

## Promiscuous Target Interactions in the *mariner* Transposon *Himar1*\*<sup>§</sup>

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We have previously characterized the early intermediates of *mariner* transposition. Here we characterize the target interactions that occur later in the reaction. We find that, in contrast to the early transposition intermediates, the strand transfer complex is extremely stable and difficult to disassemble. Transposase is tightly bound to the transposon ends constraining rotation of the DNA at the single strand gaps in the target site flanking the element on either side. We also find that although the cleavage step requires  $Mg^{2+}$  or  $Mn^{2+}$  as cofactor, the strand transfer step is also supported by  $Ca^{2+}$ , suggesting that the structure of the active site changes between cleavage and insertion. Finally, we show that, in contrast to the bacterial cut and paste transposons, *mariner* target interactions are promiscuous and can take place either before or after cleavage of the flanking DNA. This is similar to the behavior of the V(D)J system, which is believed to be derived from an ancestral eukaryotic transposon. We discuss the implications of promiscuous target interactions for promoting local transposition and whether this is an adaptation to facilitate the invasion of a genome following horizontal transfer to a new host species.

Himar1 is a synthetic *mariner* transposon reconstituted by “mixing and matching” DNA sequences from almost identical elements in the horn fly, *Hematobia irritans*, and the lacewing, *Chrysoperla plorabunda* (1, 2). *mariner* is a Class II DNA transposon that belongs to a superfamily of elements that includes Tc1 in eukaryotes and the more distantly related IS630-like<sup>1</sup> elements in bacteria (3, 4). *mariner* elements are extremely widespread in nature, but the vast majority are inactive due to large numbers of point mutations and deletions. The only active examples identified in insects to date are the Mos1 elements from *Drosophila mauritiana* and *Drosophila simulans* and a newly discovered *mariner* from an earwig (5).

DNA transposons are well adapted to bacterial hosts and

may persist in the genome for an indefinite period of time. In contrast, DNA transposons have a short life span in eukaryotic species and tend to accumulate inactivating mutations during the invasion of a genome (see Ref. 6 and references therein). Therefore, for survival, DNA transposons rely on frequent horizontal transfer between species. As a result of this unusual lifestyle, the presence or absence of *mariner* does not correlate with the established phylogeny of closely related host organisms. Conversely, almost identical *mariner* elements are found in more distantly related species (Refs. 1, 6, and 7 and references therein).

*mariner* elements are very compact and contain a single transposase gene, usually flanked by simple terminal-inverted repeats (for review see Ref. 7). They transpose in the germ line and/or soma via a “cut and paste” DNA intermediate and duplicate a TA dinucleotide upon insertion. All of the classical DNA transposons such as *mariner* are descended from a common ancestor and have an RNase H-like structural fold in the active site with a characteristic DDE(D) motif. These residues serve to coordinate the catalytic metal ion and are shared by a diverse group of proteins including DNA polymerases, RuvC, the V(D)J recombinase Rag1, and the retroviral integrases. However, there are considerable mechanistic differences, even among the transposases.

The structure of *mariner* transposons most closely resembles that of the bacterial insertion sequences (IS elements). However, there are important differences in the molecular mechanism of transposition between the prokaryotic and eukaryotic elements. For example, one apparently universal feature of the bacterial IS elements is that the first nick during the excision step generates the 3'-OH at the end of the transposon that is eventually transferred to the target site by a direct transesterification mechanism (8). In *mariner* and other eukaryotic elements such as *Activator* and *Tam3*, the polarity of the reaction chemistry is reversed and the first nick generates the 5'-phosphate at the end of the transposon, for example (9). These and other differences may be attributed to a founder effect in the eukaryotic lineage, but this seems unlikely considering other examples of horizontal transfer. More likely is that the differences between prokaryotic and eukaryotic elements are ancient adaptations favored by the different scale and organization of the respective genomes.

We have already characterized the early intermediates of *Himar1* transposition and documented further important mechanistic differences to the bacterial IS elements (6). In contrast to the bacterial elements, our results suggested that *Himar1* transposase can multimerize and initiate catalysis at a single isolated transposon end. This shows that the architecture of the *mariner* synaptic complex has more in common with V(D)J recombination, which is probably derived from an ancestral eukaryotic transposon. Here we have gone on to charac-

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<sup>1</sup> The abbreviations used are: IS, insertion sequence; STC, strand transfer complex; TBE, Tris borate-EDTA; PEG, polyethylene glycol; PEC, paired ends complex; ANOVA, analysis of variance.

terize the later steps of *Himar1* transposition when the element selects a target site for insertion.

Target interactions in the cut and paste bacterial transposons Tn7 and IS10 (Tn10) take place at a defined point in the reaction, either before or after excision from the donor site, respectively (10, 11). This difference reflects the adaptation of these elements to very different evolutionary strategies. Tn7 transposition is to a single unique site in the bacterial chromosome, which is captured before the potentially suicidal excision from the donor. In contrast, IS10 transposition is to random sites and it is probably an advantage to diffuse away from the cleaved donor site before target capture. In the present work, we find that *Himar1* is more promiscuous than Tn7 or IS10 and can interact with a target either before or after excision from the donor site. This is another case in which *mariner* has more in common with the Rag proteins in V(D)J transposition than with the bacterial IS elements (12). We discuss the structural implications of this finding and how it could account for the often-observed local transposition of DNA transposons in eukaryotic genomes.

#### EXPERIMENTAL PROCEDURES

**Plasmids and DNA**—Plasmids pKL97 and pKL99 encode 27 bases from the 5'-end of *Himar1* and are described by Lipkow *et al.* (6). pKL105 is identical to pKL97 with the exception that a BsrDI restriction endonuclease site was introduced by PCR immediately adjacent to the transposon end. pKL104 was created by digesting pBluescriptII SK<sup>+</sup> with PvuII followed by religation. This procedure removed ~0.5 kb of sequences including the polylinker region. The transposon end fragments were generated by restriction digestion as defined in the figure legends. The DNA fragments were radioactively labeled and purified from polyacrylamide gels as described by Lipkow *et al.* (6).

**Protein Purification and Transposition Reactions**—*Himar1* transposase was purified as described by Akerley and Lampe (13) and modified by Lipkow *et al.* (6). Transposition reactions described by Lipkow *et al.* (6) were performed as follows. Transposition reactions (15  $\mu$ l, unless stated otherwise) were assembled in 25 mM HEPES, pH 7.9, 10% glycerol, 100 mM NaCl, 20 mM NH<sub>4</sub>Cl, 250  $\mu$ g/ml bovine serum albumin, 2 mM dithiothreitol, and 2 mM CaCl<sub>2</sub>. Each reaction contained 500 counts per second of radioactively labeled linear transposon end DNA fragment. The absolute amount of DNA (given in the figure legends) varied slightly depending on the efficiency of the end-labeling step. Unless stated otherwise, transposase was added last and the reactions were initiated by the addition of 10 mM MgCl<sub>2</sub> and incubated at 30 °C for the indicated time. Reactions were deproteinated by the addition of one-tenth volume of 1.5% SDS + 150 mM EDTA and heated to 75 °C for 10 min. The reaction products were separated by electrophoresis on TBE-buffered agarose gels. After electrophoresis gels were stained with ethidium bromide, photographed, and dried onto a sheet of HyBond-N<sup>+</sup> membrane. Autoradiograms of dried gels were recorded on a FUJI phosphorimaging device.

#### RESULTS

**The *Himar1* Strand Transfer Complex**—*Himar1* has a non-replicative mechanism in which the transposon is completely separated from the donor site by double strand breaks at both ends (Fig. 1A). The 3'-end of the transposon is generated by a nick precisely at the end of the element. However, the 5'-end is nicked at several positions, predominantly 2 bp within the element (2, 6). To study *Himar1* target interactions, we have used an *in vitro* insertion assay in which the transposon ends are provided as short radiolabeled DNA fragments (Fig. 1B). The first step of the reaction is the transposase-mediated synapsis of two transposon ends to form a paired ends complex (PEC). The *Himar1* PEC has not been observed directly because it appears to be unstable during electrophoresis (6). However, its existence has been inferred from the concerted integration of both transposon ends on either side of a TA dinucleotide (2, 6). PEC assembly is followed by cleavage of the flanking DNA to produce the double-end break complex. In principle, complete separation from the donor site would allow

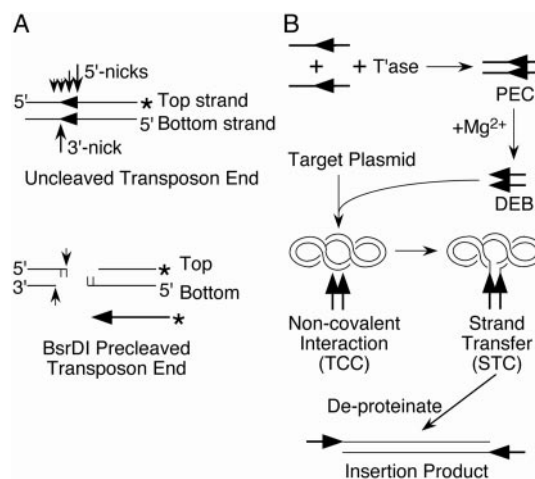


FIG. 1. *Himar1* transposon ends and insertion assay. A, top panel, the uncanceled transposon end is illustrated as double-stranded DNA. The end of the element is indicated by the horizontal arrowheads. The transposon ends are 3'-end-labeled as indicated by the asterisk. The flanking DNA is unlabeled. The vertical arrows indicate the location of the nicks introduced during the cleavage step of the reaction. *Himar1* transposase nicks the bottom strand precisely at the end of the element to generate the 3'-OH that is eventually transferred to the target site. The top strand is nicked at several positions, but the most preferred site is two base pairs inside the transposon. Bottom panel, to generate a pre-cleaved transposon end, a recognition site for the restriction endonuclease BsrDI was engineered adjacent to the transposon end. BsrDI cleaves immediately adjacent to the recognition site with a 2-bp stagger and produces a pre-cleaved transposon end with exactly the same structure as the most predominant product generated by *Himar1* excision. The transposon end is also illustrated as an arrowhead with a single thick line representing double-stranded DNA. B, the *Himar1* insertion assay. The DNA fragments encoding the transposon ends are illustrated as a single thick line with the end of the element represented by an arrowhead. The addition of transposase initiates the assembly of the PEC. If Mg<sup>2+</sup> is present, the flanking DNA is cleaved to produce a double-end break complex (DEB). The transpososome is next thought to establish a non-covalent interaction with a potential target site, illustrated as double-stranded DNA by thin lines. This complex is referred to as a target capture complex (TCC). The TCC is converted to a STC by insertion of the 3'-OH at each transposon end on either side of a TA dinucleotide in the target.

diffusion of the excised transposon and capture of a target site by random collision. Non-covalent target capture would be followed by the strand transfer step that joins the 3'-ends of the transposon to the target site (Fig. 1B). The non-replicative bacterial transposons IS10 and IS50 (Tn5) most closely resemble *Himar1* in structure, and these have been shown to transpos by this mechanism (14, 15).

*Himar1* transposition reactions were performed with a supercoiled target plasmid and analyzed by TBE-buffered agarose gel electrophoresis (Fig. 2). After electrophoresis, the gel was stained with ethidium bromide to locate the target DNA and the molecular weight markers, dried, and recorded on a PhosphorImager to locate the radiolabeled integration products. When the reaction is stopped by the addition of EDTA and SDS prior to electrophoresis, most of the integration events are located at the position of the supercoiled target (Fig. 2, lane 1). This shows that the linear insertion product has retained the supercoils originally present in the target plasmid. Transposase must therefore bind and constrain rotation of the single strand gaps on either side of the insertion site.

When the transposition reaction is extracted with phenol to remove transposase prior to electrophoresis, the insertion product runs at the position expected for linear DNA (Fig. 2, lane 4). However, the STC is extremely stable and is partially resistant to treatment with EDTA, SDS, and proteinase K at 50 °C (lane 3). Resistance to SDS and proteinase K suggests that the STC

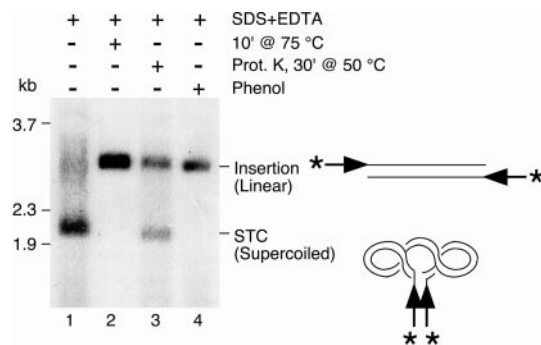


FIG. 2. **Topology of the *Himar1* strand transfer complexes.** A single large insertion reaction was set up with supercoiled pBluescript as a target and 10 mM  $MgCl_2$  to provide the catalytic metal ion. After incubating for 1.5 h at 30 °C, one-tenth of the volume of a  $\times 10$  stop mixture was added to give a final concentration of 0.15% SDS, 15 mM EDTA. Aliquots of 17.5  $\mu$ l were treated as indicated above the gel. The gel was 0.7% agarose buffered with TBE and was run at 65 V for 14 h. The gel was stained with ethidium bromide and photographed to locate the target DNA and molecular weight markers. It was then dried, and an autoradiogram was recorded on a PhosphorImager. Each lane had a 150-fmol transposon end ( $\sim 500$  counts per second), 15 fmol of transposase (1 nM), and 250 ng of pBluescript as target. The transposon end fragment was generated by digesting pKL97 with SfiI+XhoI and labeled on the transposon end by end-filling the XhoI site.

has a compact structure that is difficult to perturb. This may seem surprising in view of the instability of the PEC, which does not even survive electrophoresis in a Tris acetate-EDTA-buffered gel (6). However, this is a common feature of transposition reactions where the intermediates generally become more stable as the reaction progresses.

**The Target Commitment Assay Conditions**—Integration of a transposon into a target site occurs in at least two stages (Fig. 1B). The initial non-covalent interaction between the transpososome and the target site produces a non-covalent target capture complex. This is converted into a STC by the strand transfer step of the reaction. The capacity of a transposon to engage in productive non-covalent target interactions can be assessed in staged reactions in which different target molecules are presented sequentially (11, 12). In preliminary experiments with *Himar1* in a simple buffer system, target commitment was detected before and after cleavage of the flanking DNA (see below). However, in similar experiments on V(D)J transposition, reactions were supplemented with polyethylene glycol (PEG) (12). The authors did not discuss this point or the effect of PEG on the reaction as a whole. However, PEG is known to enhance many biological reactions *in vitro*, probably because it helps mimic the crowded environment within the cell (see “Discussion”). Preliminary experiments demonstrated that including PEG in *Himar1* reaction enhanced target commitment. However, to understand the meaning and potential pitfalls of these results, it was necessary first to determine the different effects of PEG on the *Himar1* reaction.

**Effects of Molecular Crowding on *Himar1* Transposition**—*Himar1* transposition reactions were set up with the transposon ends encoded on radioactively labeled linear DNA fragments and supercoiled pBluescript as target (e.g. Fig. 1B). Concerted insertion of a pair of transposon ends close to each other on opposite strands of the DNA gives a linear product slightly larger than the target plasmid (6). The reaction was first titrated with transposase at 0 and 10% PEG (Fig. 3A). In the presence of PEG, the optimum transposase concentration is reduced from 8 to 2 nM. The reaction is extremely sensitive to an excess of transposase and is almost completely abolished by transposase concentrations only 4-fold above the optimum. This holds true both in the presence or absence of PEG. Thus, at 10% PEG, the optimum transposase concentration and the

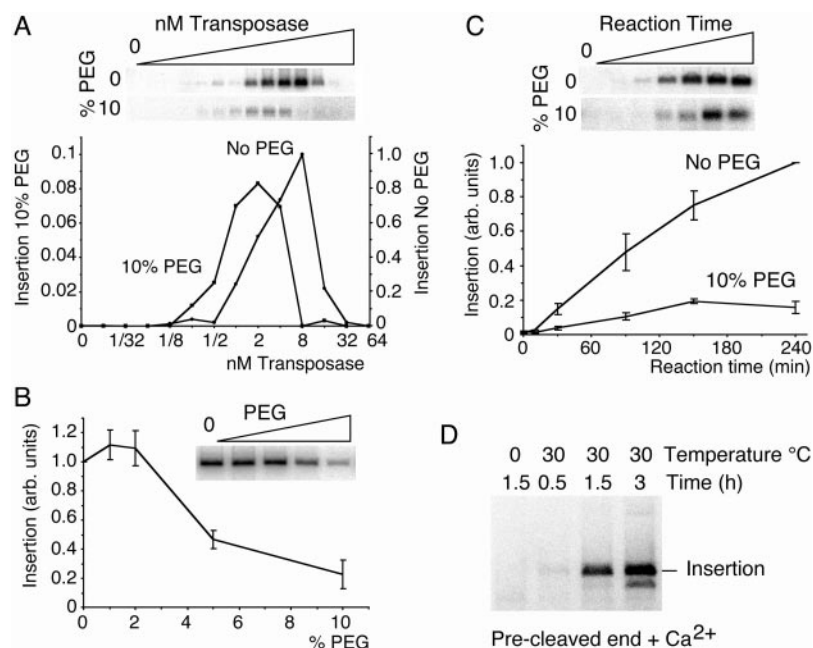
amount of transposase required to inhibit the reaction are both reduced to the same extent. This is consistent with the molecular crowding activity of PEG, which excludes large molecules from a significant proportion of the volume of the solution. The transposition reaction was titrated next with 0–10% PEG (Fig. 3B). The transposase concentration in this experiment was limited to 1 nM to preclude the inhibition of the reaction observed at higher concentrations. Under these conditions, 2% PEG has little effect on the reaction. However, at 10% PEG, the reaction is inhibited and only 20% of the insertion activity remains. Finally, to determine whether inhibition of the reaction is a kinetic effect, a time course was performed at 0 and 10% PEG (Fig. 3C). As before, 10% PEG reduced transposition to  $\sim 20\%$  but the kinetics of the reactions are similar and both are close to completion after 3 h.

**$Ca^{2+}$  Supports the Strand Transfer Step**—All of the classical DNA transposases have a DDE(D) motif in the active site, which serves to coordinate the divalent metal ions required for catalysis, for example (16). Divalent metal ions are probably also required to structure the active site, because they promote the assembly, stability, and/or conformational changes in the IS10, phage Mu, and V(D)J transpososomes (11, 12, 17–19). Although the catalytic metal ion *in vivo* is almost certainly  $Mg^{2+}$ ,  $Ca^{2+}$  can serve as a structural non-catalytic analog in some systems. For example,  $Ca^{2+}$  increased target commitment in IS10 from 14 to 43% and it was also included in all of the incubation mixtures for V(D)J target commitment assays (11, 12). Therefore, we performed insertion reactions to determine whether  $Ca^{2+}$  supported any of the steps of *Himar1* transposition. No cleavage or insertion activity was detected with uncleaved transposon ends (data not shown). However, when the transposon ends are pre-cleaved with a restriction enzyme (Fig. 1A),  $Ca^{2+}$  supports strand transfer and yields a linear insertion product (Fig. 3D). This contrasts with IS10 where none of the catalytic steps is supported by  $Ca^{2+}$  but is identical to phage Mu and V(D)J transposition when the substrates are pre-nicked and pre-cleaved, respectively (19, 20). The ability of  $Ca^{2+}$  to support integration suggests that the structure of the active site changes between the excision and strand transfer steps and represents another similarity between *mariner* and V(D)J recombination.

**Target Commitment in *Himar1* Transposition**—To assess the degree of target commitment with uncleaved and pre-cleaved transposon ends, a staged assay was set up in which two targets of distinguishable size are presented sequentially (Fig. 4, left panel). Transposase was first incubated with transposon ends to allow assembly of the PEC under non-catalytic conditions. Reactions with uncleaved transposon ends contained no  $Mg^{2+}$ , whereas reactions with pre-cleaved ends were incubated on ice to suppress the strand transfer activity supported by the  $Ca^{2+}$  present in the buffer. During Stage 1 of the reaction, Target 1 is presented and incubated for 30 min to allow target interactions to become established. At Stage 2 of the reaction, Target 2 is added together with the catalytic metal ion  $Mg^{2+}$ . After a further incubation period at 30 °C, the reactions were terminated and analyzed to determine whether insertions are biased toward the target presented during Stage 1 of the assay. This would occur if the transpososome is able to establish a stable non-covalent interaction with the first target that DNA presented and if this is a precursor to strand transfer into this target. If there is indeed a bias of insertions into the target presented during Stage 1, target commitment has occurred. Conversely, if the transposon inserts into both targets equally, no target commitment is observed.

The target plasmids were pBluescript and a derivative with a 448-bp deletion spanning the polylinker. The target plasmids





**FIG. 3. The effects of molecular crowding and metal ions on *Himar1* transposition.** 15- $\mu$ l insertion reactions were assembled with 150 ng of pBluescript as target. Reactions were incubated at 30 °C for 3 h or the time indicated. Reactions were stopped by adding one-tenth of the volume of the stop mixture to give a final concentration of 0.15% SDS and 15 mM EDTA followed by heating to 75 °C for 10 min. Samples were electrophoresed overnight at 65 V in a TBE-buffered 1.1% agarose gel. The gels were dried and recorded on a PhosphorImager. The section of the gel containing the insertion product is shown together with plots in arbitrary units. **A**, titration of transposase concentration in the presence of absence of 10% PEG 8000 is shown. Each reaction had a 39-fmol transposon end generated by digesting pKL97 with SfiI+XhoI. Transposase and the transposon end were mixed before the addition of the PEG. **B**, titration of PEG 8000 concentration. Reactions were as described in part A with the exception that there was 15 fmol (1 nM) of transposase and a 25-fmol transposon end fragment, which was generated by digesting pKL99 with SfiI+XhoI. Transposase and the transposon end fragment were first mixed together and incubated at 30 °C to allow PEC formation. The reaction was initiated by the addition of a mixture containing PEG 8000 and enough  $MgCl_2$  to provide a final concentration of 10 mM. Incubation was continued for a further 1.5 h at 30 °C. Error bars are the mean  $\pm$  S.E.,  $n = 2$ . **C**, kinetics of the insertion reaction in the presence or absence of 10% PEG 8000. Reactions were as described in parts A and B with the exception that there was a 60-fmol transposon end fragment and 15 fmol (1 nM) of transposase. Reactions were assembled on ice and then incubated at 30 °C for the indicated time. Error bars are the mean  $\pm$  S.E.,  $n = 2$ . **D**, calcium supports insertion of pre-cleaved transposon ends. Insertion reactions had a 120-fmol pre-cleaved transposon end fragment generated by digestion of pKL105 with BsrDI+XhoI. There was 60 fmol of transposase (4 nM), 250 ng of pBluescript as target, and 2 mM  $CaCl_2$  to provide the catalytic metal ion. Reactions were incubated at 30 °C for 1.5 h. The doublet band indicated as the insertion product results from incomplete deprotection of the reaction. This was occasionally observed and probably results from a slightly low temperature in the 75 °C heating block used to deprotect the reactions. After further heating, the upper band, which still has some protein attached, is shifted to the position of the lower band.

were in the supercoiled closed circular form so that insertion of a pair of transposon ends gave a linear insertion product of unique size after deprotection (Fig. 1B) (6). When the targets were presented alone or when both targets were present at Stage 1 of the assay, the short target received slightly more insertions than the long target (Fig. 4, lanes 2–4). We did not investigate this difference extensively, but it appears that *Himar1* inserted into the short target more efficiently because it contained a higher ratio of supercoiled to nicked circular DNA. This initial bias was taken into account when calculating the extent of target commitment (see supplementary material).

When the assay is performed in the absence of PEG and the targets are presented together at Stage 1, there is a slight bias toward insertion into the short target as noted above (lane 4). However, when the short target is presented first in a staged reaction, this bias is even more pronounced (lane 6). When the long target is presented first, the bias is reversed and the long target receives slightly more insertions than the short target (lane 5). These results show that, in the absence of PEG, there is a significant commitment to the target presented during Stage 1 of the assay. Commitment is observed for both types of transposon ends, but the effect was noticeably stronger for the pre-cleaved ends.

The assay was performed next with PEG added during Stage 2 of the assay in a mixture with the second target and  $Mg^{2+}$  (lanes 7–9). Under these conditions, there is strong commitment to the first target presented (compare lanes 8 and 9). Note

that the addition of PEG at Stage 2 is identical to the target commitment assay for V(D)J transposition (12).

Finally, a third set of target commitment assays was performed, but this time PEG was added at Stage 1 of the assay in a mixture with the first target (lanes 10–12). Target commitment for both types of transposon ends is extremely strong, and almost all of the insertions are made into the first target presented. We considered the possibility that the strong target commitment was due to the failure of the targets to mix freely in the presence of PEG. However, this seems unlikely as PEG is widely used in molecular biology and is not known to prevent mixing. Also, if PEG prevented mixing, we would expect the total number of insertions to be much lower when the targets are added at Stage 1 in a mixture with PEG. This is clearly not the case for the reactions with the pre-cleaved ends (Fig. 4, compare lanes 4, 7, and 10 in the bottom panel). We also considered the possibility that the observed target commitment was a consequence of nonspecific binding of DNA to the transposase protein. However, this is also very unlikely as *Himar1* transposition *in vitro* is insensitive to competitor DNA present in the reaction before the addition of transposase (6). If the transposase had a strong nonspecific DNA binding activity, as observed for many bacterial transposition systems, it would be highly likely to perturb the reaction, particularly if it was present in the reaction before transposase. Therefore, we conclude that the strong target commitment when PEG is added at Stage 1 or 2 is a true reflection of *Himar1* behavior.



TABLE I

Analysis of variance in a factorial design for influences on Himar1 target commitment

The data plotted in Fig. 5 were analyzed by the ANOVA method described by Montgomery (21). A definition of the formulas is provided in Supplemental Table S1. The *p* values were from the HyperStat Online Textbook (davidmlane.com/hyperstat/F\_table.html).

Source of variation	Sum of squares	Degrees of freedom	Mean square	<i>F</i> <sub>0</sub>	<i>p</i> value
PEG	14,126.743	2	7063.37	18.08	0.00002
End cleavage	765.214	1	765.21	1.96	0.17447
Interaction	193.957	2	96.98	0.25	0.78218
Mean ± S.E.	9377.423	24	390.73		
Total	24,463.336	29			

mine target commitment with the uncleaved and pre-cleaved transposon ends are identical.

#### DISCUSSION

**Stability and Topological Constraints on the Himar1 Complexes**—The chemical steps of transposition involve no high energy cofactors. Therefore, the reaction intermediates tend to become more stable as the reaction progresses as thermodynamic energy is lost from the system. We have shown previously that the Himar1 PEC is unstable and does not survive electrophoresis in TAE-buffered polyacrylamide gels (6). However, the STC is extremely stable and survives treatment with SDS or proteinase K (Fig. 2). This is similar to other transposition systems. For example, in phage Mu transposition, the STC is extremely stable and a specific protease must be recruited for disassembly of the complex and to allow replication (22).

**Target Commitment and the Effects of Molecular Crowding**—In the simple buffer mixture lacking PEG, Himar1 target commitment was 6 and 22% for uncleaved and pre-cleaved transposon ends, respectively (Fig. 5). This 4-fold difference is very small compared to similar experiments with IS10 where there is a 143-fold difference between target commitment with uncleaved and pre-cleaved ends (0.32 and 43% target commitment, respectively) (11).

Biochemical reactions *in vivo* can be influenced by a number of environmental factors absent from *in vitro* systems. For example, in bacteria and the eukaryotic nucleus, the concentration of the DNA alone is in the region of 10 mg/ml compared with 0.02 mg/ml in our target commitment assays. Furthermore, DNA condensation and aggregation are promoted by multivalent cations, DNA supercoiling, and molecular crowding. In the eukaryotic cytoplasm, macromolecules typically occupy 20–30% of the available volume. In some organelles such as the nucleus, the level of crowding is even higher in the range of 30–45%. All of the intracellular systems have evolved to deal with and to make use of the effects resulting from nonspecific interactions and volume exclusion. Although largely ignored in experimental biochemistry, crowding agents have proven essential for the *in vitro* reconstitution of DNA replication, transcription, cell division, and many other processes (23). The effect of crowding can be positive or negative. Solubility and diffusion rates are decreased, whereas protein folding and binding are generally increased (24). As an example, the binding of the HU protein to *Escherichia coli* DNA is increased over 10-fold in the presence of 12% PEG (25). When the Himar1 transposition assays were spiked with PEG at Stage 2 or Stage 1 of the reaction, target commitment with uncleaved and pre-cleaved transposon ends increased to almost 40 and 80%, respectively (Fig. 5). This large increase in target commitment is another example of the way in which molecular crowding preserves interactions between macromolecules.

In IS10 and V(D)J transposition, target commitment assays were accompanied by a physical demonstration that the respective PECs were engaged in a stable non-covalent interaction with the target DNA (11, 12). Himar1 also produced similar non-covalent complexes with target DNA in gel shift experiments (data not shown). However, because the Himar1 PEC is unstable and does not survive electrophoresis (6), the significance of these complexes was unclear.

**Local Transposition**—Clusters of transposons are present in many bacterial genomes. They are often found inserted within and adjacent to each other in phage sequences and pathogenicity islands, for example (26). The reason is that they may be targeted to their own ends or may tend to insert into distorted or highly bendable DNA sequences, for example (27, 28). In contrast, several different families of eukaryotic transposons have been shown to cluster over much greater distances. This finding suggests that they have a propensity to select target sites physically linked to the donor. For example, 1% of *P*-element insertions is within 128 kb of the donor site, which is 67-fold more than expected if there was no linkage between the donor and target sites (29). For the *Ac/Ds* family of elements, ~50% of new insertions are linked to the donor site in maize, *Arabidopsis*, and tobacco. In *Arabidopsis*, 35% are within 200 kb with a further 50% within 1,700 kb (see Refs. 30 and 31 and references therein).

Similar clustering is also observed for members of the *Tc1/mariner* family. For example, of 15 *Tc1* insertions isolated from the *unc-22* gene in *Caenorhabditis elegans*, 8 were from a donor site on the same chromosome (32). Local transposition in the *Sleeping Beauty* element has been even more thoroughly documented, because it is used as a tool for generating mutations in eukaryotic species. In contrast to transposition from a plasmid, which produces a random distribution of insertion sites in HeLa cells, 50% of insertions from a chromosomal donor site are made into the same chromosome (33). In the mouse germ line, 27% *Sleeping Beauty* insertions were within 200 kb of the donor site and 75% were in the same chromosome (34).

Interaction with a target before excision from the donor site is one mechanism that would account for transposition to linked loci. However, it is possible that other mechanisms contribute as well, *e.g.* if the transpososome were to remain associated with the DNA flanking the donor site after excision. Indeed, there is evidence for this in V(D)J recombination where mutations in the Rag proteins can influence whether the coding ends (the equivalent of flanking DNA) are rejoined by homologous recombination or by non-homologous end joining (35).

The difference in target selection between prokaryotic and eukaryotic elements raises the issue of whether there is a selective advantage for transposition to linked loci in eukaryotes. One possibility is that the difference is related to the lifestyle of eukaryotic DNA transposons, which rely on a high rate of horizontal transfer (Ref. 6 and references therein). The cut and paste mechanism of transposition does not produce an increase in the number of elements *per se*. Such elements can only prosper in the germ line if the donor site is restored by homologous recombination from a sister chromosome or from another copy of the element located elsewhere in the genome. This does not present a problem once the element is established. However, the first few transposition events at the start of a genome invasion are likely to be crucial for the survival of the element. A copy of the element close by on the same chromosome may therefore help to ensure restoration of the transposon during repair of the donor site. Indirect support for this model is provided by the exchange of sequences polymorphisms among *Tc1* elements (32). The pattern of polymorphisms acquired suggested that, after excision, repair is by the synthesis-



dependent-strand-annealing pathway using the sister chromosome as template. After synthesis has extended into the transposon sequences, the template is switched to a different copy of the transposon elsewhere in the genome. Because homologous recombination after *P*-element excision in *Drosophila* has a strong *cis* bias (36), it may benefit *mariner* transposons in this and other species to transpose to physically linked loci.

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