Translational Accuracy during Exponential, Postdiauxic, and Stationary Growth Phases in *Saccharomyces cerevisiae*

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When the yeast *Saccharomyces cerevisiae* shifts from rapid growth on glucose to slow growth on ethanol, it undergoes profound changes in cellular metabolism, including the destruction of most of the translational machinery. We have examined the effect of this metabolic change, termed the diauxic shift, on the frequency of translational errors. Recoding sites are mRNA sequences that increase the frequency of translational errors, providing a convenient reporter of translational accuracy. We found that the diauxic shift causes no overall change in translational accuracy but does cause a strong reduction in the frequency of one type of programmed error: Ty +1 frameshifting. Genetic data suggest that this effect may be due to changes in the relative amounts of tRNA participating in translation elongation. We discuss possible implications for expression strategies that use recoding.

Translation maintains a balance between speed and accuracy (24). Kinetic proofreading (19, 29, 44) ensures that correct aminoacyl-tRNAs are selected for each round of translation in the ribosomal A (acceptor) site and that they are in the correct frame, as defined by the previous codon present in the P (peptidyl-tRNA) site. Accurate translation requires a highly discriminating A site. Most tRNAs that enter the A site fail to form three base pairs with the codon displayed there, and so the tRNA rapidly dissociates; only cognate tRNAs are efficiently and quantitatively retained. Hyperaccurate ribosomes actually reject a large proportion of even cognate tRNAs, an event which has been demonstrated in *Escherichia coli* to increase the translation step time (2, 33). It is likely that such a costly step in gene expression is regulated by physiological conditions encountered by the cell. The cell may gain in fitness if translational accuracy varies in response to growth conditions and energy availability.

The yeast *Saccharomyces cerevisiae* is a eukaryotic microorganism able to ferment carbon sources such as glucose or galactose or to respire on nonfermentable carbon sources such as glycerol or ethanol. Fermentative growth in glucose media produces ethanol as a by-product that is consumed after glucose depletion during a second respiratory growth phase, which begins after a change in cellular metabolism that is called the diauxic shift; after the ethanol is completely consumed, yeast cells stop growing and enter a distinct stationary phase (48). During the postdiauxic phase, many genes undergo large changes in expression (12). After a short lag at the diauxic shift, cells grow much more slowly in the postdiauxic phase; the rate of protein synthesis declines at the shift and recovers only partially (17). Consistent with these changes, genes encoding the translational apparatus, ribosomal pro-

teins, and translation factors predominate among the genes most strongly repressed at the diauxic shift (12). Perhaps as importantly, late in the exponential phase, as cells approach the diauxic shift but before the shift in expression begins, the concentration of ribosomes begins to decline, reaching 25% of its initial value at the diauxic shift (22).

Previous studies with bacteria indicated that translational accuracy actually changes with the stage of growth of cells. Several reports showed 3- to 10-fold increases in frameshifting upon entrance into the stationary phase in *E. coli* (4, 16, 47) and *Salmonella enterica* serovar Typhimurium (34). One experiment showed that the effect in *E. coli* is transient as cells enter the stationary phase (47). We were interested in determining whether such effects occur in a eukaryotic system and whether other translational errors are also affected. We made use of mRNA sequences that program the ribosome to efficiently undergo particular translational errors, such as reading through termination codons (nonsense readthrough) or shifting the reading frame by 1 nucleotide (nt) in the upstream or downstream direction (−1 or +1 frameshifting). These events, termed programmed alternative decoding or recoding events, occur because the sequence of the mRNA stimulates the ribosome to commit the error. The mechanism underlying these events is quite complex but often involves a translational pause that reduces the rate of normal peptide elongation, allowing a normally inefficient error to occur (reviewed by Farabaugh [14]).

We found an effect in postdiauxic growth opposite that found in bacteria. Programmed +1 frameshifting at a site derived from the yeast retrotransposon Ty1 actually decreases as cells approach the diauxic shift and decreases further as they continue into the postdiauxic and stationary phases. The effect is not general; we found no significant effect of growth stage on two other types of programmed frameshifting or on programmed nonsense readthrough. Since programmed frameshifting is extremely sensitive to changes in tRNA availability,

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the simplest explanation for this effect is that the ratio of charged tRNAs to the number of elongating ribosomes changes as yeast cells enter postdiauxic growth. We present genetic data supporting this hypothesis.

MATERIALS AND METHODS

Yeast strains. The main yeast strain used in this work is FY1679-18B (*MATα his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52 GAL2*) (13). Yeast strain FY1679-18B Δ*hsx1* (*MATα his3-Δ200 leu2-Δ1 trp1-Δ63 ura3-52 Δhsx1::HIS3*) was constructed by deleting the gene *HSX1*, which encodes the AGG-decoding tRNA, from strain FY1679-18B by introducing by yeast transformation a PCR product encompassing the Δ*hsx1::HIS3* disruption mutation amplified from strain KK240 (23). The fragment was amplified with oligonucleotides oli1714 (ATTGATTTCCATCGT GTAAG) and oli1715 (ACAACGATAATTCTTATTTC).

Media. A synthetic medium was used to select for plasmids carrying nutritional markers introduced during yeast transformation (SD: 0.17% yeast nitrogen base without amino acids and without ammonium sulfate [Difco], 0.5% ammonium sulfate, 2% glucose, appropriate amino acids). SD or a rich glucose medium (YPD: 2% yeast extract [Difco], 1% peptone [Difco], 2% glucose) was used to perform time course and batch growth experiments.

Plasmids. The reporter plasmids used (6, 37) consist of a control reporter, the *E. coli lacZ* gene, encoding β-galactosidase, translationally fused to the firefly luciferase gene, *luc*. Recoding sites are cloned between the genes such that luciferase is expressed only if the ribosome commits a translational error (readthrough or frameshift) at a recoding site. The expression of reporter plasmids previously constructed to study Ty1 +1 frameshifting (pACTy), human immunodeficiency virus type 1 (HIV-1) −1 frameshifting (pAC1789), and tobacco mosaic virus (TMV) termination readthrough (pACTMV) (37) was compared with that of specific in-frame controls used as a reference to determine the enzymatic activity corresponding to 100% expression of the fusion protein. The control for Ty +1 frameshifting was pACTTy, that for HIV-1 −1 frameshifting was pAC1790, and that for TMV readthrough was pACTQ (37). Sets of reporter plasmids were constructed for this study based on the +1 frameshift sites of the Ty3 retrotransposon of *S. cerevisiae*. Ty3 frameshifting occurs at a 21-nt sequence encompassing a GCG-AGU-U recoding site and a downstream 14-nt stimulatory sequence (15, 25). The 21-nt region was inserted in place of the recoding signal in pAC1789 to create pACTy3; an in-frame version was constructed by mutating the recoding heptamer from GCG-AGU-U to GCA-GUU to create pACTy3FF. Plasmids were transformed into yeast cells by using a lithium acetate method (18).

Growth stage analysis. To test the effect of growth stage on recoding efficiencies, we cultured yeast transformants in two ways to obtain cells at various stages of the growth cycle. A true single-step growth curve experiment was performed by inoculating yeast cells from an overnight postdiauxic-phase culture in SD minimal medium by diluting 1:1,000 into 60 ml of fresh YPD medium. After 15 to 19 h of incubation at 30°C in shaking cultures, the cells attained early exponential phase (optical density at 600 nm [OD_{600}], 0.5; corresponding to 10^7 cells/ml). Samples were withdrawn after approximately 15, 17, 19, 21, 36, and 108 h of incubation of the YPD cultures. The first four samples were in the exponential phase, as judged by their rate of doubling and the fact that over 60% of the cells were budded (10). The last two samples corresponded to the postdiauxic and stationary phases, respectively, as determined by changes in growth rate and cell morphology (48). For reproducibility, samples were obtained at approximately matched optical densities.

The second method of culturing was batch growth. For this method, cells were diluted to various extents as separate 5-ml cultures in fresh YPD or SD medium and grown for a predetermined time of 17 to 20 h to obtain samples of cells in the exponential or postdiauxic phase. To obtain stationary-phase samples, the cultures were incubated for over 60 h. We found that the level of expression observed was determined by the final density of the culture when sampled and was unaffected by the growth stage or density of the inoculum or the length of incubation before sampling (data not shown), indicating that the results reflect differences in growth stage rather than culture history.

Enzymatic activities and recoding efficiencies. Luciferase and β-galactosidase activities were assayed by using the same crude extract as described previously (37). For each assay, extracts of three transformants grown under identical conditions were prepared by breakage with acid-washed glass beads (diameter, 0.4 to 0.5 mm) as described previously (37). The concentration of protein in the crude extract was determined by using the Bradford assay (Sigma) (8). The efficiencies of programmed translational errors were determined by calculating the ratio of luciferase activity to β-galactosidase activity expressed in the experiment and in the control (expression from the in-frame control plasmid). The ratio calculated in the control corresponds to the expression of 100% *lacZ-luc* fusion protein. This value provides the bench mark for determining recoding efficiencies, which were calculated by dividing the ratio obtained in the experiment by the ratio obtained in the control. The standard errors for recoding efficiencies were generally under 10% throughout the course of this work. Specific activities used for calculations can be obtained as supplemental data at http://www-lmgm.biotoul.fr/grpfayet/stahl/supdata2004.

RESULTS

We have been concerned with the issue of how the translational apparatus maintains an adequate level of accuracy. We were interested in determining how translational accuracy might change as cells progress from the exponential phase through the postdiauxic phase and then into the stationary phase given the drastic changes in the availability of the translational machinery during those phases of growth.

Figure 1 shows a typical single-step growth curve for yeast strain Fy1679-18B in rich YPD medium. Cells diluted 1,000-fold from a stationary-phase culture rapidly enter a phase of exponential growth that continues for almost 20 h until they reach an OD_{600} of 6. For the strain used, an OD_{600} of 1.0 is equivalent to 2×10^7 cells per ml, as measured by counting with a hemacytometer. Yeast cells grow exponentially by fermentation, doubling every 90 min. After the consumption of all fermentable carbon sources, yeast cells switch to respiration of ethanol, with the switch causing a short lag called the diauxic shift. Cells then continue in a second phase of growth termed the postdiauxic phase, visualized in Fig. 1 as a break in the slope of the growth curve, since yeast cells grow more slowly when respiring. Postdiauxic growth continues for about 30 h, after which the culture density stops increasing and unbudded cells accumulate, signs that the culture has entered the stationary phase. The postdiauxic and stationary phases should not be confused, since they are physiologically quite different (21, 48).

Programmed +1 frameshifting varies with growth phase. Programmed +1 frameshifting is a very sensitive reporter of the accuracy of A-site selection (5, 15, 31). At these sites, slow recognition of an in-frame sense codon allows the peptidyl-tRNA bound to the codon immediately upstream to stimulate a +1 shift in frames. This mechanism occurs in the Ty family of yeast retrotransposons (5, 15) and in prokaryotic (11) and eukaryotic (1, 3, 26, 27, 32, 42, 43) cellular genes. Members of our laboratory have studied the mechanism of +1 frameshifting in Ty elements for many years and have arrived at a thorough molecular understanding of the process (reviewed by Stahl et al. [39]). At one of the recoding sites derived from Ty1, frameshifting is stimulated by the slow recognition of an AGG codon by the rare tRNA (5). Ty1 +1 frameshifting therefore provides an indirect measure of the rate of AGG decoding.

To test the effect of growth stage on Ty1 +1 frameshifting, we used a plasmid in which the expression of firefly luciferase (*luc*) depends on frameshifting at the Ty1 frameshift site. The reporter plasmid actually contains two reporter genes separated by a region including the Ty1 recoding site. Translation begins at the initiator of the upstream gene, *lacZ*. Ribosomes that traverse *lacZ* encounter the Ty1 recoding site, at which a proportion of them undergo a +1 frameshift event and continue translating into *luc*. The primary translation product is a fusion of the *lacZ* product, β-galactosidase, to luciferase, the
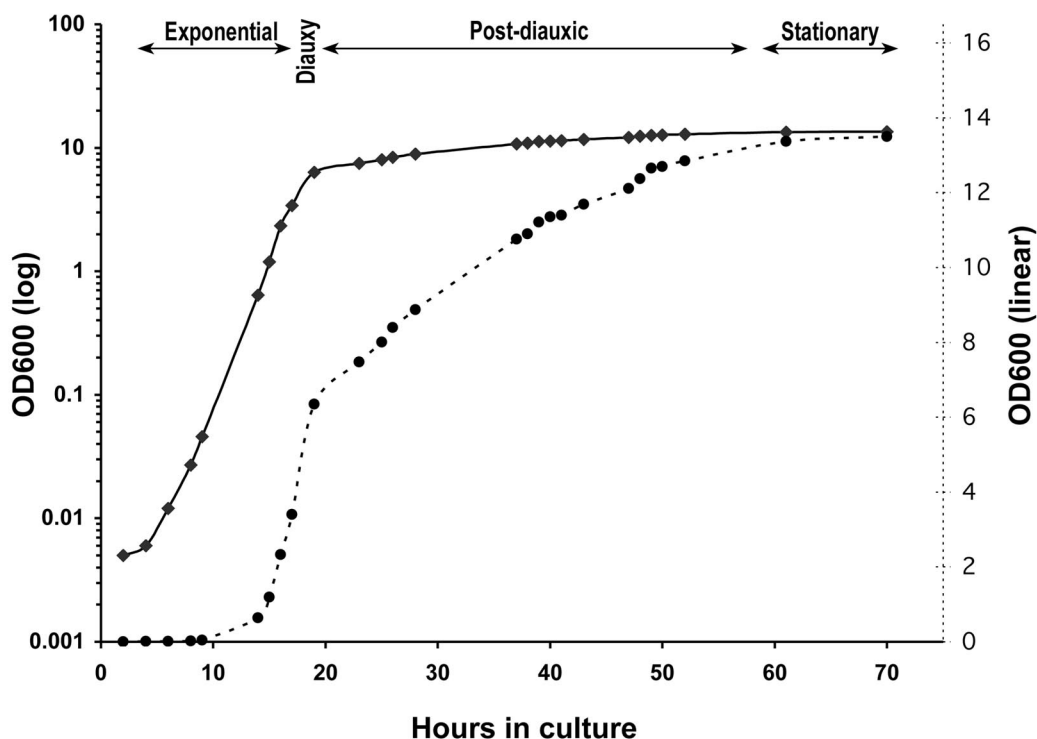
FIG. 1. Growth curves for yeast cells grown in rich YPD medium. The approximate extents of the three major growth phases are indicated by the arrows. The diauxic shift, at which glucose is exhausted and cells switch to ethanol utilization, occurs at approximately 20 h and corresponds to an obvious reduction in the growth rate.

luc product. The frequency of frameshifting is proportional to the ratio of luciferase activity to β-galactosidase activity. A construct in which the *lacZ* and *luc* genes are in the same reading frame (frame fusion plasmid) and therefore always are translated as a fusion protein provides a bench mark against which to compare expression in the frameshift reporter plasmid. The efficiency of frameshifting is determined by dividing the luciferase/β-galactosidase ratio expressed from the frameshift reporter plasmid by that expressed from the frame fusion plasmid.

A plasmid (pACTy) carrying the Ty1 frameshift site and a second reporter plasmid (pACTTy), used as the frame fusion control, were transformed into FY1679-18B. For each plasmid, cultures of three transformants that had been grown overnight (OD_{600}, 5) in SD medium with glucose as the carbon source were diluted 1:1,000 in fresh YPD medium and grown to the stationary phase. Despite the lack of selection for retention of the plasmid, which requires growth in SD minimal medium lacking leucine, the plasmid, which carries a yeast centromere to stabilize its partitioning, was quantitatively retained during culturing, as judged by plating on minimal medium with and without leucine (data not shown). Samples were periodically withdrawn, and the specific activities of both luciferase and β-galactosidase present in crude extracts were determined as described in Materials and Methods. Early during growth, frameshifting efficiency was over 50% (Fig. 2), in agreement with previous results (5, 37). As the cells continued into the late exponential phase (OD_{600}, 4.4) but before entering the postdiauxic phase, the efficiency of frameshifting began to de-

cline to 35%. As the experiment continued, the efficiency continued to decline to 22% during the postdiauxic phase and to 5% in the stationary phase. The decline in efficiency was continuous and appeared to be proportional to culture time.

We tested whether we could use a simpler batch growth experimental system, growing cells overnight in SD-glucose medium, diluting them in fresh YPD medium to various initial concentrations, and then growing them for defined periods to produce simultaneously cultures at various growth stages up to the postdiauxic phase. To achieve the stationary phase, the cultures required more extended incubation. Figure 3 shows the results of such an experiment testing variations in frameshifting at the Ty1 frameshift site with growth phase. The results strongly resemble those obtained in the single-step growth experiment illustrated in Fig. 2. Early in the exponential phase, the efficiency of frameshifting was quite high, and that level declined in cells collected at later stages of growth, with postdiauxic- and stationary-phase cultures showing about 10% frameshifting. Very similar results were obtained when cells were grown in SD-glucose selective minimal medium with the same batch culture approach (data not shown). The absolute values obtained by the two protocols were not identical, although the results obtained with each protocol were reproducible. These results suggest that there may be subtle differences in the state of cells collected at stages of growth that appear to be the same, depending on the history of culture conditions. However, the differences are slight in comparison to the overall effects of declining frameshifting efficiencies as cells approach glucose depletion and beyond.
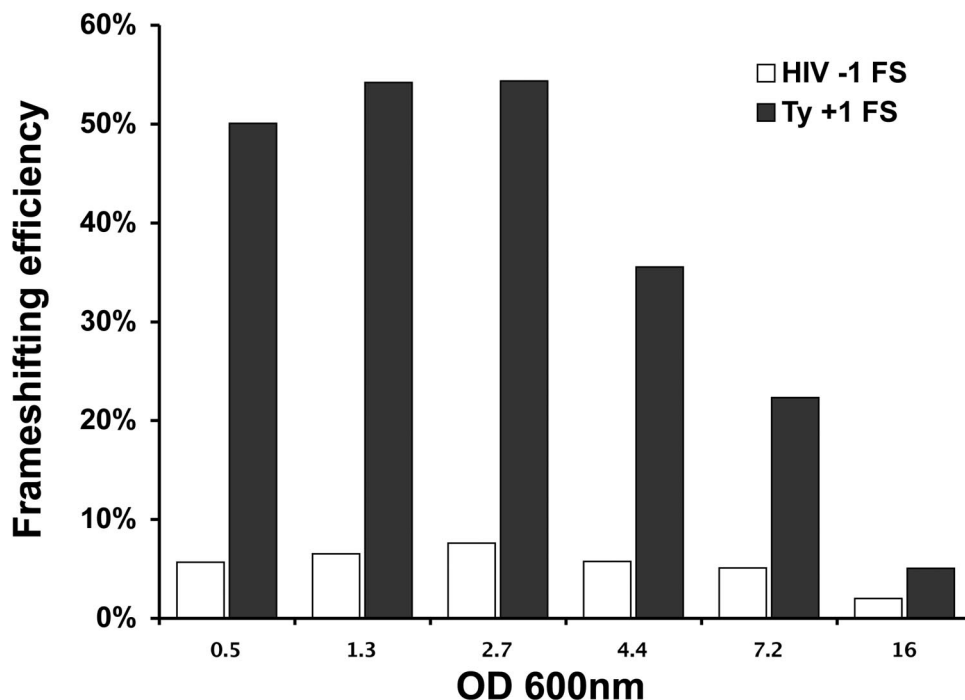
FIG. 2. Effect of growth stage on two programmed frameshifts. Cells were grown in rich YPD medium by using a single-step growth protocol. Samples were removed when the cells attained the indicated OD_{600} values. OD_{600} values of 0.5 to 4.4, 7.2, and 16 correspond to the exponential, postdiauxic, and stationary phases, respectively. FS, frameshift.

Importantly, these experiments were internally controlled to eliminate the effects of changes in gene expression occurring at levels other than frameshifting. The concept behind the dual-reporter system is that it eliminates the effects of various promoter strengths or mRNA stabilities, for example, because the upstream *lacZ* reporter acts as an internal standard. Doubling the amount of mRNA for the dual reporter would double the expression of each reporter but would leave unchanged the ratio of the two enzymatic activities.

Growth stage does not affect nonsense readthrough or programmed −1 frameshifting. Does growth stage affect other recoding events in the same way? The most versatile and best-characterized programmed error is −1 simultaneous slippage frameshifting, found ubiquitously in eukaryotic viruses (reviewed by Brierley [9]) and in cellular genes in prokaryotes, e.g., *dnaX* (45), and eukaryotes, e.g., *Edr* (35). −1 Programmed frameshifting takes place at a slippery heptamer, X XXY YYZ (X and Y can be identical), usually stimulated by a downstream structure, usually a pseudoknot in eukaryotes. Unlike the efficiency of programmed +1 frameshifting, the efficiency of simultaneous slippage −1 frameshifting is unaffected by the concentrations of the XXY and YYZ decoding aminoacyl-tRNAs, since frameshifting to XXX and YYY occurs after they have been recruited to the ribosome (reviewed by Farabaugh [14]).

We repeated the single-step growth experiment with a *lacZ-luc* dual reporter in which the HIV-1 programmed −1 frameshift site had been inserted between the two reporters so that luciferase expression required the frameshift. The HIV-1 frameshift reporter and the frame fusion control reporter were introduced into FY1679-18B as described above. Triplicate transformants for each reporter were grown to saturation in SD-glucose medium, diluted to an OD_{600} of 0.1, and cultured at 30°C. Samples were withdrawn when the cultures had attained average OD_{600} values of 0.5, 1.3, 2.7, and 4.4, all in the exponential phase, 7.2 in the postdiauxic phase, and 16 in the stationary phase. As shown in Fig. 2, the level of −1 frameshifting for the HIV-1 target was 6% in the samples taken in the early exponential phase, and that value remained virtually unchanged in samples taken throughout the course of the experiment. The experiment was repeated with the batch growth protocol. The level of frameshifting was 6 to 10% throughout the course of this experiment (Fig. 3), quite similar to the level obtained in the single-step growth experiment. The data show that the level of programmed −1 frameshifting is not significantly affected by growth stage.

TMV uses stop codon readthrough to express its polymerase (36). In yeast cells, the peptide release factor inefficiently recognizes the TMV stop codon (in bold type) in CAA**UAG**CA AUUA, allowing one in five ribosomes to read through, incorporating Tyr, Trp, or Lys at the UAG (7). The efficiency of this event depends first on slow recognition of the UAG codon by peptide termination factor, which allows for noncognate recognition of the UAG by $tRNA^{Tyr}$, $tRNA^{Trp}$, or $tRNA^{Lys}$. Since noncognate tRNAs are generally rejected by the ribosome, the second factor is the efficiency with which the translational accuracy mechanism discriminates against these noncognate tRNAs. Therefore, readthrough efficiency is a measure both of termination efficiency and of missense incorporation of aminoacyl-tRNA at noncognate codons. Using a batch growth
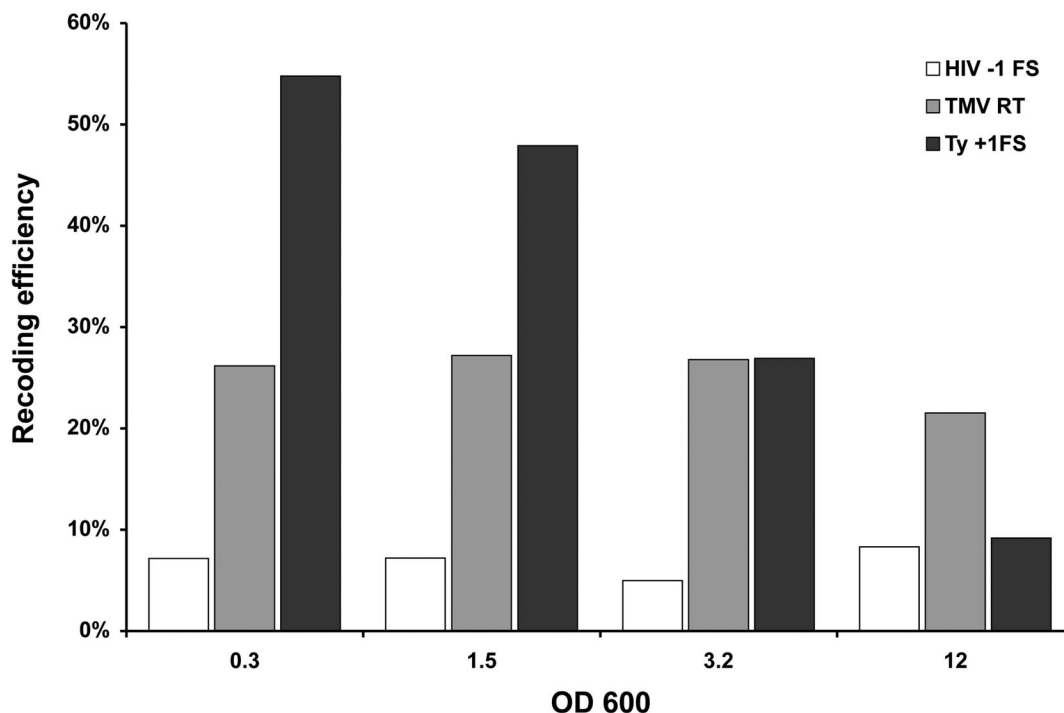
FIG. 3. Batch assay of the effect of growth stage on three recoding events. The efficiency of frameshifting in batch cultures of cells grown in rich YPD medium to the indicated OD_{600} values is shown. FS, frameshift; RT, readthrough.

approach, we tested whether the efficiency of readthrough was affected by growth stage. We detected no significant change in stop codon readthrough (Fig. 3).

Not all programmed +1 frameshifts respond to growth stage. The lack of a significant effect on either programmed −1 frameshifting or programmed nonsense readthrough suggested that the effect on programmed +1 frameshifting must be specific to that type of frameshifting. Rather than the translation system becoming generally hyperaccurate during the postdiauxic and stationary phases, an alteration in some aspect of cell physiology may directly stimulate +1 frameshifting. Recent evidence shows that +1 frameshifting in yeast cells occurs because of the presence of a noncanonical interaction between peptidyl-tRNA and the mRNA in the P site during the shift (41). This interaction can be a weak pyrimidine-pyrimidine wobble interaction, which probably does not involve any base pairing, or a purine-purine wobble clash. The presence of an unconventional pair predisposes the ribosome to shift +1 at the next step in elongation. This fact suggests that the ribosome normally uses the structure of the P-site codon-anticodon complex, especially the wobble pair, to constrain recognition in the A site to the correct reading frame (38). It is conceivable that growth stage has a general effect on the probability of errors at such a site or that its effect is more specific, affecting the probability of frameshifting only at the Ty1 frameshift site.

To examine whether the growth stage effect is general, we tested another programmed +1 frameshift that occurs in a yeast retrotransposon, Ty3, distantly related to Ty1. This frameshift site involves a different heptameric recoding signal. In Ty1, that signal is CUU-AGG-C, presented in codons of the upstream zero frame, while Ty3 uses the sequence GCG-AGU-U. The Ty3 frameshift site stimulates frameshifting quite weakly, with about 2.5% efficiency (15); a downstream mRNA sequence increases that efficiency 7.5-fold (15, 25). *lacZ-luc* dual-reporter plasmids were constructed to carry the Ty3 frameshift site with or without the adjacent stimulator. Frame fusion versions of each were constructed for each plasmid. FY1679-18B yeast cells transformed by these plasmids were grown in SD-glucose medium with the batch growth protocol. Frameshifting at the Ty3 recoding site was constant in the exponential, postdiauxic, and stationary phases. In the presence of the downstream stimulator, about 8% frameshifting occurred in each phase, while in its absence, 2% frameshifting occurred in both the exponential and the postdiauxic phases; expression in the stationary phase was too low to accurately quantify but was no higher than 2%.

Growth stage affects ribosomal pausing at AGG codons. Since the effect of growth stage on Ty1 frameshifting requires only the heptameric sequence CUU-AGG-C, it is likely that the effect can be explained by understanding how this sequence is decoded in the ribosome. Two effects combine to define the efficiency of Ty1 frameshifting: the nature of the codon-anticodon interaction at the P-site codon CUU (41) and the rate of decoding of the A-site codon AGG (5). Slippery stop frameshift sites in which the same CUU P-site codon is immediately followed by a poorly recognized termination codon also are unaffected by growth stage (data not shown). These data imply that the AGG A-site codon rather than the CUU P-site codon may be responsible for the growth stage effect.

The amount of the normally low-abundance AGG cognate $tRNA_{CCU}^{Arg}$ controls Ty1 frameshifting. Overexpressing the tRNA forces in-frame decoding and thus reduces frameshifting
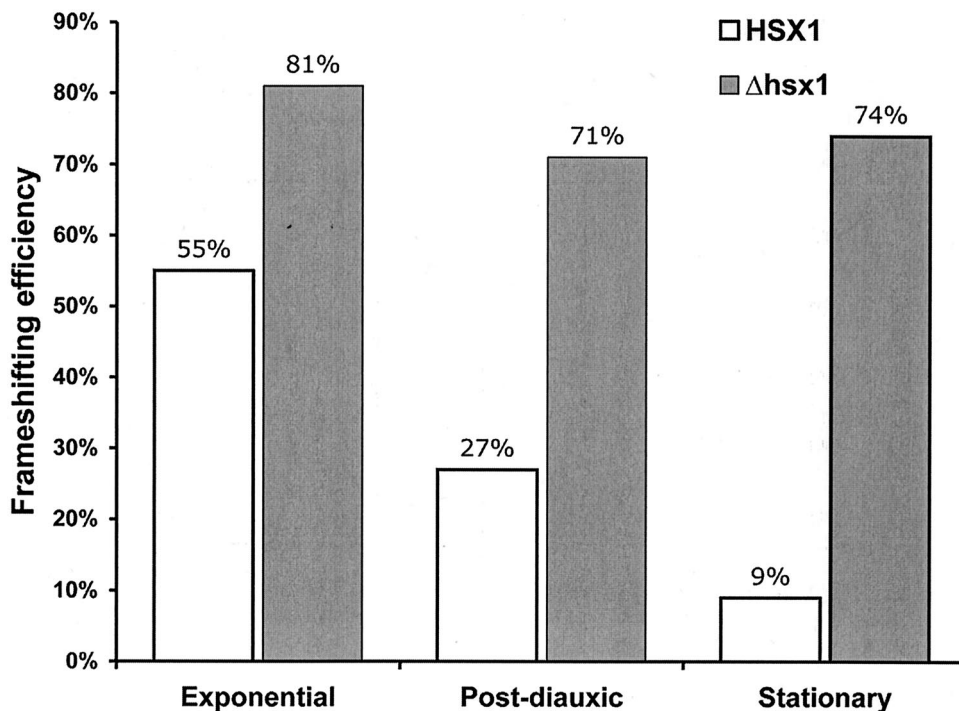
FIG. 4. Lack of tRNA$_{CCU}$^{Arg} eliminates the effect of growth stage on Ty1 +1 frameshifting. The efficiency of frameshifting in congenic strains differing in the presence of the *HSX1* gene at the three stages of growth is shown.

(5). Eliminating tRNA$_{CCU}$^{Arg} by deleting *HSX1*, the gene encoding tRNA$_{CCU}$^{Arg}, forces slow decoding of AGG by the nearly cognate tRNA$_{UCU}$^{Arg}, normally the AGA decoder (23), stimulating frameshifting. Ty1 frameshifting efficiency decreases as cells leave the exponential phase, suggesting that the availability of the tRNA may increase in the postdiauxic and stationary phases.

In a wild-type strain, the stimulatory pause results from a very slow rate of binding of the low-abundance tRNA$_{CCU}$^{Arg}. Pausing in a Δ*hsx1* strain results from a different effect. In such a strain, tRNA$_{UCU}$^{Arg}, which decodes AGG, although highly abundant, is frequently rejected by the ribosome because it is not cognate for AGG (44). The frequent dissociation of tRNA$_{UCU}$^{Arg} slows AGG recognition sufficiently and strongly stimulates frameshifting. We hypothesized that the growth stage decrease in Ty1 frameshifting results from an increase in the forward rate of binding of tRNA$_{CCU}$^{Arg} caused by a general increase in tRNA availability. Since pausing in the Δ*hsx1* strain reflects rapid dissociation rather than slow binding, passage into the postdiauxic phase in the Δ*hsx1* strain should not affect frameshifting efficiency, since the rate of dissociation should be unaffected by tRNA availability in solution.

We assayed the growth stage effect on Ty1 frameshifting in strain FY1679-18B Δ*hsx1*. The frameshifting efficiency in this strain during the exponential phase was 80%; as expected, it was increased from the level of 55% seen in the congenic parent strain FY1679-18B (Fig. 4). However, as predicted, the frameshifting efficiency in the Δ*hsx1* strain remained essentially unchanged in the postdiauxic and stationary phases, whereas that in the congenic wild-type strain declined to 27 and 9%, respectively (Fig. 4). These results strongly argue that

as cells advance through and exit the exponential phase, the normally highly limiting tRNA becomes much more available, causing a change in the way in which the ribosome reads the Ty1 recoding signal in favor of more frequent continued in-frame translation. By using specific oligonucleotide probes, we measured the yeast tRNA content in various growth phases and normalized it to that of 5S RNA (data not shown). We were not able to detect any significant change in tRNA content versus rRNA content by using this method.

DISCUSSION

There is ample evidence that the translational machinery is the aspect of yeast cellular physiology most affected by the diauxic shift. The translational machinery makes up a large proportion of cellular mass. rRNA and tRNA make up about 95% of total RNA in yeast cells, and ribosomal protein mRNAs make up almost 20% of the mRNA pool (reviewed by Warner [46]). With 200,000 ribosomes in an average cell, yeast cells must synthesize about 2,000 ribosomes per min to maintain exponential growth, doubling every 100 min (46). At the diauxic shift, the abundance of ribosomal protein mRNAs decreases an average of 4.1-fold (12). As cells approach the diauxic shift, the abundance of rRNA decreases 75%, partly because about 50% of existing cellular ribosomes are destroyed (22). The effect of these changes on yeast translational accuracy was unknown previously. Here we show that as cells shift from exponential to postdiauxic growth, there are effects on certain programmed translational errors, although there does not appear to be a general change in translational accuracy.

Ty3 programmed +1 translational frameshifting, simultaneous slippage −1 frameshifting at the HIV-1 frameshift site, and readthrough of a termination codon derived from TMV each shows little if any effect of the transit into postdiauxic growth. Programmed +1 frameshifts are sensitive indicators of A-site accuracy. These sites appear to have evolved to directly depress the kinetics of cognate, in-frame decoding in the ribosomal A site, a process which indirectly results in an increase in +1 frameshifting (reviewed by Stahl et al. [38]). Similarly, the level of TMV readthrough indicates A-site accuracy as well as the efficiency of translation termination (6). The fact that Ty3 programmed +1 frameshifting and TMV readthrough remain unchanged during the yeast growth phase suggests that A-site translational accuracy does not vary.

Despite the lack of a general effect, +1 frameshifting in the Ty1 system did respond to growth stage. Ty1 frameshifting decreased continuously as cells progressed through single-step growth from exponential to postdiauxic and finally stationary phases. The effect on Ty1 frameshifting occurred in a reporter construct that included only the heptameric recoding site, CUU-AGG-C. Frameshifting at this heptamer occurs during a pause caused by slow recognition of the in-frame codon, AGG, by its cognate $tRNA_{CCU}^{Arg}$ (5). The translational pause occurs because the supply of $tRNA_{CCU}^{Arg}$ is limiting, slowing reading of the AGG codon. The postdiauxic reduction in frameshifting at this site resembled the reduction in frameshifting that results from overexpression of the tRNA. We suspected that $tRNA_{CCU}^{Arg}$ was no longer limiting in translation after the diauxic shift. If this were true, then forcing recognition of the AGG by an abundant isoacceptor, $tRNA_{UCU}^{Arg}$, would eliminate the effect of the diauxic shift, an event which we observed. These data suggest that Ty1 frameshifting declines as cells enter the postdiauxic phase because of a change in the availability of tRNA for translation, specifically, the availability of the rare $tRNA_{CCU}^{Arg}$. Measurements of the content of this tRNA during different growth phases showed no variations relative to the cellular amounts of 5S rRNA. This finding clearly contradicts the above hypothesis. It should be noted that this experiment does not distinguish charged tRNA from uncharged tRNA, nor do we know whether the 5S rRNA content reflects the number of ribosomes actively participating in translation. Translation initiation or ribosome biogenesis could decrease after the diauxic shift.

Given these experimental limitations, we favor a hypothesis supported by the three main experimental results reported here: (i) translational accuracy, either at the level of A-site accuracy (measured at the TMV readthrough site) or for −1 frameshifting (measured at the HIV −1 frameshift site), does not vary in different growth phases; (ii) Ty3 +1 frameshifting, which is mechanistically related to that of Ty1, is not affected in different growth phases; and (iii) deletion of the scarce tRNA responsible for high levels of Ty1 frameshifting renders Ty1 frameshifting insensitive to the growth phase effect. These data suggest that the availability of $tRNA_{CCU}^{Arg}$ for translation elongation increases relative to the number of translating ribosomes as yeast cells exhaust carbon sources in the media. Why would the availability of $tRNA_{CCU}^{Arg}$ change as cells enter the postdiauxic phase? One possibility is that the massive redirection of cellular metabolism at the diauxic shift (12) reduces the demand for the tRNA. $tRNA_{CCU}^{Arg}$ is among the least abundant of all the yeast tRNAs (20), but it recognizes 21% of the Arg codons in yeast cells, or an average of 4.4 codons per structural gene (28). In contrast, $tRNA_{UCU}^{Arg}$, which is almost 10 times as abundant (20), decodes only 48% of Arg codons (28). We calculate, based on available data (30, 46), that a yeast cell contains approximately 10,000 copies of $tRNA_{CCU}^{Arg}$, or 1 copy for every 20 ribosomes. The imbalance between tRNA concentration and codon use could explain why the tRNA is so limiting.

Previous experiments showing an effect of cell growth phase on programmed frameshifting or nonsense readthrough suggested that an intrinsic change in ribosomal accuracy may be the cause of the effect. Barak et al. (4) suggested that the reduced availability of GTP in the stationary phase may cause ribosomes to stall at frameshift-inducing sequences. The data presented here clearly imply that although the efficiency of specific programmed events may change, growth phase does not affect translational accuracy in general in *S. cerevisiae*. The causes of growth phase effects on programmed translational errors likely will prove to be species dependent and to resist generalization. However, the data draw attention to the reason why genes utilize this baroque manner of expression. In general, the event accomplishes a morphogenetic task, provides an autogenous or feedback regulatory goal, or generates an alternative enzymatic activity (reviewed by Farabaugh [14]). However, no rationale is known for the use of programmed errors in several cellular genes, including the *ABP140* (3) and *EST3* (27) genes in *Saccharomyces*, the p43 protein gene in *Euplotes* (1), and the higher eukaryotic genes *Edr* (35), *headcase* (40), and others. It is possible that frameshifting in these genes conforms to the previously established types: morphogenesis, regulation, or enzymology. The data presented here suggest the possibility of another general explanation: that the frameshift provides a way to coordinate the expression of a protein product with variations in growth stage or some other general variations in cellular physiology.

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