mutant proteins promote recombination between normally disallowed configuration of res sites. Product topologies indicate that, in contrast to the −3 wild-type synapse, site alignment during the mutant reaction occurs by random collision. Thus, in both the Hin and the resolvase systems, the enhancer appears to play a similar role in organizing a unique synapse.

The Mu System—DNA supercoiling has varied pre- and postsynaptic roles in Mu transposition (see Introduction), some similar to those found in the invertase and resolvase systems. With MuA, two types of enhancer independence have been revealed. The Me_2SO-mediated enhancer independence (see Ref. 9 and under "Results" for this study) results in concomitant independence from the topological and directional restrictions, similar to that seen in the invertase and resolvase systems. Because of its amphipathic properties, Me_2SO can influence both DNA and protein conformations. By analogy with the Gin system, Me_2SO might promote functional MuA-MuA contacts and/or induce DNA distortion at the Mu ends, which is normally facilitated by negative supercoiling (22). It has been reported that even a single att site is sufficient to promote formation of the MuA tetramer under Me_2SO conditions (45). There is also evidence that, in presence of Me_2SO, strand transfer can occur from nonstandard alignment of linear att sites (see Refs. 23 and 46). Since a defined topology to the normal Mu transposition complex can now be experimentally deciphered,[3] it should be possible to test whether the Me_2SO effects include the disordering of a unique synapse into a random set of synapses, like that observed for the mutant invertases and resolvases.

We have shown in this study that enhancer independence conferred by an N-terminal deletion of MuA (MuAΔ84; see Ref. 7), unlike the Me_2SO case and contrary to the invertase/resolvase examples, does not relieve the dependence on DNA supercoiling and on the correct orientation of Mu ends. Do the contrasting properties of MuA(Δ84) with those of the enhancer-independent mutants of Gin (or Cin) and Tn3 resolvase suggest functionally distinct roles for the enhancer in the transposition and recombination reactions? Not necessarily. It is plausible that the topological and geometric features of a system are intrinsic to the target site and the cognate protein (the transposase or recombinase) of that system (for example, asymmetry of the Mu att sites and the *trans* activity of MuA) and are independent of the enhancer. The apparent role of the enhancer in each system is to use DNA supercoiling to facilitate precise assembly of the synaptic complex and/or promote the chemical competence of the corresponding DNA-protein complex. Although a potential implication of this model is that conditions that overcome topological/orientation specificities would lead to enhancer independence, the converse is not necessarily true, as demonstrated here for the MuA reaction. Available evidence from the transposition and recombination systems poses no contradiction.

[3] S. Pathania, M. Jayaram, and R. M. Harshey, unpublished results.

Acknowledgments—We thank Steve Goodman for IHF and M. Jayaram and Shailja Pathania for critical reading of the manuscript.

REFERENCES

1. Gellert, M., and Nash, H. (1987) *Nature* **325,** 401–404
2. Craigie, R., and Mizuuchi, K. (1986) *Cell* **45,** 793–800
3. Boocock, M. R., Brown, J. L., and Sherratt, D. J. (1986) *Biochem. Soc. Trans.* **14,** 214–216
4. Arnold, P. H., Blake, D. G., Grindley, N. D. F., Boocock, M. R., and Stark, W. M. (1999) *EMBO J.* **18,** 1407–1414
5. Klippel, A., Cloppenborg, K., and Kahmann, R. (1988) *EMBO J.* **7,** 3983–3989
6. Haffter, P., and Bickle, T. A. (1988) *EMBO J.* **7,** 3991–3996
7. Yang, J.-Y., Jayaram, M., and Harshey, R. M. (1995) *Genes Dev.* **9,** 2545–2555
8. Leung, P. C., Teplow, D. B., and Harshey, R. M. (1989) *Nature* **338,** 656–658
9. Mizuuchi, M., and Mizuuchi, K. (1989) *Cell* **58,** 399–408
10. Surette, M. G., Lavoie, B. D., and Chaconas, G. (1989) *EMBO J.* **8,** 3483–3489
11. Watson, M. A., and Chaconas, G. (1996) *Cell* **85,** 435–445
12. Chaconas, G., Lavoie, B. D., and Watson, M. A. (1996) *Curr. Biol.* **7,** 817–820
13. Heichman, K. A., and Johnson, R. C. (1990) *Science* **249,** 511–517
14. Kanaar, R., Klippel, A., Shekhtman, E., Dungan, J. M., Kahmann, R., and Cozzarelli, N. R. (1990) *Cell* **62,** 353–366
15. Surette, M. G., and Chaconas, G. (1992) *Cell* **68,** 1101–1108
16. Jiang, H., Yang, J.-Y., and Harshey, R. M. (1999) *EMBO J.* **18,** 3845–3855
17. Stark, W. M., Boocock, M. R., and Sherratt, D. J. (1992) *Trends Genet.* **8,** 432–439
18. Grindley, N. D. F. (1994) *Nucleic Acids Mol. Biol.* **8,** 236–267
19. Krause, H. M., and Higgins, N. P. (1986) *J. Biol. Chem.* **261,** 3744–3752
20. Kobryn, K., Lavoie, B., and Chaconas, G. (1999) *J. Mol. Biol.* **289,** 777–784
21. Wang, Z., and Harshey, R. M. (1994) *Proc. Natl. Acad. Sci. U. S. A.* **91,** 699–703
22. Wang, Z., Namgoong, S.-Y., Zhang, X., and Harshey, R. M. (1996) *J. Biol. Chem.* **271,** 9619–9626
23. Namgoong, S.-Y., and Harshey, R. M. (1998) *EMBO J.* **17,** 3775–3785
24. Williams, T. L., Jackson, E. L., Carritte, A., and Baker, T. A. (1999) *Genes Dev.* **13,** 2725–2737
25. Aldaz, H., Schuster, E., and Baker, T. A. (1996) *Cell* **85,** 257–269
26. Savilahti, H., and Mizuuchi, K. (1996) *Cell* **85,** 271–280
27. Levin, D. B., and DuBow, M. S. (1989) *Mol. Gen. Genet.* **217,** 392–400
28. Allison, R. G., and Chaconas, G. (1992) *J. Biol. Chem.* **267,** 19963–19970
29. Mizuuchi, M., Baker, T. A., and Mizuuchi, K. (1995) *Cell* **83,** 375–385
30. Spengler, S. J., Stasiak, A., and Cozzarelli, N. R. (1985) *Cell* **42,** 325–334
31. Abremski, K., and Hoess, R. (1985) *J. Mol. Biol.* **184,** 211–220
32. Beatty, L. G., Babineau-Clary, D., Hogrefe, C., and Sadowski, P. D. (1986) *J. Mol. Biol.* **188,** 529–544
33. Colloms, S. D., Bath, J., and Sherratt, D. J. (1997) *Cell* **88,** 855–864
34. Parker, C. N., and Halford, S. E. (1991) *Cell* **66,** 781–791
35. Vologodskii, A. V., and Cozzarelli, N. R. (1994) *Annu. Rev. Biophys. Biomol. Struct.* **23,** 609–643
36. Oram, M., Marko, J. F., and Halford, S. E. (1997) *J. Mol. Biol.* **270,** 396–412
37. Marko, J. F., and Siggia, E. D. (1994) *Science* **265,** 506–508
38. Kanaar, R., van de Putte, P., and Cozzarelli, N. R. (1989) *Cell* **58,** 147–159
39. Moskowitz, I. P. G., Heichman, K. A., and Johnson, R. C. (1991) *Genes Dev.* **5,** 1635–1645
40. Crisona, N. J., Kanaar, R., Gonzalez, T. N., Zechiedrich, L. E., Klippel, A., and Cozzarelli, N. R. (1994) *J. Mol. Biol.* **243,** 437–457
41. Klippel, A., Kanaar, R., Kahmann, R., and Cozzarelli, N. R. (1993) *EMBO J.* **12,** 1047–1057
42. Murley, L. L., and Grindley, N. D. (1998) *Cell* **95,** 553–562
43. Bednarz, A. L., Boocock, M. R., and Sherratt, D. J. (1990) *Genes Dev.* **4,** 2366–2375
44. Benjamin, K. R., Abola, A. P., Kanaar, R., and Cozzarelli, N. R. (1996) *J. Mol. Biol.* **256,** 50–65
45. Baker, T. A., and Mizuuchi, K. (1992) *Genes Dev.* **6,** 2221–2232
46. Yang, J.-Y., Jayaram, M., and Harshey, R. M. (1996) *Cell* **85,** 447–455

The Mu Enhancer Is Functionally Asymmetric Both in*cis* and in *trans* : TOPOLOGICAL SELECTIVITY OF Mu TRANSPOSITION IS ENHANCER-INDEPENDENT

Hong Jiang and Rasika M. Harshey

J. Biol. Chem. 2001, 276:4373-4381.
doi: 10.1074/jbc.M008523200 originally published online November 17, 2000
