Host genes that influence transposition in yeast: The abundance of a rare tRNA regulates Ty1 transposition frequency

(retrotransposon/Saccharomyces cerevisiae/translational frameshifting)

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ABSTRACT Genetic screening of a yeast genomic library in a high-copy-number vector identified the normally single-copy tRNA gene as one of the genes that reduces Ty1 transposition frequency when overexpressed. Immunoblot analyses of Ty1-encoded proteins indicate an inverse correlation between the copy number of the tRNA gene and the production of the TYB protein. Thus, TYB transposition frequency is apparently regulated by the level of tRNA in yeast cells.

Ty1 elements are members of a family of retrotransposons in the yeast Saccharomyces cerevisiae. Like many retroviruses and retrotransposons, Ty1 elements contain two overlapping, out-of-phase open reading frames (ORFs) flanked by long terminal repeats (LTRs, also called 5' elements) (1). Ty1 elements are transcribed from the 5' LTR as full-length mRNAs (2). Transposition of Ty1 elements resembles the retroviral replication process, and the functions and relationships of Ty1-encoded proteins are strikingly similar to those of many retroviral proteins (3–6). The two ORFs, TYA and TYB, encode proteins that are analogous to the products of retroviral gag and pol, respectively. The TYB gene products include enzymatic activities important for transposition such as protease, reverse transcriptase, and integrase. Previous studies indicate that TYB proteins are made initially as a TYA–TYB fusion protein, pTYA/TYB-190, via a specific frameshifting event occurring within the overlapping region of the two ORFs during translation (7–10). Subsequently, the fusion protein is processed to smaller proteins such as the integrase protein, pTYB-90 (4–6, 11), by a protease encoded in TYB.

To better understand the Ty1 transposition process, and in particular, the relationship between Ty1 elements and their host (the yeast cell), we have developed a genetic screening procedure designed to identify yeast genes whose products inhibit Ty1 transposition at a posttranscriptional level. We report here the isolation and identification of a host gene that directly affects Ty1 transposition frequency posttranscriptionally; among the genomic clones isolated was a segment containing the single-copy tRNA gene*, which was described previously (12). Overexpression of this tRNA gene from a high-copy-number plasmid decreases Ty1 transposition frequency by a factor of >1.5. A defect in transposition can be detected even when an extra copy of the gene is carried by a centromeric plasmid, suggesting that Ty1 transposition frequency is very sensitive to the abundance of this rare tRNA. At the protein level, TYA protein is produced at a normal level whereas the amount of TYB protein is greatly diminished. Consistent with our results that the production of TYB proteins and consequently the transposition process are regulated by the intracellular concentration of tRNA.

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MATERIALS AND METHODS

Strains, Plasmids, and Media. Yeast strain YH23, used for the screening of transposition-inhibitory genomic clones, is a pGTy1-H3-TRP1 (pX3) transformant of YH8 (MATa ura3-52 leu2Δ1 his3Δ200 gal1). Yeast strains YH138 and YH139 were derived from YH82 (MATa ura3-52 trp1Δ65 leu2Δ1 his3Δ200 lys2-801) by transformation with a mutagenized pGTy1-H3-neo (pD109) plasmid that contains a TYA–TYB in-frame fusion and either YEp351 or pX114. Other strains are derivatives of YH82 containing pX3 and the various plasmids listed in Table 1.

Plasmid p49A, the original clone containing the tRNA gene on a 64-kilobase (kb) fragment, was isolated from a yeast genomic library constructed in YEp351 (14). Plasmid pX113 was derived from p49A by inserting the bacterial neo gene into the unique Mlu I site inside the tRNA gene; the neo gene is in the same transcriptional orientation as the tRNA gene. Plasmids pX114 and pX116/pX117 were constructed by subcloning the 277-base-pair (bp) Kpn I–Nde I fragment from the genomic insert in p49A into the Sma I site of the polylinkers of plasmids YEp351 and the centromeric vector pRS315, which bear the LEU2 marker (15), respectively. In both pX114 and pX116, the tRNA gene is in the opposite transcriptional orientation as the lacZ gene. In pX117, the tRNA gene is in the same orientation as the lacZ gene.

Yeast media were prepared as described (16). The SC-Trp/Glu+FOA medium contained 5-fluoroorotic acid at 1 mg/ml (17).

Screening Procedure. Yeast cells from strain YH23 were first grown on SC-Ura/Glu plates for 2 days at 30°C. They were then resuspended in YPD medium at 10⁶ cells/ml and allowed to grow for 3–4 hr at 30°C before being harvested for transformation by the LiOAc procedure (18) with a yeast genomic library constructed in the LEU2-2μ vector YEp351, which was kindly provided by Janet Kurjan and Jeannie Hirsch (Columbia University). Typically 100–200 Leu+ Trp+ transformants were obtained on one plate by using about 2 μg of library DNA; ÷65% of these bore recombinant plasmids. Two days after transformation, 11,000 transformants were directly transferred to SC–Ura–Leu/Gal plates by replica plating. The galactose plates were incubated at 30°C for 2 days, an induction condition that limits transposition frequency by the marked Ty1 to an average of less than

Abbreviations: FOA, 5-fluoroorotic acid; LTR, long terminal repeat; ORF, open reading frame; VLP, virus-like particle.

*tRNA is sometimes referred to by the cDNA it recognizes, AGG. All tRNAs in this paper are referred to by their anticodons.
one transposition event per 10 cells in this strain. Then the cells were transferred sequentially to YPD and SC-Trp/Glu+FOA plates by further replica plating and then allowed to grow for <24 hr on each kind of medium. Subsequently, the colonies on SC-Trp/Glu+FOA were visually inspected for defects in growth. Cells corresponding to these colonies were recovered from the original transformation plates and subjected to further analyses. First, integrants of the pGTy1 plasmids, which also fail to grow on SC-Trp/Glu+FOA plates, were detected by their inability to grow on SC+Trp/Glu+FOA plates and they were discarded from the collection. Second, the LEU2 plasmids bearing genomic DNAs were recovered from the remaining colonies and reintroduced into YH23 for another round of screening. The LEU2 plasmids that survived the above procedure were studied further by (i) plasmid segregation tests, to determine whether the altered transposition phenotype cosegregated with the plasmid; (ii) quantitative transposition assays; (iii) restriction mapping and plasmid DNA blots (hybridized with inserts from the various clones) to group the plasmids; (iv) immunoblotting with antibodies against TYA and TYB proteins; and (v) DNA sequencing.

Quantitative Transposition Assays. Quantitative transposition assays were carried out essentially as described (19) with slight modifications. Briefly, patches of yeast cells from the strains to be analyzed were first grown for 2 days on SC–Ura–Trp–Leu/Glu plates (the temperature for growing yeast cells was maintained at 30°C throughout the assays). Then the cells were transferred to SC–Ura–Leu/Gal plates by replica plating. After the cells had been induced for 2 days, they were resuspended in water and plated out at low density on SC–Ura–Trp–Leu/Glu plates. The plates were incubated for 2 days. Single colonies were individually picked to YPD plates and the cells were allowed to grow overnight. Subsequently, the colonies were transferred to SC/Glu+FOA plates by replica plating to segregate the pGTy-H3–TRPI plasmids. Twenty-four hours later, the colonies were transferred to SC-Trp/Glu plates by replica plating and their phenotypes were scored after overnight incubation at 30°C. Transposition frequency was defined as the number of Trp"Ura" colonies divided by the total number of Ura" (FOAΔ) colonies.

Protein Ex extractions and Immunoblot Analyses. Yeast total protein extracts were prepared as follows. Cells from strains to be studied were first grown on SC–Trp–Ura–Leu/Glu plates for 2 days at 30°C. Then they were resuspended in 5 ml of SC–Ura–Leu 1%/2 rifamine medium at 2 x 10^7 cells per ml and grown for 3 hr with shaking at 30°C. Galactose was added to a final concentration of 2% and the cultures were continued for 6 hr under the same conditions. Subsequently, the cells were collected by centrifugation in Eppendorf tubes and washed once with water and once with buffer B (15 mM KCl, 5 mM EDTA/10 mM Hepes-KOH, pH 7.8) plus 3 mM dithiothreitol. Lysis of the cells was accomplished by vigorous vortex mixing of the cells resuspended in 125 μl of ice-cold buffer B/3 mM dithiothreitol/2 mM phenylmethylsulfonyl fluoride with 0.45- to 0.5-mm-diameter glass beads at 4°C for 5 min. The lysate was immediately mixed with 150 μl of 2x SDS/PAGE sample buffer [10% (vol/vol) 2-mercaptoethanol/5% (wt/vol) SDS/125 mM Tris CI, pH 6.8/20% (vol/vol) glycerol] and boiled for 5 min. The supernatant from a 15-min spin in a microcentrifuge was used for electrophoresis. Normal, 10-15 μl of the total protein extract was used for each analysis.

Immunoblotting was carried out as described (11). For detection of TYA proteins, anti-Ty1 virus-like particle (VLP) antibodies (11) were used, and for TYB proteins, anti-TyB2 antibodies (6). Ten-percent gels were used for TYA protein blots and 7.5% gels were used for TYB protein blots. The transfer of the total proteins from the gel to the nitrocellulose filter was complete as judged from complete transfer of prestained molecular weight standard of similar size to the Ty1 proteins to be analyzed.

DNA Manipulations. Plasmid constructions were carried out as described (20). DNA sequencing was performed using a Sequenase kit from United States Biochemical on the single-stranded DNA rescued from plasmid pX116.

RESULTS
Identification of Genes That Reduce Ty1 Transposition Frequency When Overexpressed. Many genes have been identified by their effects on transposition from the Ty1 LTR promoter (reviewed in ref. 21). However, very little is known about the regulation of transposition posttranscriptionally. We designed a genetic screening scheme that made use of the pGTy1-H3 plasmid (3). In this system, the transposition of the Ty1-H3 element is under the control of the GALI promoter so that cellular transcriptional controls over the Ty1 LTR promoter are bypassed and transposition can be easily induced by switching the carbon source of the medium from glucose to galactose. Furthermore, the Ty1 element is marked with a gene (TRPI) that can be followed by simple phenotypic assays (22, 23). In this system, a 10-fold difference in transposition frequency can be very easily distinguished. The screening process is summarized in Fig. 1. Briefly, a yeast strain (YH23) containing the pGTy1-H3–TRPI plasmid was transformed with a 2-μm-plasmid (2μ)-based genomic library bearing the LEU2 selectable marker. After a short induction on selective galactose-containing medium, the transposition phenotypes of the transformants were assayed by a simple replica-plating technique (19). The LEU2 plasmids, bearing segments of genomic DNA, were then isolated from the transposition-deficient transformants and again introduced into the parent strain (YH23) to retest.

![Fig. 1. Screening procedure to identify yeast genes whose products inhibit Ty1 transposition. Step 1. Transform YH23 with LEU2-2μ genomic library plasmids (phenotypes: Ura"Trp" due to presence of pGTy1-H3–TRPI; Leu" due to the genomic clones). Step 2. Induce transposition on galactose medium selective for both plasmids. Step 3. Segregate the plasmids on rich medium. Step 4. Analyse transposition of the TRPI marker from pGTy1-H3–TRPI plasmid into the genome by examining the growth of the cells on SC–Trp/Glu+FOA medium (phenotypes of the cells that were able to grow: Trp" due to transposition, Ura"/FOAΔ due to loss of the pGTy1-H3–TRPI plasmids; Leu" or Leu" depending on the genomic clones that were retained). Step 5. Recover the LEU2 plasmids from colonies that may have a defect in transposition and repeat steps 1–4. For details, see Materials and Methods.](image-url)
their ability to inhibit Ty1 transposition. Only clones that survived this second round of screening were studied further.

At this point, the transposition defect of each secondary transformant was tested quantitatively (19). Only those that had a transposition frequency at least 2-fold lower than that of the control strain carrying the LEU-2-µ vector were kept. Eight independent genomic clones were obtained after we had searched through enough transformants to cover the genome once with at least 80% certainty. They fell into three nonoverlapping groups. Two of these groups consisted of a single isolate; one of these is described in greater detail below (the other groups of genes will be discussed in detail elsewhere).

Identification of tRNA\textsuperscript{AGG} as an Inhibitor of Ty1 Transposition. One of the clones we obtained after the screening described above contains an insert of 4 kb of yeast genomic DNA that is very similar, by restriction mapping, to the tRNA\textsuperscript{AGG} locus (previously termed AGG-tRNA\textsuperscript{AGG}; ref. 12) (Fig. 2). The sequence of a 277-bp Kpn I–Nde I fragment contained within this clone was determined and is completely identical to that of the tRNA\textsuperscript{AGG} gene and its flanking regions (12, 24). To confirm that the transposition-inhibiting effect of the original plasmid was due to the tRNA\textsuperscript{AGG} gene, we subcloned this 277-bp restriction fragment into the LEU2-2µ vector used to construct the library. The resulting plasmid (pX114) retained full inhibitory activity (Table 1, compare strains YH109 and YH113). In addition, insertion of the bacterial neo gene into the Mlu I site of the tRNA\textsuperscript{AGG} gene contained within the original clone largely abolished the inhibitory effect of the original clone on Ty1 transposition frequency (Table 1, strain YH112). Furthermore, transposition frequency was decreased even when just one extra copy of the tRNA gene was introduced on a centromeric plasmid (Table 1, compare strain YH118 to strains YH116 and YH117). These results suggest that Ty1 transposition frequency is sensitive to the abundance of tRNA\textsuperscript{AGG}.

Overexpression of tRNA\textsuperscript{AGG} Reduces TyB Protein Production. To determine the molecular mechanism by which Ty1 transposition was inhibited by extra copies of the tRNA\textsuperscript{AGG} gene, we examined the relationship between the abundance of the tRNA and Ty1-encoded proteins. Northern hybridization analysis of total cellular RNA showed that the level of tRNA\textsuperscript{AGG} was significantly increased in strains bearing extra copies of the tRNA gene on a µ plasmid (data not shown). We then used immunoblotting to analyze the Ty1 proteins produced in the presence of multicopy plasmids carrying the tRNA\textsuperscript{AGG} gene, the disrupted tRNA\textsuperscript{AGG} gene, or the vector alone. The amount of the TyA proteins, which are analogous to retroviral gag-encoded proteins, was very similar in all of the strains examined (Fig. 3a), suggesting that the level of Ty1 mRNA is not affected by the abundance of this normally rare tRNA. In contrast, the amount of TyB proteins, which are similar to retroviral pol products, was greatly reduced in strains carrying extra copies of the wild-type tRNA gene (Fig. 3b, lanes 2 and 3) compared to strains containing the vector alone (lane 1) or the disrupted version of the tRNA gene (lane 4). From the immunoblot analyses, it is also apparent that the processing of the Ty1 proteins by the Ty-encoding protease was defective in transformants bearing the wild-type tRNA gene. Specifically, TyA proteins largely accumulated in the form of pTYA-58, the precursor, rather than the processed pTYA-54 form; TyB proteins accumulated in the form of pTYA/TYB-190 (precursor form), which could be detected upon prolonged exposure of the blot, rather than pTYB-90 (processed form) (Fig. 3b and data not shown). Since the Ty1 protease responsible for this processing event is encoded in TyB (4–6), its production is also necessarily decreased when overall production of TyB proteins is reduced. Consistent with the results of the quantitative transposition analyses (Table 1), an obvious decrease in the amount of TyB proteins synthesized and a partially defective processing of TyA proteins were also observed in strains containing just one extra copy of the tRNA gene on a centromeric plasmid (data not shown).

Table 1. Overexpression of tRNA\textsuperscript{AGG} inhibits Ty1 transposition

<table>
<thead>
<tr>
<th>Strain*</th>
<th>Plasmid</th>
<th>Transposition frequency†</th>
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<tr>
<td>YH107</td>
<td>Yep351, 2µ-based, no insert</td>
<td>14.3% (18/126)</td>
</tr>
<tr>
<td>YH109</td>
<td>p49A, 4-kb genomic insert containing tRNA\textsuperscript{AGG} in Yep351</td>
<td>0.8% (1/120)</td>
</tr>
<tr>
<td>YH113</td>
<td>pX114, 0.3-kb genomic insert containing tRNA\textsuperscript{AGG} in Yep351</td>
<td>0.8% (1/119)</td>
</tr>
<tr>
<td>YH112</td>
<td>pX113, 4-kb genomic insert containing tRNA\textsuperscript{AGG}, disrupted by neo in Yep351</td>
<td>8.5% (9/106)</td>
</tr>
<tr>
<td>YH118</td>
<td>pRS315, centromeric, no insert</td>
<td>8.1% (20/247)</td>
</tr>
<tr>
<td>YH116</td>
<td>pX116, 0.3-kb genomic insert containing tRNA\textsuperscript{AGG} in pRS315</td>
<td>4.3% (11/258)</td>
</tr>
<tr>
<td>YH117</td>
<td>pX117, 0.3-kb genomic insert containing tRNA\textsuperscript{AGG} in pRS315</td>
<td>4.4% (11/250)</td>
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*All the strains listed here were derivatives of YH82 (MATa ura3-52 trplΔ63 leu2Δ1 his4-539 lys2-801). They also contained the plasmid pX3 (pGTY1-H3-TRP1).
†Transposition frequency was defined as the fraction of the cells that became Trp\textsuperscript{+} after galactose induction and segregation of the pGTY1-H3-TRP1 plasmid.
from that of TYB proteins produced from a wild-type Ty1 element (compare Fig. 3b and Fig. 4). In the strains carrying the mutant Ty1, the major form of TYB proteins has an apparent molecular mass ≈160 kDa; and the mature pTYB-90 integrase protein was absent. This is probably due to the fact that in this mutant Ty1, the ratio of TYA to TYB proteins is grossly altered and the assembly of TY1 VLPs is apparently defective (D. Moore and J.D.B., unpublished data).

**DISCUSSION**

In this paper, we describe the identification of a host gene, tRNA\(^{\text{Au}}\), that affects Ty1 transposition frequency posttranscriptionally. We found that the abundance of the tRNA is one of the critical elements in determining Ty1 transposition frequency. When the copy number of the tRNA gene was artificially increased, Ty1 transposition frequency decreased significantly. The lower transposition frequency was directly correlated with reduced production of TYB proteins. The decrease in TYB protein levels could be due to decreased synthesis or increased degradation of TYB proteins. However, since the introduction of multiple copies of the tRNA gene had no effect on the amount of TYA–TYB fusion proteins synthesized from a mutant Ty1 element in which TYA and TYB ORFs were fused in frame, we conclude that the turnover rate of TYB proteins is not affected by the level of tRNA\(^{\text{Au}}\) in the cells.

Disruption of the tRNA gene on the high-copy plasmid reduced its ability to inhibit transposition, as expected. However, the reduction of inhibition was incomplete; transposition frequency was still lower than in the presence of the vector alone. It is possible that the disruption does not create a null allele and that a partially functional tRNA fragment is produced in these cells that can still inhibit frameshifting slightly. In support of this possibility, the amount of TYB protein produced is also slightly reduced in the disruption strain (Fig. 3b, compare lanes 1 and 4).

The expression of the TYB ORF poses an interesting problem from several points of view. Ty1 elements are transcribed predominantly as a single, full-length mRNA species. Normally, only the first AUG codon is used as the site of translation initiation in yeast (25). Moreover, TYB does not contain an AUG codon until 929 bp into the ORF (7, 26, 27). There is no evidence to suggest that Ty1 RNA is spliced or edited at a low frequency to allow for TYB expression in these ways (9). Therefore, Ty1 elements must use another means—namely, translational frameshifting—to produce the TYB proteins. Second, since TYB gene products are essential for transposition, it seems likely that the expression of TYB proteins can regulate Ty1 transposition frequency. The data presented here show that the abundance of tRNA\(^{\text{Au}}\) regulates transposition, apparently by regulating the efficiency of TYA–TYB frameshifting. Our results show that production of TYB protein is specifically repressed when the tRNA gene is present in high copy number, providing direct support for a proposed model for the mechanism of TYA–TYB frameshifting (13). According to this model, when translating ribosomes encounter the codons CUU-AGG (encoding Leu-Arg) near the end of the TYA ORF (where it overlaps with TYB), tRNA\(^{\text{Au}}\), which can translate all six leucine codons, recognizes the CUU codon and is then transferred to the ribosomal peptidyl (P) site. The low availability of tRNA\(^{\text{Au}}\), which would normally (in the absence of a frameshift) bind to the aminoacyl (A) site, causes a translational pause. During the pause, the tRNA\(^{\text{Au}}\) bound at the P site has an opportunity to slip and become paired to the UUA codon in the +1 frame. Subsequently, a presumably abundant tRNA\(^{\text{Gly}}\) can be bound in the A site and the ribosomal frameshifting event is locked in. Our work confirms the prediction of this model that the higher the level of
tRNA\textsubscript{ACU}, the lower the production of TYB proteins. Significantly, this is the case within the context of the Ty1 sequences themselves, with the predicted effects on Ty1 protein expression and Ty1 transposition frequency.

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