Discovery of a Gene Family Critical to Wyosine Base Formation in a Subset of Phenylalanine-specific Transfer RNAs*

William F. Waas1, Valérie de Crécy-Lagard2,3, and Paul Schimmel1,2

From the 1Department of Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California 92037 and the 2Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611

A large number of post-transcriptional base modifications in transfer RNAs have been described (Sprinzl, M., Horn, C., Brown, M., Ioudovitch, A., and Steinberg, S. (1998) Nucleic Acids Res. 26, 148–153). These modifications enhance and expand tRNA function to increase cell viability. The intermediates and genes essential for base modifications in many instances remain unclear. An example is wyebutosine (yW), a fluorescent tricyclic modification of an invariant guanosine situated on the 3′-side of the tRNAΨhe anticodon. Although biosynthesis of yW involves several reaction steps, only a single pathway-specific enzyme has been identified (Kalhor, H. R., Penjwini, M., and Clarke, S. (2005) Biochem. Biophys. Res. Commun. 334, 433–440). We used comparative genomics analysis to identify a cluster of orthologous groups (COG0731) of wyosine family biosynthetic proteins. Gene knock-out and complementation studies in Saccharomyces cerevisiae established a role for YPL207w, a COG0731 ortholog that encodes an 810-amino acid polypeptide. Further analysis showed the accumulation of N4-methylguanosine (m1G37) in tRNA from cells bearing a YPL207w deletion. A similar lack of wyosine base and build-up of m1G37 is seen in certain mammalian tumor cell lines. We proposed that the 810-amino acid COG0731 polypeptide participates in converting tRNAΨhe-m1G37 to tRNAΨhe-yW.

In all organisms, the functions of tRNAs in translation are enhanced by a series of post-transcriptional modifications (4). Over 80 modifications are known, and their presence vastly expands the structural and chemical diversity of native tRNA (2). In a putative RNA world, base modifications may have provided a way to diversify the chemical and structural properties of RNAs.) The modification-dependent structural stability and function correlate with increased cellular fitness and viability (5, 9). The importance of these modifications is underscored by a large investment of resources for their biosynthesis, with estimates suggesting nearly 1% of mammalian genes being dedicated to tRNA modification genes (4).

Wyebutosine (yW)3 of yeast phenylalanine-specific tRNA (tRNAPhe) was one of the earliest tRNA modifications to be discovered (10–14). Wyebutosine is a fluorescent, tricyclic base and a member of the wyosine family of hypermodified guanosines. All wyosine bases (Ybs) are characterized by a 1H-imidazo[1,2-α]purine core structure and a strict occurrence in archaeal and eukaryal tRNAΨhe (Figs. 1 and 2). Wyosine bases, isolated from different organisms, show variations in ring methylation and side chain structure (15–20). Generally, archaeal Yb structures are less differentiated than their eukaryotic counterparts.

The hydrophobic nature of yW37 promotes stacking with adjacent bases (A34 and A37) and restricts the conformational flexibility of the anticodon (21–25). Removal of yW produced local changes in anticodon conformation, as well as long range perturbations in tRNAΨhe tertiary structure (26). These structural changes were accompanied by subtle differences in codon specificity4 and a modest increase in retroviral ribosomal frameshifting (determined in cell-free extract) (27–29).

Most interesting, the tRNAΨhe from mouse neuroblastoma cell lacked Yb but was more efficient than the fully modified tRNAΨhe in a cell-free translation system (30–32). Although it is unclear if hypomodified tRNAs contribute to tumor-specific properties, these tRNAs support the high levels of translation required by rapidly dividing cells. Thus, despite a good understanding of its role in maintaining anticodon structure, the function of yW in translation is unclear.

Although the biosynthesis of wyebutosine has been partially characterized, the genes involved are largely unknown. Several structural components of yW were identified by metabolic labeling experiments. The purine substructure was shown as being derived from the coded guanosine (33, 34). NMR studies of13C-enriched tRNAΨhe implicated the methyl group of methionine as a source for carbon-10 (refer to Fig. 1 for numbering), and for the side chain ester and N3-methyl moieties (35). Conflicting evidence obscures understanding the origin of the 3-amino-3-carboxypropyl side chain (36, 37). The in vivo kinetics of Yb biosynthesis of Xenopus laevis was investigated (34). By using the site-specifically labeled [32P]tRNAΨhe transcript in X. laevis oocytes, Droogmans and Groesjean (34) detected N4-methylguanosine (mG) and an unknown compound “X” as intermediates, and they suggested that the pathway may involve numerous metabolites.

The recent expansion of publicly available bioinformatics tools and data bases has stimulated gene identification and functional assignment. Novel approaches, which combine public genome information with genetic context, have met with success in linking unknown genes to definitive functions (38–42). In this study, our methodology produced a single “hit” that allowed us to identify a pathway-specific polypeptide-encoding gene that acts downstream from the initial modification event (m1G37). Deletion of the gene in Saccharomyces cerevisiae produces tRNAΨhe in a modification state similar to that in certain mammalian cell types, including some tumor cell lines.

* This work was supported by Grants GM15539 and 23562 from the National Institutes of Health, a fellowship from the National Foundation for Cancer Research (to P. S.), and a Ruth L. Kirschstein National Research Service Award from the National Institutes of Health (to W. F. W.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 To whom correspondence may be addressed: Dept. of Microbiology and Cell Science, University of Florida, P. O. Box 110700, Gainesville, FL 32611-0700. Tel.: 850-392-9416; Fax: 850-392-5922; E-mail: vcrecy@ufl.edu.

2 To whom correspondence may be addressed: Dept. of Molecular Biology and The Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037. Tel.: 858-784-8970; Fax: 858-784-8990; E-mail: schimmel@scripps.edu.

3 The abbreviations used are: yW, wyebutosine; HPLC, high pressure liquid chromatography; LC-MS, liquid chromatography-mass spectrometry; RP, reverse-phase; ORF, open reading frame; Ybs, wyosine bases; m1G, N4-methylguanosine; PheRS, phenylalanyl-tRNA synthetase; Gm, 2′-O-methylguanosine.

4 It should be noted that these studies are performed with tRNA lacking any base at position 37.
Materials and Methods

Strains and Chemicals—Wild-type and deletion strains (ΔYPL207w) of S. cerevisiae were purchased from Open Biosystems (www.openbio-systems.com). Both strains were of the MATa leu2Δ0 met15Δ0 ura3Δ0 genotype. The YPL207w ORF was replaced with a Kan^R cassette in the null strain. All chemicals were obtained in high purity from Sigma unless otherwise noted.

Plasmids—YPL207w was cloned from S. cerevisiae genomic DNA by PCR (30 cycles, 1 min at 94 °C, 1 min at 55 °C, and 6 min at 68 °C) using the following oligonucleotides: 5’-ggggagccattgatatccaaacagccttatgtgattctggtctgc-3’ and 5’-cttcctctctgtctctactgctgtatggaattctggcgttcgc-3’. The gene was incorporated into pYES-DEST52 (Invitrogen) between the att1 and att2 sites by site-specific recombination using manufacturer-suggested protocols. The vector carried the LIR43 marker for auxotrophic selection and a Pga1 for protein expression. The expression vector for yeast phenylalanyl-tRNA synthetase was a gift from Dr. David Tirrell, California Institute of Technology.

Phylogenetic Queries—The occurrence of wyosine in tRNA^Phe from several organisms was determined from a data base of annotated tRNA sequences. The genomes of organisms containing (Methanococcus jannaschii, Homo sapiens, Saccharomyces cerevisiae, Schizosaccharomyces pombe, and Arabidopsis thaliana) or lacking (Drosophila melanogaster, Escherichia coli, and Bacillus subtilis) the wyosine modification were analyzed using the comparative genomics platform, Protein Link Explorer (PLEX) (43). A BLAST E-value of 10^-10 was set as a threshold for gene identification.

Bulk tRNA Purification—Bulk tRNA was isolated from yeast cells grown in synthetic complete medium (± uracil) containing 2% galactose. Cultures (2 liters) were grown at 30 °C to an absorbance at 600 nm (A600) of 0.6–1.2. Cells were pelleted by centrifugation and resuspended in 10 mM sodium acetate (pH 4.5). The pellet was resuspended in 100 ml of 0.3 M sodium acetate (pH 4.5), 10 mM EDTA before the addition of 100 ml of water-saturated phenol. After 30 min of vigorous shaking, the phases were separated by centrifugation (5,000 rpm, 20 min). Nucleic acids were precipitated from the aqueous phase with 0.4 volumes of isopropyl alcohol. Precipitate was collected as a pellet (30 min at 10,000 rpm), washed with 70% ethanol, and lyophilized.

KCl). The eluted RNA was precipitated with isopropyl alcohol, washed with Tris acetate (pH 6.3), 15% ethanol, and 400 mM KCl. The column was equilibrated in buffer RP1 (10 mM sodium phosphate (pH 4.5), 1M sodium formate, and 8m M MgCl2) prior to reverse-phase chromatography.

Thin Layer Chromatography Analysis of Acid-hydrolyzed Wybutosine—Pure, lyophilized tRNA (100 μg) was reconstituted in 500 μl of 50 mM sodium phosphate (pH 3.5) and incubated at 37 °C for 18 h to hydrolyze wybutosine from tRNA^Phe. The base was then extracted with ethyl acetate (500 μl). Ethyl acetate was removed under reduced pressure to produce a white residue. The residue was redissolved in a small volume of ethyl acetate and spotted on a Silica Gel 60 F254 TLC plate (EM Science, Gibbstown, NJ). The sample was then chromatographed using the upper layer of a 1-propyl alcohol/ethyl acetate/water (1:4:2) mixture as the mobile phase (19). Fluorescent material was visualized by excitation at 300 nm.

Fractionation of tRNA by Reverse-phase HPLC—Purified bulk tRNA (1–2 mg) was loaded onto a Vydac C4 semi-preparative HPLC column (catalog number 214TP1010) equilibrated in buffer RP1 (10 mM sodium phosphate (pH 4.5), 1 mM sodium formate, and 8 mM MgCl2). A linear gradient to 100% buffer RP2 (10 mM sodium phosphate (pH 4.5) and 15% methanol) was established over 70 min at a flow rate of 4 ml/min. Fractions were collected every 0.5 min and analyzed for phenylalanine acceptance as described above.

MALDI—Mass measurements were made using an Applied Biosystems DE system. A salt-tolerant matrix, 2,4,6-trihydroxyacetophenone containing diaminonitromethane citrate was used to analyze purified tRNA. Ions were monitored in positive mode. Masses were calculated from an average of 300 scans.

Enzymatic Digestion of tRNA^Phe—HPLC-purified tRNA (100 μg) was resuspended in 0.1 ml of 10 mM ammonium acetate (pH 5.3). The tRNA was incubated with nuclease P1 (8 units, Sigma) at 45 °C for 2 h. Fresh ammonium bicarbonate was added to a final concentration of 0.1 M before the addition of snake venom phosphodiesterase (0.008 units, New England Biolabs, Beverly, MA) for 1 h at 37 °C. Nucleosides were dephosphorylated by incubation with alkaline phosphatase (4 units, New England Biolabs, Beverly, MA) for 1 h at 37 °C. Digested RNA was lyophilized prior to LC-MS analysis.

Liquid Chromatography-Mass Spectrometry of tRNA^Phe Hydrolysates—LC electrospray ionization mass spectrometry was performed on an Agilent MSD 1100. The system was equipped with a Supelcosil LC-18-S HPLC column (25 cm × 4.6 mm, 5 μM). Prior to chromatography the column was equilibrated in LC-MS buffer A (250 ammonium acetate (pH 6.0)). Nucleosides were eluted using 0.1% formic acid, linear gradient of LC-MS buffer B (40% acetonitrile). The gradient profile was as follows: 0 min, 0% B; 6 min, 0% B; 8.8 min, 0.2% B; 11.6 min, 0.8% B; 14.4 min, 1.8% B; 17.2 min, 3.2% B; 20 min, 5% B; 50 min, 25% B; 60 min, 50% B; 68...
min, 75% B; 74 min, 75% B; 90 min, 100% B; and 96 min, 100% B. A flow rate of 0.5 ml/min was maintained during each run, and the eluate was analyzed in positive ion mode.

RESULTS

Identification of COG0731 as a Probable Wyebutosine Synthesis Gene—For the purpose of guiding comparative genomics queries, a compilation of tRNA sequence and modification data were analyzed for the occurrence of wyosine family compounds in organisms with sequenced genomes (1, 3, 47–52). Yb is absent from eubacteria and present in many archaeal and eukaryotic phenylalanine-specific tRNAs (Figs. 1 and 2). Significantly, D. melanogaster tRNA^phe harbors N\(^6\)-methylguanosine at position 37 instead of Yb (53). By using this information and a Protein Link Explorer (PLEX) algorithm (43), a phylogenetic occurrence query identified genes present in 

*Link Explorer (PLEX)* algorithm (43), a phylogenetic occurrence query position 37 instead of Yb (53). By using this information and a Protein Link Explorer (PLEX) algorithm (43), a phylogenetic occurrence query identified genes present in *M. jannaschii*, *H. sapiens*, *S. cerevisiae*, *S. pombe*, and *A. thaliana* but not in *D. melanogaster*, *E. coli*, or *B. subtilis*. A single gene family COG0731 fit the desired criteria and belongs to PACE (proteins in Archaea conserved in eukaryotes) Group 22 (54).

Although the biological role of COG0731 is generally unknown, many characterized PACE-encoding genes are implicated in the organization and processing of genetic material, including rRNA/tRNA maturation and modification (54, 55). Consequently, these genes show correlated expression with genes sharing related biological functions. Analysis of gene expression in yeast (www.yeastgenome.org) revealed that during the cell cycle and in response to DNA-damaging agents, COG0731 family members co-express with genes for ribosome biogenesis, RNA processing, and RNA metabolism (\(\mu > 10^{-5}\)).

Yeast Strains Lacking the COG0731 Ortholog Do Not Produce Aci- table Wyebutosine—YPL207w is the *S. cerevisiae* COG0731 ortholog. To assess the significance of COG0731 to Yb biosynthesis, wild-type and the \(\Delta ypl207w\) deletion strain of *S. cerevisiae* were assayed for wyebutosine production. Bulk tRNA from each strain was incubated at pH 3.5 and at 37 °C for several hours (under these conditions the wyebutosine base (yw) is hydrolyzed from tRNA\(^{phe}\) by cleavage of the N–C bond (56)). The liberated base was then isolated by extraction with ethyl acetate, and the extracts were concentrated and spotted on silica gel plates. After thin layer chromatography (Fig. 3), a single fluorescent spot (R\(_f\) = 0.47) was detected in extracts prepared from the wild-type strain, and mass spectrometric analysis of this fluorescent material gave a mass of 377.1 Da corresponding to that of yW (57). Extracts from the \(\Delta ypl207w\) deletion strain lacked the fluorescent material. Thus, the \(\Delta ypl207w\) deletion blocks Yb biosynthesis.

RP-HPLC Analysis of tRNA\(^{phe}\) from Wild-type and Gene Deletion Strains of *S. cerevisiae*—To confirm that the absence of Yb from acid-treated tRNA from the \(\Delta ypl207w\) deletion strain correlated with an alteration of the tRNA itself, the chromatographic properties of tRNA\(^{phe}\) from wild-type (tRNA\(^{phe}\)\(_{wt}\)) and null (tRNA\(^{phe}\)\(_{\Delta ypl207w}\)) strains were compared. Bulk tRNA from each strain was fractionated using RP-HPLC (Vydac C-4 column), and the presence of tRNA\(^{phe}\) was analyzed by testing each fraction for \[^3H\]phenylalanine acceptance (Fig. 4, A–C) (58). Although viable tRNA\(^{phe}\) from each strain eluted as a single peak,\(^6\) they had markedly different retention times (tRNA\(^{phe}\)\(_{wt}\) = 49.9 min, tRNA\(^{phe}\)\(_{\Delta ypl207w}\) = 18.0 min). The early elution time of tRNA\(^{phe}\)\(_{\Delta ypl207w}\) fits with a more hydrophilic, hypomodified state for that tRNA. Similar shifts have been reported for yeast tRNA\(^{phe}\) after removal of yW by acid treatment, as well as for mammalian tRNA\(^{phe}\) isolated from rat hepatomas that were hypomodified (m\(^1\)G) at position 37 (59). Because all tRNA\(^{phe}\)\(_{\Delta ypl207w}\) occurs in a single rapidly eluting peak, the \(\Delta ypl207w\) deletion appears to block completely the synthesis of yW in *S. cerevisiae*. These results confirm that the absence of yW from acid-treated tRNA from \(\Delta ypl207w\) deletion strain is due to a deficiency in tRNA\(^{phe}\) modification.

Complementation in the Null Strain by Expression of YPL207w in *Trans*—Aware that polar effects may result from disruption of YPL207w with Kan\(^\beta\), we turned to genetic complementation to provide evidence

\(^6\) A similar R\(_f\) (0.48) value was reported for yeast yW by Nakanishi et al. (19).

\(^5\) The hight specific activity of \[^3H\]phenylalanine (5,000 cpm/ pmol) used during labeling would have enabled detection of rare tRNA\(^{phe}\) species present at less than 0.5% the quantity of the major tRNA\(^{phe}\) peak.
that the phenotype of the ΔYPL207w strain is because of a single gene disruption. For these experiments, the 810-amino acid ORF of YPL207w was cloned into the pYES-DEST52 expression vector. The ΔYPL207w deletion strain was transformed with the recombinant vector and then grown under conditions that ensured continuous gene expression. Total tRNA was isolated, and chromatography of the tRNA revealed peaks (a and b) at 49.5 and 18.0 min (Fig. 4C). These retention times are the same as those of tRNA^Phe^wt and tRNA^Phe^Δypl207w, respectively. Acid treatment of peak b fractions produced a fluorescent, ethyl acetate-soluble compound with an \( RF = 0.47 \) and mass (377.1) identical to that of wyebutosine. No yW was detected in peak a fractions. The observation of two peaks was presumed to result from partial complementation. (complete complementation was achieved when cells were harvested at higher optical densities (\( A_{600} > 2.0 \)) (Fig. 4C, inset)). Thus, expression of YPL207w in the ΔYPL207w deletion strain restores wyebutosine biosynthesis and rules out downstream polar effects caused by KanR insertion.

Purification and Mass Determination of tRNA^Phe^ and Identification of a Wyebutosine Precursor by LC/MS of tRNA^Phe^ Hydrolysates—Because disruption of YPL207w produced a hypomodified and chromatographically homogenous tRNA^Phe^ species, we inferred that an intermediate or yW precursor had accumulated at position 37. To identify intermediates, transfer RNA^Phe^ was obtained at high purity (amino acid acceptance = 1969 ± 53 pmol/\( A_{260} \) (60)) using the fractionation procedure described above (TABLE ONE). By contrast, tRNA^Phe^Δypl207w co-eluted with other tRNA species (154 pmol/\( A_{260} \)) and had to be esterified with phenylalanine (using yeast PheRS) to increase the retention time (48.5 min) and resolve it (1836 ± 108 pmol/\( A_{260} \)) from other tRNA species.

MALDI mass spectroscopy was used for tRNA molecular weight determination (TABLE ONE). The observed mass for tRNA^Phe^ (24,953.8 ± 4.7 Da) was nearly identical to the value reported by Taniguchi and Hayashi (61) (24,953.4 ± 3.5 Da). The observed mass for tRNA^Phe^Δypl207w (24,745.1 ± 4.8 Da) differed from that of tRNA^Phe^wt by 208.7 ± 4.8 Da.

Tandem liquid chromatography/mass spectroscopy (LC/MS) was used to analyze tRNA nucleoside composition. For this procedure, tRNAs were extensively digested and dephosphorylated, and nucleosides were resolved by RP-HPLC, under a set of standardized conditions.
(17, 62, 63). Mass (100–600 m/z) and UV absorption (254, 280, 310 nm) data were collected from the eluents and, along with relative retention times, were used to assign nucleoside identities according to the work of Edmonds et al. (62). Absorbance and mass profiles for the tRNA from hydrolysate (Fig. 5A) were consistent with the known composition of S. cerevisiae tRNA, with masses for 14 of the 15 ribonucleosides expected for the tRNA being observed (5-methyluridine was not detected, whereas inosine (peak 3b) occurred at ~36 min and was attributed to adenosine deaminase activity in commercial preparations of nuclease P1.8 Peak 9 corresponds to yW and does not appear in the spectrum of tRNA from (Fig. 5B). The loss of yW coincided with only one other change, a substantial increase in absorbance of the peak for 2′-O-methylguanosine (Gm, peak 5*). Extensive mass scanning over the limits of the peak 5 revealed fragment ions not observed for the Gm peak of tRNA wt (5th peak, Fig. 6A). The most abundant new ion is observed at 166.0 m/z, the expected mass of a base-methylated guanosine (Fig. 6B) (62). Methylation of G37 fits with the molecular weight differences of tRNA wt and tRNA (ΔMm) = 211 Da, ΔM(36 min) = 208.7 Da, where Mm indicates molecular mass. Relative retention time and co-elution with Gm suggests these ions are produced by N,N,N,N,methylguanosine (mG). Only two other base-methylated guanosines are known to occur in RNA–7-methylguanosine (mG) and N,N,N,N,methylguanosine (mG). These ribonucleosides are accounted for in each spectrum as peaks 3a and 6, respectively, and thus can be eliminated as candidate structures. The homogeneous elution of tRNA from YPL207w during RP-HPLC chromatography and the nearly quantitative doubling (1.96-fold) of peak 5 absorbance indicate stoichiometric methylation of G37 in cells lacking YPL207w.

**DISCUSSION**

A comparative genomics analysis was used to link YPL207w to yW biosynthesis. The analysis was initially limited to organisms with completely sequenced genomes and fully characterized tRNA. The phylogenetic distribution of Yb and conservation among these organisms (which include archaea, fungi, plants, and mammals) suggests that it is among the earliest tRNA modifications to occur after branching of archaea and eukaryotes from eubacteria. Further review of the tRNA data base indicated that in Bombyx mori the tRNA contains mG, and analysis of several other complete genome sequences revealed Caenorhabditis elegans and Encephalitozoon cuniculi lack COG0731 orthologs. Like D. melanogaster, these organisms are not equipped to synthesize Yb. Phenylalanine-specific tRNAs of eubacteria also lack Yb, but instead have another highly modified purine at position 37 (2-methylthio-N,N-isopentenyladenosine). The 2-methylthio-N,N-isopentenyladenosine modification affects anticodon structure, codon recognition, and translational efficiency in a way that is similar to Yb (6, 64). Therefore, tRNA from eubacteria is distinct from that of eukaryotic organisms that harbor mG37.

Deletion of YPL207w in yeast blocked wyebutosine production and caused methylated guanosine (G37) to accumulate in tRNA. We suggested that methylation occurs at N3 of G37. This modification (mG) was reported in several cell types that produce hypomethylated tRNA. For example, in mouse neuroblastoma, 85% of tRNA contained mG (65, 66). A significant portion of rabbit reticulocyte tRNA also had mG instead of peroxowyebutosine (28). Furthermore, eukaryotic organisms that do not synthesize Yb (e.g., D. melanogaster and B. mori) produce tRNA bearing mG. These data demonstrate the natural occurrence of mG during tRNA biosynthesis and fit well with our assignment.

Droogmans and Grosjean (34) provided direct evidence that tRNA (mG) is an integral part of yW biosynthesis. They showed that the transcript synthesized with mG is a competent pathway intermediate and established mG as the obligatory first step in Yb biosynthesis in X. laevis oocytes. Subsequently, the enzyme responsible for G37 methylation was discovered to be an archaean/eukaryotic specific mG methyltransferase (8, 67). Deletion of the S. cerevisiae, methyltransferase-
encoding gene (TRMS), prevents formation of both m1G37 and yW (67). Representatives from the m1G37-methyltransferase gene family are found in all organisms that synthesize Yb. Based on the accumulation of m1G in our study, we hypothesize that YPL207w supports a biosynthetic step immediately after synthesis of m1G37.

Because COG0731 genes occur in organisms that produce “minimalist” wyosine bases such as 4-demethylwyosine (Fig. 1d), the COG0731 gene product may not be involved in formation of eukaryotic Yb side chains. This suggestion is supported by the work of Kalhor et al. (7) which demonstrated that tRNA from S. cerevisiae lacking the TRM12 gene contained 4-demethylwyosine (structure d, Fig. 1) rather than yW (7).

CONCLUSION

In this study, YPL207w was identified as essential for wybutosine biosynthesis in yeast. A ΔYPL207w deletion strain produced tRNA\textsuperscript{wy} in yeast that was fully modified except at position 37 (m1G37). N3-G37-methylated tRNA\textsuperscript{wy} is a putative intermediate of the yW biosynthetic pathway. Currently, it is not known whether the protein translated from the YPL207w ORF directly modifies tRNA\textsuperscript{wy}.m1G or contributes to tRNA\textsuperscript{wy}.yW biosynthesis by some indirect means. Thus, the biochemical activity of the YPL207w gene product requires further investigation.

Acknowledgment—We thank Dr. Henri Grosjean for sharing unpublished results and for helpful advice and discussions.

REFERENCES

1. Sprinzl, M., Horn, C., Brown, M., Ioudovitch, A., and Steinberg, S. (1998) Nucleic Acids Res. 26, 148–153
2. Grosjean, H., Sprinzl, M., and Steinberg, S. (1995) Biochimie (Paris) 77, 139–141
3. Steinberg, S., Misch, A., and Sprinzl, M. (1993) Nucleic Acids Res. 21, 3011–3015
4. Björk, G. R., Ericson, J. U., Gustafsson, C. E., Hagervall, T. G., Jonsson, Y. H., and Wikstrom, P. M. (1987) Annu. Rev. Biochem. 56, 263–287
5. Persson, B. C. (1993) Mol. Microbiol. 8, 1011–1016
6. Bouadloun, F., Stichaiyo, T., Isaksson, L. A., and Björk, G. R. (1986) J. Bacteriol. 166, 1022–1027
7. Kalhor, H. R., Penjwini, M., and Clarke, S. (2005) Biochim. Biophys. Res. Commun. 334, 433–440
8. Christian, T., Evilia, C., Williams, S., and Hou, Y. M. (2004) J. Mol. Biol. 339, 707–719
9. Björk, G. R., Durand, J. M., Hagervall, T. G., Leipouviene, R., Lundgren, H. K., Nilsson, K., Chen, P., Qian, Q., and Urbanovics, I. (1999) FEBS Lett. 452, 47–51
10. RajBhandary, U. L., and Chang, S. H. (1968) J. Biol. Chem. 243, 598–608
11. Chang, S. H., and RajBhandary, U. L. (1968) J. Biol. Chem. 243, 592–597
12. RajBhandary, U. L., Stuart, A., and Chang, S. H. (1968) J. Biol. Chem. 243, 584–591
13. RajBhandary, U. L., Faulkner, R. D., and Stuart, A. (1968) J. Biol. Chem. 243, 575–583
14. RajBhandary, U. L., Stuart, A., Hoskinson, R. M., and Khorana, H. G. (1968) J. Biol. Chem. 243, 565–574
15. McCloskey, J. A., Liu, X. H., Crain, P. F., Bruenger, E., Guymon, R., Hashizume, T., and Stetter, K. O. (2000) Nucleic Acids Symp. Ser. 267–268
16. Zhou, S., Sitaramaiah, D., Noon, K. R., Guymon, R., Hashizume, T., and McCloskey, J. A. (2004) Bioorg. Chem. 32, 82–91
17. McCloskey, J. A., Graham, D. E., Zhou, S., Crain, P. F., Ibbi, M., Konisky, J., Soll, D., and Olsen, G. J. (2001) Nucleic Acids Res. 29, 4699–4706
18. Noon, K. R., Guymon, R., Crain, P. F., McCloskey, J. A., Thomm, M., Lim, J., and Cavicchioli, R. (2003) J. Bacteriol. 185, 5483–5490
19. Nakanishi, K., Blobstein, S., Funamizu, M., Furutachi, N., Van Lear, G., Grunberger, D., Lanks, K. W., and Weinstein, I. L. (1971) Biochem. Biophys. Res. Commun. 43, 107–109
20. Blobstein, S. H., Grunberger, D., Weinstein, I. L., and Nakanishi, K. (1973) Biochemistry 12, 188–193
21. Stuart, J. W., Koshlap, K. M., Guenther, R., and Agris, P. F. (2003) J. Mol. Biol. 334, 901–918
22. Kan, L. S., Ts' ů, P. O., von der Haar, F., Sprinzl, M., and Cramer, F. (1975) Biochemistry 14, 3278–3291
23. Marlick, A., von der Haar, F., and Cramer, F. (1973) Biopolymers 12, 27–43
24. Sussman, J. L., Holbrook, S. R., Warrant, R. W., Church, G. M., and Kim, S. H. (1978) J. Mol. Biol. 123, 607–630
25. Holbrook, S. R., Sussman, J. L., Warrant, R. W., and Kim, S. H. (1978) J. Mol. Biol. 123, 631–660
Discovery of Gene Family Critical to Wyosine Biosynthesis

286–289

50. Keith, G., Ebel, J. P., and Dirheimer, G. (1974) FEBS Lett. 48, 50–52

51. Martin, R., Sibler, A. P., Schneller, J. M., Keith, G., Stahl, A. J., and Dirheimer, G. (1978) C. R. Acad. Sci. Hebd. Seances Acad. Sci. D 287, 845–848

52. Sprinzl, M., Steegborn, C., Hubel, F., and Steinberg, S. (1996) Nucleic Acids Res. 24, 68–72

53. Altwegg, M., and Kubli, E. (1979) Nucleic Acids Res. 7, 93–105

54. Matte-Tailliez, O., Zivanovic, Y., and Forterre, P. (2000) Trends Genet. 16, 533–536

55. Armengaud, J., Urbonavicius, J., Fernandez, B., Chaussinand, G., Bujnicki, J. M., and Grosjean, H. (2004) J. Biol. Chem. 279, 37142–37152

56. Xue, H., Shen, W., and Wong, J. T. (1993) J. Chromatogr. 613, 247–255

57. Zhou, S., Pomerantz, S. C., Crain, P. F., and McCloskey, J. A. (2004) Nucleosides Nucleotides Nucleic Acids 23, 41–50

58. Grunberger, D., Weinstein, I. B., and Mushinski, J. F. (1975) Nature 253, 66–67

59. Wimmer, E., Maxwell, I. H., and Tener, G. M. (1968) Biochemistry 7, 2623–2628

60. Taniguchi, H., and Hayashi, N. (1998) Nucleic Acids Res. 26, 1481–1486

61. Edmonds, C. G., Vestal, M. L., and McCloskey, J. A. (1985) Nucleic Acids Res. 13, 8197–8206

62. Angenent, G., Smith, D. L., Crain, P. F., Yamaizumi, K., Nishimura, S., and McCloskey, J. A. (1982) Eur. J. Biochem. 127, 459–471

63. Vacher, J., Grosjean, H., Houssier, C., and Buckingham, R. H. (1984) J. Mol. Biol. 177, 329–342

64. Pang, H., Smith, D. L., Crain, P. F., Yamaizumi, K., Nishimura, S., and McCloskey, J. A. (1982) Eur. J. Biochem. 127, 459–471

65. Kuchino, Y., Borek, E., Grunberger, D., Mushinski, J. F., and Nishimura, S. (1982) Nucleic Acids Res. 10, 6421–6432

66. Kuchino, Y., Kasai, H., Yamaizumi, Z., Nishimura, S., and Borek, E. (1979) Biochim. Biophys. Acta 565, 215–218

67. Bjork, G. R., Jacobsson, K., Nilsson, K., Johansson, M. J., Byström, A. S., and Persson, O. P. (2001) EMBO J. 20, 231–239