

Silent chromatin determines target preference of the *Saccharomyces* retrotransposon Ty5

SIGE ZOU AND DANIEL F. VOYTAS

Department of Zoology and Genetics, Iowa State University, Ames, IA 50011

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ABSTRACT The *HML* and *HMR* mating loci of *Saccharomyces cerevisiae* are bound in silent chromatin, which is assembled at the flanking E and I transcriptional silencers. The retrotransposon Ty5 preferentially integrates into regions of silent chromatin, and Ty5 insertions near the *HMR-E* silencer account for ≈2% of total transposition events. Most Ty5 insertions occur within 800 bp on either side of the autonomously replicating consensus sequence within *HMR-E*. Ty5 target preference is determined by silent chromatin, because integration near *HMR-E* is abolished in strains with silencer mutations that alleviate transcriptional repression. The recognition of specific DNA sequences *per se* does not direct integration, rather, it is the protein complex assembled at the silencers. As demonstrated here for Ty5, recognition of specific chromatin domains may be a general mechanism by which retrotransposons and retroviruses determine integration sites.

Retroelements, which include retrotransposons and retroviruses, are not randomly distributed in eukaryotic genomes (1, 2). One explanation for this observation is that the cDNA intermediates of retroelement replication integrate preferentially into specific genomic sites. This model is supported by the Ty1 and Ty3 retrotransposons of *Saccharomyces cerevisiae*. These elements integrate preferentially upstream of tRNA genes or other genes transcribed by RNA polymerase III (pol III) (3, 4). Targeted transposition by Ty1 and Ty3 is abolished by mutations that block the assembly of pol III transcription complexes, indicating that transcriptional competence is a requirement for targeting. *In vitro* transposition assays with Ty3 further suggest that targeting is determined by interactions between the Ty3 integration complex and components of the pol III transcription apparatus (5). The loading of transcription factors TFIIB and TFIIC onto tRNA gene templates is sufficient to direct Ty3 integration.

Interactions between retroelements and chromosomal proteins is also suggested by the finding that human immunodeficiency virus (HIV) integrase binds a host factor, Ini1, which is a human homologue of the *S. cerevisiae* protein SNF5 (6). SNF5 is a member of the SWI/SNF protein complex that is involved in shaping chromatin and activating gene expression (7). Nucleosomes affect HIV and Moloney leukemia virus integration sites (8–12), and histone mutations can alter Ty1 integration patterns (13). These data suggest that chromatin influences target choice, and recognition of chromatin components by retroelements may be a general mechanism by which integration sites are determined.

We have recently demonstrated that the *Saccharomyces* retrotransposon Ty5 integrates preferentially near telomeres or the silent mating loci *HML* and *HMR* (14, 15). These regions are bound in a well-studied chromatin, called silent chromatin,

which is involved in telomere function and the transcriptional inactivation of adjacent genes (16). Transcriptional silencers flanking *HML* and *HMR* (designated E and I) mediate silent chromatin assembly, and the *HMR-E* silencer, in particular, has been studied extensively. *HMR-E* consists of three cis-acting sequences, called A, E, and B. The A site is an autonomously replicating consensus sequence, which serves as an origin of DNA replication and binds the origin recognition complex. The E and B sites bind the transcription factors RAP1 and ABF1, respectively. Silent chromatin is formed through the interaction of these DNA-bound proteins with a number of other proteins, including four silent information regulators (SIR1–SIR4). The preference for Ty5 to integrate near regions of silent chromatin suggested that silent chromatin directs integration. We tested this by evaluating *HMR-E* mutations that influence silencing for their effects on targeted transposition.

MATERIALS AND METHODS

A Ty5 Transposition Assay. All yeast strains carried the plasmid pNK254, which has a Ty5 element under transcriptional control of the *GALI-10* promoter and a *his3AI* selectable marker gene (N. Ke and D.F.V., unpublished work). To induce transposition, yeast cells were grown as patches on synthetic complete media without uracil (SC-U) and with galactose at 23°C for 2 days. Cells were scraped and resuspended in 1 ml of sterile water. For each patch, 100 μ l of a 10^7 -fold dilution of the cell suspension was plated onto non-selective media [yeast extract/peptone/dextrose (YPD)] to calculate total cell number. One hundred microliters of a 10-fold dilution was spread on selective media [SC without histidine (SC-H)]. His⁺ colonies that arose were replica plated to SC-H media with FOA (SC-H+FOA) to remove plasmid recombinants. The frequency of Ty5 transposition was calculated by dividing the cell number on SC-H+FOA plates by that on YPD plates and the dilution factor. Yeast strains W303-1A (*MATa ade2-1 can1-100 his3-11,15 leu2-3 trp1-1 ura3-1*), W303-1B, W303-1, and their derivatives with various *HMR-E* mutations were used for all manipulations (17, 18). The mutant strains were kindly provided by David Shore (University of Geneva) and Rolf Sternglanz (State University of New York, Stony Brook). For all mutant strains, the *MATa* locus was converted to *MATa* (19).

To quantify Ty5 integration and recombination events, several hundred His⁺ colonies generated from transposition assays were randomly picked and grown as small patches (≈0.5 cm × 0.5 cm) on SC-H+FOA plates. Cells that grew were considered to have Ty5 integrated in the genome, while others were considered to have Ty5 recombined with the donor plasmid (see Fig. 1). The percentage of integration events was calculated as the number of His⁺FOA^r cells divided by the total number of His⁺ cells.

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Abbreviations: FOA, 5-fluoroorotic acid; pol III, RNA polymerase III; SIR, silent information regulators; SC-H, synthetic complete media without histidine.

A PCR Assay to Identify Ty5 Insertions Near *HMR-E* and *HML-E*. Polymerase chain reactions (PCR) were conducted using 50 ng of genomic DNA prepared from 10 His⁺FOA^r strains, representing 10 independent Ty5 transposition events. The majority of His⁺FOA^r cells were assumed to have only one insertion per genome, as supported by the characterization of more than 70 His⁺FOA^r strains (refs. 14 and 15; data not shown). Insertions near transcriptional silencers were identified and confirmed by two rounds of PCR screening. The first round used primers that amplified all Ty5 insertions near *HMR-E*. This was accomplished using a Ty5-specific primer (DVO200, 5'-CATTACCCATATCATGCT-3') and two primers that amplified a 2.3-kb window flanking *HMR-E* (DVO211, 5'-TGGTAGAAGCAGTAGTAACT-3'; DVO212, 5'-ACCAGAGAGTGTAACAACAG-3'). Since DNA from at least 1 of the 10 strains in each reaction did not have an insertion near *HMR-E*, the presence of the 2.3-kb band served as a control for the PCR reactions. One of the PCR primers (DVO212) also matched sequences flanking *HML-E*, and therefore Ty5 insertions in one orientation were recovered near this silencer. The second round of PCR screening used silencer-specific primers to confirm the number of insertions and to precisely map sites of integration. For *HMR-E*, these were DVO211, DVO200, and DVO220 (5'-CTGTGTACAAGAGTAGTACC-3'); for *HML-E* these were DVO247 (5'-CACGAGCTCATCTAGAGCC-3') and DVO220. Amplifications were performed for 30 cycles using the following program: 94°C for 1 min, 50°C for 2 min, and 72°C for 5 min, with a final extension step at 72°C for 5 min.

RESULTS

A Modified Ty5 Transposition Assay. We recently established a genetic assay to identify Ty5 transposition events in *S. cerevisiae* using a functional Ty5 element from *Saccharomyces paradoxus* (14). This assay uses a *his3AI* marker gene, which generates an His⁺ phenotype upon reverse transcription of spliced Ty5 mRNA. Analysis of His⁺ cells generated by our transposition assays indicated that they arose by two distinct pathways (Fig. 1). Some His⁺ cells were due to integration of Ty5 into the host genome and had characteristic 5-bp target site duplications (14, 15); the rest were due to recombination of Ty5 cDNA with the plasmid-borne element (N. Ke and D.F.V., unpublished work). These two events can be distinguished by taking advantage of the *URA3* gene on the vector (Fig. 1). Cells with a *HIS3* gene on the plasmid cannot grow on SC-H media containing FOA, which selects against the *URA3* gene (20). We consider Ty5 transposition frequency to be the percentage of His⁺FOA^r colonies, and the recombination frequency to be the percentage of His⁺FOA^s cells. Although it is possible that some chromosomal events arise by recombination, none have been observed in the more than 54 chromosomal events characterized (14, 15). In addition, by growing each of the strains used in this study on glucose, we determined that the frequency of His⁺ cells that arise by reversion or recombination between plasmid and chromosomal *HIS3* sequences is less than 1×10^{-8} (data not shown).

Expression of Mating Type Genes Influences the Proportion of Ty5 Integration Events. The transcriptional silencer *HMR-E* is a hot spot for Ty5 integration (14). *HMR-E* is made up of three sequence domains, called A, E and B, which bind different proteins involved in silencing (16). Mutations in these binding sites have previously been evaluated for their role in transcriptional silencing (ref. 18; Table 1). Prior to investigating their effect on Ty5 targeting, we first tested whether these mutations influence the proportions of Ty5 integration and recombination events.

Integration events accounted for approximately 70% of the His⁺ cells generated in wild-type *MAT α* and *MATa* haploid strains and about 57% of the His⁺ cells generated in the

An assay to distinguish between Ty5 integration and recombination

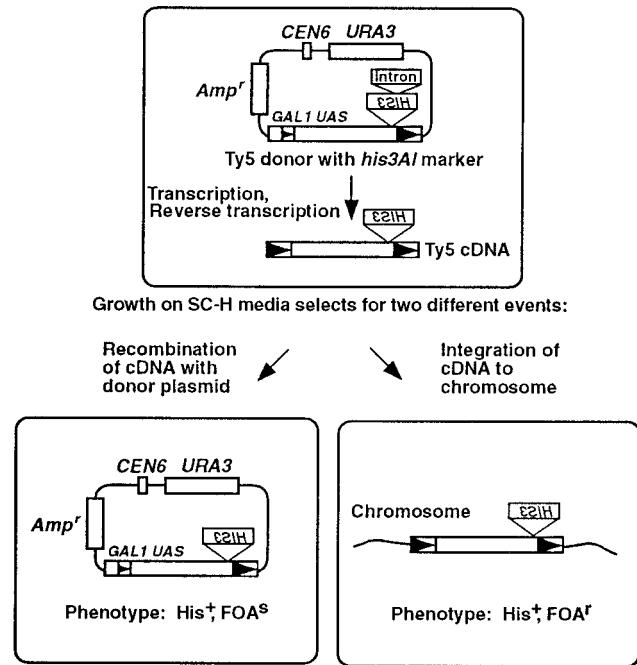


FIG. 1. An assay to distinguish between Ty5 integration and recombination events. The Ty5 donor element is on a *URA3*-based vector. Ty5 cDNA is reverse transcribed, using mRNA from the donor element, which reconstitutes a functional *HIS3* gene. The cDNA can either integrate into the host genome or recombine with the donor element to give rise to His⁺ cells. These events can be distinguished, because cells with plasmid Ty5 recombination events cannot grow on SC-H+FOA media due to the presence of the *URA3* gene.

wild-type *MAT α* /*MATa* diploid (Table 1). For the *MAT α* strain with a deletion of *HMR-E* (YSZ203), the percentage of integration events decreased dramatically to 16%. This same *HMR-E* deletion in a *MATa* strain (YSZ221), however, had integration frequencies comparable to wild type (about 70%). These two mutant strains differ only in the mating type information they express (18); the *MAT α* strain expresses both α -mating and *a*-mating type information from *MAT* and *HMR*, respectively, whereas the *MATa* strain only expresses *a*-mating type information. Gene expression of both mating type loci, therefore, decreases the ratio of Ty5 integration events relative to recombination events. This effect appears to be haploid specific, since the percentage of Ty5 integration is only slightly reduced in the diploid strain, which expresses both α -mating and *a*-mating type information.

***HMR-E* Mutations Do Not Significantly Affect Overall Frequencies of Ty5 Transposition.** Most of the strains with *HMR-E* mutations were originally constructed in a *MAT α* background. Because expression of mating-type genes influences the proportion of Ty5 integration events, we converted these strains to *MATa*. The percentage of integration events and the overall transposition frequency was determined for each *MATa* strain using the modified transposition assay described above. Ratios of integration events ranged from 57.5 to 69.7% and were comparable to wild type (Table 1). This further suggests that only the expression of α and *a* mating-type information, and not mutations in *HMR-E* *per se*, is responsible for the altered levels of integration vs. recombination. Overall transposition frequencies in the *HMR-E* mutant strains ranged from $1.5\text{--}2.4 \times 10^{-6}$ and were comparable to wild type ($\approx 3 \times 10^{-6}$; Table 1).

A PCR Assay to Measure Targeted Transposition. Based on our previous characterization of approximately 150 indepen-

Table 1. Integration and recombination of Ty5

<i>HMR-E</i> allele	Mating type	Strain	Percentage integration events	Percentage difference from wild type	Overall transposition frequency ($\times 10^{-6}$)	Fold reduction
AEB	<i>MATa</i>	YSZ202	73.0 \pm 2.3	NA	2.88	NA
AEB	<i>MATα</i>	YSZ201	75.4 \pm 1.9	2.4 \pm 4.2	2.81	1.02
AEB	Diploid	YSZ200	57.0 \pm 2.9	16.0 \pm 5.2	2.35	1.22
$\Delta A \Delta E \Delta B$	<i>MATα</i>	YSZ203	16.1 \pm 1.4	56.9 \pm 3.7	ND	ND
$\Delta A \Delta E \Delta B$	<i>MATa</i>	YSZ221	69.7 \pm 2.0	3.3 \pm 4.3	1.61	1.79
$\Delta A \Delta E B$	<i>MATa</i>	YSZ224	61.4 \pm 1.5	11.6 \pm 3.8	1.54	1.87
$\Delta A E \Delta B$	<i>MATa</i>	YSZ228	50.9 \pm 1.2	22.1 \pm 3.5	2.40	1.20
$\Delta E \Delta B$	<i>MATa</i>	YSZ222	68.6 \pm 0.9	4.4 \pm 3.2	1.80	1.60
$\Delta A E B$	<i>MATa</i>	YSZ225	68.0 \pm 1.2	5.0 \pm 3.5	1.70	1.69
$\Delta A E B$	<i>MATa</i>	YSZ227	57.5 \pm 0.9	15.5 \pm 3.2	1.58	1.82
$\Delta E \Delta B$	<i>MATa</i>	YSZ226	63.7 \pm 0.2	9.3 \pm 2.5	1.92	1.50

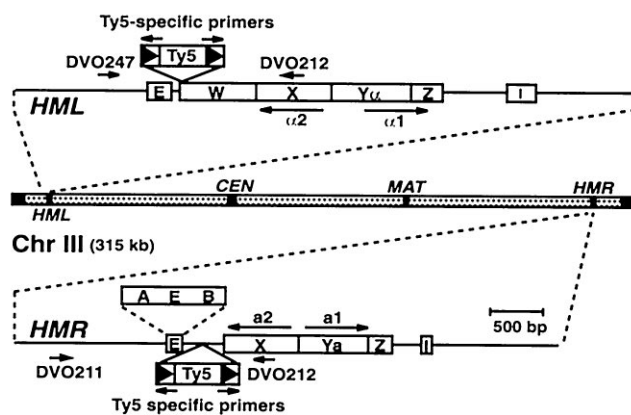
NA, not applicable; ND, not determined.

dent Ty5 transposition events, we found that $\approx 3\%$ of Ty5 insertions occurred near *HMR-E* and *HML-E* (14). This indicated that a large number of Ty5 insertions would be required to statistically evaluate targeting in the *HMR-E* mutants. To obtain the requisite numbers, we developed a PCR assay to measure targeting to *HMR-E* and *HML-E* (Fig. 2). For a given mutant, 600–1,000 His⁺FOA^r strains were randomly collected, which represent independent Ty5 transposition events. Genomic DNA samples were prepared from pools of 10 strains, and insertions near particular silencers were amplified using a Ty5-specific and two silencer-specific primers (Fig. 2). The locations of insertions were mapped by a second round of PCR using two Ty5-specific primers and a primer specific for *HMR-E* or *HML-E*. All Ty5 insertions were recovered within about a 2-kb window encompassing a given silencer, the region preferred by Ty5 for integration.

The Silencing Status of *HMR* Affects Targeted Transposition. The results of our PCR screen to evaluate targeted transposition in strains with *HMR-E* mutations are shown in Table 2. For the wild-type strain, 1.9% (17/908) of Ty5 transposition events occurred near *HMR-E* and 2.2% (20/908) occurred near *HML-E*, the latter of which served as an experimental control. Due to the nature of the primers used, insertions in only one orientation near *HML-E* were identified (see *Materials and Methods*), suggesting that the actual frequency of transposition to this locus approximates 4%. Transposition to *HMR-E* was not significantly reduced in the ΔB strain (1.0%) relative to wild type (2.2%); however, it was reduced by about 3-fold ($P < 0.05$) and 4-fold ($P < 0.01$) in the ΔA (0.68%) and ΔE (0.48%) strains, respectively. Targeting to *HMR-E* in the ΔA and ΔE mutant strains was also reduced relative to *HML-E*. This modest affect on transposition is similar to the slight derepression of *HMR* transcription observed for single-domain deletions of *HMR-E* (18).

For strains with any two or all three silencer domains deleted, no Ty5 insertions were recovered near *HMR-E* among the 672–954 transposition events screened (Table 2). This corresponds to more than a 13-fold reduction in the frequency of targeted transposition. Because deletion of any one of the three domains only slightly affects silencing at *HMR*, and deletion of any two or all three completely disrupts *HMR* silencing, targeted transposition by Ty5 is directly correlated with the assembly of silent chromatin (18). For most of these mutants, transposition frequency near *HML-E* was approximately 3-fold reduced ($P < 0.05$) compared with the wild type, indicating that the transcriptional status at *HMR* influences targeting to *HML*. This supports previous observations suggesting interactions between domains of silent chromatin; for example, *HMR* silencing is influenced by its distance from the telomere (21).

A. A PCR assay to identify Ty5 insertions near *HMR-E* and *HML-E*



B. Examples of results from first-round PCR screens

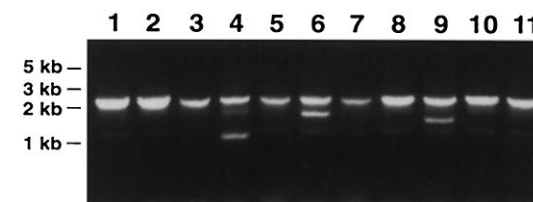


FIG. 2. (A) A PCR assay to quantitate the frequency of Ty5 transposition to *HML-E* and *HMR-E*. Shaded bar indicates chr III. Expanded views are shown for *HML* and *HMR*, and E and I designate the flanking transcriptional silencers. W, X, and Z represent homologous regions shared between *HML*, *HMR*, and *MAT*. $Y\alpha$ and $Y\alpha$ are specific for *HML* and *HMR*, respectively. Transcripts ($\alpha 1$, $\alpha 2$, $\alpha 1$, $\alpha 2$) at each mating locus are shown by the longer horizontal arrows. An expanded view is shown for *HMR-E* indicating the A, E, and B sequence domains. Hypothetical Ty5 insertions are indicated by the labeled boxes, which are not drawn to scale. Names and locations of primers used for PCR are shown by the shorter horizontal arrows. (B) Examples of PCR products obtained in the first round of screening. Each lane shows amplification products from yeast genomic DNA derived from 10 independent transposition events. The numbers at the top of each lane indicate different pools of DNA. DNA size markers are shown at the left. DNA was amplified with primers DVO200, DVO211, and DVO212. The 2.3-kb band present in all lanes is derived from sequences flanking *HMR-E* without insertions; this serves as an internal PCR control. Fragments smaller than 2.3 kb indicate Ty5 insertions near *HMR-E* or *HML-E*. Subsequently, locus-specific and Ty5-specific primers were used to confirm the location of these insertions in a second round of PCR.

Table 2. Frequency of Ty5 transposition near *HML-E* and *HMR-E*

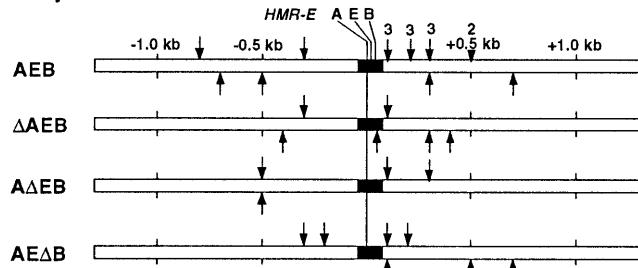
<i>HMR-E</i> allele	Strain	Total Ty5 insertions	Insertions near <i>HML-E</i> (%)	Insertions near <i>HMR-E</i> (%)	Fold reduction in targeting to <i>HML-E</i> [†]	Fold reduction in targeting to <i>HMR-E</i> [†]
AEB	YSZ202	908	20 (2.2)	17 (1.9)	1	1
ΔAEB	YSZ225	884	14 (1.6)	6 (0.68)	1.4	2.8*
ΔΔEB	YSZ227	828	21 (2.5)	4 (0.48)	0.87	3.9**
AEΔB	YSZ226	788	8 (1.0)	8 (1.0)	2.2	1.8
ΔΔEΔB	YSZ222	883	18 (2.0)	0 (<0.11)	1.1	>17***
ΔAEΔB	YSZ228	672	6 (0.80)	0 (<0.15)	2.5*	>13***
ΔAΔEB	YSZ224	754	6 (0.80)	0 (<0.13)	2.8*	>14***
ΔAΔEΔB	YSZ221	954	8 (0.84)	0 (<0.10)	2.6*	>18***

[†]Marked numbers are significantly different from the wild type at *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Ty5 Integrates Nonrandomly Within a 1.6-kb Window of *HMR-E*. We mapped the position of Ty5 insertions near *HMR-E* in strains with single-domain deletions, and found no obvious change in the integration pattern (Fig. 3). All of the insertions were within 800 bp on either side of the A site (the origin recognition complex binding site), which is consistent

with previously observed targeting patterns (14). Patterns of transposition near *HML-E* were not altered in strains with various *HMR-E* mutations. Many insertions are clustered at certain sites (e.g., to the left of *HML-E*), suggesting that targeted integration may be influenced by local chromatin structure.

A. Ty5 Insertions near *HMR-E*



B. Ty5 Insertions near *HML-E*

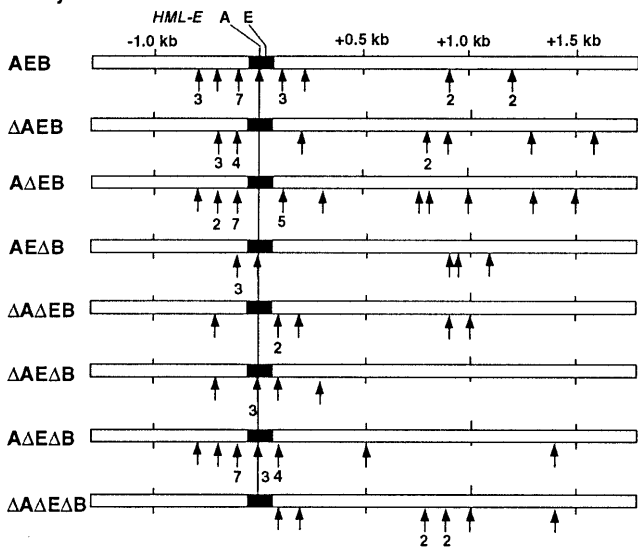


FIG. 3. (A) Location of Ty5 insertions near *HMR-E*. Ty5 insertions were identified throughout the ≈2-kb window represented by the open box. The reference point is the autonomously replicating consensus sequence (A) located within *HMR-E*. The genotype of *HMR-E* is indicated to the left. Arrows pointing down represent insertions in the same 5' to 3' orientation as the chromosome sequence, and arrows pointing up represent insertions in the opposite orientation. (B) Location of Ty5 insertions near *HML-E*. Only one orientation of Ty5 insertions was identified in this experiment, since only one primer (DVO212) matches sequence at the *HML* locus. The reference point is the A site located within *HML-E*. The genotype of *HMR-E* is indicated to the left, while the genotype of *HML-E* is wild type in all strains.

DISCUSSION

Silent Chromatin Directs Ty5 Integration. The preference for Ty5 to integrate near the *HMR-E* transcriptional silencer is determined by silent chromatin assembled at this site. Targeted integration to *HMR-E* is abolished in strains with mutated silencers that alleviate transcriptional repression of *HMR*. DNA sequences *per se* are not critical for target choice, since deletion of any one of the three *HMR-E* sequence domains does not dramatically reduce targeting. Rather, the proteins recruited by these cis sequences appear to be responsible for target specificity. The A, E, and B domains of *HMR-E* interact with origin recognition complex, RAP1 and ABF1, respectively (16). These proteins are assembled into silent chromatin along with other proteins, such as SIR1–SIR4. We predict that one or more of these components interacts with the Ty5 integration complex to direct transposition.

Targeted transposition by Ty5 correlates well with the transcriptional status of *HMR*. Deletion of two or more *HMR-E* domains completely derepresses transcription and completely eliminates targeted integration (18, 22). We observed slight decreases in targeting for single ΔE and ΔA mutations. Deletion of the E domain partially derepresses transcriptional silencing at *HMR* (18). Although deletion of A domain does not derepress transcription, this deletion appears to weaken silencing, and ΔA mutations have been shown to enhance the level of derepression caused by certain mutations in components of silent chromatin, such as the *RAP1* temperature-sensitive mutations (23). The observed reduction in Ty5 targeting for single-domain mutations, therefore, may be the consequence of a weakened silent chromatin. Whereas transcriptional silencing is sufficient for targeting to occur, it remains to be determined whether it is absolutely necessary. One or few protein factors, which by themselves cannot mediate silencing, may be capable of directing integration.

Insertion Patterns of Ty Elements. Most Ty5 insertions near *HMR-E* are within 800 bp of the A site in this silencer without any orientation specificity. The boundary of silent chromatin at *HMR* extends over 0.8 kb upstream of *HMR-E* (24). Ty5 insertions, therefore, occur within this highly packed chromatin, which is inaccessible to many protein–DNA interactions (24). This suggests that the Ty5 integration complex can negotiate silent chromatin to access target sites, or that transposition occurs when these regions are disassembled, such as during DNA replication. The silent mating loci are traversed by precisely positioned nucleosomes, and DNase I and micro-

coccal nuclease sensitive sites have been mapped across *HMR* and *HML* (25). Hotspots for Ty5 integration, particularly near the silencers, correlate loosely with sites of prominent nuclease hypersensitivity. Additional experiments, however, are required to more precisely map integration and nuclease sensitive sites to determine whether chromatin dictates local integration site preferences.

Ty5 insertions occur on either side of their targets. This pattern is in contrast to Ty1 and Ty3 (3, 4); Ty1 insertions are generally within 700 bp upstream of pol III transcribed genes, and Ty3 integrates within several bp upstream of transcription start sites of target genes. Like Ty5, neither Ty1 nor Ty3 display an orientation preference; however, Ty1 and Ty3 insertions seldom occur downstream of the promoters in their target genes. It is interesting to note that the rDNA and tRNA genes, which are preferred targets for Ty1, repress expression of pol II transcribed genes (26–28). This suggests that silencing is a common feature of Ty targets. Retroviruses preferentially insert upstream of transcriptionally active genes, and this preference is presumably due to the presence of open chromatin at these sites (1). The differences among Ty elements and retroviruses suggest that each likely uses a unique targeting mechanism, although they probably share similar targeting strategies to recognize DNA-bound proteins or protein complexes.

Chromatin and Retroelement Targeting. The proteins that make up silent chromatin carry out or influence numerous genetic processes, including DNA replication, transcriptional silencing, and telomere function (16). We demonstrate here that silent chromatin also directs transposition. We propose that targeted integration by Ty5 is mediated by interactions between the Ty5 integration complex and one or more protein component of silent chromatin. This is supported by preliminary data indicating that mutations in *SIR* genes affect targeted transposition (S. Zou and D.F.V., unpublished work). Retroelements may generally sense specific chromatin domains during integration, as supported by the observation that Ty1 and Ty3 recognize domains of pol III transcription, and that HIV integrase interacts with the chromatin-related protein In11 (3, 4, 6). Retroelements are the major class of interspersed repetitive DNAs in plant and animal genomes. Targeted integration is suggested by their nonrandom distribution, as evidenced by the clustering of retrotransposons in intergenic regions of maize and the association of some retroelements with heterochromatin and telomeres in *Drosophila* (29–32). Retroelement targeting, therefore, is likely an important factor in shaping eukaryotic genome organization.

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