The yeast rapid tRNA decay pathway competes with elongation factor 1A for substrate tRNAs and acts on tRNAs lacking one or more of several modifications

JOSHUA M. DEWE, JOSEPH M. WHIPPLE,¹ IRINA CHERNYAKOV,² LAURA N. JARAMILLO,² and ERIC M. PHIZICKY³

Department of Biochemistry and Biophysics, Center for RNA Biology, University of Rochester School of Medicine, Rochester, New York 14642, USA

ABSTRACT

The structural and functional integrity of tRNA is crucial for translation. In the yeast *Saccharomyces cerevisiae*, certain aberrant pre-tRNA species are subject to nuclear surveillance, leading to 3' exonucleolytic degradation, and certain mature tRNA species are subject to rapid tRNA decay (RTD) if they are appropriately hypomodified or bear specific destabilizing mutations, leading to 5'-3' exonucleolytic degradation by Rat1 and Xrn1. Thus, *trm8-Δ trm4-Δ* strains are temperature sensitive due to lack of m⁷G₄₆ and m⁵C and the consequent RTD of tRNA^{Val(AAC)}, and *tan1-Δ trm44-Δ* strains are temperature sensitive due to lack of ac⁴C₁₂ and Um₄₄ and the consequent RTD of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}. It is unknown how the RTD pathway interacts with translation and other cellular processes, and how generally this pathway acts on hypomodified tRNAs. We provide evidence here that elongation factor 1A (EF-1A) competes with the RTD pathway for substrate tRNAs, since its overexpression suppresses the tRNA degradation and the growth defect of strains subject to RTD, whereas reduced levels of EF-1A have the opposite effect. We also provide evidence that RTD acts on a variety of tRNAs lacking one or more different modifications, since *trm1-Δ trm4-Δ* mutants are subject to RTD of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} due to lack of m^{2,2}G₂₆ and m⁵C, and since *trm8-Δ*, *tan1-Δ*, and *trm1-Δ* single mutants are each subject to RTD. These results demonstrate that RTD interacts with the translation machinery and acts widely on hypomodified tRNAs.

Keywords: *TEF1*; *TRM1*; EF-1A; tRNA turnover; *Saccharomyces cerevisiae*

INTRODUCTION

The structural and functional integrity of tRNA is crucial for translation in all cells. Efficient and accurate translation requires that tRNAs have four features. First, to ensure their participation in translation with more or less equal efficiency, all tRNAs require a similar overall shape and structure, including the familiar clover leaf secondary structure, and tertiary interactions that are important for efficient folding of the tRNA into its usual L-shape (Kim et al. 1974; Jack et al. 1976; Westhof et al. 1985; Basavappa and Sigler 1991).

Second, to ensure specific recognition by their cognate tRNA synthetases (Ling et al. 2009), all tRNAs require unique features, including the anticodon (N₃₄-N₃₆), the discriminator base (N₇₃), several modifications, and a number of residues throughout the tRNA (Muramatsu et al. 1988; Musier-Forsyth and Schimmel 1992; Giege et al. 1998). Third, to ensure high-accuracy translation (Zaher and Green 2009; Kramer et al. 2010), all tRNAs require features to uniquely decode their cognate codon, including an extensive set of anticodon loop modifications for highly specific codon:anticodon pairing (Murphy and Ramakrishnan 2004; Murphy et al. 2004; Agris et al. 2007), and other sequence elements (Cochella and Green 2005; Ledoux et al. 2009). Fourth, all tRNAs are required to be rugged enough to survive constant use in translation but flexible enough to withstand the bending and contortions that occur during ribosome transit (Valle et al. 2003; Yarus et al. 2003; Schmeing et al. 2009). The approximately 3×10^6 molecules of tRNA in an actively growing yeast cell require synthesis of about two tRNAs/second/locus (Waldron and Lacroute 1975) and

¹Present address: Department of Biochemistry, University of Utah, Salt Lake City, UT 84112, USA

²Present address: Department of Molecular Biology and Genetics, Weill Institute for Cell and Molecular Biology, Cornell University, Ithaca, NY 14853, USA

³Corresponding author

E-mail eric_phizicky@urmc.rochester.edu

Article published online ahead of print. Article and publication date are at <http://www.rnajournal.org/cgi/doi/10.1261/rna.033654.112>.

long tRNA lifetimes, which in turn require mechanisms to ensure that tRNAs have the proper stability (Phizicky and Hopper 2010).

tRNA is subject to two quality-control steps during and after its biogenesis. During processing, a nuclear surveillance pathway acts on pre-tRNA^{Met} lacking 1-methyladenosine at A₅₈ (m¹A₅₈) and on pre-tRNA species that are aberrantly processed at their 3' ends; this occurs by oligoadenylation of the 3' end of the tRNA by the TRAMP complex, followed by 3' exonucleolytic degradation by Rrp6 and the nuclear exosome (Kadaba et al. 2004, 2006; LaCava et al. 2005; Vanacova et al. 2005; Copela et al. 2008; Wang et al. 2008; Ozanick et al. 2009). After processing, a rapid tRNA decay (RTD) pathway acts on several specific mature tRNA species that lack certain modifications; this is mediated by the 5'-3' exonucleases Rat1 and Xrn1 and by Met22, which likely regulates Rat1 and Xrn1 activity indirectly, through inhibition by its substrate pAp (Dichtl et al. 1997; Alexandrov et al. 2006; Chernyakov et al. 2008; Whipple et al. 2011). Thus, *trm8-Δ trm4-Δ* strains are temperature sensitive due to lack of m⁷G₄₆ and m⁵C in their tRNAs and the consequent RTD of tRNA^{Val(AAC)} (Alexandrov et al. 2006), and *tan1-Δ trm44-Δ* strains are temperature sensitive due to lack of ac⁴C₁₂ and Um₄₄ in their tRNAs and the consequent RTD of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}, as well as tRNA^{Leu(GAG)} (Chernyakov et al. 2008; Kotelawala et al. 2008). RTD may act on charged tRNAs, since RTD of tRNA^{Val(AAC)} in *trm8-Δ trm4-Δ* strains appears to be selective for the charged species and since suppressor mutations in *MET22*, *RAT1*, and *XRN1* that prevent RTD also restore charging (Alexandrov et al. 2006; Chernyakov et al. 2008).

We have recently shown that the RTD pathway also acts on tRNAs bearing mutations that destabilize the combined acceptor and T-stems of tRNA^{Ser} or tRNA^{Tyr} (Whipple et al. 2011). To understand how the RTD pathway selects certain substrate tRNA species lacking modifications, but not other tRNAs with the same modifications, we developed a genetic screen to examine tRNA^{Ser(CGA)} variants. From analysis of 43 tRNA^{Ser} variants, we showed that RTD substrate recognition in vivo correlates strongly with reduced predicted stability of the acceptor and T-stems, but not of the anticodon stem, and does not necessarily require hypomodified tRNA, since fully modified tRNAs are subject to RTD if appropriately destabilized. Moreover, our biochemical evidence shows a strong correlation between the increased degradation by purified Xrn1 and the weaker predicted stability of the acceptor and T-stems or the weaker stability due to lack of ac⁴C₁₂ and Um₄₄ (Whipple et al. 2011). Remarkably, tRNA^{Ser} species subject to RTD also have an increased population of molecules in which the 3' ends have CCACCA or oligo(A) instead of the usual CCA terminus (Wilusz et al. 2011).

A number of features of the RTD pathway are currently unclear, including how the RTD pathway interacts with the translation machinery and with other cellular processes, as well as the true scope of the RTD pathway with respect

to the number of different modification defects that can trigger RTD and the number of different tRNAs that are subject to RTD. To gain a better understanding of these factors, we selected and characterized high-copy suppressors of the RTD phenotype of *trm8-Δ trm4-Δ* strains and examined the role of RTD in another tRNA modification mutant. We find evidence that the translation elongation factor 1A (EF-1A) is in competition with the RTD pathway for charged tRNA substrates. We also find that RTD is triggered by *trm1-Δ trm4-Δ* mutants lacking m^{2,2}G and m⁵C, due to loss of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}, and provide evidence that the lack of a number of different individual modifications triggers RTD of the same tRNA species found for the corresponding double mutants. These results significantly broaden the scope of the RTD pathway and define an important interaction of this pathway with the translation apparatus.

RESULTS

RTD competes with EF-1A for tRNA substrates

To further define components of RTD, we screened the MORF library of yeast expression plasmids for genes that suppress the temperature-sensitive phenotype of *trm8-Δ trm4-Δ* mutants, when expressed at high levels under the control of the P_{GAL} promoter from a high-copy plasmid (Gelperin et al. 2005). In this way we found several high-copy suppressors (Fig. 1A), including *TEF1* and *TEF2*, which each encode identical copies of EF-1A.

To determine if overexpression of EF-1A suppresses the temperature-sensitive phenotype of *trm8-Δ trm4-Δ* mutants by preventing RTD of tRNA^{Val(AAC)}, we examined tRNA levels after thiolutin treatment, which inhibits further transcription by RNA polymerases including polymerase III (Jimenez et al. 1973), thereby freezing the number of tRNA species before the temperature shift. We find that, under these conditions, overexpression of *TEF1* or *TEF2* markedly prevents degradation of tRNA^{Val(AAC)} in *trm8-Δ trm4-Δ* mutants undergoing RTD at 34°C (Fig. 1B).

To determine the generality of EF-1A effects, we examined suppression of the growth defect of *tan1-Δ trm44-Δ* mutants, which are also subject to RTD and have different tRNA substrates (Chernyakov et al. 2008). Since overexpression of either *TEF1* or *TEF2* also suppresses the temperature sensitivity of *tan1-Δ trm44-Δ* mutants (Fig. 2A) and since overexpression of *TEF1* prevents RTD of its known substrates tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} after thiolutin treatment and a temperature shift to 36°C (Fig. 2B), we conclude that the overexpression of EF-1A generally prevents RTD.

One plausible mechanism by which RTD could be suppressed by overexpression of EF-1A is if there is competition between EF-1A and the RTD pathway for charged tRNAs. It is well established that EF-1A binds charged tRNA (Gromadski et al. 2007), and our evidence suggests that

FIGURE 1. Overproduction of EF-1A suppresses RTD in a *trm8-Δ trm4-Δ* strain. (A) Overexpression of *TEF1* or *TEF2* suppresses the temperature sensitivity of a *trm8-Δ trm4-Δ* strain. A *trm8-Δ trm4-Δ* strain was transformed with MORF plasmids expressing the indicated ORFs under P_{GAL} control, transformants were grown overnight in S-Ura media containing raffinose, and serial 10-fold dilutions were plated on YP media containing raffinose and galactose and incubated for 2 d at the indicated temperatures. (B) Overproduction of EF-1A prevents degradation of tRNA^{Val(AAC)} in *trm8-Δ trm4-Δ* mutants. Strains containing plasmids as indicated were grown at 28°C in S-Ura media containing raffinose and galactose to OD₆₀₀ 0.6, thiolutin (TL) was added, cells were shifted to 34°C for the times indicated, and RNA was extracted, resolved by 10% PAGE, transferred to a membrane, and hybridized to the indicated probes, as described in the Materials and Methods. RNA levels were quantified by normalizing to 5S RNA levels and then normalizing to the values in the wild type (BY4741) at time zero. Diamonds indicate wild type; squares, *trm8-Δ trm4-Δ*; open triangles, *trm8-Δ trm4-Δ [TEF1]*; and open circles, *trm8-Δ trm4-Δ [TEF2]*.

RTD of tRNA^{Val(AAC)} preferentially degrades the charged species (Alexandrov et al. 2006; Chernyakov et al. 2008). If competition between the RTD pathway and EF-1A occurs, then reduced levels of EF-1A ought to make cells more temperature sensitive, due to enhanced RTD. Consistent with this hypothesis, we find that a *tef1-Δ* mutation significantly enhances the temperature sensitivity of a *trm8-Δ trm4-Δ* mutant or a *tan1-Δ trm44-Δ* mutant (Fig. 3A). Since *tef1-Δ* mutants are not themselves temperature sensitive (Fig. 3A) and since the enhanced temperature sensitivity of a *trm8-Δ trm4-Δ tef1-Δ* mutant or a *tan1-Δ trm44-Δ tef1-Δ* mutant is completely suppressed by introduction of a *met22-Δ* mutation to prevent RTD (Chernyakov et al. 2008), we infer that the enhanced temperature sensitivity is due to the enhanced RTD

occurring in *tef1-Δ*-derivative strains. Consistent with this explanation, we find that total tRNA^{Val(AAC)} levels are reduced in a *trm8-Δ trm4-Δ tef1-Δ* mutant relative to a *trm8-Δ trm4-Δ* mutant prior to a shift from 28°C and are degraded at the same rate in both strains after a temperature shift and that levels of charged tRNA^{Val(AAC)} are reduced at an accelerated rate after a shift to 32°C (Fig. 3B) or after a shift to 30.5°C or 35°C (data not shown). Similarly, we observe somewhat accelerated and more complete RTD of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} in a *tan1-Δ trm44-Δ tef1-Δ* mutant relative to that in a *tan1-Δ trm44-Δ* mutant, after thiolutin treatment and a shift to 37°C (Fig. 3C).

FIGURE 2. Overproduction of EF-1A suppresses RTD in a *tan1-Δ trm44-Δ* mutant. (A) Overproduction of *TEF1* or *TEF2* partially suppresses the temperature-sensitive phenotype of *tan1-Δ trm44-Δ* mutants. Strains with MORF plasmids expressing *TEF1* or *TEF2* (or a vector control) were grown and plated on YP media containing raffinose and galactose as described in Figure 1A. (B) Overproduction of *TEF1* prevents degradation of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} in *tan1-Δ trm44-Δ* mutants. Strains were grown at 28°C, treated with thiolutin (TL), and shifted to 36°C for the times indicated, and RNA was extracted, resolved by PAGE, transferred, and hybridized. RNA levels were quantified by normalizing to 5.8 S RNA levels and then normalizing to the values in the wild type (BY4741) at time zero. Diamonds indicate wild type; open squares, *tan1-Δ trm44-Δ*; and triangles, *tan1-Δ trm44-Δ [TEF1]*.

These results are consistent with competition between EF-1A and the RTD pathway for charged tRNAs in the cell, which is possible because cellular levels of EF-1A approach those of tRNAs (Thiele et al. 1985). As predicted from these results, we also find that a *tef1-Δ* mutation enhances the temperature sensitivity of a *trm8-Δ pus7-Δ* mutant and a *trm8-Δ dus3-Δ* mutant (Fig. 3D), which are each subject to RTD (Chernyakov et al. 2008). Based on these results, we conclude that the enhanced sensitivity of *tef1-Δ*-derivative strains to RTD is a general feature of the pathway, presumably due to the relative lack of competing EF-1A, just as the enhanced protection from RTD caused by overexpression of EF-1A is likely due to the relative increase in competing EF-1A.

trm1-Δ trm4-Δ mutants are subject to RTD of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} due to the lack of m^{2,2}G and m⁵C

To further explore the potential of RTD to occur with different modification mutants, we focused on double mutants generated from *trm1-Δ* mutants since *trm1-Δ* mutants, like *trm8-Δ* mutants (Alexandrov et al. 2005), have a subtle growth defect at high temperature that is significantly exacerbated in double mutants (Gustavsson and Ronne 2008). Thus, we find that *trm1-Δ trm4-Δ* mutants are temperature sensitive and that this temperature sensitivity is suppressed by a *met22-Δ* mutation, indicative of an RTD phenotype (Fig. 4A). Northern analysis demonstrates reduced levels of only tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} of nine tRNA species examined (of 16 total) that are known to have both of these modifications (Fig. 4B; data not shown). Consistent with the idea that these tRNAs are the targets of RTD in *trm1-Δ trm4-Δ* mutants, we find that the temperature sensitivity of *trm1-Δ trm4-Δ* mutants is nearly fully suppressed by overexpression of tRNA^{Ser(CGA)} or tRNA^{Ser(UGA)} (Fig. 4C).

RTD occurs in *trm8-Δ*, *tan1-Δ*, and *trm1-Δ* single-mutant strains, but with reduced efficiency

We speculated that RTD might also occur in single mutants because RTD occurs in several double mutants with a common *trm8-Δ* mutation (*trm8-Δ trm4-Δ*, *trm8-Δ pus7-Δ*, and *trm8-Δ dus3-Δ*) (Alexandrov et al. 2006; Chernyakov et al. 2008) and because our analysis shows that RTD occurs with varying efficiency that corresponds to the extent of destabilization of the acceptor and T-stems (Whipple et al. 2011). Consistent with this idea, we find that RTD can be triggered by single-modification mutants, albeit with reduced efficiency relative to that in double mutants. Thus, a temperature shift of a *trm8-Δ* mutant to 37°C after thiolutrin treatment results in a distinct loss of tRNA^{Val(AAC)} levels within 8–24 h, albeit at a much reduced rate relative to that in *trm8-Δ trm4-Δ* mutants, but there is no reduction in levels of tRNA^{Phe}, which also has m⁷G (Fig. 5). Similarly, a

FIGURE 3. (Legend on next page)

temperature shift of a *tan1-Δ* mutant to 37°C after thiolutin treatment results in significant loss of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} within 3–24 h, but not as severely as in *tan1-Δ trm44-Δ* mutants (Fig. 5). In both strains, tRNA^{Leu(GAG)} is lost to the same extent, consistent with the fact that tRNA^{Leu(GAG)} is not modified by Trm44 (data not shown).

As we found for double mutants, we find that single mutants have a detectable RTD growth phenotype that is exacerbated by reduced EF-1A levels. Thus, we find that *tef1-Δ* derivatives of *trm8-Δ*, *tan1-Δ*, and *trm1-Δ* single-mutant strains are each more temperature sensitive than the corresponding single-mutant strains on YP (yeast extract, peptone) media containing glycerol (YPG) (Fig. 6A) or glucose (YPD) (Fig. 6B,C), and that a *met22-Δ* mutation completely suppresses the temperature sensitivity of *trm8-Δ*, *tan1-Δ*, and *trm1-Δ* single-mutant strains, as well as of the corresponding *tef1-Δ*-derivative strains (Fig. 6B,C).

To identify the tRNAs subject to RTD in a *trm1-Δ tef1-Δ* strain, we examined suppression of the growth defect by overexpression of individual tRNA species, and examined tRNA degradation. Our evidence suggests that the temperature sensitivity of *trm1-Δ tef1-Δ* mutants is associated primarily with loss of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}, since overexpression of either of these tRNA species substantially suppresses the temperature sensitivity on YPG media (Fig. 7A) and since Northern analysis demonstrates loss of these same tRNA species after a temperature shift to 37°C in YPG media (Fig. 7B). We note that the *trm1-Δ* single-mutant strain also undergoes RTD of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} under these conditions, in the absence of thiolutin treatment, albeit at a reduced rate relative to that observed in the *trm1-Δ tef1-Δ* strain. The observation of RTD in a *trm1-Δ* strain in the absence of thiolutin treatment emphasizes the

FIGURE 3. Reduced levels of EF-1A exacerbate the temperature sensitivity of strains subject to RTD and result in reduced levels of tRNAs subject to RTD. (A) A *tef1-Δ* mutation exacerbates the temperature sensitivity of *trm8-Δ trm4-Δ* mutants and *tan1-Δ trm44-Δ* mutants, and this growth defect is fully suppressed by a *met22-Δ* mutation. Strains were grown overnight in YPD media, serially 10-fold diluted, plated on YPD plates, and incubated for 2 d at the indicated temperatures. (B) *trm8-Δ trm4-Δ tef1-Δ* mutants have reduced levels of tRNA^{Val(AAC)} and its charged species during RTD, relative to *trm8-Δ trm4-Δ* mutants. Strains were grown at 28°C and shifted to 32°C, cells were harvested at the times indicated, and RNA was isolated under acidic conditions to preserve charging. Then RNAs were resolved by 6.5% acidic PAGE as described in the Materials and Methods, transferred, and hybridized to probes as indicated. RNA levels were quantified as described in Figure 2B. Quantification of charging was done for each individual tRNA^{Val(AAC)} sample. (C) *tan1-Δ trm44-Δ tef1-Δ* mutants have reduced levels of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} relative to *tan1-Δ trm44-Δ* mutants during RTD. Strains were grown at 28°C, treated with thiolutin (TL), and shifted to 37°C, and RNA was analyzed at the times indicated and quantified as described in Figure 1B. Bars in black indicate 0 min; light gray, 90 min; and dark gray, 180 min. (D) A *tef1-Δ* mutation exacerbates the temperature sensitivity of *trm8-Δ pus7-Δ* mutants and *trm8-Δ dus3-Δ* mutants. Strains were grown, diluted, and plated on YPD plates as described in A.

FIGURE 4. *trm1-Δ trm4-Δ* mutants are subject to RTD of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}. (A) The temperature sensitivity of *trm1-Δ trm4-Δ* mutants is suppressed by a *met22-Δ* mutation. Strains were grown, diluted, and plated on YPD plates as described in Figure 3A. (B) *trm1-Δ trm4-Δ* mutants have reduced levels of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}. Wild-type or *trm1-Δ trm4-Δ* strains were grown in YPD media at 28°C, treated with thiolutin (TL), and shifted to 37.5°C, and RNA from cells was analyzed by hybridization at the times indicated. Quantification of RNA levels was done as described in Figure 2B. Bars in black indicate 0 min; light gray, 90 min; and dark gray, 240 min. (C) The temperature sensitivity of *trm1-Δ trm4-Δ* mutants is suppressed by overproduction of tRNA^{Ser(CGA)} or tRNA^{Ser(UGA)}. Wild-type and *trm1-Δ trm4-Δ* strains bearing [2μ LEU2 tRNA^{Ser(CGA)}], [2μ LEU2 tRNA^{Ser(UGA)}], or a plasmid vector control were grown overnight in SD-Leu media, serially diluted, plated on SD-Leu media, and incubated for 4 d at the indicated temperatures.

substantial amount of RTD occurring in this single-mutant strain under these conditions.

DISCUSSION

We have provided substantial evidence here for a model in which the RTD machinery is in competition with EF-1A for

FIGURE 5. *trm8-Δ* mutants undergo slow degradation of tRNA^{Val(AAC)}, and *tan1-Δ* mutants undergo slow degradation of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}. Strains were grown at 28°C, treated with thiolutin, and shifted to 37°C, and RNAs from cells were analyzed at the times indicated, as described in Figure 1B. RNA levels were quantified by normalizing to tRNA^{Phe} levels, and then by normalizing to the values in wild type (BY4741) at time zero. Bars in black indicate 0 h; gray, 24 h.

access to tRNA substrates. We have shown that elevated levels of EF-1A suppress the temperature sensitivity of *trm8-Δ trm4-Δ* strains and *tan1-Δ trm44-Δ* strains and prevent degradation of the corresponding tRNA^{Val(AAC)} and tRNA^{Ser(CGA)} (and tRNA^{Ser(UGA)}) species. Furthermore, the reduction of EF-1A levels by a *tef1-Δ* mutation leads to exacerbated temperature sensitivity of the *trm8-Δ trm4-Δ* strains and *tan1-Δ trm44-Δ* strains (each of which is suppressed by a *met22-Δ* mutation) and results in reduced levels of tRNA^{Val(AAC)} and tRNA^{Ser(CGA)} (and tRNA^{Ser(UGA)}), respectively, before and during the temperature shift.

These effects of EF-1A extend to a number of strains subject to RTD. Thus, compared with *trm1-Δ* strains, *trm1-Δ tef1-Δ* strains are more temperature sensitive and undergo more complete degradation of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}, and the temperature sensitivity is suppressed by a *met22-Δ* mutation. Similarly, *trm8-Δ tef1-Δ* and *tan1-Δ tef1-Δ* strains are each more temperature sensitive than the corresponding *trm8-Δ* and *tan1-Δ* strains, and their temperature sensitivity is suppressed by a *met22-Δ* mutation. In addition, *trm8-Δ pus7-Δ tef1-Δ* and *trm8-Δ dus3-Δ tef1-Δ* strains are each more temperature sensitive than their *TEF1*⁺ derivatives.

The apparent competition between EF-1A and the RTD pathway is consistent with the known specificity of EF-1A for charged tRNA (Gromadski et al. 2007) and the observed selective loss of charged tRNA^{Val(AAC)} during

RTD in *trm8-Δ trm4-Δ* mutants, which suggests that charged tRNA^{Val(AAC)} may be the substrate for RTD (Chernyakov et al. 2008). Indeed, since bacterial EF-Tu, which corresponds to eukaryotic EF-1A, binds charged tRNAs with a different affinity based on the identity of the amino acid and specific base pairs in the T-stem (LaRiviere et al. 2001; Sanderson and Uhlenbeck 2007; Schrader et al. 2009), differential competition between EF-1A and RTD for specific tRNA species may explain why certain tRNAs are substrates for RTD, but other identically modified tRNA species are not, despite the reduced predicted stability of their acceptor and T-stems (Whipple et al. 2011).

Our findings also significantly extend the known range of modification defects subject to RTD. We had previously reported that the RTD pathway acts in *trm8-Δ trm4-Δ* mutants, *trm8-Δ pus7-Δ* mutants, and *trm8-Δ dus3-Δ*

FIGURE 6. The RTD phenotypes of single-modification mutants are exacerbated by a *tef1-Δ* mutation. (A) A *tef1-Δ* mutation exacerbates the temperature sensitivity of *trm8-Δ* mutants, *tan1-Δ* mutants, and *trm1-Δ* mutants. Strains were grown overnight in YPD media, serially 10-fold diluted, plated on YPG plates containing 3% glycerol (YPG), and incubated for 4 d at the indicated temperatures. (B) A *met22-Δ* mutation fully suppresses the temperature sensitivity of *trm8-Δ* mutants and *trm8-Δ tef1-Δ* mutants. Strains were grown, diluted, and plated on YPG plates as described in Figure 3A. Similar results are observed in YPG media (data not shown). (C) A *met22-Δ* mutation fully suppresses the temperature sensitivity of *tan1-Δ* and *trm1-Δ* mutants, and their *tef1-Δ*-derivative strains. Strains were grown, diluted, and plated on YPG plates as described in Figure 3A. Similar results are observed in YPG media (data not shown).

FIGURE 7. A *trm1-Δ tef1-Δ* strain is subject to RTD of tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}. (A) The temperature sensitivity of a *trm1-Δ tef1-Δ* strain is substantially suppressed by overproduction of tRNA^{Ser(CGA)} or tRNA^{Ser(UGA)}. Wild-type and *trm1-Δ tef1-Δ* strains bearing [2 μ *LEU2* tRNA^{Ser(CGA)}], [2 μ *LEU2* tRNA^{Ser(UGA)}], or a plasmid vector control were grown overnight in SD-Leu media, serially diluted, plated on YPG media, and incubated for 3 d at the indicated temperatures. (B) *trm1-Δ tef1-Δ* mutants undergo more rapid degradation of tRNA^{Ser(UGA)} than *trm1-Δ* mutants. Strains were grown in YPG media to OD₆₀₀ 0.4 and shifted to 37°C, and RNA from cells was analyzed as described in Figure 1B. Diamonds indicate wild type; squares, *trm1-Δ*; and open triangles, *trm1-Δ tef1-Δ*.

mutants (each targeting tRNA^{Val(AAC)}); in *tan1-Δ trm44-Δ* mutants (targeting tRNA^{Ser(CGA)}, tRNA^{Ser(UGA)}, and tRNA^{Leu(GAG)}); and in wild-type strains bearing mutations in tRNA^{Ser} and tRNA^{Tyr} that disrupt the predicted stability of the acceptor and T-stems (Alexandrov et al. 2006; Chernyakov et al. 2008; Whipple et al. 2011). The results described here extend the scope of RTD (based on Northern analysis and *met22-Δ* suppression of the temperature sensitivity) to *trm1-Δ trm4-Δ* mutants (lacking m^{2,2}G₂₆ and m⁵C) targeting tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} and to three single modification mutants: *trm8-Δ* mutants, targeting tRNA^{Val(AAC)}, and *tan1-Δ* mutants and *trm1-Δ* mutants, each targeting tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}.

Since each of the *trm8-Δ*, *tan1-Δ*, and *trm1-Δ* single mutants target the same tRNA species that are targeted in the corresponding double-mutant strains but in each case the RTD effect (as measured by Northern and/or growth phenotype) is more mild than in the corresponding double-mutant strains, we speculate that RTD arises in numerous other cases of hypomodified or mutated tRNAs, even under conditions where there is little, if any, distinct growth phenotype, as is especially true for *trm8-Δ* mutants (Alexandrov et al.

2005) and *trm1-Δ* mutants (Gustavsson and Ronne 2008). Indeed, it seems likely that RTD is continually monitoring the integrity of tRNA by monitoring the spectrum of modifications to the body of the tRNA, and mutations generated during transcription that impair stability of the acceptor and T-stems and the tertiary fold (Whipple et al. 2011).

It is curious that *trm1-Δ* mutants, like *tan1-Δ* mutants, only appear to target tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)}, since m^{2,2}G₂₆ is known to be present on 19 tRNA species, and is likely also present on most of the five unsequenced tRNA species with a G₂₆ residue. The sensitivity of these two tRNA^{Ser} species to RTD in both *trm1-Δ* mutants and *tan1-Δ* mutants is consistent with the fact that these tRNA species are predicted to have among the least stable combined acceptor and T-stems among the 42 cytoplasmic tRNA species (Whipple et al. 2011), and suggests that these tRNA^{Ser} species are poised at the edge of permissible stability for function in the cell, such that lack of any of several modifications targets them for destruction by RTD. Furthermore, the specificity of both *trm1-Δ* mutants and *trm1-Δ trm4-Δ* mutants for tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} are additional examples of cases in which a small specific subset of tRNAs is the main apparent biological target of a modification mutant with a distinct phenotype (Phizicky and Alfonzo 2010). This is also observed in *trm8-Δ trm4-Δ* mutants, which target only tRNA^{Val(AAC)} for RTD and not the three other tRNAs with the same modifications in the same locations (Alexandrov et al. 2006), and in *tan1-Δ trm44-Δ* mutants, which target tRNA^{Ser(CGA)} and tRNA^{Ser(UGA)} for RTD and not the two other tRNA^{Ser} species with the same modifications (Kotelawala et al. 2008). A similar result is observed in *trm6-504* mutants (which lack m¹A₅₈), which target pre-tRNA^{Met} for degradation, and not the other 17 species known to have m¹A₅₈ (Anderson et al. 1998; Vanacova et al. 2005; Schneider et al. 2007). Likewise, yeast strains carrying mutations in the ELP complex (which lack the cm⁵U moiety of mcm⁵U₃₄, ncm⁵U₃₄, ncm⁵Um₃₄, and mcm⁵s²U₃₄), have several distinctive phenotypes that are due only to tRNA^{Lys(UUU)} and tRNA^{Gln(UUG)} and not the nine other tRNA species with these modifications (Esberg et al. 2006; Johansson et al. 2008), and the lethal phenotype of *elp3-Δ tuc1-Δ* mutants (which lack both the cm⁵U and the s²U moieties of mcm⁵s²U₃₄) is due to defects in tRNA^{Lys(UUU)} and not the two other tRNA species with this modification (Bjork et al. 2007).

In summary, the results described here and previously (Whipple et al. 2011) provide substantial support for a model in which RTD acts widely on tRNAs lacking one or more of several different modifications implicated in stabilizing tertiary structure or on tRNAs with mutations that destabilize their combined acceptor and T-stems. In such strains, RTD occurs constitutively before a temperature shift, resulting in reduced initial levels of tRNAs for substrate species. As the temperature increases in the strain, the 5' ends of RTD substrate tRNAs are increasingly exposed to

TABLE 1. Parent yeast strains used in this study

Strain name	Parent strain	Genotype	Source
BY4741		WT MAT α	Giaever et al. (2002)
BY4742		WT MAT α	Giaever et al. (2002)
AA 527	BY4741	<i>trm8-Δ::natMX trm4-Δ::kanMX</i>	Alexandrov et al. (2006)
LY 1172	BY4741	<i>tan1-Δ::kanMX trm44-Δ::natMX</i>	Kotelawala et al. (2008)
ISC 576	BY4741	<i>met22-Δ::hygR</i>	This study
YJD149	BY4741	<i>tef1-Δ::bleR</i>	This study
YJD151-1	AA 527	<i>trm8-Δ::natMX trm4-Δ::kanMX tef1-Δ::bleR</i>	This study
YJD152-1	LY 1172	<i>tan1-Δ::kanMX trm44-Δ::natMX tef1-Δ::bleR</i>	This study
YJD236-1	AA 527	<i>trm8-Δ::natMX trm4-Δ::kanMX met22-Δ::hygR</i>	This study
LY 1729	LY 1172	<i>tan1-Δ::kanMX trm44-Δ::natMX met22-Δ::hygR</i>	Chernyakov et al. (2008)
YJD182-1	YJD151-1	<i>trm8-Δ::natMX trm4-Δ::kanMX tef1-Δ::bleR met22-Δ::hygR</i>	This study
YJD183	YJD152-1	<i>tan1-Δ::kanMX trm44-Δ::natMX tef1-Δ::bleR met22-Δ::hygR</i>	This study
AA 537	BY4741	<i>trm8-Δ::kanMX pus7-Δ::natMX</i>	Alexandrov et al. (2006)
AA 774	BY4741	<i>trm8-Δ::kanMX dus3-Δ::natMX</i>	Alexandrov et al. (2006)
YJD227	AA 537	<i>trm8-Δ::kanMX pus7-Δ::natMX tef1-Δ::bleR</i>	This study
YJD228	AA 774	<i>trm8-Δ::kanMX dus3-Δ::natMX tef1-Δ::bleR</i>	This study
YLN002	BY4742	<i>BY4742 trm1-Δ::kanMX</i>	This study
YLN086	YLN002	<i>BY4742 trm1-Δ::bleR trm4-Δ::kanMX</i>	This study
YLN206	YLN086	<i>BY4742 trm1-Δ::bleR trm4-Δ::kanMX met22-Δ::hygR</i>	This study
JMW678	BY4741	<i>trm8-Δ::natMX</i>	Alexandrov et al. (2006)
JMW679	BY4741	<i>tan1-Δ::kanMX</i>	This study
YJD232	JMW678	<i>trm8-Δ::natMX tef1-Δ::bleR</i>	This study
YJD233	JMW679	<i>tan1-Δ::kanMX tef1-Δ::bleR</i>	This study
YJD234	YLN002	<i>BY4742 trm1-Δ::kanMX tef1-Δ::bleR</i>	This study
YJD352	JMW679	<i>tan1-Δ::kanMX met22-Δ::hygR</i>	This study
YJD354	YLN002	<i>BY4742 trm1-Δ::kanMX met22-Δ::hygR</i>	This study
YJD361	YJD233	<i>tan1-Δ::kanMX tef1-Δ::bleR met22-Δ::hygR</i>	This study
YJD362	YJD234	<i>BY4742 trm1-Δ::kanMX tef1-Δ::bleR met22-Δ::hygR</i>	This study
YJD376-2	JMW678	<i>trm8-Δ::natMX met22-Δ::hygR</i>	This study
YJD381-3	YJD232	<i>trm8-Δ::natMX tef1-Δ::bleR met22-Δ::hygR</i>	This study

attack by Xrn1 and Rat1 exonucleases, and RTD occurs at a faster rate. The increased expression of EF-1A can compete with the RTD pathway for binding of charged tRNAs and prevent RTD, until temperatures are high enough that the tRNAs not bound by EF-1A have increased likelihood of RTD due to their more exposed 5' ends, whereas reduced levels of EF-1A cause more RTD at lower temperatures because the tRNA is less bound to EF-1A and is therefore more available for attack. Future experiments will be required to further clarify how the RTD pathway competes with EF-1A for specific tRNA substrates and how RTD interacts with other components of the translation machinery, and other cellular processes.

MATERIALS AND METHODS

Yeast strains and plasmids

Strains used for these experiments are all derived from BY4741 and BY4742 and are listed in Table 1. Deletion strains were constructed using standard methods with appropriate knockout marker cassettes (Geldener et al. 2002; Johnston et al. 2002), after PCR amplification of the cassette with gene specific primers or after PCR amplification of the flanking region of an existing knockout strain (Giaever et al. 2002), followed by linear trans-

formation and PCR confirmation of insertion. Plasmids are listed in Table 2 and were constructed using similar PCR methods with appropriate primers. Primers are listed in Table 3.

Isolation of high-copy suppressors of the temperature-sensitive phenotype of *trm8-Δ trm4-Δ* strains

Pools of DNA from the MORF collection of yeast ORFs (Gelperin et al. 2005) were transformed into a *trm8-Δ trm4-Δ* strain, plated on synthetic (S) media containing 2% raffinose, and transformants were replica plated to S media containing 2% raffinose and 2% galactose at 33°C. Plasmids were isolated and sequenced to identify the ORF associated with suppression, and plasmids were then retested by retransformation.

TABLE 2. Plasmids used in this study

Plasmid name	Genotype	Source
IC012	[2 μ URA3]	
IC037	[2 μ URA3 P _{GAL} TRM4]	Gelperin et al. (2005)
IC041	[2 μ URA3 P _{GAL} TEF1]	Gelperin et al. (2005)
IC045	[2 μ URA3 P _{GAL} TEF2]	Gelperin et al. (2005)
JW036	[2 μ LEU2]	
JW038	[2 μ LEU2 tRNA ^{Ser(CGA)}]	This study
JW044	[2 μ LEU2 tRNA ^{Ser(UGA)}]	This study

TABLE 3. Primers used in this study

Primer name	Target	Purpose	Sequence (5'-3')
Val(CCA)	tRNA ^{Val} (^{ACC})	Northern	TGGTGATTTCGCCCAGGA
Val3(UAC)D-loop	tRNA ^{Val} (^{UAC})	Northern	GACGTCCTTGAACTGGACCATGGAC
tRNA ^{Phe} loop	tRNA ^{Phe} (^{GAA})	Northern	TGTGGATCGAACACAGGACC
tRNA ^{His} P1	tRNA ^{His} (^{GUG})	Northern	GCCATCTCCTAGAATCGAACCAG
Mature-SerCGA-a	tRNA ^{Ser} (^{CGA})	Northern	GCCCAAGAGATTTTCGAGTCTCT
Ser1-UGA-2	tRNA ^{Ser} (^{UGA})	Northern	GCAGAGCCCCAACAGATTTCAGTCTG
Ser-IGA	tRNA ^{Ser} (^{IGA})	Northern	GCAAAGCCCCAAAAGATTTCCTAA
G9ArgSa	tRNA ^{Arg} (^{ACG})	Northern	ACCATTGGGCCACGAGGAA
5S	5S rRNA	Northern	TGGTAGATATGGCCGCAACC
5.8S 30-51	5.8S rRNA	Northern	CGCTGCGTTCTTCATCGATGC
snR24-39-63	snR24 RNA	Northern	GTATGTCTCATTCGGAACCTCAAAG
TEF1-up40+Phleo	<i>TEF1</i>	Gene deletion	AAAGAAAGCATAGCAATCTAATCTAAGTTTAA ATTACAAACAGCTGAAGCTTCGTACGC
TEF1-dwn38+Phleo	<i>TEF1</i>	Gene deletion	ATAAAAGATATGCAACTAGAAAAGTCTTATCAATCT CCGCATAGGCCACTAGTGGATCTG
TEF1-up80+Phleo	<i>TEF1</i>	Gene deletion	TCTTGTTCTATTACAACCTTTTTTACTTCTTGCTCATT GAAAGAAAGCATAGCAATCTA
TEF1-dwn78+Phleo	<i>TEF1</i>	Gene deletion	GATAAATTACAAAAATTAATAAGATAAGATT AAATATAAAGATATGCAACTAGA
TEF1 del conf up	<i>TEF1</i>	PCR verification	CTTCGTCGAAAAAGGCAATAAAAAATT
TEF1 del conf down	<i>TEF1</i>	PCR verification	ACATTGGACTGTCGCCTGTGA
tef1 del up304	<i>TEF1</i>	Gene deletion	TCTTTCTTCTCTAGGGTGTCGTTAATTAC
tef1 del down312 RC	<i>TEF1</i>	Gene deletion	TTTCGAACTATAAAAGACCAACGGTGAC
tef1 del conf up340	<i>TEF1</i>	PCR verification	TTCAAAACACCCAAGCACAGCATACTAAAT
tef1 del conf down369 RC	<i>TEF1</i>	PCR verification	TGGTAAAAAATACGCCCTGAACGATGAATT
Met22_Tn7_5'	<i>MET22</i>	Gene deletion	TTGAAATGCCGCAATGACTC
Met22_Tn7_3'	<i>MET22</i>	Gene deletion	GCCTACTCTTACCTCTTCAG
MET22Tn7 + 515	<i>MET22</i>	PCR verification	CCCCTAAACATGAATACTGCTC
MET22Tn7-509	<i>MET22</i>	PCR verification	GAGTATATTCTTGGATGGGAAG
tS(CGA)C_LIC_5'	tS(CGA)C	Cloning	GGGTCTGGTTCGACTGGAGGTTCTCTCTGAG
tS(CGA)C_LIC_3'	tS(CGA)C	Cloning	CTTGTTCTGTGCTGTTTATCTTCTGGTCTTTATGCGCG
tS(UGA)E_LIC_5'	tS(UGA)E	Cloning	GGGTCTGGTTCGACGGTATGATGAGGCTTTGG
tS(UGA)E_LIC_3'	tS(UGA)E	Cloning	CTTGTTCTGTGCTGTTTGTGCTCTTGAGAACTAATG
trm1-UP39+Phleo	<i>TRM1</i>	Gene deletion	GCAGTCATAAGTTGATACCTTCTCTTACAATGTA GATCAGCTGAAGCTTCGTACGC
trm1-dwn38+Phleo	<i>TRM1</i>	Gene deletion	AGTTTCTTACGTTTGTGCTCTAACCTAATCAAAATTC GCATAGGCCACTAGTGGATCTG
trm1-UP75+Phleo	<i>TRM1</i>	Gene deletion	CGCATTCAATGCTCATCGCAAAGTTACAGAT CCTGAGCAGTCATAAGTTGATACCT
trm1-dwn76+Phleo	<i>TRM1</i>	Gene deletion	TCTTTTCCGTCCATTATACCGTTCTCGGGCGGATC CTTAGTTTCTTACGTTTGTGCTCTA
TRM1 + 366	<i>TRM1</i>	PCR verification	GAGCGTTTGTGTTAGGTTGGCCTGGTGCTG
TRM1-300	<i>TRM1</i>	PCR verification	GGTGGCATCGTGTGTAATAATATATGGTCTG

Northern blot analysis

Strains were grown in media as indicated at 28°C, the culture was shifted to restrictive temperature, and ~2 OD of cells were harvested at the time indicated, washed, and quick frozen. Where indicated, thiolutin was added to 5 µg/mL, 10 min before the temperature shift. Bulk RNA was prepared using glass beads (Elder et al. 1983) or hot phenol (Alexandrov et al. 2006), and ~2 µg RNA was separated by 10% PAGE in 8 M urea and TBE buffer, transferred to Hybond N+ membrane, cross-linked with UV, and hybridized to 5' ³²P-labeled DNA probes (Table 2) according to the method previously described (Alexandrov et al. 2006), followed by visualization on a Typhoon PhosphorImager (GE Healthcare) and quantification using Imagequant. For acidic Northern blots, RNA was

prepared at pH 4.5 and was resolved on 6.5% acrylamide gels for 18 h at 4°C in 8 M urea and 0.1 M sodium acetate (pH 5) (Alexandrov et al. 2006).

ACKNOWLEDGMENTS

We thank Elizabeth Grayhack and Michael Guy for discussions and comments on the manuscript. This research was supported by NIH grant GM52347 to E.M.P. J.M.D. was partially supported by NIH Training Grant in Cellular, Biochemical, and Molecular Sciences 5T32 GM068411.

Received April 4, 2012; accepted July 3, 2012.

REFERENCES

- Agris PF, Vendeix FA, Graham WD. 2007. tRNA's wobble decoding of the genome: 40 years of modification. *J Mol Biol* **366**: 1–13.
- Alexandrov A, Grayhack EJ, Phizicky EM. 2005. tRNA m⁷G methyltransferase Trm8p/Trm82p: Evidence linking activity to a growth phenotype and implicating Trm82p in maintaining levels of active Trm8p. *RNA* **11**: 821–830.
- Alexandrov A, Chernyakov I, Gu W, Hiley SL, Hughes TR, Grayhack EJ, Phizicky EM. 2006. Rapid tRNA decay can result from lack of nonessential modifications. *Mol Cell* **21**: 87–96.
- Anderson J, Phan L, Cuesta R, Carlson BA, Pak M, Asano K, Bjork GR, Tamame M, Hinnebusch AG. 1998. The essential Gcd10p–Gcd14p nuclear complex is required for 1-methyladenosine modification and maturation of initiator methionyl-tRNA. *Genes Dev* **12**: 3650–3662.
- Basavappa R, Sigler PB. 1991. The 3 Å crystal structure of yeast initiator tRNA: Functional implications in initiator/elongator discrimination. *EMBO J* **10**: 3105–3111.
- Bjork GR, Huang B, Persson OP, Bystrom AS. 2007. A conserved modified wobble nucleoside (mcm⁵s²U) in lysyl-tRNA is required for viability in yeast. *RNA* **13**: 1245–1255.
- Chernyakov I, Whipple JM, Kotelawala L, Grayhack EJ, Phizicky EM. 2008. Degradation of several hypomodified mature tRNA species in *Saccharomyces cerevisiae* is mediated by Met22 and the 5'-3' exonucleases Rat1 and Xrn1. *Genes Dev* **22**: 1369–1380.
- Cochella L, Green R. 2005. An active role for tRNA in decoding beyond codon:anticodon pairing. *Science* **308**: 1178–1180.
- Copela LA, Fernandez CF, Sherrer RL, Wolin SL. 2008. Competition between the Rex1 exonuclease and the La protein affects both Trf4p-mediated RNA quality control and pre-tRNA maturation. *RNA* **14**: 1214–1227.
- Dichtl B, Stevens A, Tollervey D. 1997. Lithium toxicity in yeast is due to the inhibition of RNA processing enzymes. *EMBO J* **16**: 7184–7195.
- Elder RT, Loh EY, Davis RW. 1983. RNA from the yeast transposable element Ty1 has both ends in the direct repeats, a structure similar to retrovirus RNA. *Proc Natl Acad Sci* **80**: 2432–2436.
- Esberg A, Huang B, Johansson MJ, Bystrom AS. 2006. Elevated levels of two tRNA species bypass the requirement for elongator complex in transcription and exocytosis. *Mol Cell* **24**: 139–148.
- Gelperin DM, White MA, Wilkinson ML, Kon Y, Kung LA, Wise KJ, Lopez-Hoyo N, Jiang L, Piccirillo S, Yu H, et al. 2005. Biochemical and genetic analysis of the yeast proteome with a movable ORF collection. *Genes Dev* **19**: 2816–2826.
- Giaever G, Chu AM, Ni L, Connelly C, Riles L, Veronneau S, Dow S, Lucau-Danila A, Anderson K, Andre B, et al. 2002. Functional profiling of the *Saccharomyces cerevisiae* genome. *Nature* **418**: 387–391.
- Giege R, Sissler M, Florentz C. 1998. Universal rules and idiosyncratic features in tRNA identity. *Nucleic Acids Res* **26**: 5017–5035.
- Gromadski KB, Schummer T, Stromgaard A, Knudsen CR, Kinzy TG, Rodnina MV. 2007. Kinetics of the interactions between yeast elongation factors 1A and 1B α , guanine nucleotides, and aminoacyl-tRNA. *J Biol Chem* **282**: 35629–35637.
- Gueldener U, Heinisch J, Koehler GJ, Voss D, Hegemann JH. 2002. A second set of loxP marker cassettes for Cre-mediated multiple gene knockouts in budding yeast. *Nucleic Acids Res* **30**: e23. doi: 10.1093/nar/30.6.e23.
- Gustavsson M, Ronne H. 2008. Evidence that tRNA modifying enzymes are important in vivo targets for 5-fluorouracil in yeast. *RNA* **14**: 666–674.
- Jack A, Ladner JE, Klug A. 1976. Crystallographic refinement of yeast phenylalanine transfer RNA at 2-5 Å resolution. *J Mol Biol* **108**: 619–649.
- Jimenez A, Tipper DJ, Davies J. 1973. Mode of action of thiolutin, an inhibitor of macromolecular synthesis in *Saccharomyces cerevisiae*. *Antimicrob Agents Chemother* **3**: 729–738.
- Johansson MJ, Esberg A, Huang B, Bjork GR, Bystrom AS. 2008. Eukaryotic wobble uridine modifications promote a functionally redundant decoding system. *Mol Cell Biol* **28**: 3301–3312.
- Johnston M, Riles L, Hegemann JH. 2002. Gene disruption. *Methods Enzymol* **350**: 290–315.
- Kadaba S, Krueger A, Trice T, Krecic AM, Hinnebusch AG, Anderson J. 2004. Nuclear surveillance and degradation of hypomodified initiator tRNA^{Met} in *S. cerevisiae*. *Genes Dev* **18**: 1227–1240.
- Kadaba S, Wang X, Anderson JT. 2006. Nuclear RNA surveillance in *Saccharomyces cerevisiae*: Trf4p-dependent polyadenylation of nascent hypomethylated tRNA and an aberrant form of 5S rRNA. *RNA* **12**: 508–521.
- Kim SH, Suddath FL, Quigley GJ, McPherson A, Sussman JL, Wang AH, Seeman NC, Rich A. 1974. Three-dimensional tertiary structure of yeast phenylalanine transfer RNA. *Science* **185**: 435–440.
- Kotelawala L, Grayhack EJ, Phizicky EM. 2008. Identification of yeast tRNA^{Ura4} 2'-O-methyltransferase (Trm44) and demonstration of a Trm44 role in sustaining levels of specific tRNA^{Ser} species. *RNA* **14**: 158–169.
- Kramer EB, Vallabhaneni H, Mayer LM, Farabaugh PJ. 2010. A comprehensive analysis of translational missense errors in the yeast *Saccharomyces cerevisiae*. *RNA* **16**: 1797–1808.
- LaCava J, Houseley J, Saveanu C, Petfalski E, Thompson E, Jacquier A, Tollervey D. 2005. RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. *Cell* **121**: 713–724.
- LaRiviere FJ, Wolfson AD, Uhlenbeck OC. 2001. Uniform binding of aminoacyl-tRNAs to elongation factor Tu by thermodynamic compensation. *Science* **294**: 165–168.
- Ledoux S, Olejniczak M, Uhlenbeck OC. 2009. A sequence element that tunes *Escherichia coli* tRNA^{Ala}_{GGC} to ensure accurate decoding. *Nat Struct Mol Biol* **16**: 359–364.
- Ling J, Reynolds N, Ibba M. 2009. Aminoacyl-tRNA synthesis and translational quality control. *Annu Rev Microbiol* **63**: 61–78.
- Muramatsu T, Nishikawa K, Nemoto F, Kuchino Y, Nishimura S, Miyazawa T, Yokoyama S. 1988. Codon and amino-acid specificities of a transfer RNA are both converted by a single post-transcriptional modification. *Nature* **336**: 179–181.
- Murphy FV 4th, Ramakrishnan V. 2004. Structure of a purine-purine wobble base pair in the decoding center of the ribosome. *Nat Struct Mol Biol* **11**: 1251–1252.
- Murphy FV 4th, Ramakrishnan V, Malkiewicz A, Agris PF. 2004. The role of modifications in codon discrimination by tRNA^{Lys}_{UUU}. *Nat Struct Mol Biol* **11**: 1186–1191.
- Musier-Forsyth K, Schimmel P. 1992. Functional contacts of a transfer RNA synthetase with 2'-hydroxyl groups in the RNA minor groove. *Nature* **357**: 513–515.
- Ozanick SG, Wang X, Costanzo M, Brost RL, Boone C, Anderson JT. 2009. Rex1p deficiency leads to accumulation of precursor initiator tRNA^{Met} and polyadenylation of substrate RNAs in *Saccharomyces cerevisiae*. *Nucleic Acids Res* **37**: 298–308.
- Phizicky EM, Alfonzo JD. 2010. Do all modifications benefit all tRNAs? *FEBS Lett* **584**: 265–271.
- Phizicky EM, Hopper AK. 2010. tRNA biology charges to the front. *Genes Dev* **24**: 1832–1860.
- Sanderson LE, Uhlenbeck OC. 2007. The 51–63 base pair of tRNA confers specificity for binding by EF-Tu. *RNA* **13**: 835–840.
- Schmeing TM, Voorhees RM, Kelley AC, Gao YG, Murphy FV 4th, Weir JR, Ramakrishnan V. 2009. The crystal structure of the ribosome bound to EF-Tu and aminoacyl-tRNA. *Science* **326**: 688–694.
- Schneider C, Anderson JT, Tollervey D. 2007. The exosome subunit Rps44 plays a direct role in RNA substrate recognition. *Mol Cell* **27**: 324–331.
- Schrader JM, Chapman SJ, Uhlenbeck OC. 2009. Understanding the sequence specificity of tRNA binding to elongation factor Tu using tRNA mutagenesis. *J Mol Biol* **386**: 1255–1264.
- Thiele D, Cottrelle P, Iborra F, Buhler JM, Sentenac A, Fromageot P. 1985. Elongation factor 1 α from *Saccharomyces cerevisiae*. Rapid

- large-scale purification and molecular characterization. *J Biol Chem* **260**: 3084–3089.
- Valle M, Zavialov A, Li W, Stagg SM, Sengupta J, Nielsen RC, Nissen P, Harvey SC, Ehrenberg M, Frank J. 2003. Incorporation of aminoacyl-tRNA into the ribosome as seen by cryo-electron microscopy. *Nat Struct Biol* **10**: 899–906.
- Vanacova S, Wolf J, Martin G, Blank D, Dettwiler S, Friedlein A, Langen H, Keith G, Keller W. 2005. A new yeast poly(A) polymerase complex involved in RNA quality control. *PLoS Biol* **3**: e189. doi: 10.1371/journal.pbio.0030189.
- Waldron C, Lacroute F. 1975. Effect of growth rate on the amounts of ribosomal and transfer ribonucleic acids in yeast. *J Bacteriol* **122**: 855–865.
- Wang X, Jia H, Jankowsky E, Anderson JT. 2008. Degradation of hypomodified tRNA_i^{Met} in vivo involves RNA-dependent ATPase activity of the DExH helicase Mtr4p. *RNA* **14**: 107–116.
- Westhof E, Dumas P, Moras D, Romby P. 1985. Crystallographic refinement of yeast aspartic acid transfer RNA. *J Mol Biol* **184**: 119–145.
- Whipple JM, Lane EA, Chernyakov I, D'Silva S, Phizicky EM. 2011. The yeast rapid tRNA decay pathway primarily monitors the structural integrity of the acceptor and T-stems of mature tRNA. *Genes Dev* **25**: 1173–1184.
- Wilusz JE, Whipple JM, Phizicky EM, Sharp PA. 2011. tRNAs marked with CCACCA are targeted for degradation. *Science* **334**: 817–821.
- Yarus M, Valle M, Frank J. 2003. A twisted tRNA intermediate sets the threshold for decoding. *RNA* **9**: 384–385.
- Zaher HS, Green R. 2009. Fidelity at the molecular level: Lessons from protein synthesis. *Cell* **136**: 746–762.

RNA

A PUBLICATION OF THE RNA SOCIETY

The yeast rapid tRNA decay pathway competes with elongation factor 1A for substrate tRNAs and acts on tRNAs lacking one or more of several modifications

Joshua M. Dewe, Joseph M. Whipple, Irina Chernyakov, et al.

RNA 2012 18: 1886-1896 originally published online August 15, 2012

Access the most recent version at doi:[10.1261/rna.033654.112](https://doi.org/10.1261/rna.033654.112)

References

This article cites 52 articles, 28 of which can be accessed free at:
<http://rnajournal.cshlp.org/content/18/10/1886.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

To subscribe to *RNA* go to:

<http://rnajournal.cshlp.org/subscriptions>