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The *Saccharomyces* Ty5 retrotransposon family is associated with origins of DNA replication at the telomeres and the silent mating locus *HMR*

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ABSTRACT **We have characterized the genomic organization of the Ty5 retrotransposons among diverse strains of *Saccharomyces cerevisiae* and the related species *Saccharomyces paradoxus*. The *S. cerevisiae* strain S288C (or its derivatives) carries eight Ty5 insertions. Six of these are located near the telomeres, and five are found within 500 bp of autonomously replicating sequences present in the type X subtelomeric repeat. The remaining two *S. cerevisiae* elements are adjacent to the silent mating locus *HMR* and are located within 500 bp of the origin of replication present in the transcriptional silencer *HMR-E*. Although the *S. cerevisiae* Ty5 elements no longer appear capable of transposition, some strains of *S. paradoxus* have numerous Ty5 insertions, suggesting that transposition is occurring in this species. Most of these elements are adjacent to type X telomeric repeats, and regions flanking four of five characterized *S. paradoxus* insertions carry autonomously replicating sequences. The genomic organization of the Ty5 elements is in marked contrast to the other *S. cerevisiae* retrotransposon families (Ty1–4), which are typically located within 500 bp of tRNA genes. For Ty3, this association reflects an interaction between Ty3 and the RNA polymerase III transcription complex, which appears to direct integration [Chalker, D. L. & Sandmeyer, S. B. (1992) *Genes Dev.* 6, 117–128]. By analogy to Ty3, we predict that Ty5 target choice is specified by interactions with factors present at both the telomeres and *HMR* that are involved in DNA replication, transcriptional silencing, or the maintenance of the unique chromatin structure at these sites.**

Retrotransposons are mobile genetic elements that replicate by reverse transcription of an RNA intermediate. The final step in the retrotransposon life cycle is the integration of an element into a new site in the host genome. Integration may be deleterious to the host cell because it can cause mutations or lead to chromosome rearrangements. Mechanisms that minimize this damage preserve the host's genetic integrity and also allow retrotransposons to replicate and maintain viable populations. In the yeast, *Saccharomyces cerevisiae* mechanisms have evolved that limit the negative consequences of integration by directing transposition to specific sites in the genome.

Target preference of the *S. cerevisiae* retrotransposons has been made strikingly evident by the recent genome sequencing efforts. Insertions of the four best-characterized retrotransposon families (Ty1–4) are found within the nucleotide sequences of chromosomes III and XI (chr III and chr XI, respectively) (1, 2). Of the 28 insertions representing the closely related Ty1 and Ty2 retrotransposons, 27 are found within 500 bp of tRNA genes (or 500 bp from a Ty element in cases of multiple insertions at a single tRNA gene) (3). For the Ty3 element family, a single insertion is found on chr XI within the promoter region of a tRNA gene. The association of Ty3 with tRNA genes has been well documented, and most Ty3 insertions are located within a few bases of the transcription start site of genes transcribed by RNA polymerase III (pol III) (4). The fourth retrotransposon family, Ty4, is represented by a single insertion on chr III. This element is within 300 bp of a tRNA gene, and tRNA genes are associated with 10 of 12 Ty4 insertions currently in the sequence data base (GenBank, release 83.0). Thus, a target bias, and in particular a preference for tRNA genes, is readily apparent from the genomic organization of endogenous Ty1–4 elements.

Transposition assays have made it possible to monitor the target choice of *de novo* Ty1 or Ty3 transposition events. For example, Ty1 target preference was analyzed by determining integration sites for 32 independent transposition events onto chr III (3). Over half (57%) were within 400 bp upstream of tRNA genes. Similarly, Ty3 integrates within 1–4 nt of the transcription start site of genes transcribed by pol III (5–7). Target specificity for Ty3 requires the assembly of the pol III transcription complex, and the transcription factor TFIIIB has been directly implicated in directing Ty3 integration (6, 7). The target bias observed from the genomic organization of Ty1 and Ty3 insertions, therefore, apparently reflects a mechanism that directs these elements to particular sites in the genome and, at least for Ty3, to the pol III transcription complex.

An additional *S. cerevisiae* retrotransposon family, designated Ty5, was discovered upon examination of the DNA sequence of chr III (8). In contrast to the other Ty element families, none of the three Ty5 insertions on chr III resides near tRNA genes (3). We have analyzed additional Ty5 target sites among *S. cerevisiae* strains and species closely related to *S. cerevisiae* to determine whether the Ty5 family has a target site bias and to characterize the nature of such sites.†

MATERIALS AND METHODS

Strains. Table 1 lists yeast strains used in this study, with the exception of YH51 (*MAT***a**, *his4-539, lys2-801, spt3-202, ura3-52*) (gift of J. Boeke, Johns Hopkins School of Medicine, Baltimore), which was used to assay replication activity.

DNA Manipulations. Southern hybridization filters containing yeast genomic DNA or yeast chromosomes that had been separated on pulsed-field gels were prepared as described (9). Filters were hybridized as in ref. 10 with DNA fragments that had been radiolabeled by random priming (Promega). Hybridization probes included either restriction fragments of the Ty5-*1* element (see Fig. 1*A*) or long terminal repeat (LTR) and type X repeat probes generated by PCR amplification (9). The LTR probes were generated from the Ty5-*1* element of *S. cerevisiae* (DVO115 = AGTAATGCTTTAGTATTG;

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Abbreviations: chr, chromosome; pol III, RNA polymerase III; LTR, long terminal repeat; ARS, autonomously replicating sequence; ACS, autonomously replicating consensus sequence.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. U19263 and U19264).

DVO116 = TAGTAAGTTTATTGGACC) or the Ty5–5 element from *Saccharomyces paradoxus* (DVO182 = GGGTAATGTTTCAGT; DVO116). The X probe was amplified with primers whose sequences were derived from the X repeat at the left telomere of chr III (DVO196 = TGCTCGAGTATATACCATCTCAAA; DVO197 = AGGTCGACTCATATCATGCTATTGG). Filters of overlapping phage λ clones of *S. cerevisiae* strain AB972 were obtained from the American Type Culture Collection. An *S. paradoxus* minilibrary was constructed in pBluescript (Stratagene) with size-selected DNA from strain NRRL Y-17217. Ligation products were introduced into *Escherichia coli* by electroporation, and desired recombinants were identified by colony hybridization using the internal probe from Ty5–1 (see Fig. 1*A*) (9). DNA sequences were determined with the fmol DNA sequencing kit (Promega), or by the Nucleic Acid Facility of Iowa State University. Sequence analysis was performed by using Genetics Computer Group computer programs (11).

Test for DNA Replication Activity. Several Ty5 elements and their flanking sequences were tested for the ability to sustain plasmid replication in *S. cerevisiae.* For Ty5–5, the 3′ flanking region was subcloned into the *URA3*-derived integration plasmid pRS406 (12) to create the test plasmid pSZ107. Plasmids identified from the minilibrary that carried the Ty5–6, Ty5–12, and Ty5–14 elements were directly modified by the addition of the *URA3* gene, resulting in test plasmids pSZ120, pSZ119, and pSZ121, respectively. Test plasmids were introduced into the *ura3 S. cerevisiae* strain YH51 by electroporation (9). Yeast cells were plated on synthetic medium without uracil, and Ura^+ colonies were counted after 48 hr at 30°C.

RESULTS

Distribution of the Ty5 Elements in *S. cerevisiae*. Eleven *S. cerevisiae* strains were analyzed by Southern hybridization to determine Ty5 copy number. Although many of these strains are designated as separate species, recent assessments of DNA divergence indicate that all are likely to be isolates of *S. cerevisiae* (Table 1 and data not shown) (13). Nine of the 11 strains carry the telomeric Ty5–1 insertion, as indicated by the presence of the 4.4-kb *Eco*RI restriction fragment that hybridizes to internal Ty5 probes (Table 1). This fragment is located on chr III (e.g., S288C, Fig. 1 *B* and *C*) and its size correlates precisely with that predicted from the chr III sequence (Fig. 1*A*). The second element that hybridizes to internal probes is located on chr XI and appears to have arisen by a telomeric duplication that included the Ty5–1 insertion and its flanking sequences. Such a duplication is evident from the chr III and chr XI nucleotide sequences (2) and is further supported by the conservation of restriction sites within and flanking the two elements. These insertions are either indistinguishable on Southern hybridization filters (e.g., A364a; Fig. 1*B*) or differ by 0.4 kb (e.g., *S. hienipiensis*; Fig. 1*B*). This polymorphism is likely due to a deletion, as it is observed regardless of the restriction endonucleases used (data not shown).

Sequences homologous to the Ty5 LTRs are more abundant than the internal Ty5 sequences (Fig. 1 *D* and *E*). The number of LTRs ranges from approximately 5 to more than 20 copies among the strains tested and likely represents solo LTRs that have lost internal sequences through recombination between the direct repeats.

***S. cerevisiae* Ty5 Elements Are Associated with Autonomously Replicating Sequences (ARSs).** To determine whether Ty5 elements have a target bias, we characterized all insertions in the strain S288C or its derivatives. S288C has Ty5 insertions on chr III, VII, VIII, and XI (Fig. 1*E*), and the nucleotide sequences for three of these chromosomes (III, VIII, and XI) have recently been completed (1, 2, 14). The chr VII insertion was identified by screening a phage library that has been used to assemble a physical map of the *S. cerevisiae* genome (15). A single phage (70640), whose insert sequence is located at the left telomere, was identified. The Ty5 insertion within this phage clone (Ty5–15) was localized within the terminal restriction fragment (500 bp) of the chr VII physical map. This terminal restriction fragment also hybridized with a probe specific for the type X telomeric repeats, which reside at the ends of all *S. cerevisiae* chromosomes (data not shown) (16, 17).

The genomic organization of the Ty5 insertions in S288C is summarized in Fig. 2. Six elements (Ty5–1, Ty5–4, Ty5–7, Ty5–8, Ty5–9, and Ty5–15) are located in close proximity to the telomeres, and five are adjacent to telomeric type X repeats. X repeats are ≈500 bp in length (17). They function as ARSs and confer to plasmids the ability to replicate extrachromosomally (16). An ACS present in X repeats is essential for ARS function (18). Of the five characterized Ty5 insertions, four are found within 500 bp of this ACS (Fig. 2*A* and Table 2). The fifth element (Ty5–9) is a half-LTR near the chr VIII telomere. This element is located among subtelomeric repeat sequences from a variety of *S. cerevisiae* chromosomes, suggesting that its flanking sequences have been scrambled through recombination (14).

Table 1. Yeast strains used in this study

Species	Strain designation	Ty5 copy no.*	Source†
Saccharomyces cerevisiae	Y-12632	0	NRRL
S. cerevisiae	A364a	2	J. Boeke
S. cerevisiae	S288C	1	J. Boeke
S. cerevisiae	SK1	1	J. Boeke
Saccharomyces chevalieri‡	Y-12633	0	NRRL
Saccharomyces italicus‡	Y-12649	2	NRRL
Saccharomyces norbensis‡	Y-12656	2	NRRL
Saccharomyces oleaceus‡	Y-12657	1	NRRL
Saccharomyces diastaticus‡	Y-2416	2	NRRL
Saccharomyces hienipiensis‡	Y-6677	2	NRRL
Saccharomyces oleaginosus‡	Y-6679	2	NRRL
Saccharomyces heterogenicus	Y-1354	0	NRRL
Saccharomyces bayanus	Y-12624	0	NRRL
Saccharomyces kluyveri	Y-12651	0	NRRL
Saccharomyces dairensis	Y-12639	0	NRRL
Saccharomyces exiguus	Y-12640	0	NRRL
Saccharomyces unisporus	Y-1556	0	NRRL
Saccharomyces servazzii	Y-12661	0	NRRL
Saccharomyces castellii	Y-12630	0	NRRL
Saccharomyces paradoxus	Y-17217	5–8	NRRL
S. paradoxus	Y-17218	1	NRRL
S. paradoxus	2980	0	CBS
S. paradoxus	7400	0	CBS
S. paradoxus	6303	0	DBVPG
S. paradoxus	6304	0	DBVPG
S. paradoxus	6489	3	DBVPG
S. paradoxus	6490	3	DBVPG
S. paradoxus	6492	3	DBVPG
Candida albicans	YDV84	0	F. Spencer
Candida utilis	F608	0	J. Boeke
Kluyveromyces lactis	F645	0	J. Boeke
Pichia rhodanensis	F612	0	J. Boeke
Saccharomycopsis lipolytica	F613	0	J. Boeke
Torulaspora delbrueckii	Y-866	0	NRRL
Zygosaccharomyces bailii	Y-2227	0	NRRL
Zygosaccharomyces rouxii	Y-229	0	NRRL

*As revealed by hybridization with internal Ty5–1 sequences.
†NRRL, Northern Regional Research Laboratory, Peoria, IL; CBS, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; DBVPG, Dipartimento di Biologia Vegetale, Universita di Perugia, Italy; J. Boeke, Johns Hopkins School of Medicine, Baltimore; F. Spencer, Johns Hopkins School of Medicine, Baltimore.
‡Original name for strain now considered synonymous with *S. cerevisiae* (13).

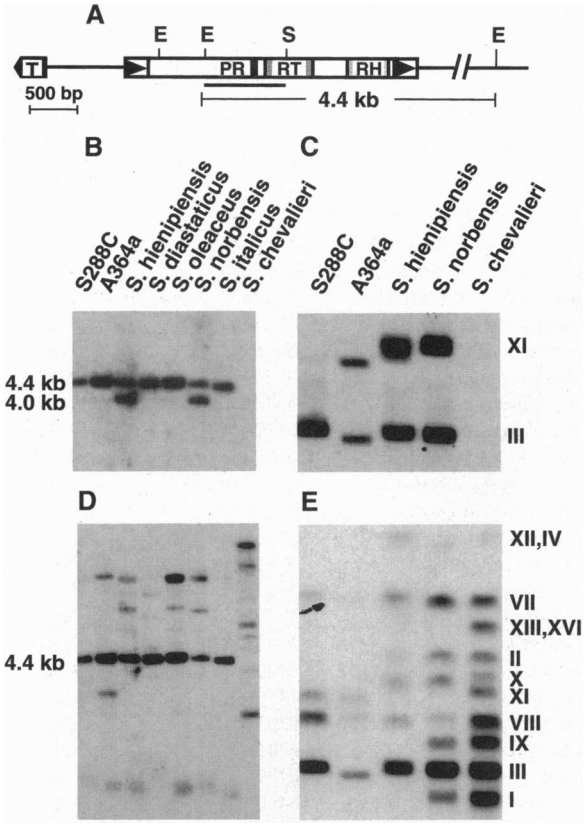

FIG. 1. Distribution of the Ty5 elements in *S. cerevisiae*. (*A*) Structural organization of Ty5–*1* at the left telomere (T) of chr III. Boxes with arrowheads indicate LTRs. Shaded boxes in the internal domain depict conserved amino acid sequences shared among retrotransposons and retroviruses (8); PR, protease; RT, reverse transcriptase; RH, RNase H. The restriction fragment used as a hybridization probe in *B* and *C* is underlined; E, *Eco*RI; S, *Sma* I. The 4.4-kb *Eco*RI fragment identified in *B* is shown below the element. (*B*) Southern hybridization analysis to determine Ty5 copy number in the listed strains whose DNA was digested with *Eco*RI. (*C*) Southern hybridization analysis of chromosomes separated by pulsed-field gel electrophoresis, with chromosome designations shown at the right. (*D* and *E*) Hybridization analyses as in *B* and *C*, except with an LTR-specific probe.

The remaining two *S. cerevisiae* Ty5 elements, Ty5–*2* and Ty5–*3*, reside at internal sites on chr III adjacent to the transcriptionally silent mating locus *HMR* (Fig. 2*A*). Transcriptional repression at *HMR* is largely determined by cis-acting sequences adjacent to this locus, designated *HMR-E* (19). An ACS is present in *HMR-E* that serves as an origin of DNA replication and is also involved in transcriptional silencing. Ty5–*2* and Ty5–*3* are found within 500 bp of this ACS (Table 2). The only example of a Ty*1* insertion on either chr III or chr XI that is not associated with a tRNA gene also resides in close proximity to *HMR-E* (Fig. 2*A*).

Ty5 Elements Are of High Copy Number in *S. paradoxus*. Ty5–*1* (or its duplicated version on chr XI) is the only *S. cerevisiae* element that carries internal coding sequences, yet this insertion has accumulated mutations and an internal deletion that likely render it incapable of further transposition (8). The search for active Ty5 elements, therefore, was extended to include other related yeast species (Table 1). Evidence of Ty5 was found only in the closely related species *S. paradoxus* (20). By using an internal element probe, Ty5 copy number was found to vary from zero to more than five in various *S. paradoxus* strains (Fig. 3*A*). When this same Southern hybridization membrane was hybridized with an LTR-specific probe (Fig. 3*B*), multiple LTR sequences were observed, suggesting that Ty5 is actively transposing in this species.

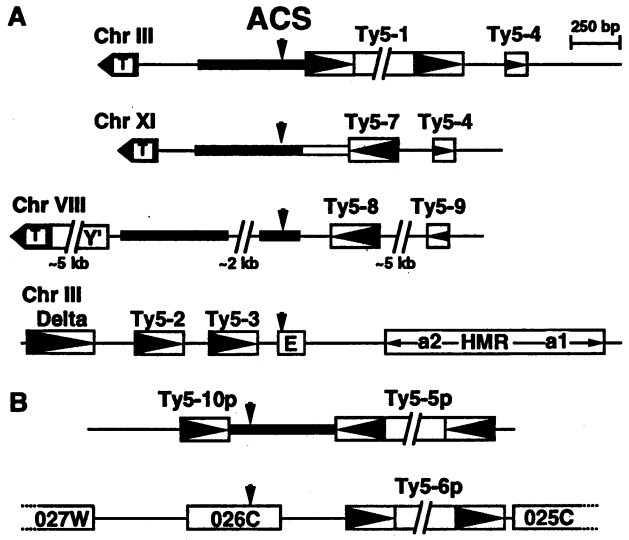

FIG. 2. Ty5 elements are associated with ARSs. (*A*) Genomic organization of the *S. cerevisiae* Ty5 retrotransposons. ACS denotes the ARS consensus sequence, T denotes the telomeric repeats (TG_{1-3}), and boxes with arrowheads indicate the Ty5 LTRs. Narrow black boxes indicate type X subtelomeric repeats. Part of the duplicated region between the chr III and chr XI telomeres is shown. The unfilled box represents sequences unique to chr XI. The chr XI Ty5 element has been designated Ty5–*7* because it is in the opposite orientation with respect to Ty5–*1*. Delta depicts a Ty*1* LTR, and Y′ is a class of subtelomeric repeats. On chr III, E depicts the transcriptional silencer adjacent to the *HMR* locus. (*B*) Genomic organization of the *S. paradoxus* Ty5 retrotransposons. Open boxes with labels indicate sequences that show similarity to *S. cerevisiae* open reading frames on the left arm of chr XI (2).

Table 2. Ty5 insertions and flanking ACS or ARS activity

Ty5 element identification	Adjacent ACS*	Distance to ACS, bp	Location/ orientation†	ARS activity‡
S. cerevisiae				
Ty5–*1*	TTTTATGTTTA	118	5′/−	ND§
Ty5–*2*	TTTTATATTTA	495	3′/−	ND¶
Ty5–*3*	TTTTATATTTA	273	3′/−	ND¶
Ty5–*4*	TTTTATGTTTA	280/426 ‖	5′/−	ND§
Ty5–*7*	ATTTATGTTTA	273	3′/+	ND§
Ty5–*8*	TTTTATATTTA	240	3′/+	ND§
Ty5–*9*	TTTTATGTTTT	3161	5′/−	ND
Ty5–*15*	ND	ND	ND	ND
S. paradoxus				
Ty5–*5p*	ATTTATGTTTT	437	3′/−	4888
Ty5–*6p*	ATTTGTATTTT	466	5′/+	815
Ty5–*10p*	ATTTATGTTTT	92	3′/+	4888
Ty5–*11p*	ND	ND	ND	ND
Ty5–*12p*	ND	<2200	ND	6213
Ty5–*13p*	ND	ND	ND	ND
Ty5–*14p*	ND	ND	ND	0

ND, not determined.
*The ACS for *S. cerevisiae* is (A/T)TTTAT(A/G)TTT(A/T).
†Refers to location and orientation of the ACS with respect to the Ty5 element. +, same orientation; −, opposite orientation.
‡Numbers represent the number of Ura^+ colonies arising from transformation with 0.5 μg of plasmid DNA.
§The closest ACS to these insertions is in the type X telomeric repeat, which has previously been shown to support plasmid replication (18).
¶The closest ACS to these insertions is in *HMR-E*, which has previously been shown both to support plasmid replication and to serve as a chromosomal origin of replication (19).
‖Refers to the position of chr III and chr XI elements, respectively.

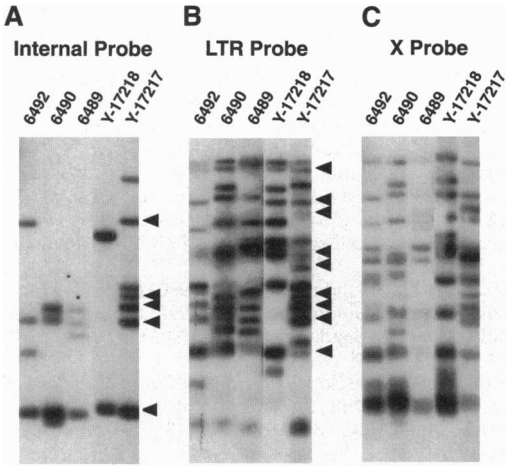

FIG. 3. Distribution of the Ty5 elements in *S. paradoxus*. Southern hybridization analysis of *S. paradoxus* strains with an internal Ty5 probe (*A*), an LTR-specific probe (*B*), or an X repeat probe (*C*). For Y-17217, Ty5 bands are noted with arrowheads if they comigrate with restriction fragments that also hybridize to X sequences. Strain designations are as in Table 1. *A*, *B*, and *C* show the same set of hybridization membranes, which have been stripped and reprobed.

***S. paradoxus* Ty5 Elements Are Associated with X Repeats.** Six different Ty5 elements with internal sequences were cloned from *S. paradoxus* strain Y-17217, which has the highest number of Ty5 insertions (Table 1). These elements are designated Ty5–*5p*, Ty5–*6p*, Ty5–*11p*, Ty5–*12p*, Ty5–*13p*, and Ty5–*14p* (the letter p is used to distinguish the *S. paradoxus* insertions from the *S. cerevisiae* insertions). The nucleotide sequence flanking Ty5–*5p* revealed a solo LTR (Ty5–*10p*) ≈600 bp from the 3′ LTR (Fig. 2*B*). The sequence between these elements shares more than 75% nucleotide identity with the X repeats found at the telomeres of *S. cerevisiae* chr XI and chr VIII (data not shown). The ACS is perfectly conserved between the X repeats of these two species, and both Ty5 elements are located within 500 bp of this ACS (see Fig. 2*B* and Table 2). Additional evidence for the association of *S. paradoxus* Ty5 elements with X repeats is provided by Southern hybridization analysis. For example, in strain Y-17217, 9 of the ≈15 restriction fragments that hybridize to LTR probes comigrate with fragments that also hybridize to the X probe (Fig. 3*C*). This degree of association is likely to be an underestimate, since the second LTR of full-length elements would not be predicted to be adjacent to X sequences.

***S. paradoxus* Ty5 Elements Are Associated with ARS Activity.** The flanking sequences of several *S. paradoxus* Ty5 elements were tested for ARS activity by determining whether they could support plasmid replication. Four *URA3*-based plasmids, each lacking a yeast origin of replication but carrying a Ty5 insertion and its flanking sequence, were introduced into a *ura3 S. cerevisiae* strain (Table 2). The plasmids that carry Ty5–*5p*, Ty5–*6p*, and Ty5–*12p* were found to transform *S. cerevisiae* with high efficiency and replicate as extrachromosomal plasmids. These same plasmids were also found to replicate in *S. paradoxus* (data not shown), suggesting that the ARS activity is conserved between the two species. In separate controls, no ARS activity was detected for the Ty5 internal domain or the Ty5 LTR, indicating that ARS activity is not due to sequences carried within the elements (data not shown). The plasmid vector alone also failed to confer a Ura^+ phenotype.

The Ty5–*6p* ARS was found to reside in a 1.6-kb fragment upstream of the 5′ LTR. Both the 5′ and 3′ flanking sequences of Ty5–*6p* share more than 80% nucleotide identity with a region in the middle of the left arm of *S. cerevisiae* chr XI (Fig. 2*B*) (2). The 1.6-kb fragment upstream of the Ty5–*6p* insertion has several A+T-rich sequences that may be responsible for ARS function, including a 10 of 11 match with the *S. cerevisiae* ACS (Table 2). This sequence is also found at the corresponding position on chr XI in *S. cerevisiae* (2).

For Ty5–*5p*, the ARS activity was localized to a 3.5-kb restriction fragment that included the flanking X repeat. A more precise location of the ARS adjacent to the Ty5–*12p* element was not determined, although the flanking sequences at either the 5′ or 3′ ends are less than 2.2 kb. Although the Ty5–*14p* element did not show any ARS activity, this element was found to have a deletion of at least its 3′ LTR and possibly flanking sequences that may have resulted in loss of an adjacent ACS (data not shown).

DISCUSSION

The genomic organization of the *S. cerevisiae* retrotransposons clearly indicates a bias for specific regions of the genome. For example, all but one of the 30 Ty*1*, Ty*2*, Ty*3*, and Ty*4* insertions on chr III and chr XI are within 500 bp of tRNA genes. In this study we have shown that the genomic organization of the Ty5 elements is strikingly different. Seven of eight Ty5 insertions present in one *S. cerevisiae* strain are located within 500 bp of origins of DNA replication associated with the telomeric X repeats or the silent mating locus *HMR*. The eighth element is also telomeric, but resides in a region of sequences that have been scrambled, likely through recombination between multiple chromosome ends (14).

The genomic organization of the *S. cerevisiae* Ty5 elements likely reflects a targeting bias. Although their structure suggests that these elements are incapable of further transposition, they are comparable in terms of degeneracy to the chr III Ty*1* elements, whose association with tRNA genes reflects the target preference of *de novo* transposition events (3). In addition, the sequences immediately flanking all but the duplicated chr III and chr XI elements show no discernible similarity, indicating that the Ty5 elements are the result of independent integration events and not reiterative duplication of a few ancient insertions.

The association of Ty5 with ARSs is also observed in the closely related species *S. paradoxus*, where the high Ty5 copy number suggests that they are transpositionally active. Two *S. paradoxus* elements are found within 500 bp of ACSs in type X repeats, consistent with Southern hybridization analysis showing that most Ty5 insertions are likely adjacent to X sequences. The *S. paradoxus* X repeats support plasmid replication as do sequences flanking two of three other *S. paradoxus* insertions, including one element that is located at an internal site on chr XI. The only characterized element in *S. paradoxus* that is not associated with ARS activity has suffered a deletion, which may have removed an ACS from its original flanking sequences.

Possible Mechanisms for Ty5 Targeting. The association of Ty*3* elements with tRNA genes reflects an interaction between transposition intermediates and the pol III transcription complex (6, 7). By analogy, the association of Ty5 with X repeats and *HMR* suggests a possible targeting mechanism. The telomeres and silent mating loci are bound in a unique chromatin structure that serves to repress the expression of adjacent genes (19). The assembly of this chromatin is directed by several well-characterized, cis-acting sequences, including sequences involved in DNA replication. For example, transcriptional repression of *HMR* is mediated by the silencer *HMR-E*, which has an origin of DNA replication and binding sites for transcription factors ABF1 and RAP1. Also contributing to transcriptional silencing are four silent information regulatory proteins that act at this locus (SIR1, SIR2, SIR3, SIR4). Telomeres of *S. cerevisiae* bind many of these same proteins; the X repeats carry an ARS and ABF1 binding site, and RAP1 binds the telomeric repeats (TG_{1-3}) at the ends of chromo-

somes. In addition, the SIR2–4 proteins play a role in the assembly of telomeric chromatin. We predict that integration of Ty5 is directed through interactions with one or more of the common proteins bound at the telomeres and *HMR*.

The Association of Ty5 with Telomeres. An intriguing aspect of the genomic organization of Ty5 is its preference for subtelomeric repeats. Reverse transcription plays a key role in telomere structure, and the enzyme telomerase is a reverse transcriptase that uses an RNA template to synthesize G-rich repeats onto the ends of most eukaryotic chromosomes (21). In *Drosophila melanogaster*, the non-LTR retrotransposons HeT and TART transpose preferentially to native and broken chromosome ends (22–26). Reiterative transposition results in tandem arrays of transposons that buffer internal genic sequences from loss through recombination or replication. These telomeric retrotransposons, therefore, appear to have assumed the role of telomerase-catalyzed repeats in protecting chromosome ends (26). In *S. cerevisiae*, a second class of subtelomeric repeats, the Y′ elements, bear similarity to mobile elements in that they vary in copy number and location among strains (27). It has recently been demonstrated that Y′ sequences can rescue *S. cerevisiae EST1* mutations, which cannot replenish telomeric (TG_{1-3}) repeats (28). Rescue occurs by recombinational amplification of Y′ elements to form tandem arrays that buffer chromosome ends in a manner similar to the *D. melanogaster* retrotransposons. Although amplification of Y′ elements is known to occur only by recombination, occasional insertion at the ends of chromosomes by transposition or reverse transcription cannot be excluded.

The association of the yeast Ty5 elements with telomeres further strengthens the link between retrotransposons, reverse transcription, and telomere structure. Although Ty5 elements do not appear to play a crucial role in the maintenance of yeast telomeres, mechanisms that target transposition to chromosome ends may be widespread among eukaryotes. The development of a *de novo* Ty5 transposition assay will make it possible to test Ty5 integration specificity directly and to dissect this targeting mechanism.

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