In vivo misreading by tRNA overdose

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ABSTRACT

Rpb5-H147R is an AT-GC transition replacing CAC(His) by CGC(Arg) at a conserved and critical position of ABC27 (Rpb5p), one of the five common and essential subunits shared by all three eukaryotic RNA polymerases. This mutation is viable at 25 °C, but has a lethal phenotype at 34 °C. A search for dosage-dependent suppressors identified five distinct clones that all bear a copy of the tRNA^{His}_{GUG} gene. Suppression was also observed with a small genomic insert bearing this tRNA gene and no other coding sequences, under conditions where there is a sevenfold increase in the cellular concentration of tRNA^{His}_{GUG}. Overexpressing tRNA^{Arg}_{ICG}, which normally decodes the suppressed CGC codon, counteracted suppression. Suppression is codon specific because it was abolished when replacing CGC by its synonymous codons CGA, CGU, or AGA, but was not detectably affected by several nucleotide substitutions modifying the surrounding sequence and is thus largely insensitive to the nucleotide context. It is proposed that overexpressing tRNA^{His}_{GUG} extends its decoding properties from CAC(His) to the noncognate CGC(Arg) codon through an illegitimate U•G pairing at the middle base of the anticodon. Accordingly, tRNA^{His}_{GUG} would compete with tRNA^{Arg}_{ICG} for chain elongation and generate a significant level of misreading errors under normal growth conditions.

Keywords: anticodon; genetic code; RNA polymerase; *Saccharomyces cerevisiae*; suppressors; tRNA modification

INTRODUCTION

The genetic code is based on defined codon/anticodon pairing rules, such that the first two codon bases undergo Watson–Crick pairing with the last two anticodon bases, whereas the third codon base can be read by wobble base pair geometries (Crick, 1966). Codon/anticodon mispairing, combined with the occasional mischarging of a noncognate tRNA by a given amino acyl tRNA synthetase (Jakubowski & Goldman, 1992), generates an inevitable level of errors during protein synthesis. Processivity errors correspond to frameshifting mistakes, stop codon read-through, or abortive termination. They can be programmed to play a key role in the reproductive cycle of several bacteriophages, viruses, retroviruses, or transposons. In rarer cases, they are also important for the expression of genes encoding cellular functions (Farabaugh, 1996; Gesteland & Atkins, 1996). Missense errors, on the other hand, involve the mistranslation of a given codon into a noncognate amino acid and are thus true misreading

defects. They do not contribute to any known form of programmed recoding, and can therefore be viewed as an unpleasant yet affordable price to pay for maintaining a sufficiently fast rate of chain elongation. Their spontaneous frequency in vivo is not precisely known but, in *Escherichia coli*, could reach 10^{−3} at some codons (Parker, 1989).

Our understanding of misreading errors owes much to the properties of informational suppressor mutants. In bacteria, several mutants affecting the spontaneous level of missense errors have been identified, most of which alter ribosomal proteins (Gorini, 1971; Kurland et al., 1996, and references therein). Bacterial missense tRNA suppressor mutants have also been reported (Murgola, 1995). They are generally a result of a change in the anticodon sequence, leading to the recognition of another codon. In one case, however, suppression resulted from a change in the aminoacyl accepting stem and was thus caused by a misacylation effect (Prather et al., 1984). In yeast, early reports on missense suppressors (Sherman, 1982) have not been followed by any molecular characterization, except for a C → U change in the third base of the tRNA^{Glu}_{UUC} anticodon that decodes AAA(Lys) into Glutamate (Su et al., 1990). Similarly, an A → C change in the middle position of the anticodon enables tRNA^{Leu}_{CAG} to misread the CGG(Pro) in *Aspergillus nidulans* (Chiu & Morris, 1997).

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Abbreviations: i⁶A: N⁶-isopentenyladenosine; m₁G: 1-methylguanosine; mcm⁵s²U: 5-methoxycarbonylmethyl-2-thiouridine; mcm⁵U: 5-methoxycarbonylmethyluridine.

Base modifications at or near the anticodon are also known to affect the efficiency and specificity of decoding (Curran, 1998, and references therein). In mitochondria, changes in the modification pattern of anticodons contribute to the diversification of the genetic code by altering the wobble properties of the first anticodon base (position 34) (Heckmann et al., 1980; Matsuyama et al., 1998) or by pseudouridylation at the middle position (Tomita et al., 1999). Differences in uridine modification at position 34 also make the decoding of eukaryotic nuclear genes somewhat different than in bacteria (Curran, 1998, and references therein). Moreover, yeast mutants lacking i^6A at position 37 (Janner et al., 1980) or mcm^5s^2U at position 34 weaken nonsense suppression in vivo (Grossenbacher et al., 1986; Heyer et al., 1984).

Since the seminal papers of Ninio (1971, 1974), it has become widely accepted that tRNAs have a non-negligible probability of pairing with noncognate codons, and that ribosomes discriminate between cognate and noncognate pairing by a kinetic proofreading system (Thompson, 1988). This study underscores the key role played by the cellular concentration of individual tRNAs in maintaining the accuracy of gene expression in vivo. We show that, in the physiological context of genetically wild-type yeast cells, a sevenfold increase in the steady state level of $tRNA^{His}_{GUG}$ suffices to generate substantial in vivo misreading at the CGC(Arg) codon, and that increasing the cellular level in $tRNA^{Arg}_{ICG}$ (decoding CGC) counteracts this effect. These data strongly suggest a dosage-dependent U•G mispairing at the middle base of the $tRNA^{His}_{GUG}$ anticodon.

RESULTS

Rpb5-H147R is suppressed by a high gene-dosage of $tRNA^{His}_{GUG}$

RPB5 encodes a polypeptide subunit called ABC27 (or Rpb5p) that is shared by all three yeast RNA polymerases and is essential for growth (Woychik et al., 1990). While studying the physiological role of ABC27 in transcription (F. Navarro, J.F. Briand, and P. Thuriaux, in prep.), we introduced amino acid substitutions at various conserved positions of the coding sequence. One of the resulting mutants, *rpb5-H147R*, is the result of a single AT-GC transition replacing CAC(His) by CGC(Arg). This mutant allele has a strong temperature-sensitive growth defect.

In an attempt to identify putative partner proteins of ABC27, strain YFN6 (bearing *rpb5-H147R* on a multicopy plasmid) was transformed by a wild-type genomic DNA library of *Saccharomyces cerevisiae* (strain FL100) borne on the multicopy plasmid pFL44L (Stettler et al., 1993). Nineteen clones were selected based on their ability to support growth at 37°C. Fourteen contained *RPB5* itself and thus acted by genetic complementa-

tion. As summarized in Figure 1, the remaining five clones all harbored a copy of the $tRNA^{His}_{GUG}$ gene that decodes the CAC and CAU triplets, because G at the first anticodon position pairs with C (Watson-Crick pairing) and U (wobble pairing). The *S. cerevisiae* genome contains seven copies of the $tRNA^{His}_{GUG}$ gene (Goffeau et al., 1996), all of which encode an identical pre- $tRNA^{His}$ transcript corresponding to the $tRNA^{His2}$ (Keith et al., 1983). $tRNA^{His1}$, a slightly different isoaccepting sequence, has no counterpart in the standard genomic sequence of *S. cerevisiae* and is thus presumably a sequencing artefact.

To firmly establish that suppression was due to the $tRNA^{His}$ gene itself, we constructed pFL44-t(His)K, a pFL44L derivative where the tRNA gene is spanned by 183 bp upstream and 134 bp downstream of the pre-tRNA sequence. This insert contains no gene other than the $tRNA^{His}_{GUG}$ coding sequence. It was sequenced to rule out the possibility of cryptic mutation(s) that might have been present in the FL100 strain background used to construct the genomic library. pFL44-t(His)K effectively suppressed *rpb5-H147R* (Fig. 1). This tRNA is therefore the only factor responsible for the effect observed in the original suppressor clones. In liquid medium, strain YFN16-H (bearing pFL44-t(His)K) had a doubling time of 140 min compared to 90 min for the isogenic wild-type control strain YFN16-R5, confirming the strong degree of suppression observed on solid medium. Finally, we checked that the centromeric pFL38-t(His)K plasmid (where the tRNA gene is present at a low copy number) fails to restore growth at 34°C, and that suppression thus strictly depends on a high copy number of the t(His)K gene.

These data show that *rpb5-H147R* suppression is mediated by an increased copy number of $tRNA^{His}_{GUG}$, and thus presumably results from an increase in the steady-state level of that tRNA. We therefore measured the steady-state level of $tRNA^{His}$, by Northern hybridization with a $tRNA^{His}$ -specific probe. pFL44L has an estimated copy number of 40 (Bonneaud et al., 1991), and there are seven chromosomal copies of the $tRNA^{His}$ gene (Goffeau et al., 1996). Assuming that gene expression is proportional to the number of gene copies present in the cell, one expects an increase of about sevenfold in the steady-state level in $tRNA^{His}$. This is consistent with our hybridization data (Fig. 2) that actually indicate a 7.4-fold increase, suggesting that the chromosomal and plasmid-borne $tRNA^{His}$ genes are transcribed at essentially the same rate.

Suppression is specific to the CGC(Arg) codon

Rpb5-H147R replaces the wild-type CAC(His) codon by CGC(Arg). We have shown that this mutation is suppressed under conditions of $tRNA^{His}_{GUG}$ overproduction. Moreover, no other tRNA gene was isolated from our suppression screen in the yeast genomic li-

FIGURE 1. Overexpression of $\text{tRNA}^{\text{His}}_{\text{GUG}}$ suppresses the *rp5-H147R* temperature sensitive mutant. **A:** Schematic representation of the chromosomal inserts harbored by the five multicopy plasmids (derived from pFL44L) that suppress the temperature-sensitive phenotype of strain YFN6(*rp5-H147R*). Black squares indicate $\text{tRNA}^{\text{His}}_{\text{GUG}}$ encoding genes. The open square denotes a $\text{tRNA}^{\text{His}}_{\text{GUG}}$ encoding gene. Striped and open rectangles denote complete or partial protein coding sequences, respectively. **B:** Multicopy suppression of the *rp5-H147R* mutation. Strain YFN6 was transformed with the indicated plasmids and grown on YPD plates for 4 days at 25°C and 34°C.

brary, implying that suppression is specific to that tRNA. These observations strongly suggest that suppression is due to the misreading of the CGC(Arg) codon into histidine, thus restoring a wild-type gene product of RPB5. In other words, it is proposed that a U•G mis-

pairing becomes possible at the second base of the anticodon when $\text{tRNA}^{\text{His}}_{\text{GUG}}$ is overproduced.

To examine whether suppression is specific to the CGC(Arg) triplet, several synonymous *rp5-H147R* alleles were created by introducing three other Arg co-

FIGURE 2. t(his)K transcript accumulation. Strain YFN16(*rpb5-H147R*) transformed with the pFL44-t(His)K or pFL44-RPB5 was cultivated in liquid YPD medium at 34 °C until midexponential phase (DO₆₀₀ 0.4). Hybridizations were carried out at 41 °C using oligonucleotides complementary to the t(his)K and t(Arg)2 genes (see Materials and Methods).

dons (AGA, CGA, and CGU) at position 147. AGA is decoded by tRNA^{Arg}_{mcm5UCU}, the most abundant tRNA^{Arg} species. CGU and CGC are decoded by tRNA^{Arg}_{ICG} due to I•U, I•C pairing at the wobble base. CGA is possibly decoded by the same tRNA species, assuming I•A pairing. As summarized in Table 2, all *rpb5-H147R* mutants are phenotypically identical, irrespective of whether the Arg triplet is abundant (AGA), moderately abundant (CGU), or extremely rare (CGC, CGA) in highly expressed genes (Sharp & Cowe, 1991). Yet, suppression is restricted to the CGC triplet. This is not due to differences in codon usage, as both CGC and CGA are rare codons. Moreover, CGU is not suppressible, although it is decoded by the same tRNA species as CGC, confirming that suppression operates at the level of codon recognition. In summary, our data show that the overexpression of tRNA^{His}_{GUG} allows it to decode in vivo the noncognate CGC codon, in addition to the two histidine codons CAC and CAU. In other words, increasing the local concentration in tRNA^{His}_{GUG} generates U•G mispairing at the middle base of the GUG anticodon.

Effect of the surrounding nucleotide sequence

Work in *E. coli* has shown that missense suppression can be notably affected by changes in the nucleotide sequence immediately upstream or downstream of the suppressed codon (Murgola et al., 1984). In the case of *rpb5-H147R*, there is another CAC (His) triplet at position 146 that might conceivably favor histidine misreading at position 147. We thus created a *rpb5-H146V*

H147R double mutant by changing the 3 nt of the CAC codon into GTG at position 146 (see Table 2). The corresponding strain has a somewhat more pronounced growth defect than *rpb5-H147R* at the restrictive temperature, but, as shown in Table 2, was effectively suppressed by the overproduction of tRNA^{His}_{GUG}. The presence of a CAC(His) triplet immediately upstream of the mutated CAC(His) triplet of *rpb5-H147R* is thus immaterial to the suppressibility of that mutant. Similarly, replacing CAC(His) at position 146 by CAU(His), or replacing GAA(Glu) at position 148 by the synonymous GAG codon did not affect suppression. Therefore, nucleotide changes in the immediate vicinity of position 147 have no detectable effect on suppression.

Overexpressing tRNA^{Arg}_{ICG} antagonises suppression by tRNA^{His}_{GUG}

Suppression is specific to CGC(Arg), a codon that is normally decoded by tRNA^{Arg}_{ICG}. This obviously suggests a competition between these two tRNAs at the CGC(Arg) codon, allowing CGC to be misread into histidine, provided that there is a sufficiently large excess in tRNA^{His}_{GUG}. Under this hypothesis, increasing the input of the tRNA^{Arg}_{ICG} species should reduce suppression. Accordingly, we cloned one of the tRNA^{Arg} genes, t(Arg)2, in the multicopy plasmid pASZ12 (with *ADE2*⁺ as selective marker), along with 39 bp upstream and 269 bp downstream of the pre-tRNA sequence. The histidine and arginine tRNA plasmids can be easily identified by their different selective markers (*URA3*⁺ and *ADE2*⁺, respectively).

Strain YFN16-HR is a *rpb5-H147R* mutant, where tRNA^{His}_{GUG} and tRNA^{Arg}_{ICG} are expressed from two distinct multicopy plasmids, pFL44-t(His)K and pASZ12-t(Arg)2, bearing *URA3*⁺ and *ADE2*⁺ as selective markers, respectively. This strain was tested for its ability to grow at 34 °C in a medium that lacks adenine and uracil and thus imposes the simultaneous presence of both plasmids. As illustrated in Figure 3A, the tRNA^{Arg}_{ICG} plasmid has an antagonistic effect on suppression by tRNA^{His}, with a distinctly delayed growth at 34 °C. Colonies were nevertheless formed upon prolonged incubation. They were quite heterogeneous in size and corresponded to a plating efficiency of about 50% compared to the permissive temperature. This suggested an adaptive effect due to a decrease in the average tRNA^{Arg}_{ICG} plasmid copy number, relieving the competition with tRNA^{His}_{GUG}.

This interpretation is further supported by the instability displayed by the tRNA^{Arg}_{ICG} plasmid when YFN16-HR is grown on YPD (Fig. 3B). The YPD medium contains adenine and thus does not impose the presence of the *ADE2*⁺ plasmid overexpressing tRNA^{Arg}_{ICG}. Moreover, the spontaneous loss of the *ADE2*⁺ plasmid can be scored by the characteristic red pigmentation of the corresponding *ade2* colonies

TABLE 1. Plasmids and yeast strains.

Plasmids			
Name	Yeast genetic markers	Promoter used to express <i>RPB5</i>	Origin
pGEN-RPB5	ORI(2μm) <i>TRP1 RPB5</i>	<i>pPGK1</i>	Cloning of the <i>RPB5</i> ORF between <i>Bam</i> HI and <i>Kpn</i> I in pGEN
pGEN-RPB5(H147R)	ORI(2μm) <i>TRP1 rpb5-H147R</i>	<i>pPGK1</i>	PCR-mediated mutagenesis of pGEN-RPB5
pCM185-RPB5	CEN <i>TRP1 RPB5</i>	<i>pTET</i>	Cloning of <i>RPB5</i> ORF in the <i>Bam</i> HI site of pCM185
pCM185-RPB5(H147R)	CEN <i>TRP1 rpb5-H147R</i>	<i>pTET</i>	PCR-mediated mutagenesis of pCM185-RPB5
pCM185-RPB5(H147R1)	CEN <i>TRP1 rpb5-H147R</i>	<i>pTET</i>	PCR-mediated mutagenesis of pCM185-RPB5
pCM185-RPB5(H147R2)	CEN <i>TRP1 rpb5-H147R</i>	<i>pTET</i>	PCR-mediated mutagenesis of pCM185-RPB5
pCM185-RPB5(H147R3)	CEN <i>TRP1 rpb5-H147R</i>	<i>pTET</i>	PCR-mediated mutagenesis of pCM185-RPB5
pCM185-RPB5(H146VH147R)	CEN <i>TRP1 rpb5-H146VH147R</i>	<i>pTET</i>	PCR-mediated mutagenesis of pCM185-RPB5
pCM185-RPB5(H146HH147R)	CEN <i>TRP1 rpb5-H146HH147R</i>	<i>pTET</i>	PCR-mediated mutagenesis of pCM185-RPB5
pCM185-RPB5(H147RE148E)	CEN <i>TRP1 rpb5-H147RE148E</i>	<i>pTET</i>	PCR-mediated mutagenesis of pCM185-RPB5
PFL44-t(His)K	ORI(2μm) <i>URA3 t(his)K</i>		Cloning of the tRNA ^{His} _{GUG} gene into the <i>Bam</i> HI site of pFL44L
PFL38-t(His)K	CEN <i>URA3 t(his)K</i>		Cloning of the tRNA ^{His} _{GUG} gene into the <i>Bam</i> HI site of pFL38
PASZ12-t(Arg)2	ORI(2μm) <i>ADE2 t(Arg)2</i>		Cloning of the tRNA ^{Arg} _{ICG} gene into the <i>Bam</i> HI site of pASZ12
PASZ11-RPB5	CEN <i>ADE2 RPB5</i>		Cloning of a 1.1-kb <i>Eco</i> RV- <i>Spe</i> I <i>RPB5</i> fragment into the <i>Bam</i> HI site of pASZ11
Strains			
Name	Genotype	Origin	
YPH501	<i>MATα/MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1</i>	(Sikorski & Hieter, 1989)	
YFS11	<i>MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb5::URA3 //pASZ11-RPB5</i>	This work	
YFN2	<i>MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb5::URA3::LEU2 //pPL44-RPB5</i>	This work	
YFN6	<i>MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb5::URA3::LEU2 //pGEN-rpb5(H147R)</i>	This work	
YFN13	<i>MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb5::URA3::LEU2 //pCM185-RPB5</i>	This work	
YFN16	<i>MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb5::URA3::LEU2 //pCM185-rpb5(H147R)</i>	This work	
YFN16-HR	<i>MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb5::URA3::LEU2 //pCM185-rpb5(H147R)//pFL44-t(His)K//pASZ12-t(Arg)2</i>	This work	
YFN16-H12	<i>MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb5::URA3::LEU2 //pCM185-rpb5(H147R)//pFL44-t(His)K//pASZ12</i>	This work	
YFN16-R5	<i>MATa ade2-101 lys2-801 ura3-52 trp1-Δ63 his3-Δ200 leu2-Δ1 rpb5::URA3::LEU2 //pCM185-rpb5(H147R)//pFL44-RPB5</i>	This work	

or colony sectors. As shown in Figure 3B, the simultaneous presence of the tRNA^{Arg}_{ICG} (*ADE2*⁺) and tRNA^{His}_{GUG} (*URA3*⁺) plasmids generated a high instability of the tRNA^{Arg}_{ICG} plasmid at 34 °C, as monitored by the frequent formation of red colonies, indicating that tRNA^{Arg}_{ICG} overexpression is strongly counterselected under these conditions.

Overproduction of tRNA^{His}_{GUG} has no strong adverse effect on growth

We wondered if tRNA^{His} overproduction might adversely affect growth, because of a general misreading effect of the CGC(Arg) triplet. To examine this possibility, the pFL44-t(His)K plasmid was introduced in an *RPB5*⁺

TABLE 2. Effect of the nucleotide context on the suppressibility of *rpb5-H147R*.

RPB5 mutations	Codons at positions 146–148	Growth	Suppression by pFL44-t(His)K
wt	CAC CAC GAA	+	nd ^a
H147R	CAC CGC GAA	ts ^b	+
H147R1	CAC CGA GAA	ts	–
H147R2	CAC CGU GAA	ts	–
H147R3	CAC AGA GAA	ts	–
H146V H147R	GUG CGC GAA	ts	+
H146H H147R	CAU CGC GAA	ts	+
H147R E148E	CAC CGC GAG	ts	+

Suppression was tested as in Figure 1. See Table 1 for the construction of the *rpb5* mutant alleles. The mutated codon is indicated in bold case.

^and: not determined.

^bts: temperature sensitive.

FIGURE 3. Competition between t(his)K and t(Arg)2 in the in vivo suppression of *rbp5-H147R*. **A:** Strains YFN16-HR and YFN16-H12 were grown in liquid minimal medium until a DO_{600} of about 1.0, spread on agar-minimal medium after appropriate dilution, and incubated at 34 °C. **B:** YFN16-HR and YFN16-H12 were grown on YPD plates at 34 °C and monitored for the formation of red sectors, corresponding to the loss of the pASZ12 (*ADE2*⁺) plasmid.

background (strain YFN13), and its spontaneous loss, which restores uracil auxotrophy, was monitored by replica plating on FOA medium (Boeke et al., 1984). There was no significant difference in plasmid loss compared to a control strain containing the void pFL44L vector. Thus, the tRNA^{His} plasmid is not appreciably counterselected, as would be expected if the high dosage in tRNA^{His} did markedly impair growth. However, minor adverse effects may remain undetected under these conditions. Moreover, living cells are probably equipped with mechanisms that rehabilitate or destroy aberrant proteins (see Kurland et al., 1996, for a cogent discussion of that point).

DISCUSSION

This work is based on the unusual genetic suppression pattern of *rbp5-H147R*, a semilethal CAC(His) →

CGC(Arg) substitution that prevents growth at 34 °C or above. *rbp5-H147R* is strongly responsive to an increased gene dosage in tRNA^{His}_{GUG} or tRNA^{Arg}_{IGA}, the tRNA species decoding the wild-type (CAC) and mutant (CGC) codons, respectively. A high gene dosage in tRNA^{His}_{GUG} restores a quasi wild-type growth at 34 °C, whereas a concomitant increase in tRNA^{Arg}_{IGA} antagonises suppression. We propose that tRNA^{His}_{GUG} illegitimately pairs with CGC(Arg) in the physiological context of growing yeast cells, thereby establishing a non-canonical G•U pairing at the middle base of the anticodon. tRNA^{His}_{GUG} would thus compete in vivo with the cognate tRNA^{Arg}_{IGA} species of CGC. As a result, a comparatively modest sevenfold increase in the tRNA^{His}_{GUG}/tRNA^{Arg}_{IGA} ratio would suffice to generate a substantial amount of CGC misreading.

Dosage-dependent suppression by overexpressed tRNA is not without precedent, as it was previously reported that yeast nonsense mutants (UAG and UAA) are partly suppressed by overexpressing tRNA^{Gln} (Weiss et al., 1987; Edelman & Culbertson, 1991). Increasing the gene dosage of tRNA^{Arg}_{CCU} also modulates the programmed frameshift recoding of the yeast Ty1 element (Belcourt & Farabaugh, 1990; Xu & Boeke, 1990), whereas some *E. coli* −1 frameshift mutants are bypassed by an increased gene dosage of tRNA^{Gly}_{CCC} (O'Connor, 1998). All these cases are related to processivity defects. As far as misreading defects are concerned, a UCG(Ser) → UUG(Leu) missense mutation was found to be suppressed by the heterologous expression of *Lactobacillus bulgaricus* tRNA^{Ser}_{CGA} in *E. coli* (Hottinger et al., 1987; O'Connor, 1998), but this may reflect intrinsic structural differences between the *L. bulgaricus* and *E. coli* tRNAs rather than a gene dosage effect.

Our results are in good agreement with the kinetic proofreading hypothesis (Ninio 1971, 1974; Thompson 1988), which states that a given codon always has a certain probability of pairing with some noncognate tRNAs. The discrimination between cognate and noncognate tRNAs (before they are irreversibly engaged in elongation) will then depend on their respective residence time at the decoding site of ribosomes, and therefore on their cellular concentration. tRNA^{His}_{GUG} normally decodes CAC and CAU by G•C and G•U pairing at the wobble base and, at a higher concentration, would thus gain the ability to form a U•G pair at the central base of the anticodon. This pairing may be stabilized by the two thermodynamically stable flanking G•C pairs. Indeed, overexpressing tRNA^{His}_{GUG} does not extend its decoding ability from CAU(His) to CGU(Arg), indicating that U•G pairing at the middle position is ineffective when G•C is replaced by G•U at the third anticodon position. The formation of G•U pairs at the middle anticodon position was already noted in vitro, using tRNAs paired with complementary anticodons (Grosjean et al., 1978).

Yeast tRNA^{Arg}_{ICG} and tRNA^{His}_{GUG} are extensively modified (Keith et al., 1983; Weissenbach et al., 1975), and these modifications may well contribute to the misreading detected in the present study. Thus, the inosine of tRNA^{Arg}_{ICG} may generate an intrinsically weak pairing to CGC, therefore facilitating the misreading of this codon by tRNA^{His}_{GUG}. Although inosine pairs with U, A, and C (Crick, 1966), CGA and CGC are strikingly rare codons in highly expressed genes (Sharp & Cowe, 1991), and $\bullet$ A pairing was directly shown to be inefficient in vivo (Munz et al., 1981; Curran, 1995). Our data suggest that this may also hold for the $\bullet$ C pair. tRNA^{His}_{GUG} is not modified at the anticodon, but has a 1-methylguanosine (m₁G) at position 37 (Keith et al., 1983). tRNA overproduction could accumulate an undermodified form that may conceivably alter the specificity of codon–anticodon pairing and lead to mispairing. No yeast mutant lacking m₁G has been reported so far, but the corresponding bacterial mutants have an increased level of frameshifting (Björk et al., 1999). This could be indicative of enhanced misreading because, as discussed by Farabaugh and Björk (1999), the presence of a noncognate tRNA at the A-site of ribosomes might well be at the origin of most spontaneous frameshifting events.

MATERIALS AND METHODS

Plasmids

All plasmids are listed in Table 1. pCM185-RPB5 and pGEN-RPB5 were constructed by expressing the *RPB5* coding sequence from the doxycycline-repressible Tet promoter of pCM185 (Gari et al., 1997) or from the strong constitutive PGK1 promoter of pGEN (Shpakovski et al., 1995), respectively. The other plasmids derive from pFL44L(URA3) (Bonneaud et al., 1991), pASZ12 (equivalent to pFL44L but having *ADE2*⁺ instead of *URA3*⁺ as selective marker), and pASZ11, a centromeric version of pASZ12 (Stotz & Linder, 1990).

Plasmids containing *Rpb5* mutant alleles were obtained by PCR-directed mutagenesis of pCM185-RPB5 or pGEN-RPB5, using complementary oligonucleotide primers containing the desired mutation and amplified with Pfu DNA polymerase (Stratagene). pFL44-t(His)K is a pFL44L derivative bearing a 378-bp *Bam*HI insertion containing the t(His)K gene that was prepared by PCR amplification from one of the plasmids obtained in the suppression experiments (pFL44-SUP13, see Fig. 1A), using GGCGGATCCGCCTTTAGCAGCAATGG and GGCGGATCCGAAGAGGTTCTATAACC as 5' and 3' primers. The insert was sequenced and found to be identical to the sequence predicted from the standard yeast genome (Goffeau et al., 1996), thus ruling out the presence of cryptic mutations originating from the FL100 strain background used to construct the genomic library. pASZ12-t(Arg)2 is a pASZ12 derivative bearing a 399-bp *Bam*HI insertion containing the t(Arg)2 gene that was prepared by PCR amplification of yeast genomic DNA from strain YPH500, using CGCGGATCCCATAGGCATACCTTGCCC and CGCGGATCCCGATTTGGGTGACACGG as 5' and 3' primers.

Five suppressor plasmids bearing wild-type copies of the tRNA^{His}_{GUG} gene (SUP8, SUP13, SUP14, SUP22, and SUP23; see Fig. 1A) were originally isolated at 37 °C in a *rpb5-H147R* context. To this end, we used a pFL44L genomic DNA library prepared from strain FL100 (Stettler et al., 1993) and introduced by transformation in strain YFN6, where *rpb5-H147R* is on the multicopy plasmid pGenRPB5-H147R. We then noted that *rpb5-H147R* has a more pronounced temperature-sensitive defect when borne on the centromeric vector pCM185-RPB5(H147R) (strain YFN16). All subsequent work was therefore done in the latter context, at a restrictive temperature of 34 °C.

Yeast strains

Standard yeast media, growth conditions, and genetic techniques were used throughout (see Shpakovski et al., 1995). All strains (see Table 1) were derived from the same isogenic stock (Sikorski & Hieter, 1989), but the suppressor plasmid bearing the tRNA^{His}_{GUG} gene derived from another laboratory strain, FL100 (Stettler et al., 1993). Strain YFS11 was constructed by inactivating one copy of the *RPB5*⁺ gene of the diploid strain YPH501 (Sikorski & Hieter, 1989) with a previously described *rpb5::URA3* disruption cassette (Baur et al., 1993). This diploid was transformed with the *ADE2*⁺ vector pASZ11-RPB5, and the YFS11 segregant was recovered by tetrad analysis. The *rpb5::URA3* allele of YFS11 was then mutagenized into *rpb5::ura3::LEU2* by integrative transformation with the *ura3::LEU2* disrupting cassette from plasmid pUL9 (Cross, 1997), and pASZ11-RPB5 was exchanged for the *URA3*⁺ plasmid pFL44-RPB5, to generate strain YFN2. *Rpb5* mutants (including strain YFN16, our reference host strain used in monitoring suppression; see above) were then derived from YFN2 by exchanging pFL44-RPB5 for pGEN or pCM185 plasmids bearing the appropriate *rpb5* mutant allele.

tRNA quantification

Total yeast RNA was transferred to nylon membranes (Boehringer Mannheim) and hybridized against two tDNA probes, 5'-TGCCATCTCCTAGAA-3' (complementary to tRNA^{His}_{GUG}) and 5'-CTTCCCCGCCAGGAC-3' (complementary to tRNA^{Arg}_{ICG}). They were labeled by phosphorylation with bacteriophage T4 polynucleotide kinase, using γ -32-ATP. Hybridization signals were quantified using a Phosphor-Imager, with the ImageQuant software (Molecular Dynamics).

tRNA nomenclature

tRNAs are designated by the cognate amino acid and by the relevant anticodon sequence, using standard symbols for base modification, based on a recent compilation of tRNA sequences (Sprinzl et al., 1998) (<http://www.uni-bayreuth.de/departments/biochemie/trna/>).

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