Introduction

DNA transposons are specialized genetic entities that mobilize and amplify within a host genome. Their success as genomic parasites is highlighted by the abundance and ubiquity of transposase genes in nature [1]. The impact that transposons have had on the evolution of genomes is such that they are considered as one of the major forces driving evolution [2,3].

DNA transposons are useful tools to manipulate genomes. They have been used in a variety of biotechnological applications including transgenesis and insertional mutagenesis and offer promises for gene therapy [4]. The Tc1-mariner transposons are amongst the most extensively exploited elements because they involve simple and versatile components and they are active in a wide range of hosts, including vertebrates [5,6].

Tc1-mariner transposons mobilize via a cut-and-paste mechanism (Figure 1A). Transposition is preceded by the assembly of the paired-ends complex (PEC), where the two ends of the transposon are brought together into a higher-order complex also called the transpososome [7]. Upon PEC formation the transposase catalyses the formation of a double-strand break at both transposon ends [8]. This excises the transposon from the donor sequence. The 3′-ends of the transposon are subsequently transferred 5′ of a TA target site, generating single-strand gaps flanking the newly inserted transposon [3,9]. These gaps are repaired by host-encoded machinery and lead to the duplication of the TA target dinucleotide at either side of the element.

Except for the stringent requirement for a TA dinucleotide, target site selection in Tc1-mariner is considered essentially random [10,11,12,13,14]. The immediate vicinity of the TA dinucleotide usually provides little specificity. Nevertheless, biased target selection has been observed for several Tc1-mariner elements, both in vivo and in vitro [10,13,15,16,17]. Correlations between preferred target sites and predicted physical properties of the local DNA revealed that DNA bendability is likely a factor favoring target interactions of the Sleeping Beauty and Hsmar1 transposons [11,13,16,18]. In contrast, Mos1 integration into a plasmid carrying the Tn9 chloramphenicol acetyltransferase (cat) gene hot spot did not correlate with DNA bendability but required supercoiling [17].

The functional state of the target may also affect transposon integration. For example, some transposons display a tendency to integrate into active genes. This is the case of the P-element that preferably integrates near transcriptional start sites [19]. Other transposons like Mu or Tn10 avoid integrating into transcriptionally active regions, which helps reducing their disruptive effects to the host [20]. The specificity of Tc1-mariner integration has not been thoroughly characterized on a genomic scale, but studies with Sleeping Beauty and Hsmar1 transposons [11,13,16,18] and the Tj1 retrotransposon, while others specifically target nucleosome-occupied sequences, like the Ty1 retrotransposon [21,22,23].

Another source of integration bias commonly observed with chromosomal copies of transposons is their tendency to integrate...
target sites located in close proximity of their original position. This phenomenon, referred to as 'local hopping', most likely reflects the elevated probability for a transposon to interact with target DNA located nearby the excision site due to rapid interactions with the target after excision and slow diffusion of the excised transposon due to macromolecular crowding. The Sleeping Beauty and the hAT elements Tol2 and Hermes are prone to local hopping, whereas PiggyBac appears to be less so [11,24,25,26].

Hsmar1 was active in the human genome from about 50 to 37 million years ago [27]. During that time about 200 copies of full-length element were generated together with thousands of copies of a non-autonomous miniature derivative [28]. In addition, one copy of the transposase gene was domesticated by the human genome and evolved under purifying selection as part of the SETMAR gene [29]. SETMAR is involved in DNA repair but its precise function remains unclear [30]. Nevertheless, several activities have been reported including activities derived from the ancestral Hsmar1 transposase [31]. Here, we are working with a resurrected copy of the Hsmar1 transposase, which was deduced by computational analysis of the fossil Hsmar1 elements [12]. This resurrected Hsmar1 transposase is highly active in vitro [32]. Taking advantage of this system, we have previously shown that DNA supercoiling accelerates Hsmar1 transposition by bringing the transposon ends in a favorable configuration for synapsis [7]. This provides the transposase with a 'topological filter' that allows the transposase to selectively synapse transposon ends that are in the appropriate inverted repeat configuration. We proposed that this topological filter serves to limit transposon-induced genome instability and to couple transposition to cellular events.

Here, we have investigated the effect of DNA supercoiling on the integration step of Hsmar1 transposition. We show that the topology of both the transposon donor and the target affect integration. Negatively supercoiled DNA is the preferred target for insertion and helps drive the otherwise slow integration of a relaxed transposon. Like other Tc1-mariner elements, Hsmar1 shows little sequence specificity in the vicinity of the target TA. Nevertheless, some target sites are preferred. Supercoiled and nicked transposon donors have similar biases for intramolecular integration sites suggesting that the distribution of integration events is unaffected by the topology of the target.
Materials and Methods

Media and Bacterial Strains

Bacteria were grown in Luria-Bertani (LB) media at 37°C. The following antibiotics were used at the indicated concentrations: ampicillin (Amp), 100 µg/ml; kanamycin (Kan), 50 µg/ml; chloramphenicol (Cm), 34 µg/ml. The following Escherichia coli strains were used: RC5024 (identical to DH5α [endA1 hsdR17 glnV44 thi-1 recA1 gyrA relA1 ΔlacZΔM15]), RC5081 (identical to S17.1Δ pir), and RC5036 (identical to BL21(DE3) (F- ompT hsdS dcm lon hsdSB(B- mB-) λDE3 [lacI lacUV5-T7 gene 1 ind1 sam7 nin5]).

Expression Vector and Plasmid Substrates

Plasmid pRC880 is a pMAL-c2x based expression vector that contains the gene encoding the reconstructed ancestral version of the Hsmar1 transposase [32]. Standard transposition reactions contained the plasmid pRC650 or pRC704. The genetic ‘hop’ assay used pRC704 as transposon donor and a dimeric form of pACYC184 (chloramphenicol and tetracycline) as a target. pRC704 encodes a 2.3 kb kanamycin-resistance Hsmar1 transposase and a 0.8 kb plasmid backbone encoding the R6K conditional origin of replication. When the transposon donor or the target was relaxed the corresponding plasmid pRC650 or pRC704 (donors) or pACYC184 (target) were digested with the nicking endonuclease NbBsrDI (New England Biolabs). NbBsrDI cuts the donor plasmids at several sites located hundreds of base pairs away from the transposon ends. When the target was positively supercoiled, the pACYC184 plasmid was treated with reverse gyrase as described previously [7,33]. After treatment with reverse gyrase or NbBsrDI, the DNA was phenol-chloroform extracted, ethanol precipitated and resuspended in TE buffer. The DNA was quantified using a Nanodrop spectrophotometer before sample preparation.

Transposase Purification and Standard Transposition Assay

Protocols for the purification of the Hsmar1 transposase and the in vitro transposition assay have been described previously [32]. Briefly, the Hsmar1 transposase was expressed as a maltose-binding protein fusion in E. coli cells harboring the pRC880 vector and purified by affinity chromatography on amylose resin (New England Biolabs), followed by ion-exchange chromatography on a MonoS HR5/5 column (Amersham Pharmacia). Transposition reactions with the standard substrate pRC650 contained 6.7 nM transposon donor and 20 nM Tris-HCl buffer pH 8, 100 mM NaCl, 10% glycerol, 2 mM DTT and 2.5 mM MgCl2. Transposase was diluted in reaction buffer and was always the last component added to the reaction mixture. After incubating at 37°C for up to 24 h, reactions were made 20 mM in EDTA, 0.1% SDS and heated at 75°C for 30 minutes. DNA was recovered by ethanol precipitation and analyzed by agarose gel electrophoresis.

Genetic ‘hop’ Assay

The genetic hop assay was also described previously [32]. The reaction conditions were as above, except for the following modifications. Unless stated otherwise, 20 µl reactions contained 43 ng pRC704 transposon donor (1 nM), 112 ng dimeric pACYC184 target (1 nM) and 10–15 nM transposase. Reactions were incubated at 37°C for up to 24 h then stopped and extracted with phenol-chloroform. DNA was recovered by ethanol precipitation and resuspended in TE buffer. One tenth of the reaction was transformed into DH5α competent cells. After transformation, 1/100 of the mixture was spread on LB-chloramphenicol plates.

The number of colonies provided a measure of the total amount of target DNA recovered. The remainder of the transformation mixture was spread on LB-chloramphenicol plus kanamycin plates and the number of colonies was used to quantify the proportion of the target plasmids that had received a transposon insertion. The donor plasmid has a conditional origin of replication that does not function in the recipient strain. This serves to eliminate the confounding effect of double transformation events with donor and target. No colonies were obtained if the transposon donor, the target or the transposase were omitted. DNA sequencing analysis of the junction between the transposon donor and the target confirmed that the colonies represent true transposition events. All of the 54 insertions tested were precise and carried a duplicated target TA dinucleotide. Half of the transposition reaction was also analyzed on a 1.1% agarose gel. After electrophoresis the gel was stained with SYBR Green I and recorded on a fluorimager.

Results

Donor DNA Topology Affects Integration

We used an in vitro ‘hop’ assay to quantify intermolecular transposition of Hsmar1 from supercoiled (SC) and nicked (N) donor plasmids (Figure 1B). In this assay, the transposon donor (pRC704) encodes a kanamycin resistance marker flanked by Hsmar1 transposon ends. The reaction is performed in the presence of a target plasmid (dimeric pACYC184) that encodes a chloramphenicol resistance marker. Transposition events, from donor to target, are subsequently recovered by genetic transformation in E. coli.

Transposition reactions with a supercoiled or a nicked transposon donor were initiated at time zero and aliquots for transformation were withdrawn at the indicated times (Figure 1C and D). Initially, the rate of transposition was higher with a supercoiled donor than with a nicked donor. The reaction had reached 50% completion after 4 h with a supercoiled substrate, but only after 8 h with a nicked substrate. However, after 24 h the total amount of intermolecular events obtained with the nicked donor was almost twice that with the supercoiled donor.

In the in vitro assays, almost 100% of the substrate is converted into products [32]. Since the transposon donor and target plasmids are equimolar, the proportion of target plasmids that receive insertions is equivalent to the proportion of the transposon that goes on to perform intermolecular integration in the target. The remainder of transposons, which corresponds to 94 and 97% for the nicked and supercoiled substrates, respectively, integrates into other DNA sequences present. These include unreacted donor plasmids or the transposon DNA itself. The vast majority of integration events are intramolecular [32].

Increasing Target Concentration Drives Intermolecular Integration

The Hsmar1 reaction, with a supercoiled or a nicked substrate, was titrated with increasing amounts of a supercoiled target plasmid and analyzed by gel electrophoresis (Figure 2A). For these reactions the standard incubation time of 6 to 8 hours was extended to 24 h to allow the slow reaction with the nicked donor to reach completion. Increasing the target DNA concentration over a 500-fold range did not significantly affect the extent of excision, judging from the amount of vector backbone produced in each case. However, the amount of intermolecular transposition increased with increasing concentration of the target. This suggests that the high proportion of intramolecular
The 2.3 kb linear excised transposon fragment (ETF) is not detected with the supercoiled transposon donor because transposon integration with this donor is delayed due to the supercoiling of the transposon ends. Nevertheless, the ETF can be detected at early time points in synchronized transposition reactions [7,32]. BMS is an intramolecular integration product of lowest electrophoretic mobility is the unknotted inversion circle (IC), which is trapped zero supercoiling nodes. The diffuse band running between the IC and the SC donor represents the first catenane, which linked two supercoiling nodes. Each catenane consists of a pair of gapped circles together that are composed of the size of the transposon. Although all the products within this diffuse band have the same molecular weight and that the gel is able to resolve a pair of circles of similar sizes from a pair of circles of very different sizes. Trapping a third supercoiling node leads to the formation of the first knot, which runs as a discrete band below the SC donor. Knots and catenanes of increasing topological complexity, the gel is able to resolve a pair of circles of similar sizes from a pair of circles of very different sizes. Trapping a third supercoiling node leads to the formation of the first knot, which runs as a discrete band below the SC donor. Knots and catenanes of increasing topological complexity then form an array of unknotted circles together that are composed of the size of the transposon. With a nicked donor the predominant intramolecular integration products are the IC and the ETF donor, which is evident from the persistence of the ETF at elevated levels but disappeared with increasing target concentration. It results from bimolecular synapsis (BMS) between transposon ends. Cleavage linearizes both donor and target plasmids used for the electrophoresis assay because of the background smear near the top of the gel. However, the donor and target plasmids used for the electrophoresis assay were identical to those used for the in vitro assay. Small aliquots of the reaction were therefore retained for transformation. The resulting quantification confirmed that intramolecular integration events are higher with the nicked donor, which is evident from the presence of elevated levels of supercoiled target.

The nicked donor also generates an integration product not present in the reactions with supercoiled substrate. It results from bimolecular synapsis (BMS) between transposon ends. Cleavage linearizes both donors and the target site upon insertion. BMS events are not recovered with supercoiled substrates because DNA supercoiling favors intramolecular synapsis by juxtaposing the transposon ends in the plectosome [7]. In the absence of DNA supercoiling, BMS events can be recovered provided that reaction times are long.

It is difficult to quantify the intramolecular reaction using the electrophoresis assay because of the background smear near the top of the gel. However, the donor and target plasmids used for the electrophoresis assay were identical to those used for the in vitro assay. Small aliquots of the reaction were therefore retained for transformation. The resulting quantification confirmed that intramolecular integration events increase with target concentration and that all target concentrations a nicked donor performs more intramolecular integrations than a supercoiled donor (Figure 2B). This difference was low (less than 2-fold), but reproducible. However, since the overall efficiency of integration is lower with the nicked donor, which is evident from the persistence of the ETF, the actual fraction of intramolecular integration events over intramolecular integration events is higher with the nicked donor than what is detected in this assay.
Target DNA Topology Affects Integration

The target DNA in the experiments described so far was in the supercoiled form. To investigate whether the topology of the target is a significant factor for transposon integration, the in vitro hop assay was used to quantify the relative efficiency of negatively supercoiled, nicked and positively supercoiled targets (Figure 3). Intermolecular integration events obtained with a negatively supercoiled target were over 10-fold more numerous than with a nicked and positively supercoiled target, indicating that negatively supercoiled DNA is a much better target than nicked and positively supercoiled DNA. The increased intermolecular events obtained with the negatively supercoiled target are presumably at the expense of the intramolecular events, which are the majority of the products. This helps to explain why an ETF from a nicked donor, which has a low level of supercoiling, if any, is less reactive and performs less intramolecular integrations than that from a supercoiled donor (Figure 2A). It also suggests that the higher levels of intermolecular integrations obtained after 24 h with a nicked substrate results from a failure to perform intramolecular integration owing to the lack of supercoiling in the ETF.

Target Sequence Bias

Quantification in the in vitro hop assay is achieved by counting bacterial colonies that result from transformation with intermolecular insertion products. To determine the sequence specificity of \textit{Hsmar1} insertion we selected 54 clones at random from the experiment with the negatively supercoiled target in Figure 3 and sequenced the junctions (Figure 4A). All of the insertions were in a TA dinucleotide. The sequences of the 15 bp on either side of the insertion sites were used to produce a sequence logo (Figure 4B). This revealed that the local DNA sequences immediately surrounding the TA dinucleotide confer very little target specificity. To determine the target specificity over longer distances, the insertion points were plotted on a map of the target plasmid (Figure 4C). Most of the insertions were well distributed throughout the plasmid. However, some sites received more than one insertion. To address whether the observed distribution is statistically different from what would be expected if target site selection was random we compared the data to a Poisson distribution. There are 188 TA dinucleotides within the pACYC184 target. Therefore, each TA has a probability of 0.29 to be present in the set of 54 target sites. According to the Poisson distribution, the probability that any single target site will receive 3 or 4 randomly selected integration events in a set of 54 is 0.36 and 0.04, respectively. Since we recovered one example of each type, it appears that target selection is not entirely random.

Intramolecular Target Selection

It is conceivable that some of the bias towards intramolecular transposition could arise from the presence of an insertion hot spot in the artificial mini-\textit{Hsmar1} transposons, which all contain a kanamycin resistance marker. To address this issue, the transposon inversion circle product was purified from a gel and analyzed by restriction digestion (Figure 5). The transposon contains a single XhoI recognition site and cleavage with this enzyme linearizes the inversion circle. The transposon also has two BamHI sites located close to the transposon ends. If the intramolecular insertion points are completely random, digestion with BamHI will produce a continuous smear of fragments of different lengths. Any bands within this smear will reveal insertion hot spots. Several bands were evident within the smear (Figure 5). However, these were well distributed, indicating that no major hot spots were present. Finally, the pattern of bands was similar for inversion circles derived from supercoiled or nicked donors, indicating that supercoiling in the target is not a major determinant of site bias.

Discussion

Donor and Target Topology Affect Integration

DNA supercoiling accelerates synapsis of the \textit{Hsmar1} transposon ends by increasing the probability of productive collision events between ends that have the appropriate inverted repeat configuration [7]. This is consistent with the experiment in Figure 1C where intermolecular transposition events accumulated more quickly with a supercoiled donor than a nicked donor. However, the effect of the transposon donor topology on the reaction has two features that cannot be explained by the accelerating effects of DNA supercoiling on synapsis. Firstly, if allowed sufficient time to reach completion, a nicked donor yields higher levels of intermolecular integrations than a supercoiled donor (Figures 1C and 2B). Secondly, a transposon excised from a nicked donor suffers from an integration defect (ETF, Figure 2A). Clearly, the integration step is affected by the topology of the transposon donor. However, we also found that negatively supercooled DNA is the preferred target of the \textit{Hsmar1} transposon (Figure 3). Since the majority of integration events in the in vitro reaction are intramolecular, it is likely that the absence of significant levels of supercoiling in excised transposon derived from a nicked donor is at least partially responsible for the integration defect. Indeed, the integration defect of the nicked transposon could be partially restored in the presence of elevated levels of supercoiled target (see ETF with nicked donor, Figure 2A). Furthermore, the target preference for negatively supercoiled DNA suggests that the propensity of the nicked donor to perform intramolecular integration events is the result of the nicked transposon having a lower tendency to integrate into itself than the supercoiled transposon. Indeed, the decrease of intramolecular insertion events with the nicked transposon at high concentrations of negatively supercoiled target was clearly observable (see Intra. with nicked donor, Figure 2A).

Figure 3. The topology of the target affects integration. Transposition reactions containing 1 nM supercoiled transposon donor (pRC704) were performed in the presence of a 1 nM target plasmid (dimeric pACYC184) that was either negatively supercoiled (SC), nicked, or positively supercoiled. Intermolecular insertions of the transposon into the target plasmid were recovered by genetic transformation in \textit{E. coli}. The percentage of target plasmids integrated by a transposon is plotted.
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Integration of the Hsmar1 Transposon

A

5′-TGATATCTGGCTTACTATGTGGGACTGATGTA-3′
5′-AGGGACACAGGATTATTATTCGCAATGAGT-3′
5′-TTATATCTGGCTTACTATGTGGGACTGATGTA-3′
5′-CAGATATCTGGCAATGATGTAAGAATTGCCCG-3′
5′-ACATATCTGGCCATATGGTAAGAATTGCCCG-3′
5′-TGGATATCTGGCTTACTATGTGGGACTGATGTA-3′
5′-AGGCACTTATCGGTAAAATGACTGTCATATTAC-3′
5′-CCATCTTATCGGTAAAATGACTGTCATATTAC-3′
5′-TGATATCTGGCTTACTATGTGGGACTGATGTA-3′
5′-AGGCACTTATCGGTAAAATGACTGTCATATTAC-3′
5′-TGATATCTGGCTTACTATGTGGGACTGATGTA-3′
5′-CCATCTTATCGGTAAAATGACTGTCATATTAC-3′

B

![Graph showing bits distribution](image)

C

![Graph showing insertion events](image)

Position within the target (kb)

rep tet cat
Figure 4. Target site bias. A. 54 integration events into a supercoiled pACYC184 target were selected at random from the experiment in Figure 3. The junctions between the transposon and the target were analyzed by DNA sequencing. All 54 insertions were precise and the transposon ends were flanked by duplicated TA dinucleotides. The sequence of the integration sites were centered on the target TA and aligned. B. The target sites shown in A were used to generate a logo representing the bias in target site selection in the vicinity of the TA. The logo was created by the program WebLogo [44]. C. The position of the 54 integration sites was plotted on a map of the pACYC184 target. cat, chloramphenicol resistance marker; tet, tetracycline resistance marker; rep, origin of replication. Integration events are represented by vertical bars. The target plasmid was a dimeric version of the pACYC184 plasmid, which allowed the detection of integration events into the cat gene and the origin of replication.
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Regulation of Mariner Transposition by DNA Supercoiling

Hsmar1 transposition is sensitive to DNA topology, both at the level of transposon excision and integration (ref. [7] and this work). We have previously suggested that the stimulatory effects of DNA supercoiling on transposon excision may represent a mechanism to couple transposition to cellular events [7]. This is a well-established property of bacterial transposons, as exemplified by Tn10, which is sensitive to supercoiling as well as the global regulators IHF and H-NS [34]. In the case of Hsmar1, excision of a transposon located in front of a replication fork would be inhibited by positive supercoiling. This could promote transposon amplification by favoring transposition after the passage of the replication machinery. Transposon excision may also be activated by free negative supercoils formed behind transcription bubbles or by eviction of nucleosomes associated with episodes of chromatin remodeling.

The target preference for negatively supercoiled DNA suggests that transposon integration may also be regulated in response to the dynamics of the nucleus. It is conceivable that the excised transposon preferably interacts with free supercoiled DNA that transiently forms during DNA metabolic processes. However, Sleeping Beauty and Hsmar1 do not appear to have an insertion bias for active genes, suggesting that transcription is not a predominant factor for transposon integration [11,35]. Moreover, transposons that target transcribing DNA are likely to be more disruptive than transposons that target silent genomic regions. If a transposon were to target negative supercoils formed during DNA replication, it would be expected to integrate behind replication forks.

However, in order for a transposon to increase its copy number it would be more advantageous if it were to integrate in front of replication forks. Whether free supercoiled DNA provides in vivo targets for Tc1-mariner transposons is unknown but it is intriguing to ask whether the preference for supercoiled targets could provide a selective advantage.

An alternative possibility is that the effect of DNA supercoiling on target interactions described here is not related to a mechanism to activate transposon integration in response to chromatin dynamics but instead reflects a fundamentally different aspect of the reaction. Transposon integration is in principle reversible because the strand transfer reaction is isoenergetic. It has been suggested that transposases and integrases ensure the irreversibility of the integration step by introducing a bend in the target [36,37]. The mechanical strain in the target would be released during the strand transfer reaction and produce a displacement of the newly formed phosphodiester bond away from the active site to prevent the reverse reaction. This was first suggested by the crystal structure of integration intermediates of the foamy virus intasome [38]. A comparison of the pre- and post-catalytic integration complexes showed that the position of the scissile phosphodiester bond is shifted away from the active site after catalysis, making it an unlikely substrate for subsequent nucleophilic attack [36].

The propensity to bend the target site appears to be a common theme in the DDE/D family of integrases and transposases. A similar DNA bend is observed in the recent structure of the Mu target capture complex [39]. Moreover, biochemical analyses have shown that the target site of Tn10 is bent upon capture by the

Figure 5. Target site selection during intramolecular integration is not affected by the topology of the transposon. Transposition reactions were performed using a negatively supercoiled or nicked transposon donor pRC650, which encodes a 1.7 kb transposon. The unknotted inversion circle (IC) was gel purified, digested by the restriction enzyme XhoI or BamHI and analyzed by gel electrophoresis. XhoI linearizes the IC (Lin IC). Two BamHI sites are situated close from the transposon ends. Random target site selection will generate a smear while bands into the smear represent hot spots of integration into the transposon. SYBR Green I stained 1.1% agarose gels are shown. The pattern of target site selection was similar with the supercoiled and the nicked donor DNA.
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transpososome [40]. Furthermore, the Mos1 post-cleaveage paired-ends-complex structure suggests that the mariner transpososome may also bind the target [41]. Indeed, the structure displays an additional pair of transposon ends that interacts in a non-sequence specific manner with the transpososome and has an angular geometry similar to the foamy virus intasome target. Finally, correlations drawn between preferred targets of Sleeping Beauty and predicted physical properties of integration sites suggested that DNA bendability favors target site selection [11]. However, no correlation could be drawn between preferred targets of Hsmar1 and the predicted physical properties of these sites [14,17]. By a similar approach, we could find no correlation between the preferred target sites of Hsmar1 in vitro and the predicted curvature and bendability of the target, although this analysis was probably hampered by the lack of statistical power (not shown).

Another interesting question that remains to be investigated with Tc1-mariner elements is how nucleosomes influence target site selection. Nucleosomes have different impacts on the integration bias of viruses and transposons. For example, the human immunodeficiency virus and murine leukemia virus integrate preferentially nucleosome-occupied DNA in vivo, presumably due to their propensity to target bendable DNA or sites that are bent by protein complexes [42]. The Tyl retrotransposon also preferentially integrates into nucleosome-occupied targets whereas the kAT transposon Homs and the Tyl retrotransposon integrate preferentially into nucleosome-free regions [21,22,23].

Supercoiling can affect DNA transactions by several mechanisms. Supercoiling increases the local concentration between any two sites. The right-handed intertwining of plectonemic negative supercoils can juxtapose two sites in a specific angular configuration. Negative supercoiling also underwinds the DNA and supercoils can juxtapose two sites. The right-handed intertwining of plectonemic negative supercoiling on transposon integration is likely to be due to the underwinding or the bendability of DNA. However, our observations do not allow us to clearly discriminate between these two factors. Because positive supercoiling does not promote target interactions, we would expect the underwinding of the DNA to be a predominant factor. In this case we would expect nucleosomal DNA to provide poor targets for mariner insertions because DNA is probably overwound at the surface of nucleosomes [43].

**Target Sequence Bias**

The nucleotide sequence at the vicinity of the target TA usually provides little specificity for Tc1-mariner transposons [11,12,13,14]. Nevertheless, consensus integration sites have been reported for Mos1 (‘TATA’ or ‘TxA1A’) and Sleeping Beauty (palindromic AT repeat ‘ATATATAT’ [11,14,17]. Hsmar1 also appears to target preferentially TA dinucleotides that are flanked by A-T base pairs (Figure 4B). However, as in the case of Hsmar1, this preference is mild and no consensus integration site can be derived [13]. Intriguingly, the alignment of Hsmar1 target sites revealed some sequence bias for the nucleotides located about one helix turn away from the TA target, possibly reflecting interactions between the transpososome and these nucleotides. Although the distribution of Hsmar1 integration events was not random, no major integration hotspots were detected (Figures 4C and 5). Mos1 appears to display a stronger integration bias than Hsmar1. The chloramphenicol acetyltransferase (cat) gene from Tn9 was reported to be a strong hotspot for Mos1 insertions [17]. In contrast, Hsmar1 does not display an integration bias for this gene and integration events within a plasmid that contained the cat gene were well distributed throughout the plasmid (Figure 4C). The cat gene requires DNA supercoiling to provide an integration hot spot for Mos1 [17]. In contrast, the stimulatory effect of DNA supercoiling on Hsmar1 target interactions appears to be general. The pattern of intramolecular integration sites also suggested that the target selection bias is unaffected by the topology of the target (Figure 5).

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**Author Contributions**

Conceived and designed the experiments: CCB RC. Performed the experiments: CCB. Analyzed the data: CCB RC. Contributed reagents/materials/analysis tools: CCB RC. Wrote the paper: CCB RC.

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