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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 readthrough 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 *Ty* 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 eRF3 caused a slight decrease, if any effect. A mutant form of the other release factor, eRF1, also had no effect on frameshifting. Our data suggest that *Ty* frameshifting is under the control of the cellular translational machinery. Surprisingly we find that translational suppressors can affect *Ty* frameshifting in either direction, whereas antisuppressors have either no effect or cause a decrease.

Keywords: programmed misreading; rDNA; retrotransposon; translational accuracy; yeast

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; Gesteland & Atkins, 1996). In prokaryotes, *Escherichia coli* release factor-2 is produced via a +1 frameshift after codon 25 (Craigen & Caskey, 1986), wheras 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

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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 *Ty1* system, but no slippage in the *Ty3* 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 *Ty* 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 $+1$ *Ty1* frameshifting (Dinman & 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 (Dinman & 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, 1990). 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 $+1$ *Ty1* and the -1 L-A programmed frameshifts (Dinman & 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 *Ty1* 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 *Ty* frameshifting in the same manner. Rather, some translational suppressors and an allosuppressor increase *Ty* frameshifting whereas others decrease it. Likewise, antisuppressors have either no effect or cause a decrease in *Ty* frameshifting.

RESULTS AND DISCUSSION

Ribosomal protein suppressor and antisuppressor mutations affect *Ty1* 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 (Andersson 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; Stoeffler & Stoeffler-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 *Ty1* 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. *Ty1* 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 *Ty* 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).

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

Strain/mutation	Translational affect	*Ty1* frameshifting (%)
L1354 wt	NA[a]	21 ± 5
L1354 *SUP44*	suppressor	46.5 ± 10
SL1000-1A wt	NA	25 ± 5
SL1000-1A *rps28-5*	suppressor	25 ± 4
SL1000-1A *rps28-12*	antisuppressor	30 ± 2.5
GF432	NA	23 ± 12
GF432 *sal6*	allosuppressor	43 ± 9[c]
L1491 wt	NA	29 ± 12
L1491 *rdn-1T*	antisuppressor	3.9 ± 1.6
L1491 *rdn-2*	antisuppressor	1.5 ± 0.7
L1491 *rdn-4*	antisuppressor	3.9 ± 0.7
L1491 *rdn-1A*	suppressor	55 ± 21
L1494 wt	NA	33 ± 4
L1494 *rdn-5*	suppressor	6.6 ± 4
GF-275 [*PSI*$^+$]	suppressor	21 ± 4
GF-275 [*psi*$^-$] [=L1384]	NA	29 ± 6
33-G [*PSI*$^+$]	suppressor	26 ± 5
33-G [*psi*$^-$]	NA	31.5 ± 13
L-1489 [*PSI*$^+$] [=L1609]	suppressor	18 ± 6
L-1489 [*psi*$^-$]	NA	30 ± 13
74-D694 [*PSI*$^+$]	suppressor	20 ± 10
74-D694 [*psi*$^-$]	NA	28 ± 9
74-D694 [*psi*$^-$][b]	NA	23 ± 6
74-D694 [*psi*$^-$][b] *sup45*	suppressor	34 ± 19

Measurements were performed on triplicates of a minimum of three independent transformants of each construct.
[a]NA: not applicable.
[b]These data are from an experiment performed at a different time than the other 74-D694 measurements in the table.
[c]Statistical significance was determined by ANOVA analysis ($p < 0.05$).

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

Strain/mutation	Translational affect	*Ty3* frameshifting (%)
L1491 wt	NA[a]	28 ± 7.5
L1491 *rdn-1T*	antisuppressor	14 ± 3.8
L1491 *rdn-2*	antisuppressor	25.5 ± 3.3
L1491 *rdn-4*	antisuppressor	31 ± 5.6
L1491 wt[b]	NA	30 ± 7
L1491 *rdn-1A*[b]	suppressor	11 ± 5

Measurements performed on triplicates of a minimum of three independent transformants of each construct.
[a]NA: not applicable.
[b]These data are from an experiment performed at a different time than the others in the table.

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 2). 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, 1996). 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.,

1993; Vincent et al., 1994). 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 allosuppressor (for review, see Cox, 1994), but it is now clear that it also has omnipotent suppressor activity of its own (Liebman & 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; Liebman & 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 *Ty* 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

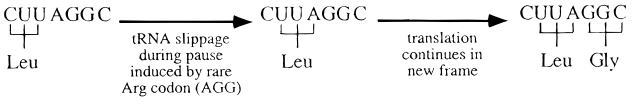

FIGURE 1. *Ty1* frameshifting mechanism. Frameshifting requires a pause induced by a limiting tRNA. The pause results in slippage of the 0-frame leucyl tRNA, in the P-site, to the +1 frame. (Belcourt & Farabaugh, 1990).

TABLE 3. Summary of translational accuracy mutation effects on *Ty1* and *Ty3* frameshifting.

Suppressors and allosuppressors	Effects[a] on *Ty1*	Effects on *Ty3*	Antisuppressors	Effects on *Ty1*	Effects on *Ty3*
SUP44	↑	NT	*rps28-12*	none	NT
sal6	↑	NT	*rdn-1T*	↓	↓
rps28-5	none	NT	*rdn-2*	↓	none
rdn-1A	none or ↑	↓	*rdn-4*	↓	none
rdn-5	↓	NT			
[*PSI*$^+$]	none or ↓	NT			

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

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 affect 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* retrotransposon 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 retrotransposon 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 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 OD_{600} of 0.01. The assay cultures were grown to an OD_{600} 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 $CHCl_3$ 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 $MgCl_2$, 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 s 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: $Z = P/v(OD)$, where Z = β-galactosidase activity, P = luminometer reading in photons, v = 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* Ty1 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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