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Genetics

Initiator methionine tRNA is essential for Ty*1* transposition in yeast

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ABSTRACT **The yeast retrotransposon Ty*1* transposes through an RNA intermediate by a mechanism similar to that of retroviral reverse transcription and integration. Ty*1* RNA contains a putative minus strand primer binding site (−PBS) that is complementary to the 3′ acceptor stem of the initiator methionine tRNA ($tRNA_i^{Met}$). Here we demonstrate that the $tRNA_i^{Met}$ is used as a primer for Ty*1* reverse transcription. Mutations in the Ty*1* element that alter 5 of 10 nucleotides that are complementary to the $tRNA_i^{Met}$ abolish Ty*1* transposition, even though they are silent with regard to Ty*1* protein coding. We have constructed a yeast strain lacking wild-type $tRNA_i^{Met}$ that is dependent on a mutant derivative of $tRNA_i^{Met}$ that has an altered acceptor stem sequence, engineered to restore homology with the Ty*1* −PBS mutant. The compensatory mutations made in the $tRNA_i^{Met}$ alleviate the transposition defect of the Ty*1* −PBS mutant. The mutant and wild-type $tRNA_i^{Met}$ are enriched within Ty*1* virus-like particles irrespective of complementarity to the Ty*1* −PBS. Thus, complementarity between the Ty*1* −PBS and $tRNA_i^{Met}$ is essential for transposition but is not necessary for packaging of the tRNA inside virus-like particles.**

The Ty*1* retrotransposon in *Saccharomyces cerevisiae* transposes through an RNA intermediate by a mechanism similar to that of retroviral reverse transcription and integration (1). In several retroviral systems that have been examined, reverse transcription of minus strand DNA is initiated using a cellular tRNA as a primer. These elements typically have an 18-nucleotide region located adjacent to the 5′ long terminal repeat (LTR) that is complementary to a cellular tRNA (2). This region is called the minus strand primer binding site (−PBS). The initiation of reverse transcription by a cellular tRNA presumably involves packaging of the tRNA in the viral particle, formation of the primer–template complex, and extension of the primer by reverse transcriptase. For avian retroviruses, reverse transcriptase appears to be necessary for packaging of the primer tRNA into the viral particle. Avian myeloblastosis virus reverse transcriptase forms a stable complex with its cognate $tRNA^{Trp}$ primer (3). Murine retrovirus reverse transcriptases bind tRNAs, but without any obvious specificity [reviewed by Varmus and Swanstrom (4)]. The human immunodeficiency virus type 1 (HIV-1) reverse transcriptase specifically binds its primer, $tRNA^{Lys}$, through the anticodon stem (5). For Rous sarcoma virus, HIV-1, and murine leukemia virus, the nucleocapsid proteins, encoded by the *gag* gene and containing a structure that may be related to a Zn^{2+} finger, are required to position primer tRNA on genomic RNA (5, 6).

The Ty*1* element has a putative −PBS, a 10-nucleotide region complementary to the 3′ acceptor stem of the initiator methionine tRNA ($tRNA_i^{Met}$) (7). Several retrotransposons contain shorter putative −PBSs with complementarity to various tRNAs (8) but there is no direct evidence that these sites actually serve as regions for priming by tRNAs. However, for some *Drosophila* elements there is biochemical evidence consistent with a tRNA-sized RNA primer (9). For the *Drosophila copia* element, the 5′ half-molecule of $tRNA_i^{Met}$ appears to prime reverse transcription (10). Here we demonstrate that the $tRNA_i^{Met}$ is essential for Ty*1* transposition.

The Ty*1* retrotransposon of the yeast *S. cerevisiae* provides a uniquely accessible system in which to study these processes. The yeast initiator methionine tRNA genes (*IMT* genes) are cloned and mapped (11, 12) and can be manipulated *in vitro* and *in vivo*. Furthermore, the transposition frequency of a Ty*1* element marked with a genetic marker such as *neo* can be quantitated *in vivo* (13, 14). Transposition of Ty*1-neo* elements fused to the *GAL1* promoter can be induced to high levels by growth on medium containing galactose as the sole carbon source. Transposition of Ty*1-neo* elements into the host genome confers resistance to G418, allowing transposition events to be quantitated by a genetic assay.

Here we demonstrate genetically that the $tRNA_i^{Met}$ is essential for Ty*1* transposition. Mutations made in the $tRNA_i^{Met}$ that allow for viability in the absence of wild-type $tRNA_i^{Met}$ abolish Ty*1* transposition *in vivo*. Transposition is restored by the introduction of compensatory mutations in the −PBS of the Ty*1* element.

MATERIALS AND METHODS

Strains and Media. All yeast strains used in this study are listed in Table 1. Media were prepared as described (15).

RNA Isolation and Blotting. Total RNA preparation from whole cells and RNA blotting from polyacrylamide gels was carried out as described (16). Ty*1* virus-like particles (VLPs) were isolated from 500-ml cultures on 20%/30%/70% sucrose step gradients as described (17) except for the experiment in Fig. 1*B*, in which they were subsequently recentrifuged on a 20–50% linear sucrose gradient. To extract VLP RNA, pelleted VLPs were resuspended in 0.3 ml of RNA buffer [100 mM NaCl/100 mM Tris base/30 mM EDTA/1% *N*-lauroylsarcosine (adjusted to pH 8.9 with HCl)], 0.15 ml of phenol, and 0.15 ml of $CHCl_3$ followed by brief agitation in a Vortex and centrifugation. The aqueous phase was extracted with phenol/chloroform and precipitated with 0.1 M NaOAc (pH 5.5) and 2.5 volumes of ethanol. RNA pellets were resuspended in 100 μl of distilled H_2O and quantitated by absorbance at 260 nm. Five to 10 μg of RNA was ethanol precipitated, resuspended in formamide loading buffer, and heated to 65°C prior to electrophoresis on 5% polyacrylamide/8 M urea gels.

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Abbreviations: 5-FOA, 5-fluoroorotic acid; −PBS, minus strand primer binding site; $tRNA_i^{Met}$, initiator methionine tRNA; VLP, virus-like particle; LTR, long terminal repeat; ^{r}, resistant; HIV-1, human immunodeficiency virus type 1.

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Table 1. Strains used in this study

Strain	Plasmid(s)
ASB217-32C	pCGS42
KC141	pKC10
KC142	pKC35
KC149	pKC10, pKC66
KC150	pKC10, pJEF1105
KC151	pKC35, pKC66
KC152	pKC35, pJEF1105

Genotype of all strains listed is *MATα ura3-52 trp1Δ1 leu2-3,112 imt1::TRP1 imt2::TRP1 imt3::TRP1 imt4::TRP1*

Construction of Mutants. The Ty*1* −PBS mutant and the *imt4-9* mutant were constructed using the method of Kunkel (18). To construct the Ty*1* −PBS mutation, a 2.6-kilobase (kb) *Eco*RI–*Sal* I fragment from plasmid pJEF724 (19) was cloned into M13mp8 digested with *Eco*RI and *Sal* I, generating plasmid mp8-724-3. The −PBS mutation was made with oligonucleotide JB33: 5′-CGAAGCACACGCCGACCCATGAGAATT-3′, generating plasmid KCf1. To generate the *imt4-9* mutation, the 0.2-kb *Bam*HI–*Hin*dIII fragment from plasmid pBY140 (20) containing the *IMT4* gene was cloned into M13mp18 digested with *Bam*HI and *Hin*dIII. The *imt4-9* mutant was made using two mutagenic oligonucleotides: JB37, 5′-AGAAATGAAAAATGTCGGCGGCTCGGTTTC-3′, and JB38, 5′-CTGCGCCACCGCCGATAGCCAACTTG-3′, generating KCf10. Two additional mutations, *imt4-4* and *imt4-5*, bearing substitutions only on the 5′ side (oligonucleotide JB38) and the 3′ side (JB37), respectively, of the acceptor stem, were also constructed.

Plasmid Constructions. Plasmid pKC66 was constructed by ligating a 7.3-kb *Kpn* I–*Xba* I fragment from plasmid pJEF1105 with a 6.9-kb *Kpn* I–*Xba* I fragment from pKC7, which contains the −PBS mutation. Plasmid pKC7 was constructed by a three-piece ligation: a 2.6-kb *Eco*RI–*Sal* I fragment from KCf1 (described above), a 4.6-kb *Sal* I–*Bam*HI fragment from pX3 (21), and a 6.7-kb *Bam*HI–*Eco*RI fragment from pX3. Plasmid pKC10 was constructed by ligating the 0.2-kb *Bam*HI–*Hin*dIII fragment from plasmid KCf10 (described above) with vector Yep351 (22) digested with *Bam*HI and *Hin*dIII. Plasmid pKC35 was constructed by ligating a 0.2-kb *Bam*HI–*Hin*dIII fragment containing the wild-type *IMT4* gene from plasmid pBY140 with vector Yep351 digested with *Bam*HI and *Hin*dIII.

Hybridization Probes. The wild-type $tRNA_i^{Met}$ probe is a 0.2-kb *Bam*HI–*Hin*dIII fragment from plasmid pKC35. The mutant $tRNA_i^{Met}$ probe is a 0.2-kb *Bam*HI–*Hin*dIII fragment from plasmid pKC10. The $tRNA^{Tyr}$ probe is a 0.8-kb *Bam*HI fragment containing the *SUP11* gene from plasmid p355 (kindly provided by Philip Hieter, Johns Hopkins University). Probes were made by random hexamer priming (23).

Transposition Assay. Yeast strains KC149, KC150, KC151, and KC152 contain either plasmid pJEF1105 or its derivative, pKC66; both of these plasmids contain a Ty*1-neo* element fused to the *GAL1* promoter. This marked Ty*1* element is on a high-copy-number, 2-μm vector marked with *URA3* (13). Transposition of Ty*1-neo* into the host genome can be detected by the acquisition of a G418-resistant phenotype in plasmid-free derivatives of the host cell. Transposition was quantitated as follows. Yeast strains KC151 (harboring the mutant Ty*1* element on pKC66) and KC152 (harboring the wild-type Ty*1* element on pJEF1105) were grown as a patch on SC−Ura medium (synthetic complete medium lacking uracil) containing 2% galactose at 22°C for 5 days. Growth on medium containing galactose as the sole carbon source induces transcription of the marked Ty*1* element from the *GAL1* promoter. These cells were replica-plated to nonselective medium, YPD, to allow for plasmid loss. To select for cells that have lost the plasmid, these patches were replica-plated to SC medium containing 1 mg of 5-fluoroorotic acid (5-FOA) per ml. The 5-FOA selects against cells expressing the *URA3* gene on the plasmid (24). Following 1 day of growth at 30°C, these plates were again replica-plated to SC plus 5-FOA and grown at 30°C overnight. For a qualitative estimate of transposition frequency, this plate is replica-plated to YPD medium containing 75 μg of G418 per ml; cells that have undergone a transposition event will papillate as G418-resistant ($G418^r$) colonies. To quantitate transposition frequencies in these strains, cells from the 5-FOA plate were scraped into water, plated on YPD medium, and allowed to form single colonies. These plates were replica plated to SC−Ura and YPD plus G418. Transposition frequency is calculated as the number of $Ura^-/G418^r$ colonies divided by the total number of Ura^- colonies.

RESULTS

A Putative tRNA Primer. Initiation of retroviral minus strand synthesis involves the utilization of a cellular tRNA as a primer for DNA synthesis. Reverse transcription initiates from the 3′ OH of the tRNA primer, which base pairs to the −PBS, located adjacent to the 5′ LTR. This initiation event results in the synthesis of minus strong stop DNA, a molecule consisting of the primer tRNA covalently attached to the newly synthesized complementary-strand DNA; specifically, the U5 and R sequences of the 5′ LTR. We have directly examined Ty*1* transposition intermediates for the presence of minus strong stop DNA. Such a molecule is predicted to have 96 nucleotides of DNA covalently attached to a tRNA primer 75 nucleotides in length.

To examine Ty*1* transposition intermediates, Ty*1* VLPs were purified from strain JB224, a strain in which high levels of transposition and transposition intermediates (VLPs) can be induced by growth on galactose (19, 25). Nucleic acid transposition intermediates were labeled *in vitro* in two ways. In the first, [α-^{32}P]dTTP was added to preparations of VLPs under conditions that allowed for reverse transcription. Since the first nucleotide on the Ty*1* transcript that is adjacent to the −PBS is an adenosine, incubation of VLPs in the presence of TTP as the only nucleotide triphosphate should result in the addition of a single T residue to the putative tRNA primer. As predicted for a tRNA-sized primer, we observe a labeled species of the appropriate size (76 nucleotides) that is sensitive to RNase A (Fig. 1*A*).

In the second labeling experiment, all four deoxyribonucleotide triphosphates were included, as well as [α-^{32}P]dTTP. The full-length minus strong stop has a predicted length of 171 nucleotides. We observe such a molecule, which is sensitive to RNase A. In the RNase-treated reaction products, we observe a product of 96 nucleotides, the expected size of the minus strong stop DNA without the 75-nucleotide tRNA primer. These observations are consistent with a tRNA-sized RNA primer for reverse transcription of Ty*1* minus strand. End-labeled minus strong stop molecules have also been treated with sequence-specific ribonucleases and have patterns of nuclease sensitivity similar to those of purified $tRNA_i^{Met}$ (A.S.B. and Gerald R. Fink, unpublished data).

The $tRNA_i^{Met}$ Is Localized to Ty*1* VLPs. Ty*1* RNA contains a putative −PBS that is complementary to the $tRNA_i^{Met}$. If the $tRNA_i^{Met}$ is used as a primer for reverse transcription, we would expect to observe preferential packaging of this tRNA in Ty*1* VLPs. We have directly examined Ty*1* VLPs for the presence of $tRNA_i^{Met}$. Ty*1* VLPs were purified as described (17) and RNA was extracted. This RNA was fractionated on an 8% polyacrylamide gel, electrophoretically transferred to a membrane, and probed with radioactive $tRNA_i^{Met}$ DNA (Fig. 1*B*). The $tRNA_i^{Met}$ is detectable in Ty*1* VLPs and total RNA. A control tRNA, $tRNA^{Tyr}$, is present in total RNA but is not readily detectable in the VLP RNA. Densitometric

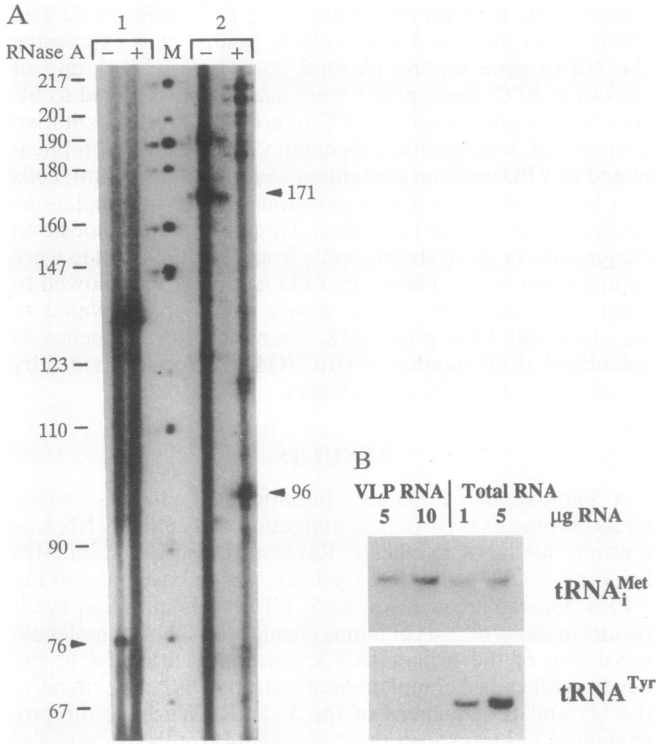

FIG. 1. A putative tRNA primer for Ty*1* reverse transcription. (*A*) Ty*1* reverse transcription reaction identifies a tRNA-sized primer. Ty*1* VLPs were incubated with [α-^{32}P]TTP [reaction 1 (1)] or [α-^{32}P]TTP plus all four unlabeled nucleotide triphosphates [reaction 2 (2)]. Reaction products were deproteinized, half of the sample was treated with RNase A, and the reaction products were run on a DNA sequencing gel. Size markers (M; sizes indicated in nucleotides) derive from an *Msp* I digest of pBR322 labeled by treatment with Klenow fragment plus [α-^{32}P]dCTP. (*B*) tRNA$_i^{Met}$ is localized in Ty*1* VLPs. Total RNA was extracted from whole cells or Ty*1* VLPs; the indicated amount of RNA was electrophoresed on an 8% polyacrylamide gel and transferred to a membrane. RNA blot analysis was carried out using the tRNA$_i^{Met}$ probe; the same blot was stripped and probed with the tRNA^{Tyr} probe.

scanning of these autoradiograms indicates that the tRNA$_i^{Met}$ is enriched 44-fold relative to tRNA^{Tyr} in Ty*1* VLPs.

A Mutation in the −PBS Eliminates Transposition of Ty*1*. To investigate the role of the −PBS sequence in the mechanism of Ty*1* transposition, we have introduced specific nucleotide changes in the −PBS that do not disrupt protein coding but that change 5 of 10 nucleotides of complementarity with the tRNA$_i^{Met}$ (Fig. 2). This −PBS mutation was incorporated in a GAL-Ty*1* element marked with *neo* to quantitate transposition. Transposition of Ty*1-neo* into the host genome confers resistance to G418. As shown in Fig. 3, cells containing the wild-type Ty*1-neo* element (KC152; wt Ty*1* −PBS) exhibit a high level of Ty*1* transposition; growth on YPD/G418, which indicates transposition, is nearly confluent. In contrast, cells containing the −PBS Ty*1* mutant (KC151; mut Ty*1* −PBS) are markedly reduced in the number of G418^r colonies, suggesting that transposition of the mutant Ty*1-neo* element was either greatly reduced or abolished. The transposition frequency of the mutant Ty*1* element is reduced by at least 30-fold (Table 2). These results suggest that the nucleotide sequence of the −PBS region in Ty*1* is essential for transposition.

Mutation of the Acceptor Stem of tRNA$_i^{Met}$ Results in a Functional tRNA. To investigate the role of the tRNA$_i^{Met}$ in Ty*1* transposition, we have introduced specific nucleotide changes in the portion of the *IMT* gene that encodes the 3′ acceptor stem of the tRNA. These changes disrupt complementarity with the wild-type Ty*1* −PBS (diagrammed schematically in Fig. 2). In addition, the 5′ end of the tRNA$_i^{Met}$ was mutated to restore complementarity with the 3′ side of the acceptor stem. This mutant tRNA$_i^{Met}$ gene (*imt4-9*) was cloned on a high-copy-number (2 μm) plasmid marked with *LEU2* to derive plasmid pKC10.

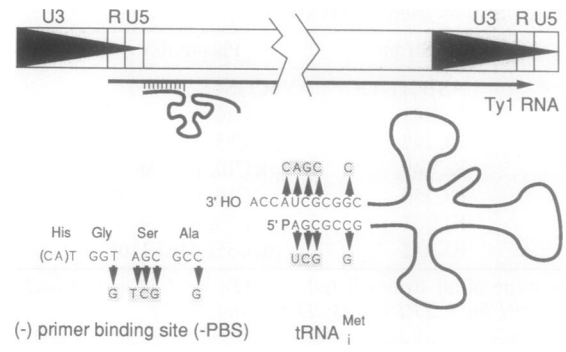

FIG. 2. Construction of a mutant Ty*1* −PBS and compensatory mutations in the tRNA$_i^{Met}$. Ty*1* RNA contains 10 nucleotides of complementarity (the −PBS) to the 3′ acceptor stem of the tRNA$_i^{Met}$. The mutations in the Ty*1* −PBS do not disrupt TYA protein coding but disrupt 5 of 10 base pairs of complementarity with the wild-type tRNA$_i^{Met}$. Five base changes in the 3′ acceptor stem of the tRNA$_i^{Met}$ restore base-pairing to the mutant −PBS; 4 nucleotide changes in the 5′ end of the tRNA restore base-pairing of the acceptor stem. Boxed triangles, LTR sequences; open box, coding region of Ty*1*; vertical lines, boundaries of LTR domains U3, R, and U5; heavy lines, RNA; plain letters, wild-type sequences; shaded letters, mutant sequences. Drawing is not to scale.

Wild-type yeast strains typically contain four or five copies of the *IMT* gene (12). At least one copy of the *IMT* gene is essential for viability. To test the role of the tRNA$_i^{Met}$ in Ty*1* transposition, we sought to construct a yeast strain that expressed the mutant tRNA in the absence of wild-type tRNA$_i^{Met}$. A yeast strain, ASB217-32C, was constructed in which all four genomic *IMT* genes are deleted and a wild-type *IMT4* is present on a high-copy-number plasmid marked with *URA3* (20). To replace the wild-type *IMT4* with the mutant, *imt4-9*, contained on pKC10, a plasmid shuffle technique (26) was used (diagrammed in Fig. 4). Yeast strain ASB217-32C was transformed with pKC10 or pKC35 (containing the wild-type *IMT4* in Yep351), selecting Leu$^+$ transformants. Ura$^-$ derivatives were obtained by selection on 5-FOA. The Leu$^+$ Ura$^-$ derivatives represent cells that have lost the *URA3* marked plasmid containing the wild-type tRNA$_i^{Met}$ and are surviving on either the mutant tRNA supplied by pKC10 (yeast strain KC78) or the wild-type tRNA$_i^{Met}$ supplied by pKC35 (yeast strain KC79). When the same tRNA$_i^{Met}$ muta-

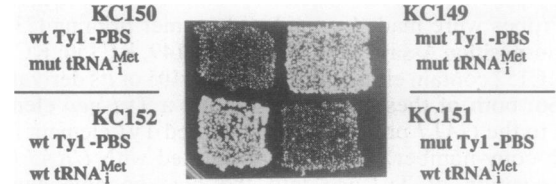

FIG. 3. Transposition defect of Ty*1* −PBS mutant is alleviated by compensatory mutations in the tRNA$_i^{Met}$. The transposition phenotype of yeast strains containing various combinations of wild-type (wt) or mutant (mut) Ty*1* −PBS and tRNA$_i^{Met}$ is shown on YPD plates containing 75 μg of G418 per ml. Transposition of GAL-Ty*1-neo* was induced by growth on SC−Ura galactose medium; cells were then replica-plated to YPD to allow for plasmid loss. These replicas were replica-plated to SC medium containing 1 mg of 5-FOA per ml for two successive rounds of growth, selecting for cells that have lost the GAL-Ty plasmid, which contains the *URA3* marker. Cells were finally replica-plated to YPD containing 75 μg of G418 per ml in order to identify colonies that had undergone transposition of Ty*1-neo*.

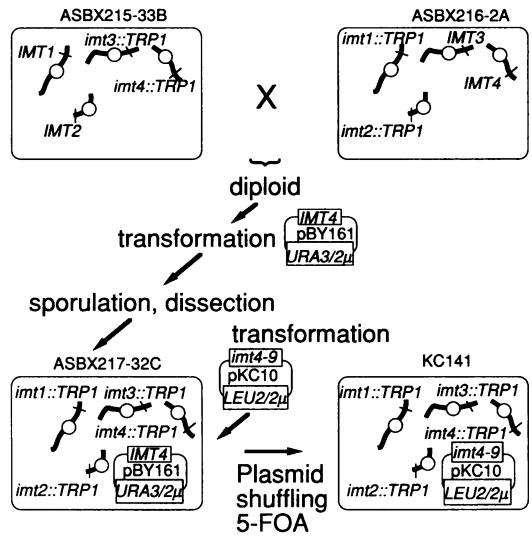

FIG. 4. Construction of a yeast strain expressing exclusively mutant $tRNA_i^{Met}$. The four chromosomal *IMT* genes were deleted by the procedure illustrated and described in detail elsewhere (20). Strain ASBX217-32C has all four copies of the *IMT* gene disrupted by *TRP1*; wild-type $tRNA_i^{Met}$ is supplied by the *IMT4* gene on plasmid pBY161. To construct yeast strain KC78, ASBX217-32C was transformed with plasmid pKC10 containing a mutant *IMT4* gene (*imt4-9*, Fig. 2). Leu^+ transformants were selected. Ura^- derivatives were selected by replica-plating to medium containing 5-FOA, resulting in strain KC78, which is expressing exclusively the mutant $tRNA_i^{Met}$ encoded by *imt4-9*.

tions were introduced on one side of the acceptor stem only (disrupting the stem structure) the resulting mutant tRNAs were not able to substitute for the wild-type $tRNA_i^{Met}$, as evidenced by the lack of any Leu^+ Ura^- derivatives in the plasmid shuffle assay. Interestingly, these extensive nucleotide changes introduced in the acceptor stem of the $tRNA_i^{Met}$ do not appear to be deleterious as long as complementarity of the acceptor stem is maintained; we do not observe any growth defect in yeast strain KC78, which survives exclusively on the mutant $tRNA_i^{Met}$.

Mutation of the Acceptor Stem of $tRNA_i^{Met}$ Eliminates Ty*1* Transposition. To investigate the role of the $tRNA_i^{Met}$ in Ty*1* transposition, we assayed transposition of wild-type Ty*1* in yeast strain KC78, which expresses exclusively the mutant $tRNA_i^{Met}$ that lacks extensive complementarity to the Ty*1* −PBS. This yeast strain was transformed with plasmid pJEF1105, which contains a wild-type *GAL*-Ty*1*-*neo* element to assay transposition frequency. The resultant strain (KC150) has at least a 78-fold reduction of transposition frequency relative to wild type (Fig. 3 and Table 2), demonstrating that the $tRNA_i^{Met}$ is essential for transposition.

Mutant $tRNA_i^{Met}$ Complements Transposition Defect of Ty*1* −PBS Mutant. To unambiguously demonstrate that this complementarity between the Ty*1* −PBS and the $tRNA_i^{Met}$ is essential for transposition, the Ty*1* −PBS mutant was combined with the mutant $tRNA_i^{Met}$, restoring 10 base pairs of perfect complementarity in the −PBS region. Either the Ty*1* −PBS mutation or the $tRNA_i^{Met}$ mutation alone virtually abolishes transposition (Fig. 3 and Table 2). However, in strain KC149, which combines the Ty*1* −PBS mutation with the mutant $tRNA_i^{Met}$, transposition is restored (Fig. 3). The level of transposition is reduced (2.6% compared to 15.7% in wild type) but still readily detectable at >5-fold above background (Table 2). These results demonstrate that complementarity between the $tRNA_i^{Met}$ and the Ty*1* −PBS is essential for transposition. Furthermore, the ability of the mutant $tRNA_i^{Met}$ to complement the Ty*1* −PBS mutation suggests that the mutant $tRNA_i^{Met}$ is packaged in the Ty*1* VLPs.

Table 2. Transposition frequencies

Strain	Ty*1* −PBS	$tRNA_i^{Met}$	Neo^r Ura^- colonies/Ura^- colonies Exp. 1	Exp. 2	Transposition frequency, %
KC152	wt	wt	157/789	64/615	15.7
KC151	mut	wt	0/459	6/875	<0.5
KC150	wt	mut	1/775	1/875	<0.2
KC149	mut	mut	29/965	7/411	2.6

wt, Wild type; mut, mutant.

The $tRNA_i^{Met}$ Is Packaged in Ty*1* VLPs in the Absence of Extensive Complementarity to the Ty*1* −PBS. The ability of the mutant $tRNA_i^{Met}$ to suppress the Ty*1* −PBS mutation suggested that the mutant tRNA is packaged in Ty*1* VLPs. We have directly examined the packaging of mutant and wild-type tRNA in VLPs containing either wild-type Ty*1* RNA or the Ty*1* −PBS mutant. Ty*1* VLPs were isolated from strains KC149, KC150, KC151, and KC152. Nucleic acids were extracted from the VLPs and were fractionated on a 5% polyacrylamide gel. The RNA species were transferred to a membrane and hybridized to probes for the wild-type and the mutant $tRNA_i^{Met}$ (Fig. 5). The mutant $tRNA_i^{Met}$ probe only detects a signal in VLPs derived from KC149 and KC150, the strains that express exclusively the mutant $tRNA_i^{Met}$. Interestingly, there is no detectable difference in the amount of $tRNA_i^{Met}$ in KC149 and KC150 VLPs, even though KC150 harbors exclusively wild-type Ty*1*, which does not share extensive complementarity with the mutant $tRNA_i^{Met}$. In addition, similar amounts of wild-type $tRNA_i^{Met}$ are detected in strains KC151 and KC152, which harbor the mutant and the wild-type Ty*1* −PBS, respectively. These results suggest that packaging of the primer tRNA is by a mechanism that is independent of sequence complementarity to the −PBS.

DISCUSSION

The use of a cellular tRNA as a primer for reverse transcription is an integral aspect of the retroviral life cycle. Here, this type of priming mechanism is extended to the yeast retrotransposon Ty*1*. Unlike retroviruses, which have 18 base pairs of perfect complementarity between the −PBS and priming tRNA, the Ty*1* element contains a −PBS that shares only 10 nucleotides of complementarity to $tRNA_i^{Met}$. Recent experiments with HIV-1 indicate that −PBS mutants with less extensive com-

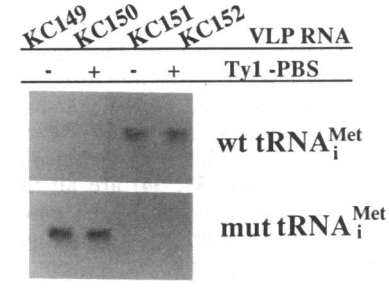

FIG. 5. Packaging of wild-type (wt) and mutant (mut) $tRNA_i^{Met}$ in Ty*1* VLPs occurs irrespective of homology with the Ty*1* primer binding site. VLP RNA extracted from the indicated strains was analyzed by RNA blotting with a wild-type *IMT4* probe or the mutant *imt4-9* probe. Yeast strains KC149 and KC150 express exclusively the mutant $tRNA_i^{Met}$. Strain KC149 harbors the mutant Ty*1* −PBS, which base-pairs to the mutant $tRNA_i^{Met}$; strain KC150 harbors the wild-type Ty*1*. Yeast strains KC151 and KC152 are expressing exclusively wild-type $tRNA_i^{Met}$. KC151 has the mutant Ty*1* −PBS, whose RNA lacks substantial complementarity to the wild-type $tRNA_i^{Met}$; strain KC152 harbors the wild-type Ty*1* element.

plementarity have a severe impact on the kinetics of viral infection (27). The ability to alter the $tRNA_i^{Met}$ genes and the Ty*1* element provides a unique opportunity to examine this priming mechanism in yeast.

The ability to introduce specific nucleotide changes in the acceptor stem of the $tRNA_i^{Met}$ that retain the ability to function in translation has allowed the construction of a yeast strain expressing exclusively an altered $tRNA_i^{Met}$. Although many tRNAs are recognized by their synthetases in the aminoacyl stem (28, 29), there is evidence that the initiator tRNA in *Escherichia coli* is recognized primarily, if not exclusively, in the anticodon (30, 31). Nevertheless, we were surprised that a $tRNA_i^{Met}$ with five altered base pairs in the acceptor stem shows no growth defect.

The nucleotide changes in the altered $tRNA_i^{Met}$ disrupt 5 of the 10 base pairs of complementarity to the Ty*1* −PBS. These nucleotide changes are sufficient to abolish detectable levels of Ty*1* transposition *in vivo*, demonstrating that $tRNA_i^{Met}$ is an essential host gene product for Ty*1* transposition. Compensatory mutations made in the Ty*1* −PBS, which restore 10 base pairs of complementarity to the mutant $tRNA_i^{Met}$, also restore Ty*1* transposition, demonstrating that complementarity between Ty*1* and its primer tRNA is an essential aspect of Ty*1* transposition.

Interestingly, restoration of transposition to the Ty*1* −PBS mutant by the compensatory mutations in the $tRNA_i^{Met}$ results in transposition frequency at a reduced level as compared to the wild-type frequency. Although it is conceivable that this reduced frequency of transposition is due to inefficient packaging of mutant $tRNA_i^{Met}$ in Ty*1* VLPs, this possibility seems unlikely as the mutant $tRNA_i^{Met}$ is readily detected in Ty*1* VLPs. It remains possible that additional steps in the formation of the active priming complex—i.e., the melting of the tRNA acceptor stem or the positioning of the tRNA on the −PBS—may involve the formation of specific interactions with sequence or base-composition specificity. One A-T base pair in the wild-type/wild-type combination is exchanged for a G-C base pair in the mutant/mutant combination, potentially resulting in more stable acceptor stem and tRNA/−PBS interactions. Alternatively, these differences in frequency could be due to differences in expression, modification, or stability of the two forms of the tRNA. Among 11 $tRNA_i^{Met}$ mutants studied (20), most had normal levels of tRNA, and a few had slightly elevated levels, suggesting that mutant tRNAs can readily be expressed at normal levels in yeast.

The observation that nine base changes in the acceptor stem of the tRNA do not appear to have an impact on packaging into VLPs suggests that the macromolecule(s) responsible for this packaging has recognition determinants outside of the acceptor stem region. Perhaps, as in the case with methionyl tRNA synthetases and also apparently with HIV-1 reverse transcriptase (5), a special sequence or structure around the anticodon is recognized. Reverse transcribing elements from yeast (Ty*1*), insects (10), a plant virus (32), and plant retrotransposons (33, 34) are all thought to use $tRNA_i^{Met}$ as primers. Perhaps, as in the case of the synthetases, the tRNAs are recognized and packaged in a similar fashion in these diverse systems.

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1. Boeke, J. D. (1989) in *Mobile DNA*, eds. Berg, D. E. & Howe, M. M. (Am. Soc. Microbiol., Washington), pp. 335–374.
2. Chen, H. R. & Barker, W. C. (1984) *Nucleic Acids Res.* **12,** 1767–1778.
3. Panet, A., Haseltine, W. A., Baltimore, D., Peters, G., Harada, F. & Dahlberg, J. E. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2535–2539.
4. Varmus, H. E. & Swanstrom, R. (1984) in *RNA Tumor Viruses*, eds. Weiss, R., Teich, N., Varmus, H. & Coffin, J. (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 369–512.
5. Barat, C., Lullien, V., Schatz, O., Keith, G., Nugeyre, M., Gruninger-Leitch, F., Barre-Sinoussi, F., LeGrice, S. & Darlix, J. (1989) *EMBO J.* **8,** 3279–3285.
6. Prats, A. C., Sarih, L., Gabus, C., Litvak, S., Keith, G. & Darlix, J. L. (1988) *EMBO J.* **7,** 1777–1783.
7. Eibel, H., Gafner, J., Stotz, A. & Philippsen, P. (1981) *Cold Spring Harbor Symp. Quant. Biol.* **45,** 609–617.
8. Boeke, J. D. & Corces, V. G. (1989) *Annu. Rev. Microbiol.* **43,** 403–433.
9. Arkhipova, I. R., Mazo, A. M., Cherkasova, V. A., Gorelova, T. V., Schuppe, N. G. & Ilyin, Y. V. (1986) *Cell* **44,** 555–563.
10. Kikuchi, Y., Ando, Y. & Shiba, T. (1986) *Nature (London)* **323,** 824–826.
11. Cigan, M. & Donahue, T. F. (1986) *Gene* **41,** 343–348.
12. Byström, A. S. & Fink, G. R. (1989) *Mol. Gen. Genet.* **216,** 276–286.
13. Boeke, J. D., Xu, H. & Fink, G. R. (1988) *Science* **239,** 280–282.
14. Xu, H. & Boeke, J. D. (1991) *Mol. Cell. Biol.* **11,** 2736–2743.
15. Sherman, F., Fink, G. R. & Hicks, J. B. (1986) *Methods in Yeast Genetics* (Cold Spring Harbor Lab., Cold Spring Harbor, NY).
16. Chapman, K. B. & Boeke, J. D. (1991) *Cell* **65,** 483–492.
17. Eichinger, D. J. & Boeke, J. D. (1988) *Cell* **54,** 955–966.
18. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 488–492.
19. Boeke, J. D., Garfinkel, D. J., Styles, C. A. & Fink, G. R. (1985) *Cell* **40,** 491–500.
20. von Pawel-Rammingen, U., Åstrom, S. & Byström, A. S. (1992) *Mol. Cell. Biol.* **12,** in press.
21. Xu, H. & Boeke, J. D. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8553–8557.
22. Hill, J. E., Myers, A. M., Koerner, T. J. & Tzagoloff, A. (1986) *Yeast* **2,** 163–167.
23. Feinberg, A. P. & Vogelstein, B. (1983) *Anal. Biochem.* **137,** 266–267.
24. Boeke, J. D., Lacroute, F. & Fink, G. R. (1984) *Mol. Gen. Genet.* **197,** 345–346.
25. Garfinkel, D. J., Boeke, J. D. & Fink, G. R. (1985) *Cell* **42,** 507–517.
26. Sikorski, R. S. & Boeke, J. D. (1990) *Methods Enzymol.* **194,** 302–318.
27. Rhim, H., Park, J. & Morrow, C. D. (1991) *J. Virol.* **65,** 4555–4564.
28. Hou, Y.-M. & Schimmel, P. R. (1988) *Nature (London)* **333,** 140–145.
29. Normanly, J. & Abelson, J. (1989) *Annu. Rev. Biochem.* **58,** 1029–1049.
30. Shulman, L. H. & Pelka, H. (1988) *Science* **242,** 765–768.
31. Ghosh, G., Pelka, H. & Schulman, L. H. (1990) *Biochemistry* **29,** 2220–2225.
32. Pfeiffer, P. & Hohn, T. (1983) *Cell* **33,** 781–789.
33. Voytas, D. F. & Ausubel, F. M. (1988) *Nature (London)* **336,** 242–244.
34. Grandbastien, M. A., Spielmann, A. & Caboche, M. (1989) *Nature (London)* **337,** 376–380.