Translational suppressors and antisuppressors alter the efficiency of the Ty1 programmed translational frameshift

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ABSTRACT

Certain viruses, transposons, and cellular genes have evolved specific sequences that induce high levels of specific translational errors. Such “programmed misreading” can result in levels of frameshifting or nonsense codon read-through that are up to 1,000-fold higher than normal. Here we determine how a number of mutations in yeast affect the programmed misreading used by the yeast Ty1 retrotransposons. These mutations have previously been shown to affect the general accuracy of translational termination. We find that among four nonsense suppressor ribosomal mutations tested, one (a ribosomal protein mutation) enhanced the efficiency of the Ty1 frameshifting, another (an rRNA mutation) reduced frameshifting, and two others (another ribosomal protein mutation and another rRNA mutation) had no effect. Three antisuppressor rRNA mutations all reduced Ty1 frameshifting; however the antisuppressor mutation in the ribosomal protein did not show any effect. Among nonribosomal mutations, the allosuppressor protein phosphatase mutation enhanced Ty1 frameshifting, whereas the partially inactive prion form of the release factor-2 is produced via a +1 programmed frameshift after codon 25 (Craigen & Caskey, 1986), whereas ribosomes make a 50-nt hop during translation of bacteriophage T4 gene 60 (Huang et al., 1988; Weiss et al., 1990). A large number of eukaryotic viruses use programmed misreading to control the amounts of catalytic and structural proteins that are translated from the same mRNA species. Many retroviruses fall into this group and utilize a −1 programmed frameshift or programmed readthrough (suppression) of an in-frame stop codon in the translation of the pol proteins. Retroviruses that frameshift include HIV (Jacks et al., 1988), mouse mammary tumor virus (Jacks et al., 1987), Rous sarcoma virus (Jacks & Varmus, 1985), and feline immunodeficiency virus (Morikawa et al., 1991). In addition, the yeast L-A dsRNA virus uses a −1 programmed frameshift for the synthesis of its GAG-POL fusion protein (for review, see Wickner, 1992).

The Saccharomyces cerevisiae Ty retrotransposons require a +1 programmed frameshift for the translation of their POL proteins. Furthermore, conditions or mutations that alter the relative levels of translation of the Ty1-encoded TYA (analogous to retroviral gag) and TYA-TYB (analogous to retroviral gag-pol fusion) proteins have been shown to interfere with transposition (Farabaugh, 1995). The cis elements of the frameshifting systems of the yeast Ty1 and Ty3 retrotransposons

INTRODUCTION

High levels of translational misreading in response to specific mRNA encoded sequences (programmed misreading) have been described in both prokaryotes and eukaryotes (for reviews, see Farabaugh, 1996; Gesto-
have been studied (Clare et al., 1988; Belcourt & Farabaugh, 1990; Farabaugh et al., 1993; Kawakami et al., 1993), and both shift at a 7-nt site. Slippage occurs when a translational pause is induced by a rare codon in the A-site of the ribosome. It is because the codon is recognized by a rare tRNA that the A-site may remain empty long enough for this shift to occur. The two systems differ slightly; each uses a different codon to induce pausing, and there is slippage of the peptidyl-tRNA in the P-site of the Tyl system, but no slippage in the Tyl3 system (the first frameshifted peptidyl-tRNA just binds in the new frame). The identity of the tRNAs that are involved in these frameshifting events is important, as only some tRNAs allow high-level frameshifting to occur (Vimaladithan & Farabaugh, 1994; Pande et al., 1995).

Mutations that affect translational accuracy might be expected to influence Tyl frameshifting. Ribosomal protein alterations could affect codon recognition, the configuration of the A- and/or P-sites, or the ability of other factors, such as release factor, to bind to the ribosome. In fact, mutant alleles of some translation factors, for example, EF-1α and SUP35, suppress certain frameshift mutations (Culbertson et al., 1982; Sandbaken & Culbertson, 1988). EF-1α mutations also affect programmed −1 L-A or +1 Tyl frameshifting (Dimman & Kinzy, 1997) and frameshifting at the minimal Ty3 retrotransposon frameshift site (Farabaugh & Vimaladithan, 1998). In contrast, several mutants that enhance translational misreading, SUP42, SUP43, SUP44, SUP45, SUP46, and [PSI+], had no effect on the efficiency of the −1 frameshift associated with translation of the S. cerevisiae L-A dsRNA virus (Dimman & Wickner, 1994).

The RNA components of the ribosome have also been shown to play important roles in translational accuracy. Ribosomal RNA (rRNA) can catalyze the peptidyl transferase reaction, even when most of the ribosomal proteins have been stripped away (Noller et al., 1992; Noller, 1993) and it is also a primary target of translational antibiotics (Cundliffe, 1999). Mutations in the yeast and E. coli large and small rRNAs have been shown to have activities that either enhance translational misreading (suppressors; Murgola et al., 1988; O’Connor et al., 1992; Chernoff et al., 1996; Liu & Liebman, 1996; Pagel et al., 1997; Arkov et al., 1998) or increase translational accuracy (antisuppressors; Melançon et al., 1992; Chernoff et al., 1994, 1996). In addition, 5S rRNA has been shown to influence the efficiency of the +1 Tyl and the −1 L-A programmed frameshifts (Dimman & Wickner, 1995).

In the studies described here we have examined the effects of both suppressor and antisuppressor mutations as well as a mutation that enhances the efficiency of several suppressor mutations (an allosuppressor) on Tyl frameshifting. These mutations occur in a number of different components of the translational apparatus, from ribosomal proteins to ribosomal RNA and release factors. Surprisingly, we find that not all translational suppressors affect Tyl frameshifting in the same manner. Rather, some translational suppressors and an allosuppressor increase Tyl frameshifting whereas others decrease it. Likewise, antisuppressors have either no effect or cause a decrease in Tyl frameshifting.

RESULTS AND DISCUSSION

Ribosomal protein suppressor and antisuppressor mutations affect Tyl frameshifting

The yeast SUP44 and RPS28 genes respectively encode ribosomal small subunit proteins S4 and S28 (Eustice et al., 1986; Alksne & Warner, 1993). S4 and S28 are equivalent to the E. coli S5 and S12 ribosomal proteins (All-Robyn et al., 1990b; Alksne & Warner, 1993). In E. coli, the S5, S4, and S12 proteins and the 16S rRNA are proposed to be involved in the proofreading of incoming aminoacyl-tRNAs (Anderson et al., 1986; Allen & Noller, 1989). The E. coli S4, S5, and S12 proteins have been localized to a cluster on the opposite side of the 30S subunit from the site of the codon–anticodon interaction (Oakes et al., 1986; Stoeflfer & Stoeflfer-Meilicke, 1986; Capel et al., 1987). Recent studies in yeast, using in vitro translation, have confirmed the role of S28 and S4 in optimizing translational accuracy (Synetos et al., 1996).

We have assessed Tyl frameshifting in strains carrying a SUP44 dominant omnipotent suppressor mutation (All-Robyn et al., 1990a, 1990b). Omnipotent suppressors are capable of misreading a broad range of codons. Strains carrying either the rps28-5 suppressor mutation (Anthony & Liebman, 1995) or the rps28-12 antisuppressor mutation (Anthony & Liebman, 1995) were also tested. Tyl frameshifting increased more than twofold in the SUP44 strain (Table 1), whereas no significant changes in frameshifting were seen in either the rps28-5 suppressor mutant or the rps28-12 antisuppressor mutant (Table 1).

Suppressor and antisuppressor mutations in ribosomal RNA affect Tyl programmed frameshifting

The RDN locus consists of tandem repeats of the 9-kb yeast rDNA unit. This 9-kb region, in which the coding regions for all four rRNAs are located, is repeated ~100–200 times in the RDN locus on chromosome XII. Suppressor and antisuppressor mutations in 18S rRNA have been previously characterized (Chernoff et al., 1994, 1996). Using the E. coli numbering system, these mutations change nt 1054 of the 18S rRNA from a C to a T (rdn-1T), causing antisuppression, or a C to an A (rdn-1A), causing suppression (Chernoff et al., 1996).
Antisuppression was also caused by changes of nt 517 from a G to an A (rdn-2; Chernoff et al., 1994), or nt 912 from a U to a C (rdn-4; Chernoff et al., 1994). These mutations are located in rRNA regions that are functionally important in E. coli translation (Murgola, 1996).

The rdn-2 antisuppressor decreased Ty1 programmed frameshifting about 20-fold (Table 1) and the rdn-1T and rdn-4 antisuppressors decreased Ty1 frameshifting approximately sevenfold (Table 1). The rdn-1A mutation did not decrease Ty1 frameshifting (Table 1), but it did decrease Ty3 frameshifting approximately threefold (Table 1). Because the rdn-2 and rdn-4 mutations do not appear to affect Ty3 frameshifting (Table 2), whereas the rdn-1T and rdn-1A mutations do decrease frameshifting in Ty3 (Table 2), rdn-2 and rdn-4 appear to be involved in some part of the frameshifting process that differs between Ty1 and Ty3. Correspondingly, the rdn-1A mutation could affect a Ty3-specific part of the process.

Because the rdn-1T and rdn-4 antisuppressors decreased Ty1 frameshifting, we asked if they altered the Ty1 transposition rates using the HIS3AI transposition assay (Curcio & Garfinkel, 1991; see Materials and Methods). We did not examine the effect of rdn-2 on Ty1 transposition due to the poor growth associated with this mutation. Transposition was induced in 40 same-size patches of transformants containing rdn-1T, rdn-4, or the control wild-type RDN plasmid. When transposition was induced at 30 °C, the average number of His + colonies (His + colonies are indicative of a transposition event) was about 50 per patch regardless of the RDN plasmid present. When transposition was induced at 20 °C, the number of His + colonies per patch was too high to count, but again no differences due to the rdn-1T or rdn-4 mutations were detected.

The rdn-5 suppressor mutation is located in the sarcin/ricin domain of the rDNA 25S rRNA (Liu & Liebman, 1993). This domain is universally conserved and is composed of a stem and loop with a GAGA tetraloop (Szewczak et al., 1993; Gluck et al., 1994). The sarcin/ricin domain has been suggested to have important functions in translation and to interact with E. coli elongation factors (Moazed & Noller, 1988). It is believed that conformational changes involving the GAGA tetraloop and its putative closing GC pair occur during the elongation cycle (Wool et al., 1992). The yeast rdn-5 mutation tested here changes the wild-type CG closing pair of the tetraloop to a UG pair (Liu & Liebman, 1996). The rdn-5 mutation decreased Ty1 frameshifting about fivefold (Table 1).

**The effects of nonribosomal protein suppressors and allosuppressors on Ty programmed frameshifting**

Another translational accuracy mutation that had an effect on Ty1 and Ty3 frameshifting was sal6-1. The sal6-1 mutation was originally identified as an allosuppressor that enhanced the efficiency of sup35 and sup45 suppressor mutations (Song & Liebman, 1987) and was cloned by complementation (Vincent et al., 1994). A disruption in SAL6 (also called PPQ1) was later shown to have a reduced rate of protein synthesis (Chen et al., 1993). The cloned SAL6 gene has homology to type 1 serine/threonine protein phosphatases (Chen et al.,

### TABLE 1. Effects of mutations on Ty1 programmed frameshifting.

<table>
<thead>
<tr>
<th>Strain/mutation</th>
<th>Translational affect</th>
<th>Ty1 frameshifting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1354 wt</td>
<td>NA</td>
<td>21 ± 5</td>
</tr>
<tr>
<td>L1354 SUP44</td>
<td>suppressor</td>
<td>46.5 ± 10</td>
</tr>
<tr>
<td>SL1000-1A wt</td>
<td>NA</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>SL1000-1A rps28-5</td>
<td>suppressor</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>SL1000-1A rps28-12</td>
<td>antisuppressor</td>
<td>30 ± 2.5</td>
</tr>
<tr>
<td>GF432</td>
<td>NA</td>
<td>23 ± 12</td>
</tr>
<tr>
<td>L1491 wt</td>
<td>NA</td>
<td>29 ± 12</td>
</tr>
<tr>
<td>L1491 rdn-1T</td>
<td>antisuppressor</td>
<td>3.9 ± 1.6</td>
</tr>
<tr>
<td>L1491 rdn-2</td>
<td>antisuppressor</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>L1491 rdn-4</td>
<td>antisuppressor</td>
<td>3.9 ± 0.7</td>
</tr>
<tr>
<td>L1491 rdn-1A</td>
<td>suppressor</td>
<td>55 ± 21</td>
</tr>
<tr>
<td>L1494 wt</td>
<td>NA</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>L1494 rdn-5</td>
<td>suppressor</td>
<td>6.6 ± 4</td>
</tr>
<tr>
<td>GF-275 [PSI’]</td>
<td>suppressor</td>
<td>21 ± 4</td>
</tr>
<tr>
<td>GF-275 [PSI’] [-L1384]</td>
<td>NA</td>
<td>29 ± 6</td>
</tr>
<tr>
<td>33-G [PSI’]</td>
<td>suppressor</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>33-G [psi’]</td>
<td>NA</td>
<td>31.5 ± 13</td>
</tr>
<tr>
<td>L-1489 [PSI’]</td>
<td>suppressor</td>
<td>18 ± 6</td>
</tr>
<tr>
<td>L-1489 [psi’]</td>
<td>NA</td>
<td>30 ± 13</td>
</tr>
<tr>
<td>74-D694 [PSI’]</td>
<td>suppressor</td>
<td>20 ± 10</td>
</tr>
<tr>
<td>74-D694 [psi’]</td>
<td>NA</td>
<td>28 ± 9</td>
</tr>
<tr>
<td>74-D694 [psi’] b</td>
<td>NA</td>
<td>23 ± 6</td>
</tr>
<tr>
<td>74-D694 [psi’] b sup45</td>
<td>suppressor</td>
<td>34 ± 19</td>
</tr>
</tbody>
</table>

Measurements were performed on triplicates of a minimum of three independent transformants of each construct.

*NA: not applicable.

a These data are from an experiment performed at a different time than the other 74-D694 measurements in the table.

b Statistical significance was determined by ANOVA analysis ($p < 0.05$).

### TABLE 2. Effects of rDNA antisuppressor mutations on Ty3 frameshifting.

<table>
<thead>
<tr>
<th>Strain/mutation</th>
<th>Translational affect</th>
<th>Ty3 frameshifting (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1491 wt</td>
<td>NA</td>
<td>28 ± 7.5</td>
</tr>
<tr>
<td>L1491 rdn-1T</td>
<td>antisuppressor</td>
<td>14 ± 3.8</td>
</tr>
<tr>
<td>L1491 rdn-2</td>
<td>antisuppressor</td>
<td>25.5 ± 3.3</td>
</tr>
<tr>
<td>L1491 rdn-4</td>
<td>antisuppressor</td>
<td>31 ± 5.6</td>
</tr>
<tr>
<td>L1491 wt b</td>
<td>NA</td>
<td>30 ± 7</td>
</tr>
<tr>
<td>L1491 rdn-1A b</td>
<td>suppressor</td>
<td>11 ± 5</td>
</tr>
</tbody>
</table>

Measurements performed on triplicates of a minimum of three independent transformants of each construct.

*NA: not applicable.

b These data are from an experiment performed at a different time than the others in the table.
The protein also contains an N-terminal, serine-rich region that has no significant homology to other proteins in the database. It has been postulated that the accuracy of protein synthesis is affected by the levels of phosphorylation of a target(s) of SAL6 (Vincent et al., 1994). In the Ty1 frameshifting assays, the sal6-1 mutation increased frameshifting almost twofold.

We have also assayed [PSI+] strains to determine if this factor influences programmed frameshifting. The yeast [PSI+] factor was originally described as an allo-suppressor (for review, see Cox, 1994), but it is now clear that it also has omnipotent suppressor activity of its own (Lieberman & Sherman, 1979). Evidence from numerous reports supports the hypothesis (Wickner et al., 1995) that the [PSI+] factor is a self-propagating alternative conformation (for reviews, see Tuite & Lindquist, 1996; Lieberman & Derkatch, 1999; Wickner & Chernoff, 1999; Wickner et al., 1999) of the translational release factor eRF3, encoded by the SUP35 gene (Stansfield et al., 1995; Zhouravleva et al., 1995).

We examined the effects of [PSI+] on Ty1 programmed frameshifting, in four independent sets of strains. In all cases, programmed frameshifting occurred at lower levels in the [PSI+] strain as compared with its isogenic [psi-] strain. Although the trend in the data is clear, the amount of the difference is quite small and within the experimental error. This limits our confidence that the [PSI+] factor actually affects programmed frameshifting at the Ty1 sites. This is perhaps not surprising because, as an altered form of a translational release factor, [PSI+] was not expected to have a general effect on translation elongation, but only on termination. Repeated attempts to measure the effects of a suppressor mutation in SUP35 failed to give reproducible results because of a high variation among transformants. We attribute this to the poor growth caused by the sup35 mutation, which may lead to the frequent accumulation of faster-growing modifiers with altered translational accuracy that take over the population. Ty1 frameshifting was tested in a strain with a mutation in the SUP45 gene, which encodes the translational release factor 1 (eRF1) that interacts with eRF3 (Stansfield et al., 1995; Zhouravleva et al., 1995), and no significant effect on Ty1 frameshifting was detected (Table 1).

How do the effects of translational accuracy mutations on suppression of nonsense codons correlate with their effects on Ty1 frameshifting?

Ty1 frameshifting has been shown to depend on the presence of tRNA-Leu in the P-site that is capable of slipping into the +1 frame (Belcourt & Farabaugh, 1990). This slippage occurs when there is a translational pause and the A-site remains empty because the codon in the A-site is recognized by a rare tRNA (Fig. 1).

Translational accuracy mutations of several types might be expected to affect Ty1 frameshifting. Elongation rates in the presence of antisuppressors have been measured in some cases and were shown to be slower than in the wild type (Andersson et al., 1986; Ehrenberg et al., 1986; Bilgin et al., 1988). In contrast, in some instances the slower rate of translation in an antisuppressor has been seen to increase in the presence of a suppressor (Andersson et al., 1986). Because of the importance of a translational pause in Ty1 frameshifting, a prediction is that suppressors—which speed up translation—would reduce Ty1 frameshifting. Using this same logic, antisuppressors should increase Ty1 frameshifting because they slow translation. Although some of our results support this hypothesis, others do not (see Table 3). Some of the exceptions are mutations that have no effect, like the rps28 mutants on Ty1 and the rdn-2 and rdn-4 mutants on Ty3. More significant exceptions are rdn-5 on Ty1 and rdn-1A on Ty3, which both have effects that are opposite to that predicted by the model.

There are several types of ribosomal alterations that could cause nonsense codon suppression. The simplest type may arise because mutant ribosomes cannot bind release factor as well as wild-type ribosomes. If this were the only change in the ribosomal activity, Ty frameshifting should not be affected. The rps28 suppressor could be of this type.

Other ribosomal alterations may cause a reduced affinity for release factor, while at the same time enhancing the ability of noncognate tRNA to decode codons. Such alterations would promote noncognate decoding of the rare AGG at the Ty1 slip site, thus reducing the translational pause and the associated frameshift. The reduction in Ty1 frameshifting associated with the rdn-5 large subunit rRNA suppressor mutation can be explained in this way. As rdn-5 does not affect Ty3 frameshifting, which requires another tRNA for pausing, different tRNAs may vary in the specificity of their interactions with ribosomal components.

Some ribosomal alterations may affect the conformation of the A-site so that both release factor and cognate tRNA binding is impaired. Such alterations should cause ribosomes to pause at stop codons long enough for a noncognate tRNA to bind. If noncognate tRNA...
binding was not adversely affected, or proofreading was adversely affected, by the changes in the A-site, these tRNAs may be able to effectively compete with the release factor during mistranslation of nonsense codons. Likewise, the pause at the AGG site would be lengthened because the cognate tRNA binding is impaired due to competition with noncognate tRNA (which is eventually rejected by the ribosome) providing additional time for the frameshift to occur. The small subunit ribosomal protein nonsense suppressor mutation SUP44, which enhances Ty1 frameshifting, can be explained by this model.

Similar models can be invoked to predict the effects of antisuppressor mutations that cause ribosomes to efficiently terminate translation at stop codons. The simplest type of antisuppressor may arise because mutant ribosomes bind release factor better than wild-type ribosomes. If this is the only change in ribosomal activity, there should be no effect on Ty1 frameshifting. The rps28 antisuppressor could be of this type. Another type of antisuppressor could result from an alteration in the ribosomal A-site that makes it more accessible for binding both release factors and tRNAs. The antisuppressor activity might arise because release factor binding is enhanced more than the binding of miscognate tRNA at stop codons is enhanced, or because the efficiency of peptidyl-tRNA hydrolysis is affected. Such antisuppressor ribosomal alterations would enhance the binding of noncognate tRNA at the rare AGG Ty1 pause site, thereby reducing the pause time and the level of Ty1 frameshifting. If peptidyl-tRNA hydrolysis occurs more quickly in the antisuppressor strains, the pause time would also be reduced, and therefore the level of frameshifting. The results obtained for small subunit rRNA antisuppressor mutations rdn-1T, rdn-2, and rdn-4 can be explained by these scenarios.

Quite remarkably, we have observed different effects of some rRNA mutations on Ty1 and Ty3 frameshifting sites. For example, rdn-2 and rdn-4 antisuppressors inhibited frameshifting in Ty1 but not in Ty3, whereas the rdn-1A mutation inhibited frameshifting in Ty3 but not in Ty1, and the rdn-1T mutation inhibited frameshifting in both. These variations could be explained either by a differential specificity of interactions between various tRNAs and ribosomal components, or by a mechanism where some rRNA mutations affect the ribosomal “slippage” that is involved in Ty1 but not in Ty3 frameshifting. It is possible that rdn-2 and rdn-4 specifically affect the slippage step, or “Ty1-specific” ribosomal conformation. Further experiments are needed to test this hypothesis.

Finally, the effects of these mutations may also reflect variations in the ability to accept near-cognate tRNA. Frameshifting in Ty1 occurs when a near-cognate tRNA in the ribosomal P-site slips +1 during a translational pause (Belcourt & Farabaugh, 1990). Similarly, frameshifting in the Ty3 retrotranspon occurs when a near-cognate tRNA is present in the P-site, though in this case it induces out-of-frame binding of aminoacyl-tRNA (Farabaugh, 1996; S. Anuradha & P.J. Farabaugh, unpubl. results). Given the importance of errant near-cognate decoding, any mutations that increase or decrease near-cognate decoding relative to cognate decoding should increase or decrease frameshifting, respectively.

The fact that translational accuracy mutations alter the efficiency of programmed frameshifting suggests that retrotranspon and retroviral proliferation can be regulated at the translational level by the cellular ribosome apparatus. Surprisingly, we found that not all translational suppressors affect Ty1 frameshifting in the same manner. Some suppressors increase frameshifting, others decrease it, whereas still others have no effect. Likewise, some antisuppressors decrease Ty1 frameshifting whereas others have no effect. These differential effects suggest differences in the molecular mechanisms of each suppressor or antisuppressor.

### MATERIALS AND METHODS

**Frameshift reporter plasmids**

Plasmids carrying the minimal Ty1 and Ty3 frameshift sites fused to the E. coli lacZ gene were used to assay frameshifting. The plasmids were transformed into isogenic strains that were wild-type or mutant for the desired translational accuracy locus and therefore the level of frameshifting measured Ty1 and Ty3 frameshifting are based on the same vector as those for Ty1, with the Ty1 frameshift site replaced by the Ty3 site. The vector pMB38-Ty3 requires that a +1 frameshift take place for lacZ expression and the in-frame control is pMB38-Ty3FF (Farabaugh et al., 1993).

<table>
<thead>
<tr>
<th>Suppressors and antisuppressors</th>
<th>Effects* on Ty1</th>
<th>Effects on Ty3</th>
<th>Effects on Ty1</th>
<th>Effects on Ty3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SUP44</td>
<td>↑ ↑ NT</td>
<td>rps28-12 none</td>
<td>NT</td>
<td>none</td>
</tr>
<tr>
<td>sal6</td>
<td>↑ ↑ NT</td>
<td>rdn-1T ↓ ↓</td>
<td>↓ ↓</td>
<td>none</td>
</tr>
<tr>
<td>rps28-5</td>
<td>none ↑ NT</td>
<td>rdn-2 ↓</td>
<td>↓ none</td>
<td>none</td>
</tr>
<tr>
<td>rdn-1A</td>
<td>none or ↓ ↓</td>
<td>rdn-4 ↓ ↓</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>rdn-5</td>
<td>↓ ↓ NT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[PSI]+</td>
<td>none or ↓ ↓</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*↑ and ↓: an increase or decrease in Ty1 frameshifting, respectively. None: no effect on frameshifting was detected. NT: not tested.

**MATERIALS AND METHODS**

**Frameshift reporter plasmids**

Plasmids carrying the minimal Ty1 and Ty3 frameshift sites fused to the E. coli lacZ gene were used to assay frameshifting. The plasmids were transformed into isogenic strains that were wild-type or mutant for the desired translational accuracy locus and β-galactosidase activity levels were compared. Two plasmids for measuring Ty1 frameshifting were used. In one, a +1 frameshift in the Ty1 frameshift region is required for lacZ expression (pMB38-9merWT; Belcourt & Farabaugh, 1990). In another, lacZ is fused in frame to the Ty1 frameshift sequence (100% expression control; pMB38-9mer-fusion; Belcourt & Farabaugh, 1990). The plasmids for measuring Ty3 frameshifting are based on the same vector as those for Ty1, with the Ty1 frameshift site replaced by the Ty3 site. The vector pMB38-Ty3 requires that a +1 frameshift take place for lacZ expression and the in-frame control is pMB38-Ty3FF (Farabaugh et al., 1993).
**β-galactosidase assays**

β-galactosidase assays were performed using a chemiluminescent system (Jain & Magrath, 1991) with reagents purchased from Tropix, Inc. Three to five independent transformants of each plasmid were grown in liquid synthetic minimal medium (15–50 mL volumes; in a given experiment all cultures were the same volume) with the appropriate supplements and with dextrose as the carbon source. These transformants were first grown in precultures of 10 mL, overnight, and then diluted into the larger assay cultures to an OD₆₅₀ of 0.01. The assay cultures were grown to an OD₆₅₀ of ~1. Yeast cells were harvested at room temperature, resuspended in 20% glycerol, 0.1 M Tris-HCl, pH 8, 1 mM dithiothreitol (DTT), 2 mM phenyl-methyl-sulfonyl-fluoride (PMSF), at a ratio of 0.2 mL of buffer per 5 mL of cell culture, and permeabilized with 20 µL each of CHCl₃ and 10% sodium dodecyl sulfate (SDS) per 5 mL of culture. Each culture was assayed in triplicate. One to 20 µL of diluted or undiluted extract were added to 200 µL of reaction buffer [0.1 M sodium phosphate, pH ~7.0, 1 mM MgCl₂, 10 µg/mL AMPGD (Tropix, Inc., Bedford, Massachusetts)] and incubated at room temperature for 30 to 50 min. Within any given experiment, all tubes were incubated for the same length of time. The reactions were stopped with injections of 0.3 mL of 10% Emerald Enhancer (Tropix, Inc.) in 0.2 M NaOH at the time that readings were taken in a Turner TD-20e luminometer. The delay and integration settings on the luminometer were 5 and 5 s, respectively, or 5 s and 10 s, respectively. Within any given experiment the delay and integration settings were the same; comparisons of assays on the same extracts using the two different integration settings showed no effects on the activity determinations. The formula used to calculate activity in arbitrary units was: Z = P/ν(OD), where Z = β-galactosidase activity, P = luminometer reading in photons, ν = volume of extract measured (µL) and OD = OD of culture at 600 nm.

**Transposition assays**

Transposition frequency was examined using a previously described assay (Curcio & Garfinkel, 1991). Strains to be tested were transformed with plasmid pGTy1-H3mH153AI (kindly provided by M.J. Curcio and D. Garfinkel), which carries the HIS3-AI Ty element driven by a GAL promoter. The HIS3-AI Ty element is marked with a HIS3 gene. The HIS3 gene is inactivated by an intron in the antisense strand relative to HIS3, but the sense strand relative to Ty1. The antisense orientation of the HIS3-AI marker relative to the Ty1 element allows the intron to be spliced out of the Ty1, but not the HIS3AI, mRNA. Therefore the HIS3-AI gene is activated when the HIS3-AI-marked Ty1 element transposes. Individual transformants were streaked out on minimal medium lacking uracil, to select for the plasmid. Once grown, these plates were replica-plated to minimal medium lacking uracil and with galactose as a carbon source to induce transposition, and incubated for 2 days at either 20 or 30 °C. The plates were then replica-plated to minimal medium lacking histidine to detect transposition events, which were scored after a week of incubation. pGTy1-H3H153mAI was the negative control plasmid (kindly provided by M.J. Curcio and D. Garfinkel), where HIS3-AI is in the same orientation as the Ty1, but the intron is in the antisense orientation and is therefore unable to be spliced out.

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Mutations affecting Ty1 programmed frameshifting


