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Localization of Sequences Required in *cis* for Yeast Ty1 Element Transposition near the Long Terminal Repeats: Analysis of Mini-Ty1 Elements

HUA XU AND JEF D. BOEKE*

Department of Molecular Biology and Genetics, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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In order to identify and characterize sequences within Ty1 elements which are required in *cis* for transposition, a series of mini-Ty1 plasmids were constructed and tested for transposition. Mini-Ty1s are deletion mutants of the Ty1-H3 element; Ty1 gene products required for transposition are supplied in *trans* from a helper Ty1 which has intact open reading frames but lacks a 3′ long terminal repeat (LTR) and therefore cannot transpose itself. Up to 5 kilobase pairs of internal sequences of the 6-kilobase-pair-long Ty1 element can be deleted without a significant effect on transposition. The smallest mini-Ty1 element capable of transposition contains the 3′ LTR and the transcribed portion of the 5′ LTR, 285 base pairs (bp) of internal sequence 3′ to the 5′ LTR, and 23 bp of internal sequence 5′ to the 3′ LTR. We conclude that Ty1-encoded proteins can act in *trans* and that *cis*-acting sequences in Ty1-H3 are all within or near the LTRs. Further deletion of the 285-bp internal sequence adjacent to the 5′ LTR significantly reduced transposition frequency, and the mini-Ty1 RNA produced failed to be packaged into the viruslike particles efficiently. Surprisingly, several nonhomologous cellular mRNAs were also associated with viruslike particles.

Ty1 elements are a family of transposable sequences in the yeast *Saccharomyces cerevisiae*. They are similar to retroviruses in many aspects (3). Like retroviruses, they consist of a central coding region containing two open reading frames (ORFs) (*TYA* and *TYB*) corresponding to retroviral *gag* and *pol* genes and two directly repeated sequences (the long terminal repeats [LTRs]) at each terminus. The Ty1 gene products are functionally and structurally analogous to those of retroviruses (1, 20, 30). Ty1 transposes via an RNA intermediate, and viruslike particles (VLPs) have been observed in yeast cells overexpressing Ty1 gene products (5, 12, 19).

In retroviruses, *cis* elements that are important for retroviral replication have been identified and characterized (reviewed in reference 27). Retroviral *cis* elements are located near the ends of the viral genomes.

In this report, we describe a system designed to study the *cis*-acting sequences required for Ty1 transposition. Mini-Ty1 elements are constructed by replacing portions of the ORFs of a Ty1 element, Ty1-H3, with a selectable marker, the yeast *TRP1* gene (and, in some cases, the *HIS3* gene). Ty1-encoded gene products required for transposition are provided in *trans* from a helper Ty1 which is defective for transposition but has intact ORFs. We show that mini-Ty1 elements lacking functional gene products can be complemented in *trans* by the helper Ty1. The minimal sequences required for transposition have been identified by constructing and testing mini-Ty1s bearing various deletions. Our results suggest that, as is true for retroviruses, all Ty1 sequences required in *cis* for transposition are located within or near the LTRs. Also, a 120-base-pair (bp)-long region contained within the *TYA* gene is shown to be directly or indirectly involved in the efficient encapsidation of mini-Ty1 RNA into the VLPs. Finally, several cellular mRNAs have been observed to be associated with the VLPs.

* Corresponding author.

MATERIALS AND METHODS

Yeast strains and media. The *S. cerevisiae* strains used in this study are listed in Table 1. The plasmids were originally transformed into strain YH8 for transposition assays. They were later introduced into another strain (YH61) for biochemical studies because this strain background consistently gave higher yields of VLPs. Media were prepared as described elsewhere (24). 5-Fluoro-orotic acid (5-FOA) medium contained 1 mg of 5-FOA per ml (6).

Transposition assay. Quantitative assays were carried out as follows. Patches of yeast cells containing the mini-Ty plasmid to be tested were replica plated from complete synthetic medium minus uracil plus glucose (SC−Ura+GLU) to SC−Ura+GAL (where GAL is galactose) and grown for 5 days at 22°C. The cells were then suspended in water and plated at low density on SC−Ura+GLU. After 2 days at 30°C, hundreds of colonies were individually picked onto YPD plates and allowed to grow overnight at 30°C. Subsequently they were transferred by replica plating to SC containing 5-FOA for 1 to 2 days at 30°C so that only plasmid-free segregants could grow. In the final step of the procedure, the phenotypes of the segregants in regard to transposition were determined by replica plating the cells to SC−Trp+GLU. A qualitative assay for Ty1 transposition was also utilized (8). Patches of cells grown on selective glucose medium (SC−Ura+GLU) were replica plated onto SC−Ura+GAL plates to induce transposition. Following incubation for 3 days at 22°C, they were replica plated to YPD (rich) medium and allowed to grow overnight at 30°C. Then the cells were transferred to SC−Trp+GLU plates containing 5-FOA and grown for 2 days at 30°C. The host strains bearing the mini-Ty1 plasmids are *ura3 trp1*. If the mini-Ty1 tested is capable of transposition, some cells in the patch become Trp^+ independent of the existence of the plasmid and therefore can grow on the 5-FOA medium. Similarly, absence of growth indicates a defect in transposition by the mini-Ty1. Under these conditions, a transposi-

TABLE 1. *S. cerevisiae* strains

Strain	Parent[a]	Plasmid[b]
YH24	YH8	pX4 (helper-independent Gal-Ty1 marked with *TRP1*)
YH15	YH8	pX22 (maxi-Ty1 with helper) *TYB*::*TRP1* (*1703*)[c]
YH17	YH8	pX24 (mini-Ty1 with helper) Δ*1703-5562*
YH18	YH8	PX25 (mini-Ty1 with helper) Δ*818-3302*
YH19	YH8	pJEF1254 (mini-Ty1 with helper) Δ*818-5562*
YH88	YH8	pX100 (mini-Ty1 with helper) Δ*620-5562*
YH22	YH8	pX28 (mini-Ty1 with helper, transposition [−][d]) Δ*478-5562*
YH33	YH8	pX40 (mini-Ty1 with helper, transposition [−]) Δ*478-3947*
YH86	YH8	pX97 (mini-Ty1 with helper, transposition [−]) *TYA*::*TRP1* (*478*), Δ*818-5562*
YH79	YH61	pJEF1254 (mini-Ty1 with helper) Δ*818-5562*
YH91	YH61	pX100 (mini-Ty1 with helper) Δ*620-5562*
YH80	YH61	pX28 (mini-Ty1 with helper, transposition [−]) Δ*478-5562*
YH81	YH61	pX40 (mini-Ty1 with helper, transposition [−]) Δ*478-3947*
YH89	YH61	pX97 (mini-Ty1 with helper, transposition [−]) *TYA*::*TRP1* (*478*), Δ*818-5562*
YH103	YH61	pX112 (p*GAL-TRP1* with helper) (no Ty1 sequences)
YH129	YH61	pX136 (mini-Ty1 with helper, *HIS3* marked) Δ*818-5562*
YH130	YH61	pX135 (mini-Ty1 with helper, *HIS3* marked, transposition [−]) Δ*478-5562*
YH131	YH61	pX132 (p*GAL-HIS3* with helper) (no Ty1 sequences)
YH136	YH61	pX110 (p*GAL-TRP1* without helper) (no Ty1 sequences)
YH137	YH61	pX129 (p*GAL-HIS3* without helper) (no Ty1 sequences)
JB584	JB503	pJEF1202 (mini-Ty1 without helper) Δ*818-5562*
JB590	JB503	pJEF1254 (mini-Ty1 with helper) Δ*818-5562*
YH59	JB198	pJEF1254 and pAB100 (target for transposition)

[a] The genotype of YH8 is α *ura3-167 his3Δ200 leu2Δ1 trp1Δ1*. It was derived from GRF167 (5) by integration and excision of plasmids YRp14-*trp1Δ1* and YRp15-*leu2Δ1* (25). The genotype of YH61 is **a** *ura3-52 his4-539 lys2-801 trp1Δ63*. It was derived from strain CH332 of Connie Holm by integration and excision of plasmids pAA1 (making it *GAL2* [A. Antebi and G. R. Fink, personal communication]) and YRp14-*trp1Δ63* (25). The genotype of JB503 is **a** *ura3-52 his3Δ200 lys2 trp1Δ1*. The *trp1Δ1* mutation was introduced into the parent strain JB183 (5) as described above for YH8. The genotype of JB198 is **a** *ura3-52 his3Δ200 lys2 trp1-289 spt3-101* (7). All of the strains are Gal$^+$.

[b] Maps of the mini-Ty1 plasmids are shown in Fig. 1. Insertions into Ty1 sequences are indicated by ::; deletions are indicated by Δ; all numbers refer to the sequence of Ty1-H3 (4). The mini-Ty1s are marked with the *TRP1* gene unless otherwise indicated.

[c] Numbers in parentheses indicate positions at which *TRP1* is inserted when it interrupts *TYA* or *TYB*.

[d] Indicates that the mini-Ty1 element is defective in transposition.

tion frequency of less than 0.5% would be scored as negative. In the case of *HIS3*-marked mini-Ty1 elements, SC−His plates were used in place of SC−Trp plates. In each transposition assay, at least two independent and nonintegrant transformants of the same mini-Ty1 plasmid were tested.

Construction of mini-Ty1 plasmids. A helper-Ty1 plasmid, pJEF938, was derived from pGTy1-H3 (5) as follows. The restriction fragment extending from the *Bgl*II site at position 5562 to the *Bam*HI site in flanking DNA was removed; the remaining DNA was filled in and ligated to a synthetic *lacO* *Eco*RI (filled-in) fragment (5). An *Mlu*I linker was inserted into the unique *Sma*I site in pJEF938 to give pX6. As the final result, the 3′ LTR (the natural Ty1 transcriptional terminator) was removed and pBR322 sequences were fused to the 3′ region of the helper Ty1 element.

The structures of the final mini-Ty1 plasmids are shown in Fig. 1. Each construction was done by a two-step procedure. First, mutations (insertion of the *TRP1* gene combined, in most cases, with a deletion) were made within the Ty1-H3 element present on a pGAL-Ty1 plasmid by utilizing preexisting restriction sites. Second, the resulting mini-Ty1 element was cloned into a unique site in one of the helper-Ty1 plasmids described above. All mini-Ty1 plasmids studied carried the mini-Ty1 and the helper Ty1 in opposite orientations. Thus, any homologous recombination events occurring between the two Ty1s in the plasmids cannot result in deletion formation, which would give a high background of Ura$^-$ Trp$^+$ cells in transposition assays.

Two *HIS3*-marked mini-Ty1 plasmids were constructed similarly. The *HIS3* fragment used includes sequences starting at position −170 from the transcription initiation site to about 20 bp after the stop codon (kindly provided by D. Garfinkel) (13). For the constructs which contain the *HIS3* structural gene driven by the *GAL1* promoter, the sequence 5′ to position −7 of the *HIS3* gene (26; J. Li and P. Hieter, personal communications) has been replaced by the *GAL1* promoter.

Other techniques. DNA sequencing of double-stranded plasmid was carried out as described previously (14). For VLP purification, yeast cells were directly transferred from selective plates to 100 ml of liquid SC−Ura containing 1% raffinose as the carbon source at a density of 10^7 cells per ml. After the cells had grown for 3 h at 30°C, galactose was added to the culture at a final concentration of 2% and the cells were allowed to grow for another 14 h at 25°C. VLPs were isolated from the culture as described elsewhere, using sucrose step gradients (9). To determine whether the RNA from a specific construct could be packaged into the VLPs, RNAs from the total cell extract and purified VLPs were isolated and subjected to Northern (RNA) blot analyses (28). Hybridization probes used were ^{32}P-labeled *TRP1* and *HIS3* DNAs, the 1.1-kilobase-pair (kb) *Bam*HI-*Ava*I pBR322 fragment, and part of the yeast actin gene (from position −40 to 1083), all made by the random hexamer labeling method (10). The *TRP1* and *HIS3* probes hybridize only to the mini-Ty1 RNAs, whereas the pBR322 probe specifically detects the helper Ty1 RNAs. Quantitation of the Northern blots was accomplished by cutting out the bands from the filters and counting the radioactivity (*C*) in a scintillation counter. The level of the mini-Ty1 RNA in the total cell extract was normalized to the amount of helper-Ty1 RNA. The level of mini-Ty1 RNA in the VLPs was normalized to the amount of reverse transcriptase activity, determined by using exogenous oligo(dG)-poly(rC) as the primer and template (12). Normalization to the amount of TYA proteins gave similar

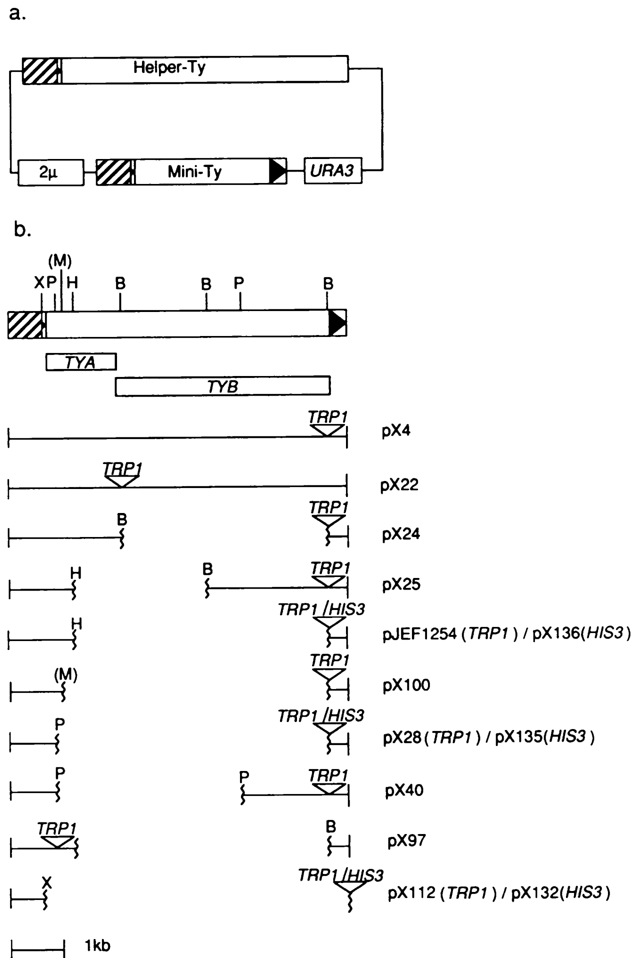

FIG. 1. Structures of the mini-Ty1 plasmids. Ty1-H3 sequences (▭) and LTRs (▶) are indicated. The U3 portion of the 5′ LTR (the Ty1 promoter) has been removed; the *GAL1* promoter (▨) is shown. 2μ is a piece of the yeast 2μm plasmid containing the replication origin. pBR322 sequences (—) are indicated. Restriction sites are abbreviated as follows: B, *Bgl*II; H, *Hpa*I; M, *Mlu*I; P, *Pvu*II; X, *Xho*I. The marker gene (*TRP1* or *HIS3*) in all the constructs has the same orientation as the mutant Ty1 elements. (a) Overall structure of the plasmids shown schematically. Notice that the 3′ LTR has been deleted from the helper-Ty1 element. As a result, part of the pBR322 sequences is fused to it. (b) Mutations made within the mini-Ty1 part of the plasmids. The endpoints of each deletion are indicated (⌇). The sites of *TRP1* (or the *HIS3* gene) insertions are shown (▽). The *TRP1* gene used in all constructs except pX112 lacked its own transcriptional terminator and is in the same orientation with respect to Ty1 for all constructs tested. The *HIS3* gene in pX136 and pX135 has about 20 bp of 3′-flanking sequences left, and the *HIS3* gene in pX132 lacks its own promoter. An *Mlu*I site at position 620 created by linker insertion mutagenesis (J. D. Boeke and G. M. Monokian, unpublished data) was utilized to construct pX100. This *Mlu*I site is not present in any of the mini-Ty1 elements. *TYA* and *TYB* refer to the two ORFs of the Ty1 element. Ty1 sequences include nucleotides 239 to 5919. The positions of the restriction sites utilized to make the mutations (left to right) are as follows: X, 239; P, 478; M, 620; H, 818; B, 1703; B, 3302; P, 3947; B, 5562. Plasmid pX4 contains no helper-Ty1 element (28).

results. The packaging efficiency (P) is defined as the ratio of the amount of the mini-Ty1 RNA in the VLPs versus that in the corresponding total RNA after normalization (i.e., C_{VLP}/C_{Tot}). The packaging efficiency of the mini-Ty1 in strain YH79(pJEF1254) was arbitrarily assigned the value 100%, and the numbers for other strains were adjusted accordingly [hence the item $(1/P_{79}) \times 100$ in the formula]. The packaging efficiency of a given mini-Ty1 RNA into VLPs was calculated according to the formula $[(C_{VLP}/C_{Tot})/P_{79}] \times 100$, where $C_{Tot} = C_{Trp} \times (C_{pBR.79}/C_{pBR})$ and $P_{79} = C_{VLP}/C_{tot}$ for strain YH79(pJEF1254). The specific parameters are defined as follows. C_{VLP} is the radioactivity (counts per minute) of the *TRP1* probe from the band excised from the VLP lane; C_{Trp} and C_{pBR} are radioactivity (counts per minute) from the bands excised from the total RNA lane using the *TRP1* and pBR322 probes, respectively; $C_{pBR.79} = C_{pBR}$ for strain YH79(pJEF1254). The packaging efficiencies for *HIS3*-marked mini-Ty1s were determined in the same way except that a *HIS3* rather than a *TRP1* probe was used.

RESULTS

Mini-Ty1 elements and assays for their transposition. In order to identify the sequences required in *cis* for Ty1 transposition, deletion mutants of Ty1-H3, i.e., mini-Ty1 elements, were constructed in a vector which contains the *URA3* selectable marker and the 2μm replication origin. Their structures are shown in Fig. 1. To facilitate the transposition assays, a second selectable marker, the yeast *TRP1* gene, lacking its transcriptional terminator (8) was inserted into the mini-Ty1 elements in the same orientation. Because mini-Ty1s do not encode all the Ty1 proteins, a helper Ty1 which lacks the 3′ LTR but has complete ORFs was also included in each construct. As a control, a maxi-Ty1 element was made in the event that all Ty1 sequences were necessary for transposition. The maxi-Ty1 contains no deletion but instead contains as insertion of the *TRP1* gene in *TYB*. The transcription of both mini- and helper-Ty1 elements is controlled by the *GAL1* promoter. To induce transposition, strains containing these plasmids were grown on selective galactose medium at 22°C for 5 days. Following induction, the plasmids were segregated from the cells and their phenotypes were assayed. If transposition occurred, the cell should have the *TRP1* gene in the genome and thus remain Trp^+ even in the absence of the plasmid. Therefore, by scoring the fraction of cells which become Ura^- Trp^+ following galactose induction and plasmid segregation, one can conveniently determine the frequency of transposition.

Quantitative transposition analyses of the mini-Ty1s. The results of transposition assays of the mini-Ty1 elements are summarized in Table 2. The smallest mini-Ty1 element (pX100) constructed which could still transpose contains only 285 bp of internal Ty1 sequences. Further deletions (pX28 and pX40) extending to the 5′ *Pvu*II site, which is about 120 bp downstream from the 5′ LTR, abolished transposition. This result was confirmed by using *HIS3*-marked mini-Ty1s of similar structures. As insertion at this *Pvu*II site (pX97) also reduced transposition frequency by at least 10-fold. All mini-Ty1s capable of transposition transposed at similar frequencies (approximately 2 to 6%), a rate that is about 10-fold lower than that seen for a similarly marked helper-independent Ty1 element (pX4) (28). One possible explanation is that some sequences important for high-frequency transposition had been deleted even in the largest mini-Ty1. However, a maxi-Ty (pX22) which has no deletion but instead bears an insertion of the *TRP1* gene

TABLE 2. Transposition and RNA packaging of mutant Ty1 elements

Mini-Ty1 plasmid	Transposition frequency[a]	Phenotype[b]	Packaging efficiency (%)[c]
Group 1 (*TRP1*-marked)			
pX4	42.9 (121/282)	++	
pX22	3.9 (8/205)	+	
pX24	2.4 (9/369)	+	
pX25	1.5 (5/336)	+	
pJEF1254	6.2 (13/211)	+	100
pX100	4.5 (12/269)	+	81
pX28	0.19 (1/537)	−	14
pX40	<0.27 (0/372)	−	14
pX97	0.36 (2/556)	−	43
pX112	NA	NA	45
Group 2 (*HIS3*-marked)			
pX136	ND	+	100
pX135	ND	−	23
pX132	NA	NA	141

[a] Defined as the percentage of independent colonies which become Trp^+ in the absence of the plasmid after induction of transposition on galactose medium. Transposition frequency measurements were made in host strain YH8. ND, Not determined; NA, not applicable.

[b] The phenotypes listed here refer to the results of the qualitative transposition assays as described in Materials and Methods. ++, Very strong growth on 5-FOA−Trp plates.

[c] These numbers are derived from quantitation of the Northern blots similar to those shown in Fig. 4. Packaging efficiency is the ratio of the counts per minute of the *TRP1* (or *HIS3*) probe in the VLP RNA versus those in the total RNA band after normalization to the amount of the helper-Ty1 RNA. The packaging efficiency of the mini-Ty1 RNA from pJEF1254 (for *TRP1*-marked mini-Ty1s) or pX136 (for *HIS3*-marked mini-Ty1s) is arbitarily assigned the value 100% (which represents recovery of 10 to 15% of the total cellular mini-Ty1 RNA in the VLP fraction if one assumes that the recovery throughout the experiment is 100%), and the packaging efficiencies of other mini-Ty1 RNAs are expressed as the percentage of that of pJEF1254 or pX136. RNA packaging measurements were made in host strain YH61.

inside the *TYB* ORF also transposed at a frequency very close to that of the mini-Ty1 elements. Therefore, it appears that there are no particular sequences whose loss could account for the relatively low transposition frequency of the mini-Ty1 elements; rather, the overall structure of the mini-Ty1 family of plasmids may be responsible.

Structural analysis of transposed mini-Ty1. When a wild-type Ty1 element transposes, it generates 5-bp duplications of the target sequences. Homologous recombination or gene conversion between episomal mini-Ty1s and genomic Ty1 elements could also result in the integration of the *TRP1* gene; it was, therefore, important to demonstrate directly the ability of the mini-Ty1s to transpose. Since transposition of Ty1 elements into a centromeric plasmid harboring a promoterless *HIS3* gene (*his3Δ4*) can activate the expression of the *HIS3* message and therefore revert the His^- phenotype of the cells (5, 7), individual transposition events can be recovered and studied in detail. Two such transposition events into plasmid pAB100 by the mini-Ty1 in pJEF1254 were isolated from a *spt3* strain, in which genomic Ty1s are not transcribed normally and, consequently, do not transpose (7). Restriction analyses of the recovered plasmids showed that each contained a mini-Ty1 element just 5′ to the *his3Δ4* gene, inserted in DNA that has no homology to Ty1 sequences. The orientation of these mini-Ty1 elements with respect to *HIS3* sequences is the same as that observed when full-length Ty1 elements activate *his3* expression; i.e., they are transcribed in the opposite direction of the *HIS3* message (5, 23). Furthermore, sequence analysis of the DNA

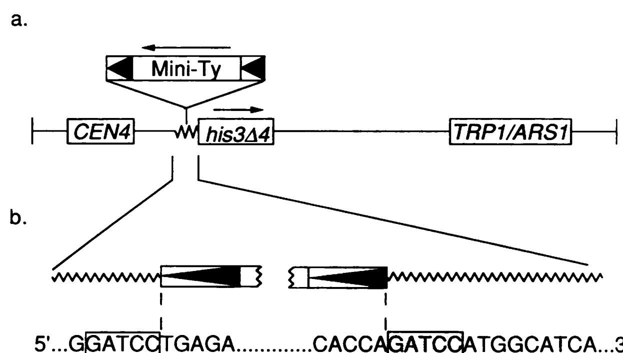

FIG. 2. A 5-bp duplication in the target sequences resulting from transposition of a mini-Ty1 element into a nonhomologous plasmid. (a) Two independent transposition events by the mini-Ty1 in pJEF1254 into target plasmid pAB100 were recovered and mapped by restriction analyses. Their structures are shown schematically here. *CEN4* is the centromere segment from chromosome 4; *TRP1* is the yeast *TRP1* gene; *ARS1* is a yeast autonomous replicator; *his3Δ4* is the yeast *HIS3* gene without its promoter. The Ty1 LTRs are indicated (◀). pBR322 sequences (—) and sequences from phage λ where both mini-Ty1 elements integrated (∿∿∿) are shown. Arrows show the direction of transcription. (b) Sequences at the junction between the mini-Ty1 element and the target DNA. The 5 bp of λ DNA which were duplicated are shaded.

flanking the LTRs in one of these plasmids revealed the 5-bp duplication of the target sequence characteristic of transposition by wild-type Ty1s (Fig. 2). This shows that mini-Ty1s are capable of de novo transposition.

Transposition of mini-Ty1 elements requires a helper Ty1. There are about 25 copies of Ty1 elements per haploid yeast genome. Thus it is possible that the mini-Ty1 elements could be complemented by the genomic Ty1s rather than the helper Ty1. To test this hypothesis, two plasmids containing identical mini-Ty1 elements but with or without a helper Ty1 were constructed and tested for transposition by the qualitative assay. The mini-Ty1 failed to transpose in the absence of the helper (Fig. 3). Therefore, the Ty1 proteins used for mini-Ty1 transposition are supplied mainly by the helper, not by the genomic Ty1 elements.

Deletion of a short sequence near the 5′ LTR reduces the amount of mini-Ty1 RNAs found within the VLPs and abolishes transposition. The mini-Ty1 element shown in pX28 (Fig. 1) has a defect in transposition, as assayed both qualitatively and quantitatively, though it is only 142 bp shorter than the smallest mini-Ty1 (pX100) which is capable of transposition (Table 2). A second mini-Ty1 element (pX40), which is larger than several transposition-proficient mini-Ty1s but still contains the upstream deletion in pX28 (positions 478 to 600 of Ty1-H3), also failed to transpose. Insertion of *TRP1* at the *Pvu*II site at position 478 (pX97) also resulted in a marked decrease in transposition. When RNAs isolated from total cell extracts and from VLPs were analyzed by Northern hybridization, it was found that the RNA molecules from the transposition-defective mini-Ty1s in pX28 and pX40 were underrepresented in the VLPs, though the insertion mutant (pX97) seemed to be only slightly defective for RNA packaging (Fig. 4 and Table 2). Similar results were obtained with mini-Ty1 RNAs from plasmids pX135 and pX136, which are similar to the critical *TRP1*-marked mini-Ty1s but carry instead the *HIS3* marker (Table 2). It is unlikely that the decreased levels of defective mini-Ty1 RNAs in the VLPs are due to RNA degradation occurring during VLP purification and RNA extraction,

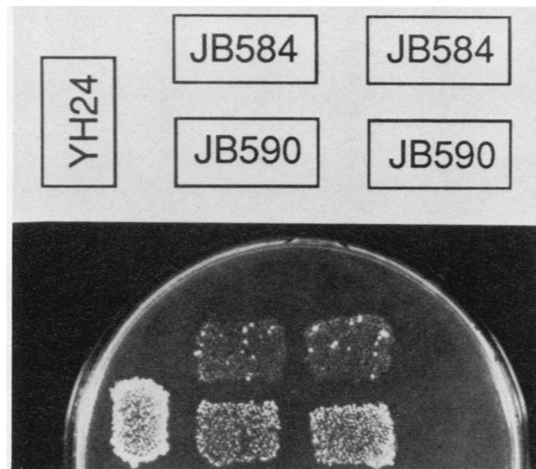

FIG. 3. Helper-dependent transposition of the mini-Ty1 elements. Qualitative transposition was performed on strains JB584 and JB590 as described in Materials and Methods. Absence of growth indicates a defect in transposition by the mini-Ty1 element. Each strain includes two independent transformants of the same plasmid. The mini-Ty1 elements in these two plasmids are identical. The plasmid in JB584(pJEF1202) does not contain the helper-Ty1 element, while the plasmid in JB590 does. The arrangement of the strains on the SC−Trp+5-FOA plate is shown in the diagram above the picture. Strain YH24(pX4) was used as the positive control for transposition.

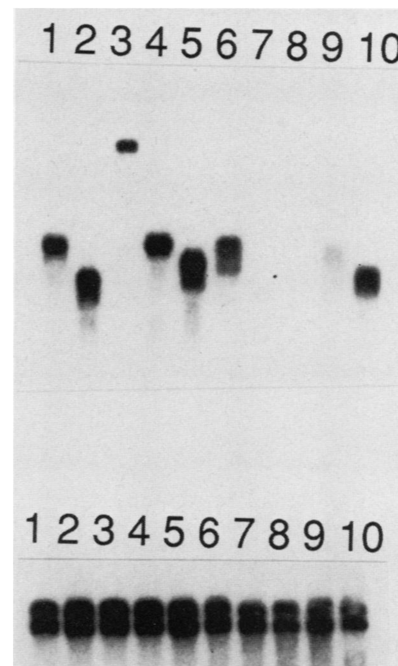

FIG. 4. Inefficient packaging of the mini-Ty1 RNAs caused by mutations in the sequences near the 5′ LTR. RNAs isolated from total cell extracts and VLPs were subjected to Northern analyses as described in the text. The blot was first probed with ^{32}P-labeled *TRP1* probe which hybridized to the mini-Ty1 RNAs (top). Then the blot was boiled in water and reprobed with ^{32}P-labeled pBR322 probe (bottom). pBR322 probe hybridized to two bands which correspond to helper messages whose sizes suggest that they end at the two transcriptional terminators 5′ and 3′ to the *URA3* gene (29) since the Ty1 transcriptional terminator (31) has been deleted from the helper-Ty1 element. Lanes 1 to 5, total RNAs, 10 μg each; lanes 6 to 10, VLP RNAs, each containing the same amounts of reverse transcriptase activity and TYA proteins. Lanes 1 and 6, YH79 (pJEF1254); lanes 2 and 7, YH80(pX28); lanes 3 and 8, YH81(pX40); lanes 4 and 9, YH89(pX97); lanes 5 and 10, YH91(pX100).

since the much larger helper-Ty1 RNAs were still largely intact in the same samples. Moreover, mixing experiments in which RNA was extracted from VLPs isolated from an equal-amount mixture of yeast cells containing transposition-proficient and -deficient mini-Ty1 plasmids (pJEF1254 and pX28, for example) produced the same results (data not shown). The small amount of the mini-Ty1 RNAs found in VLPs was indeed reverse transcribed into DNA and could be detected by Southern blots. Figure 5 shows the result of a Southern blot on the VLP DNAs for the mini-Ty1s in pX97 and pJEF1254 as an example of such analyses. There are multiple bands smaller than the size expected (about 1.9 kb) for the linear, full-length, double-stranded mini-Ty1 DNA. This heterogeneity is probably due to the relatively slow kinetics of VLP production in strains containing mini-Ty1 plasmids. These species may be the immature reverse transcripts of the mini-Ty1 RNAs and probably are only partially double-stranded. The results for the other two defective mini-Ty1 elements (pX28 and pX40) are similar, although their reverse transcripts were much less abundant.

Copurification of certain non-Ty1 mRNAs with the Ty1 VLPs. We next tested whether the sequences identified by the deletion analyses were necessary and sufficient for targeting a non-Ty1 mRNA into the VLPs. As a result of these studies, we were surprised to find that *TRP1* RNA with its natural transcriptional terminator, transcribed from the *GAL1* promoter (pX112), was present in VLP fractions (Fig. 6 and Table 2). It is unlikely that the *TRP1* message found in the VLPs was due to general contamination of the VLP preparations by cellular messages since actin RNA, *GAL1* RNA, and pyruvate kinase RNA, all abundant messages, were not present in significant amounts in the same VLP preparation (Fig. 6 and data not shown). On the basis of these findings, we decided to construct the set of mini-Ty1 elements bearing *HIS3* as the selectable marker (see above). Remarkably, *HIS3* RNA (expressed from the *GAL1* promoter) was present in the VLPs at an even higher level than the *HIS3*-marked mini-Ty1 capable of transposition (pX132, Table 2). To rule out the possibility that the *TRP1* and *HIS3* RNAs were associated with the VLP fractions merely because they interact with some factor(s) unrelated to Ty1, we repeated these experiments, using strains which carry p*GAL1-TRP1* and p*GAL1-HIS3* constructs with and without the helper-Ty1 element. In this experiment, the amount of RNA loaded was normalized to the optical density at 280 nm of the VLP fractions. The results showed that in the absence of the helper Ty1, the amounts of *TRP1* and *HIS3* RNAs in the VLP-containing fractions were greatly diminished, as would be expected because of the reduced amount of VLPs (data not shown). This suggests that the *TRP1* and *HIS3* messages are at least associated with, if not packaged into, the VLPs. In another experiment, we also found the presence of native *RP51* RNA in the VLPs.

DISCUSSION

In this article, we report deletion analyses of Ty1 elements in regard to transposition. We demonstrate directly that the Ty1-encoded proteins can act in *trans* to complement defective Ty1 elements which lack both ORFs. The results show that all *cis* elements necessary for Ty1 transposition, with

FIG. 5. Southern analysis of nucleic acids isolated from VLPs, showing that the RNAs from a mini-Ty1 element defective in transposition are reverse transcribed. Nucleic acids isolated from the VLP preparations containing the same amounts of reverse transcriptase activities were treated with 200 μg of RNase A per ml at 37°C for 5 min. Then sodium dodecyl sulfate was added to a final concentration of 1% and the samples were heated at 65°C for 5 min before being loaded on an agarose gel for electrophoresis. Southern blot was carried out as described previously (17). The probe was ^{32}P-labeled *TRP1* DNA. Lane 1, YH79(pJEF1254); lane 2, YH89(pX97). The sizes indicated at the right were calculated from DNA standards (λ DNA, *Hin*dIII digest) run on the same gel.

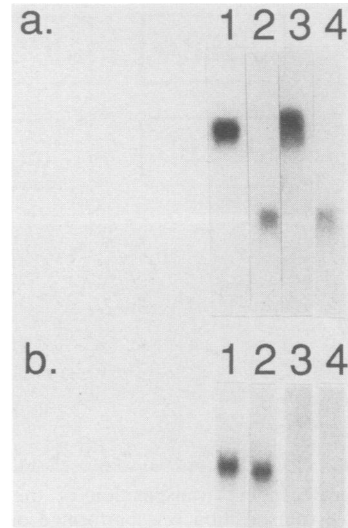

FIG. 6. Packaging of the *TRP1* mRNA into VLPs. RNAs isolated from total cell extracts and VLPs were subjected to Northern analyses. (a) The blot was probed with ^{32}P-labeled *TRP1* DNA which picked up the *TRP1* mRNA and the mini-Ty1 RNA marked with the *TRP1* gene. (b) The blot was reprobed with the ^{32}P-labeled actin probe after removal of the *TRP1* probe. Lanes 1 and 2, total RNAs from strains YH79(pJEF1254) and YH103(pX112), respectively, 10 μg each; lanes 3 and 4, RNAs from equal amounts of VLPs purified from strains YH79 and YH103, respectively.

the possible exception of a packaging signal, are within or near (less than 285 bp away from) the LTR sequences. These signals include the previously identified or proposed important *cis* elements, such as primer binding sites and the U3, U5, and R regions of the LTRs (3).

Our studies uncovered a region necessary for transposition which is 142 bp long and located just downstream from the 5′ LTR. When this segment was deleted, the amount of the mini-Ty1 RNA associated with VLPs was decreased. A simple explanation for this result is that these sequences form part of a packaging signal which targets the Ty1 RNA into the VLPs. However, the situation is clearly more complicated than this, because the constructs we have made include marker segments which are themselves (surprisingly) relatively efficiently packaged into VLPs. Nevertheless, in the context of terminal LTR sequences, these same marker sequences are poorly packaged. Inclusion of the 142-nucleotide segment allows efficient packaging; hence, it appears that this segment serves to reverse the inhibitory effect on packaging of placement of LTR sequences adjacent to the *HIS3* or *TRP1* sequences. Another possible role for this region is that it is involved in the putative dimerization of Ty1 RNAs (7); the mini-Ty1 RNAs may be packaged passively by virtue of heterodimerization with helper or genomically derived Ty1 RNAs. Moreover, since mini-Ty1 elements which contain mutations in this region have a more severe phenotype in transposition than in RNA packaging, it is possible that this region may in addition have other functions. The residual packaging activity seen in the mutants may not represent functional, productive encapsidation of the mini-Ty1 RNA; i.e., these VLPs may not have the correct structure to carry out all the processes necessary for transposition. Compared with certain retroviral packaging mutants, the deletions in pX28 and pX40 retain relatively efficient packaging (18, 21). Perhaps part of the encapsidation signal still remains in these mutants, or maybe the mRNAs of the marker genes (*TRP1* and *HIS3*) act as pseudo signals, since they are packaged quite well into VLPs by themselves. This problem will only be resolved when a suitable marker gene whose mRNA is not associated with the VLPs and which does not interfere with transposition is found. The discrepancy between defects in transposition and RNA packaging could also be partly due to the fact that transposition assays did not take into the account the differential stabilities of the mini-Ty1 RNAs, whereas the measurements of the packaging efficiency did.

A surprising finding from our studies is that at least several cellular non-Ty1 mRNAs, such as the *TRP1* and *HIS3* messages, are associated with the VLPs. At present, the functional significance of this phenomenon is unknown. On the one hand, the Ty1 VLPs do not seem to be very specific at selecting the RNAs to be packaged, since these RNAs do not share any obvious sequence homology or common structural motif. On the other hand, not all cellular messages are present in the VLPs, suggesting that the packaging process is not completely random. In some retroviral systems, specific cellular mRNAs have been shown to be packaged in virions presumably by virture of the weak or pseudo packaging signals present in these mRNAs (2, 15). To the best of our knowledge, however, there is no evidence that a given retrovirus can package several different types of cellular messages which are apparently unrelated. In a

theory previously proposed by G. R. Fink (11), it was suggested that the relatively few introns in the *S. cerevisiae* genome and their positions being near the 5′ termini indicated that most, if not all, of the *S. cerevisiae* genes might be pseudogenes derived by reverse transcription of cellular messages and subsequent homologous recombination between the cDNAs and their homologs in the genome. Our results, showing that significant amounts of three of six cellular mRNAs examined are found in the VLPs, are relevant to this theory. Those messages which are not found within Ty1 VLPs may be packaged by other families of Ty elements.

Although many of the mini-Ty1s constructed are capable of transposition, they do not transpose at a frequency nearly as high as that of a wild-type helper-independent Ty1 marked with the *TRP1* gene. This could be due to the facts that each mini-Ty1 plasmid contains two copies of the *GAL1* promoter and that there are many copies of the 2μm plasmid per cell. The *GAL1* promoters might simply titrate out some activator(s) such as the GAL4 protein. Consequently, there would be less transcription from both the helper Ty1 and the mini-Ty1s compared with the transcription from the normal (helper-independent) Ty1 fused to the *GAL1* promoter. Thus, the amounts of both Ty1-encoded proteins (derived from the helper mRNA) and transposition-proficient template (mini-Ty1 RNA) could be significantly reduced. Moreover, since helper-Ty1 RNA is packaged into VLPs and may be copackaged with mini-Ty1 RNA, there may be further inhibition of mini-Ty1 transposition if priming of negative-strand reverse transcription requires an intermolecular transfer of sequence information, as is true in at least some retroviral systems (22). Alternatively, complementation between the mini- and the helper-Ty1 elements might not be very efficient; i.e., the proteins could be somewhat *cis* acting and preferentially form particles around the RNA molecules from which they were derived. Third, it is possible that the site in the Ty1 element which was used to construct the maxi-Ty1 happens to be located in a *cis*-acting region which is important for high-frequency transposition. The mini-Ty1s all have deletions including or ending at this position. As a result, the present study may have missed such a sequence. Further work will be needed to distinguish between the above possibilities.

The mini-Ty1 system may be useful for introducing large foreign genes into the yeast genome. Non-Ty1 sequences of up to about 2 kb can be transposed efficiently when inserted into a Ty1 element at a site outside the Ty1 ORFs. However, larger inserts do seem to interfere with transposition (16, 28). The size of the smallest transposable mini-Ty1 is only 1.2 kb. Therefore, theoretically at least 6.5 kb of marker sequences could be inserted into the mini-Ty1 without a deleterious effect on its transposition.

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LITERATURE CITED

1. **Adams, S. E., K. M. Dawson, K. Gull, S. M. Kingsman, and A. J. Kingsman.** 1987. The expression of hybrid HIV:Ty virus-like particles in yeast. Nature (London) **329**:68–70.
2. **Adkins, B., and T. Hunter.** 1981. Identification of a packaged cellular mRNA in virions of Rous sarcoma virus. J. Virol. **39**:471–480.
3. **Boeke, J. D.** 1989. Transposable elements in *Saccharomyces cerevisiae*, p. 335–374. *In* D. Berg and M. Howe (ed.), Mobile DNA. American Society for Microbiology, Washington, D.C.
4. **Boeke, J. D., D. Eichinger, D. Castrillon, and G. R. Fink.** 1988. The *Saccharomyces cerevisiae* genome contains functional and nonfunctional copies of transposon Ty1. Mol. Cell. Biol. **8**: 1432–1442.
5. **Boeke, J. D., D. J. Garfinkel, C. A. Styles, and G. R. Fink.** 1985. Ty elements transpose through an RNA intermediate. Cell **40**:491–500.
6. **Boeke, J. D., F. Lacroute, and G. R. Fink.** 1984. A positive selection for mutants lacking orotidine 5′-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. Mol. Gen. Genet. **197**:345–346.
7. **Boeke, J. D., C. A. Styles, and G. R. Fink.** 1986. *Saccharomyces cerevisiae SPT3* gene is required for transposition and transpositional recombination of chromosomal Ty elements. Mol. Cell. Biol. **6**:3575–3581.
8. **Boeke, J. D., H. Xu, and G. R. Fink.** 1988. A general method for the chromosomal amplification of genes in yeast. Science **239**: 280–282.
9. **Eichinger, D. J., and J. D. Boeke.** 1988. The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: cell-free Ty1 transposition. Cell **54**:955–966.
10. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **137**:266–267.
11. **Fink, G. R.** 1987. Pseudogenes in yeast? Cell **49**:5–6.
12. **Garfinkel, D. J., J. D. Boeke, and G. R. Fink.** 1985. Ty element transposition: reverse transcriptase and virus-like particles. Cell **42**:507–517.
13. **Garfinkel, D. J., M. F. Mastrangelo, N. J. Sanders, B. K. Shafer, and J. N. Strathern.** 1988. Transposon tagging using Ty elements in yeast. Genetics **120**:95–108.
14. **Huibregtse, J. M., and D. R. Engelke.** 1986. Direct identification of small sequence changes in chromosomal DNA. Gene **44**: 151–158.
15. **Ikawa, Y., J. Ross, and P. Leder.** 1974. An association between globin messenger RNA and 60S RNA derived from Friend leukemia virus. Proc. Natl. Acad. Sci. USA **71**:1154–1158.
16. **Jacobs, E., M. Dewerchin, and J. D. Boeke.** 1988. Retrovirus-like vectors for *Saccharomyces cerevisiae:* integration of foreign genes controlled by efficient promoters into yeast chromosomal DNA. Gene **67**:259–269.
17. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Mann, R., R. C. Mulligan, and D. Baltimore.** 1983. Construction of a retrovirus packaging mutant and its use to produce helper-free defective retrovirus. Cell **33**:153–159.
19. **Mellor, J., M. H. Malim, K. Gull, M. F. Tuite, S. McCready, T. Dibbayawan, S. M. Kingsman, and A. J. Kingsman.** 1985. Reverse transcriptase activity and Ty RNA are associated with virus-like particles in yeast. Nature (London) **318**:583–586.
20. **Müller, F., K.-H. Brühl, K. Freidel, K. V. Kowallik, and M. Ciriacy.** 1987. Processing of Ty1 proteins and formation of Ty1 virus-like particles in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **207**:421–429.
21. **Murphy, J. E., and S. P. Goff.** 1989. Construction and analysis of deletion mutations in the U5 region of Moloney murine leukemia virus: effects on RNA packaging and reverse transcriptions. J. Virol. **63**:319–327.
22. **Panganiban, A. T., and D. Fiore.** 1988. Ordered interstrand and intrastrand DNA transfer during reverse transcription. Science **241**:1064–1069.
23. **Scherer, S., C. Mann, and R. W. Davis.** 1982. Reversion of a promoter deletion in yeast. Nature (London) **298**:815–819.
24. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
25. **Sikorski, R. S., and P. Hieter.** 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics **122**:19–27.

26. **Struhl, K.** 1982. Regulatory sites for *his3* gene expression in yeast. Nature (London) **300**:284–287.
27. **Varmus, H. E.** 1983. Retroviruses, p. 411–503. *In* J. A. Shapiro (ed.), Mobile genetic elements. Academic Press, Inc., New York.
28. **Xu, H., and J. D. Boeke.** 1987. High frequency deletion between homologous sequences during retrotransposition of Ty elements in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA **84**:8553–8557.
29. **Yarger, J. G., G. Armilei, and M. C. Gorman.** 1986. Transcription terminator-like element within a *Saccharomyces cerevisiae* promoter region. Mol. Cell. Biol. **6**:1095–1101.
30. **Youngren, S. D., J. D. Boeke, N. J. Sanders, and D. J. Garfinkel.** 1988. Functional organization of the retrotransposon Ty from *Saccharomyces cerevisiae*: the Ty protease is required for transposition. Mol. Cell. Biol. **8**:1421–1431.
31. **Yu, K., and R. T. Elder.** 1989. Some of the signals for 3′ end formation in transcription of the *Saccharomyces cerevisiae* Ty-D15 element are immediately downstream of the initiation site. Mol. Cell. Biol. **9**:2431–2444.