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Nonsense-mediated decay mutants do not affect programmed –1 frameshifting

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ABSTRACT

Sequences in certain mRNAs program the ribosome to undergo a noncanonical translation event, translational frameshifting, translational hopping, or termination readthrough. These sequences are termed recoding sites, because they cause the ribosome to change temporarily its coding rules. *Cis* and *trans*-acting factors sensitively modulate the efficiency of recoding events. In an attempt to quantitate the effect of these factors we have developed a dual-reporter vector using the *lacZ* and *luc* genes to directly measure recoding efficiency. We were able to confirm the effect of several factors that modulate frameshift or readthrough efficiency at a variety of sites. Surprisingly, we were not able to confirm that the complex of factors termed the surveillance complex regulates translational frameshifting. This complex regulates degradation of nonsense codon-containing mRNAs and we confirm that it also affects the efficiency of nonsense suppression. Our data suggest that the surveillance complex is not a general regulator of translational accuracy, but that its role is closely tied to the translational termination and initiation processes.

Keywords: HIV; PSI; recoding; release factor; reporter; UPF

INTRODUCTION

Protein synthesis must accurately translate the information encoded in nucleic acids, as a sequence of three nucleotide codons, into a sequence of amino acids. Many components of the translational apparatus ensure correct decoding including ribosomal RNA (rRNA), ribosomal proteins (R-proteins), elongation factors, and other ribosome-associated factors. Together these factors reduce the frequency of both missense errors and errors leading to premature cessation of elongation (processivity errors) to about 5×10^{-4} per codon (Kurland, 1992). This estimate was derived from studies looking for spontaneous misincorporation or termination of translation at fortuitous sites within normal structural genes. However, error frequency is sequence dependent. At some sites missense or processivity errors are common whereas at others they are probably extremely rare. Sites that allow abnormally high frequencies of errors have evolved in many genes to allow the expression of two distinct proteins, one expressed according to the normal rules of translation and a second that requires a change in those rules at the site of high error. These sequences can stimulate efficient readthrough of termination codons or shifts in reading frames, either frameshifting between overlapping codons or hopping, where the ribosome shifts to a codon far downstream in the mRNA. Because they cause a change in the rules of coding they have been termed recoding sites. Because proteins expressed under the control of these sites depend on a nonstandard coding event they are sensitive monitors of translational accuracy.

Recoding sites program the ribosome to undergo a noncanonical translation step by reducing the rate of canonical decoding, increasing the probability of the noncanonical event, or both (reviewed by Farabaugh, 1996; Gesteland & Atkins, 1996). Stimulatory sequences in and around the recoding site can reduce normal translation elongation by introducing a poorly recognized codon (a termination codon in an inappropriate sequence context or a sense codon recognized by a low-abundance tRNA) or by interposing a stable RNA structure (a pseudoknot or hairpin loop). Stimulatory sequences can also directly promote the noncanonical event. For example, in prokaryotes programmed frame-

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shift sites often include Shine–Dalgarno interaction sites; formation of mRNA•rRNA pairing can actually force slippage of the mRNA to promote frameshifting (e.g., see Weiss et al., 1988).

Studies of translational recoding sites use one of several reporter systems. The simplest of these systems uses a readily assayed protein, for example, the β-galactosidase enzyme of *Escherichia coli*, whose expression depends on ribosomes undergoing the recoding event (readthrough or frameshift). To estimate the efficiency of the recoding event, expression is standardized using a second version of the reporter construct that expresses the protein without the need for a recoding event. Parallel experiments performed with the recoding and the normal reporter can be compared to determine indirectly the efficiency of recoding. These single-gene vector systems suffer from the fact that the control comes not only from a separate gene construct, but from a separate experiment. Because one of the goals of studying recoding events is to determine what factors affect their efficiency, the separation of the experiment and control has the potential to allow unknown variables to confuse the analysis of recoding efficiency. For example, a gene including a recoding site resembles a gene including a nonsense or frameshift mutation. Such transcripts are known to be subject to nonsense-mediated RNA degradation. Because the control plasmid is not sensitive to degradation, it is not necessarily clear how to assess the effect of RNA degradation on the expression of the recoding reporter construct.

Alternative systems have been developed that attempt to simplify the analysis of recoding events by providing an internal control as part of the recoding reporter gene (Reil et al., 1993; Stahl et al., 1995). In these systems an upstream gene is encoded using the normal rules of translation. A downstream gene is expressed as a fusion to the upstream control gene through an intervening recoding sequence. Using such a system, expression of the upstream gene serves as a control to assess the efficiency of expression of the downstream reporter. Because any process affecting the downstream gene will affect the upstream gene, for example, translation initiation or RNA degradation, this reporter system has the potential to report more accurately the effect of *trans*-acting factors.

We have designed such a dual-gene reporter using the *E. coli lacZ* gene encoding β-galactosidase as the upstream control reporter and the firefly luciferase gene, *luc*. The system has been used to assess the in vivo importance of a stem-loop structure downstream of the −1 frameshift signal of the retrovirus HIV-1 (Stahl et al., 1995). Using this vector we showed that the efficiency of frameshifting depends sensitively on the stability of the structure with increasing frameshift frequencies being induced by increasingly stable structures (Bidou et al., 1997). These results show that the system can sensitively monitor even small changes in recoding efficiencies. We have used the same system to test the effect of a variety of potential *trans*-acting regulators of recoding efficiency. Some of these *trans*-acting factors clearly modulate the efficiency of frameshifting as shown by this dual-gene reporter system, but some have no effect despite published results using single-reporter systems suggesting that they affect recoding efficiency. The difficulty inherent in interpreting results with single reporters seems to have led to incorrect identification of these factors as regulators of recoding efficiency.

RESULTS

Nature of the dual-gene reporter assay

The *lacZ-luc* dual-gene construct expresses two proteins: β-galactosidase and a translational fusion of β-galactosidase and luciferase (Fig. 1). Ribosomes that terminate upon encountering the recoding site express β-galactosidase whereas those that read through the terminator also express luciferase. A control plasmid in which *lacZ* and *luc* are in the same reading frame provides a way to equate the activity of β-galactosidase and luciferase when they are expressed in equimolar quantities. This ratio can be used to determine the relative molar expression of the two enzyme's activities expressed in any other condition. In this article it is used to determine the efficiency of recoding events. In the simplest hypothetical example, in which all ribosomes read through the terminator, the ratio of the two enzymes expressed should be identical to that produced by the control gene fusion plasmid. Note that the only use of the control plasmid bearing a translational fusion of *lacZ* and *luc* is to establish the relative activities of β-galactosidase and luciferase when expressed in equimolar amounts. In the frameshift reporter constructs the relationship between the activities of the two enzymes would be unaffected by any factor other than the frequency of frameshifting.

The dual-gene reporter system quantifies and confirms reported effects of some *trans*-acting factors

Previously, we have shown that the dual-gene reporter can accurately assess the efficiency of recoding at programmed readthrough and frameshift sites (Stahl et al., 1995; Bidou et al., 1997). Here we use the same system to quantitate the effect of reported *trans*-acting regulators of these events. The first test involves the effect of variation in release factors (RF) availability on programmed translational readthrough. The efficiency of readthrough depends on the ability of aminoacyl–tRNA to compete with RF for the ribosomal A site (reviewed by Tate et al., 1996). Those effects that reduce the RF recognition will tend to stimulate readthrough whereas

those that increase RF recognition will tend to reduce it. Similarly, those effects that tend to increase the ability of aminoacyl–tRNA to bind will tend to increase readthrough. In the simplest example, a nonsense suppressor mutation in a tRNA increases its ability to compete with RF because it recognizes the termination codon as a cognate. Missense mutations affecting RF reduce its effective concentration and thus encourage readthrough whereas overexpressing RF reduces readthrough.

We measured readthrough efficiency in yeast *Saccharomyces cerevisiae* during overexpression or depletion of RF. In yeast, eRF1 and eRF3, encoded respectively by *SUP45* and *SUP35* genes, compose RF. To increase the availability of RF, and thus increase termination efficiency, requires overexpression of both proteins (Stansfield et al., 1995). We achieved overexpression by cloning both *SUP45* and *SUP35* together on a multicopy plasmid (see Materials and methods). Certain strains carry a prion form of Sup35p that oligomerizes in vivo creating an epigenetic state termed [*PSI*$^+$] (Wickner, 1994; Patino et al., 1996; Paushkin et al., 1996). The [*PSI*$^+$] factor causes increased readthrough of termination codons (Liebman & Sherman, 1979; Liebman & Derkatch, 1999). Presumably the increase in readthrough results from sequestering of RF in inactive oligomers, reducing the availability of free monomers. Thus we are able to directly increase availability of RF or to indirectly reduce its availability so as to test for an effect on readthrough of termination codons. As a simple assay of readthrough we measured the frequency of nonsense readthrough on a 12-nt sequence from the Tobacco Mosaic Virus (TMV) replicase gene. The sequence, a termination codon flanked by CAA codons, promotes a high level of spontaneous readthrough in yeast, with the UAG codon being read as a mixture of Tyr, Trp, and Lys (Fearon et al., 1994; Bonetti et al., 1995; Stahl et al., 1995). The natural TMV sequence is CAA–UAG–CAA–UUA. We replaced the UAG termination codon with either UGA or UAA codons.

In the wild-type Y349 strain of yeast, which expresses normal amounts of eRF1 and eRF3, the sites including each of the three termination codons drove significant levels of readthrough. Readthrough efficiency varied among the three termination codons, and was least at the UAA codon (Fig. 2A,B) in agreement with previous studies that showed more efficient termination at ochre termination codon (Bonetti et al., 1995). Overexpressing both eRF1 and eRF3 reduced readthrough on all three nonsense codons by approximately a factor of 2 (Fig. 2A). This is consistent with our expectation that increasing the availability of RF would increase the efficiency of termination and therefore reduce readthrough. Conversely, a [*PSI*$^+$] strain, containing prion form of eRF3, increased readthrough efficiency approximately from 3.6- (for the UAG codon) to 5.8-fold (for the UAA codon) compared to a isogenic [*psi*$^-$] strain (Fig. 2B).

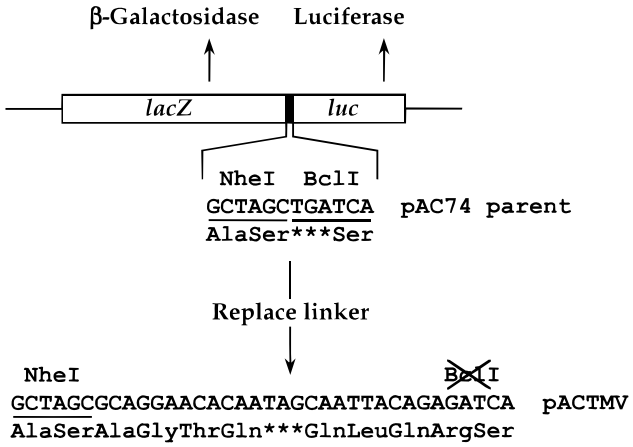

FIGURE 1. Schematic of the *lacZ-luc* reporter system. The reporter consists of tandem *lacZ* and *luc* genes separated by a short linker. The ends of the linker are *Nhe*I and *Bcl*I restriction sites that can be used to insert oligonucleotides bearing recoding sites. The figure shows the introduction of an oligonucleotide carrying the TMV termination readthrough site. When inserting such an oligonucleotide, the sequence of the *Bcl*I site was changed to eliminate an in-frame TGA termination codon that would otherwise block continued translation into *luc*.

This is also consistent with our expectation that reducing RF availability would reduce the efficiency of termination and thus increase readthrough efficiency. It is important both that the dual-gene reporter confirmed the effect of increasing and decreasing availability of RF, but also that it was able to quantitate even these rather subtle effects.

The term "+1 shifty stops" denotes programmed frameshift sites that consist of a slippery codon (e.g., CCC) followed by an in-frame termination codon (de Smit et al., 1994). Frameshifting results when slow recognition of the termination codon allows +1 slippage of the peptidyl–tRNA in the ribosomal P site. This was demonstrated in vivo in *E. coli* using partially functional release factor mutants (Donly et al., 1990) and in vitro (Adamski et al., 1993). We tested whether restricting availability of RF in *S. cerevisiae* would stimulate frameshifting at slippery stops.

Reporter constructs were introduced into congenic strains that carried either the [*PSI*$^+$] or [*psi*$^-$] trait. The reporters carried a site prone to +1 frameshifting consisting of the slippery codon CUU followed by the sequence UAA-G (FSt1), UAA-C (FSt2), or UGA-C (FSt3). Each of these constructs promoted low but measurable amounts of +1 frameshifting in the [*psi*$^-$] strain, which is wild type for RF activity (Fig. 3). Frameshifting in two of the three constructs was significantly higher in a [*PSI*$^+$] strain, increasing over fivefold for FSt2 and over almost eightfold for FSt3 (Fig. 3). Expression of the reporter carried by the FSt1 construct was not significantly affected by PSI. Again, these results demonstrate that the dual-gene reporter construct sensitively measures changes in recoding efficiency.

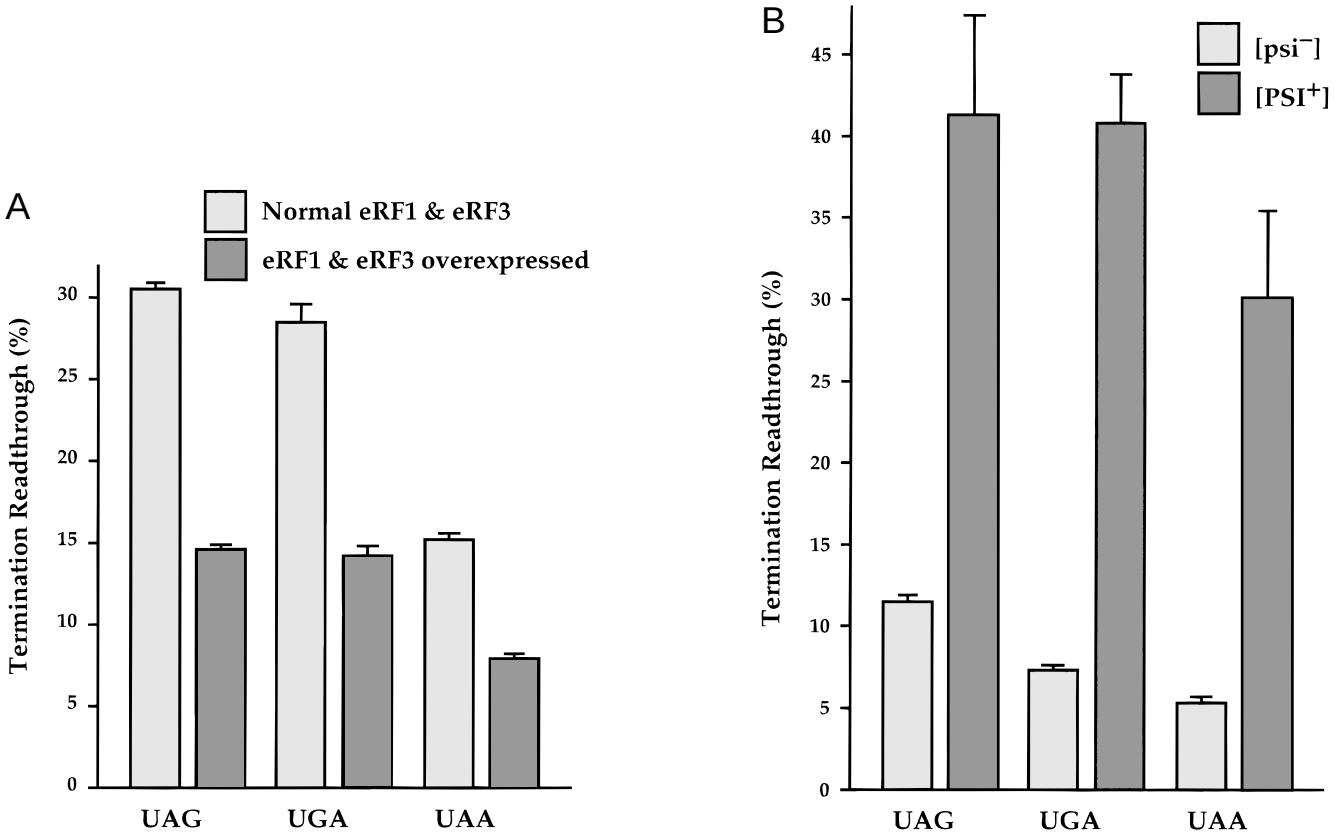

FIGURE 2. **A**: Wild-type yeast strain Y349 was transformed with multicopy vectors pFL44L (vector alone) or pSP35-45 (overexpressing eRF1 and eRF3), and cotransformed with either pACTMV (UAG), pACTGA, pACTAA, or pACTQ. **B**: [*psi*$^-$] strain 74-D694 and its [*PSI*$^+$] derivative strain were transformed with pACTMV (UAG), pACTGA, pACTAA, or pACTQ. Termination readthrough is expressed as the luciferase/β-galactosidase ratio of a test construct normalized to the control ratio obtained with the in-frame control (pACTQ). Note that the measured ratio in the wild-type strain ([*psi*$^-$]) is much lower than that measured in strain Y349. This difference reflects uncharacterized background differences between these unrelated yeast strains.

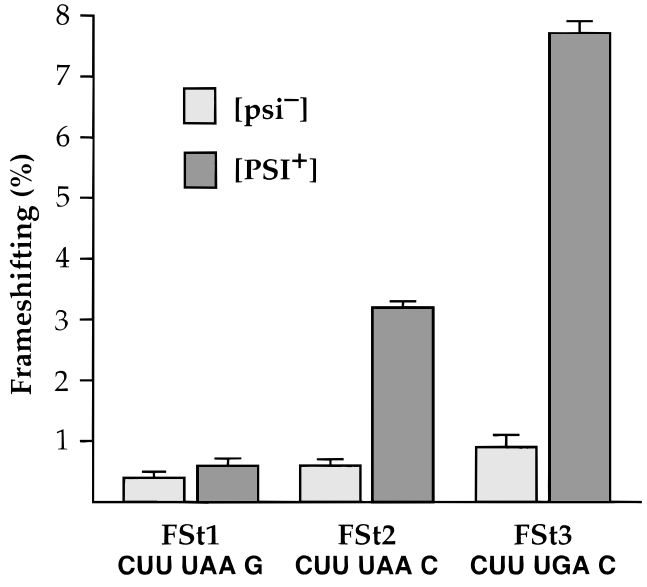

FIGURE 3. [*psi*$^-$] strain 74-D694 and its [*PSI*$^+$] derivative strain were transformed with pACFSt1, pACFSt2, pACFSt3, or pACTQ. Frameshifting efficiency is expressed as in Figure 2.

Another type of programmed frameshift site similar to shifty stops are those that use a poorly recognized sense codon to stimulate +1 frameshifting (Belcourt & Farabaugh, 1990; Peter et al., 1992; Farabaugh et al., 1993). At such sites the slow recognition of an in-frame sense codon allows the peptidyl–tRNA bound to the immediately upstream codon to stimulate a +1 shift in frames. The recoding sites derived from the yeast retrotransposon Ty1 contain such a site stimulated by the slow recognition of an AGG codon by the rare $tRNA^{Arg}_{CCU}$ (Belcourt & Farabaugh, 1990). Using a single-gene reporter system, we have previously shown that deleting the single gene encoding $tRNA^{Arg}_{CCU}$ (Δ*hsx1*) to force decoding by the near-cognate tRNA greatly stimulates +1 frameshifting (Kawakami et al., 1993; Vimaladithan & Farabaugh, 1994). Using the dual-gene reporter system we tested the effect of Δ*hsx1* on frameshifting on the Ty1 programmed frameshift site, CUU–AGG–C. In accord with our previous results, Δ*hsx1* strongly stimulates +1 frameshifting. Remarkably, the value we determine for frameshifting in the

strain KK240 (Δ*hsx1*) is 100%. This means that essentially all ribosomes that encounter the frameshift signal shift into the +1 reading frame, whereas in the congenic strain KK242 (*HSX1*), which has a normal concentration of $tRNA^{Arg}_{CCU}$, frameshifting as expected is much lower, 30%. The fact that the system measures the genes both upstream and downstream of the frameshift site eliminates the uncertainty about the meaning of the high level of expression that we had from our previous study using the single-gene reporter. Apparently in this genetic background, the ribosome is virtually incapable of properly decoding CUU–AGG–C.

The dual-gene reporter shows no effect on frameshifting of mutants of the surveillance complex

None of these reported results is unexpected. They all serve to demonstrate that the dual-reporter system sensitively records the effect of *trans*-acting factors on two types of recoding events: nonsense codon readthrough and programmed +1 frameshifting. The system is sensitive enough to record quite subtle changes in efficiency and is still effective recording even very large changes.

Recent work has suggested that a complex of proteins termed the surveillance complex associates with the ribosome. The complex appears to be involved in a variety of cotranslational processes: nonsense-mediated mRNA decay and translational termination (reviewed by Czaplinski et al., 1999), and perhaps −1 translational frameshifting (Cui et al., 1996; Ruiz-Echevarria et al., 1998). The fact that the complex may regulate several aspects of protein synthesis introduces a complication in analyzing its effect on frameshifting. One needs either to adjust the raw results obtained by analysis of expression of reporter constructs or to attempt to eliminate the effect of changes in either decay or translation initiation. Previous analysis has depended on adjusting observed differences in protein production dependent on frameshifting by introducing a correction factor determined by differences in mRNA stability or abundance. Corrections for the abundance of mRNA do not take into account the possibility that the surveillance complex might also regulate other aspects of translation. In fact, recent data suggest that the surveillance complex may directly regulate translational initiation (Muhlrad & Parker, 1999).

We felt that our dual-reporter construct offered a different approach whereby we could eliminate the effect of either differences in mRNA stability or translational competence. Because the assay involves establishing the ratio of expression of two proteins expressed from a single mRNA, the absolute level of mRNA is not important. The fact that both proteins depend on a single translation initiation also eliminates differences in initiation as a concern.

The surveillance complex does appear to regulate the efficiency of translational termination as assayed by our dual-reporter system, though the effect of the deleting *UPF* genes varied among the constructs (Fig. 4). Each of the three deletions—Δ*upf1*, Δ*upf2*, and Δ*upf3*—increased readthrough of a UAA codon 2- to 3.4-fold. The effect on readthrough of UGA was smaller for each strain, a 66–93% increase. With UAG, only the Δ*upf1* and Δ*upf3* strain showed statistically significant increases in readthrough (26 and 63% increases, respectively) whereas there was no significant change in the Δ*upf2* strain. Interestingly, the least efficient readthrough site evidenced the most pronounced effect of the *UPF* deletions, and the overall effect decreased with increasing efficiency. Apparently the state of the surveillance complex is most relevant in cases where termination is relatively more efficient, and as efficiency of termination decreased, the relevance decreases.

The effect of surveillance complex mutations on frameshifting was remarkably different. We tested the effect of Δ*upf* mutations on three types of frameshift events: −1 simultaneous slippage frameshifting at either the HIV-1 or L-A sites, and +1 frameshifting at the Ty1 site, or at the three slippery-stop sites. As shown in Figures 5 and 6, none of these events showed evidence of dependence on any of the three *UPF* genes tested. The frameshift sites vary widely in intrinsic activity, from a low of around 1% for the two slippery stops to a high of over 40% for the Ty1 site. It is notable that there was no effect even for the slippery stops using UAA and UGA as pause-inducing codons. The previous experiment showed that the Upf proteins are regulators of functional recognition of these codons. The fact that Δ*upf* mutations do not stimulate frameshifting is puzzling.

Though we were able to confirm several other effects on programmed frameshifting, including some very subtle ones, we could not confirm the approximately twofold increase in frameshifting associated with Δ*upf3* (Ruiz-Echevarria et al., 1998) and the effect reported for Δ*upf1* (Cui et al., 1996).

DISCUSSION

The *lacZ-luc* dual-reporter system sensitively reported the effect of several *trans*-acting factors on a variety of recoding sites. Some effects were quite strong; forcing near-cognate recognition of the AGG pause codon caused essentially all ribosomes that encountered the Ty1 programmed +1 frameshift site to shift frames. These results confirmed those obtained using a *lacZ* single-reporter system (Vimaladithan & Farabaugh, 1994). Other results were nearly as dramatic, such as the approximately four- to sixfold increase in nonsense codon readthrough in a [*PSI*$^+$] strain. In each of these cases we can explain the large increase as the result of

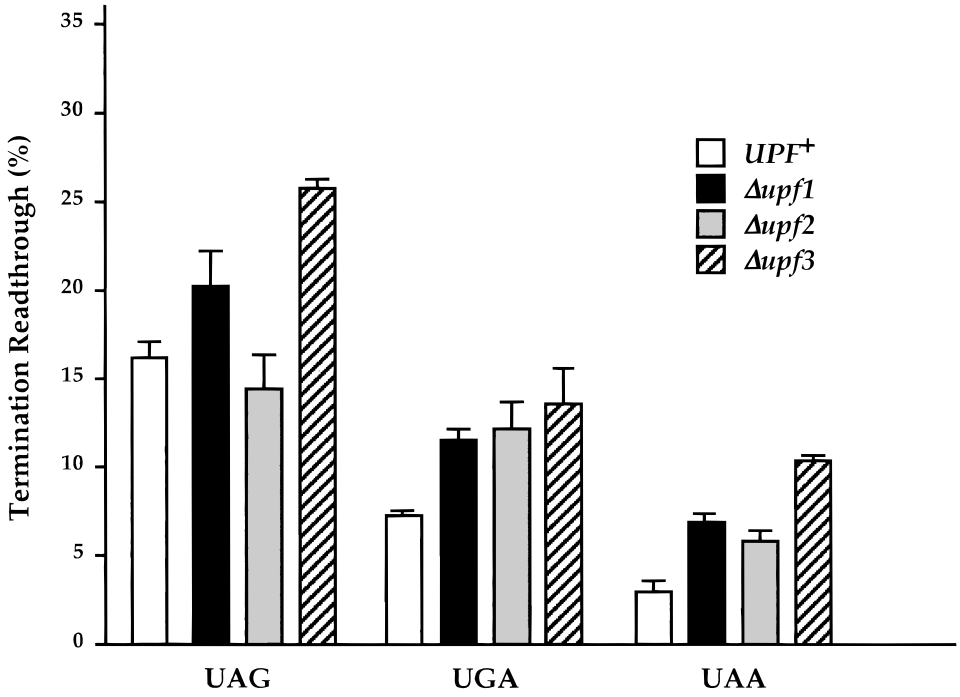

FIGURE 4. Wild-type strain HFY1200 and the congenic HFY870 (Δ*upf1*), HFY1300 (Δ*upf2*), and HFY863 (Δ*upf3*) strains were transformed with pACTMV (UAG), pACTGA, pACTAA, or pACTQ. Readthrough efficiency is expressed as in Figure 2.

restricting normal decoding in the A site. The near-cognate $tRNA^{Arg}_{UCU}$ probably reads an AGG codon very inefficiently because the wobble uridine is modified to recognize nucleotides other than A very poorly (as discussed by Vimaladithan & Farabaugh, 1994). Similarly, in a [*PSI*$^+$] strain the availability of eRF is restricted because an inactive complex sequesters it, making it unavailable to promote termination. Overexpression of

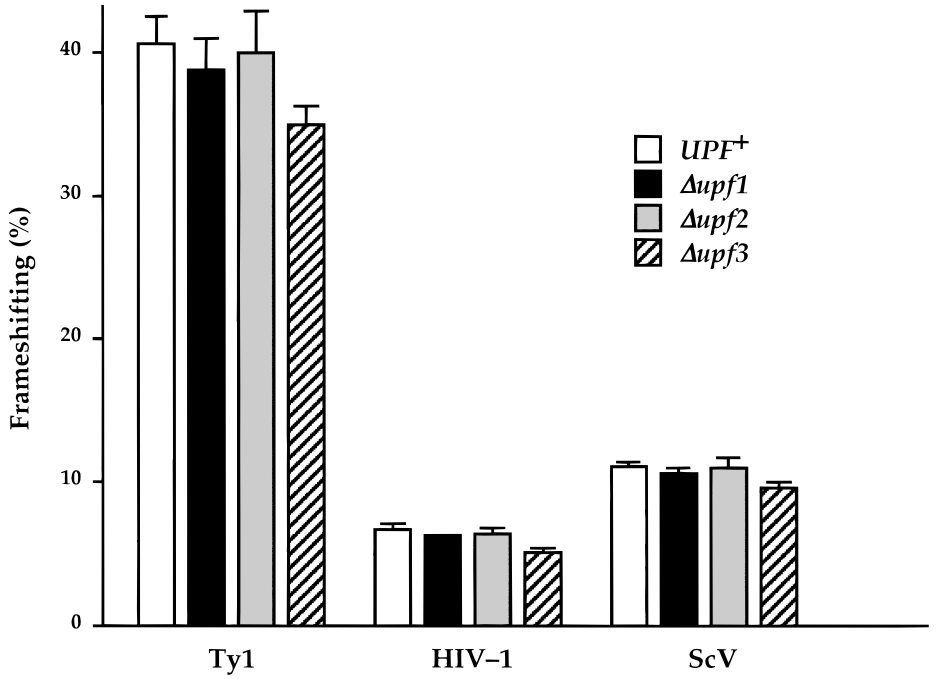

FIGURE 5. Wild-type strain HFY1200 and the congenic HFY870 (Δ*upf1*), HFY1300 (Δ*upf2*), and HFY863 (Δ*upf3*) strains were transformed with either pACTy, pACTTy, pAC1789 (HIV-1), pACLA (yeast L-A virus), or pAC1790. Frameshifting efficiency is expressed as in Figure 2.

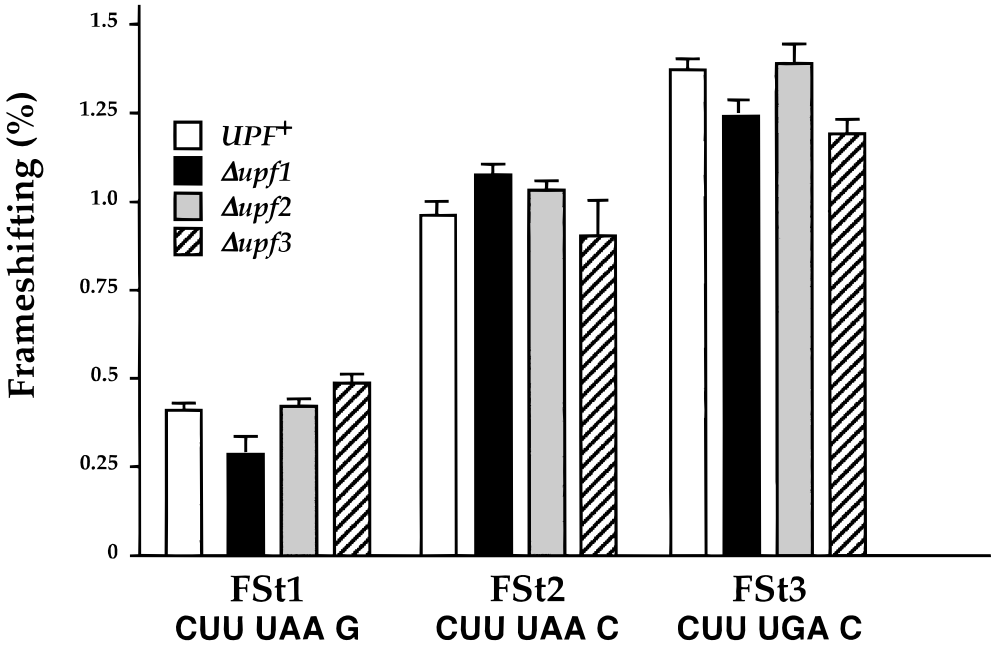

FIGURE 6. Wild-type strain HFY1200 and the congenic HFY870 (Δ*upf1*), HFY1300 (Δ*upf2*), HFY863 (Δ*upf3*) strains were transformed with pACFSt1, pACFSt2, pACFSt3, or pACTQ. Frameshifting efficiency is expressed as in Figure 2.

eRF has the opposite effect, reducing nonsense readthrough about twofold at each of the termination codons when they are present in an inefficiently recognized context. These data are consistent with the data reported previously by Stansfield et al. (1995). As expected, a [*PSI*$^+$] strain also shows an increase in frameshifting at slippery-stop sites, programmed sites in which a codon particularly prone to allowing +1 slippage by peptidyl–tRNA is immediately followed by a poorly recognized termination codon. The effect was approximately five- to eightfold for two of the slippery-stop constructions tested. This is consistent with the idea that slow recognition of the termination codon allows +1 slippage of the peptidyl–tRNA. Indeed, UAA-C (FSt2) and UGA-C (FSt3) are inefficient for termination (Bonetti et al., 1995), and are also highly sensitive to a PSI$^+$ context. Conversely, the UAA-G (FSt1) is more efficient for termination (Bonetti et al., 1995) and is not sensitive to a PSI$^+$ context.

The surveillance complex also appears to regulate termination. Each of the Δ*upf* mutations caused an increase in programmed termination readthrough, though the effects were small and variable. Weak readthrough of an efficient UAA termination codon was stimulated approximately twofold by each of the Δ*upf* mutations. The Δ*upf* mutations had no effect or marginal effects on the stronger readthrough at less efficient terminators. This suggested that perturbation of the surveillance complex could have an effect on readthrough, but that its importance diminishes as the efficiency of eRF at a termination codon decreased. This difference might suggest that the effect of the surveillance complex on termination is distinct from the effect of sequence context on eRF activity. Where the sequence context causes eRF to have high intrinsic activity, a poor surveillance complex can lengthen pausing to stimulate readthrough, but as intrinsic activity is reduced, the ability of the *UPF* mutations to stimulate pausing further is reduced or lost.

More surprisingly, the *UPF* deletions had no effect on programmed −1 frameshifting. These data directly contradict conclusions drawn by Cui et al. (1996) and Ruiz-Echevarria et al. (1998). They found evidence that mutations affecting the surveillance complex increased the efficiency of programmed −1 frameshifting. For example, Ruiz-Echevarria et al. (1998) showed an approximately twofold increase in relative expression of reporter constructs that required programmed −1 frameshifting. Recent data from Muhlrad and Parker (1999) have shown that a Δ*upf* mutation causes a two- to threefold increase in the rate of translational initiation on mRNAs normally subject to nonsense mediated decay (NMD). The *lacZ* single-reporter system used in the Cui et al. (1996) and Ruiz-Echevarria et al. (1998) experiments determines frameshift efficiency by comparing the expression of a reporter gene requiring frameshifting for expression of the *lacZ* product, β-galactosidase, to that of a reporter in which its expression does not require frameshifting. It appears that the presence of a programmed frameshift site in the reporter makes the mRNA subject to NMD, whereas the reporter lacking such a site is not. After adjusting for differences in mRNA stability, there appeared to be an excess increase in protein expression from the frameshift-reporter construct,

which was interpreted as an increase in frameshift efficiency. However, it is possible that this increase resulted from an increase in intrinsic translation initiation on the frameshift reporter mRNA caused by the effect seen by Muhlrad and Parker (1999).

The fact that our dual-reporter construct fails to report any effect of the *UPF* deletions tends to support the conclusion that the effect measured by Cui et al. (1996) and Ruiz-Echevarria et al. (1998) results from an increase in translation initiation rather than an increase in frameshifting. Although Ruiz-Echevarria et al. (1998) emphasize the threefold increase in expression of their −1 frameshift reporter caused by a Δ*upf3* mutation, they actually found that both Δ*upf1* and Δ*upf2* also caused an almost twofold increase in expression. The effect noted by Muhlrad and Parker (1999) can explain these results. At a minimum the conclusion that the surveillance complex regulates programmed −1 frameshifting must be considered suspect and attempts must be made to clearly show that the effect observed is a direct result of a change in frameshift efficiency. The experiments of Cui et al. (1996) and Ruiz-Echevarria et al. (1998) do not directly address this point, as they contain no direct test of the effect of the *upf* mutations on translation initiation.

Because the data presented here show that the surveillance complex does not modulate translational frameshifting, one could conclude that it is not involved in general translational accuracy. The data clearly show that the complex does modulate the efficiency of translational readthrough, but is this the effect of altered accuracy or simply reducing the efficiency of termination? The data do not provide a clear answer to this question. Spontaneous readthrough of termination codons increased in mutants affecting the surveillance complex and in a [*PSI*$^+$] strain. One explanation of these data is that, like PSI, the surveillance complex mutations reduce the efficiency of translational termination. Reducing the efficiency of termination would indirectly increase the probability that the nonsense codon would be read by a noncognate tRNA, allowing readthrough, because these two reactions directly compete with each other in the ribosomal A site (reviewed by Farabaugh et al., 2000). The efficiency of frameshifting at slippery-stop sites also depends on competition between termination at the in-frame nonsense codon in the ribosomal A site and slippage by the peptidyl–tRNA allowing recognition of the +1 frame codon. As expected, the efficiency of frameshifting at a slippery stop is increased in a [*PSI*$^+$] strain relative to the isogenic [*psi*$^-$]. Surprisingly, the surveillance complex mutations had no effect on slippery-stop frameshifting. This result implies that the surveillance complex may not affect suppression by modulating nonsense codon recognition by peptide release factor because that model would predict an effect on frameshifting as well as suppression. An alternative explanation may be that the surveillance complex alters some step prior to entrance of peptide release factor into the A site, a step that affects nonsense suppression but not frameshifting. Whether this step is concerned with translational accuracy or not remains to be seen.

MATERIALS AND METHODS

Yeast strains and plasmids

Yeast strains used in this work are listed in Table 1.

Reporter plasmids were constructed by inserting synthetic oligonucleotides in cloning sites present between *lacZ* and *luc* in plasmid pAC74 or derivatives (Stahl et al., 1995). For the measurement constructs, *luc* will be out of frame considering *lacZ* initiation codon. For each type of translational error tested (frameshift +1, frameshift −1 and readthrough) an in-frame control was used that allows production of 100% fusion protein. All plasmids have been sequenced in the surrounding region. The sequence in the region between the *lacZ* and *luc* genes from each of the plasmids is shown in Table 2.

Overexpression plasmid

A multicopy yeast expression plasmid, pSP35-45, was constructed using *SUP35* and *SUP45* under control of their own promoter. The genes were obtained from the pRS316-sup35 and pEMBLyex4-sup45 plasmids respectively, kindly provided by Dr. Ter-Avanesian. The *SUP35 Pvu*II-*Xba*I fragment

TABLE 1. Yeast strains used in this study.

Strains	Genotype	Reference
74-D694	*MAT***a** *ade1–14 trp1–289 his-3Δ200 leu2–3,112 ura3–52 [psi−]*	Derkatch et al., 1998
74-D694 [PSI$^+$]	*MAT***a** *ade1–14 trp1–289 his-3Δ200 leu2–3,112 ura3–52* [PSI$^+$]	Derkatch et al., 1998
KK242	*MATα his3 leu2 trp1 ura3 HSX1*	Kawakami et al., 1993
KK240	*MATα his3 leu2 trp1 ura3 Δhsx1::HIS3*	Kawakami et al., 1993
HFY1200	*MAT***a** *ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1 UPF1 NMD2 UPF3*	He & Jacobson, 1995
HFY870	*MAT***a** *ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1 Δupf1::HIS3 NMD2 UPF3*	He et al., 1997
HFY1300	*MAT***a** *ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1 UPF1 Δnmd2::HIS3 UPF3*	He & Jacobson, 1995
HFY863	*MAT***a** *ade2–1 can1–100 his3–11,15 leu2–3,112 trp1–1 ura3–1 UPF1 NMD2 Δupf3::HIS3*	He et al., 1997
Y349	*MATα lys2Δ201 leu2–3,112 his3Δ200 ura3–52*	Dang et al., 1996

TABLE 2. Schematic view of the reporter and plasmids used in the study.

Plasmids	Sequences[a]
−1 Frameshifting	
pAC1789	----------> <----------
	gctagcCAGGCTAATTTTTTAGGGAAGATCTGGCCTTCCTACAAGGAAGGCCAGGGAAggatca
	...AlaSerGlnAlaAsnPheLeu
	ArgGluAspLeuAlaPheLeuGlnGlyLysAlaArgGluGlySer...
pACFSt2	------S1-----> --S2-><-----S1------ <-S2--
	gctagcTGGCAGCAGGGTTTAGGAGTGGTAGGTCTTACGATGCCAGCTGTAATGCCTACCGGAGAACCTACAGCTGGCGCTGCccacca
	...AlaSerTrpGlnGlnGlyLeu
	ArgSerGlyArgSerTyrAspAlaSerCysAsnAlatyrArgArgThrTyrSerTrpArgCysProPro...
+1 Frameshifting	
pACFSt1	gctagcACACTTTAAGagatca
	...AlaSerThrLeu
	LysArgSer...
pACFSt2	gctagcACACTTTAACagatca
	...AlaSerThrLeu
	AsnArgSer...
pACFSt3	gctagcACACTTTAGCagatca
	...AlaSerThrLeu
	SerArgSer...
pACTy	gctagcACACTTAGGCcgatca
	...AlaSerThrLeu
	GlyArgSer...
Termination readthrough	
pACTMV	gctagcGCAGGAACACAATAGCAATTACAGagatca
	...AlaSerAlaGlyThrGln***GlnLeuGlnArgSer...
pACTGA	gctagcGCAGGAACACAATGACAATTACAGagatca
	...AlaSerAlaGlyThrGln***GlnLeuGlnArgSer...
pACTAA	gctagcGCAGGAACACAATAACAATTACAGagatca
	...AlaSerAlaGlyThrGln***GlnLeuGlnArgSer...
Controls	
pAC1790	* ----------> <----------
	gctagcCAGGCTAATTTTTTTAGGGAAGATCTGGCCTTCCTACAAGGGAAGGCCAGGGAAggatca
	...AlaSerGlnAlaAsnPheLeuArgGluAspLeuAlaPheLeuGlnGlyLysAlaArgGluGlySer...
pACTQ	*
	gctagcGCAGGAACACAACAGCAATTACAGagatca
	...AlaSerAlaGlyThrGlnGlnGlnLeuGlnArgSer...
pACTTy	ΔC\
	gctagcACATTAGGCcgatca
	...AlaSerThrLeuGlyArgSer...

[a]Sequences cloned between *lacZ* and *luc.* Upper case text indicates inserted sequences and lower case indicates sequences from flanking pAC74 restriction sites :*Nhe*I in 5′ and a destroyed *Bcl*I site in 3′ (Fig. 1). The sequence of the primary translation product from each construct is indicated below with frameshifts indicated by shifted lines and sites of terminators by triple asterisks. Base pairing interactions of secondary structures are indicated by arrows above the DNA sequences, a stem-loop for HIV-1 (pAC1789, 1790), and a pseudoknot for L-A virus (pACLA). Changes made in controls are indicated above the sequence, asterisks indicating inserted nucleotides. The plasmids pAC1789, pAC1790, pACTMV, pACTMV, pACTQ, pACTy, and pACTTY have all been described (Stahl et at., 1995). The other plasmids are from this study.

was first ligated into an Ecl136II-XbaI-digested pBSK(+) (Stratagene). A *Xba*I-*Sna*BI *SUP45* fragment was inserted downstream of *SUP35*, after *Xba*I-*Sma*I digestion. A *Pvu*II fragment containing *SUP35* and *SUP45* was then inserted in *Pvu*II-digested pFL44L (Bonneaud et al., 1991).

Enzymatic activities and recoding efficiency

Plasmids described in Table 2 were transformed into the yeast strains described in Table 1 (refer to figure legends), using the method of Ito et al. (1983). In each case, at least three transformants cultivated in the same conditions were assayed. Cells were broken using acid-washed glass beads, as described (Stahl et al., 1995). Luciferase and β-galactosidase activities were assayed in the same crude extract, as described previously (Stahl et al., 1995).

Recoding efficiency is defined as the ratio of luciferase activity to β-galactosidase activity. To establish the relative activities of β-galactosidase and luciferase when expressed in equimolar amounts, the luciferase/β-galactosidase ratio from an in-frame control is taken as reference. Recoding efficiency, expressed in percentage, was determined by dividing the luciferase/β-galactosidase ratios obtained from each test construct by the same ratio obtained with an appropriate in-frame control.

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