Isolation of a Temperature-sensitive Mutant with an Altered tRNA Nucleotidyltransferase and Cloning of the Gene Encoding tRNA Nucleotidyltransferase in the Yeast *Saccharomyces cerevisiae*

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We have isolated a yeast mutant, ts352, that is temperature-sensitive for growth. The mutation has a general effect on mRNA metabolism and a specific effect on tRNA biosynthesis. Cells shifted to the nonpermissive temperature accumulate tRNAs that are shorter than mature tRNAs. The increased ability of these tRNAs to accept ATP demonstrates that growth of the ts352 mutant at the nonpermissive temperature results in accumulation of tRNA with defective 3' ends. The activity of ATP (CTP):tRNA-specific tRNA nucleotidyltransferase can readily be measured in extracts from wild type but not mutant cells. We have cloned and sequenced the wild type allele of the ts352 gene and find significant similarity between the yeast protein sequence predicted from the DNA sequence and the protein predicted from the sequence of the *Escherichia coli* tRNA nucleotidyltransferase gene. Expression of the yeast gene on a multicopy plasmid increases the activity of the tRNA nucleotidyltransferase in extracts. We conclude that the defect in the ts352 mutant is in the gene coding for yeast tRNA nucleotidyltransferase and that we have isolated the yeast gene that codes for this enzyme.

ATP (CTP):tRNA nucleotidyltransferase is the enzyme that catalyzes the incorporation of CMP and AMP residues into tRNAs that have an incomplete CCA sequence at their 3' end (1). The enzyme activity has been identified from both prokaryotic and eukaryotic sources but the role of the enzyme is best understood in *Escherichia coli*. The gene that encodes the *E. coli* enzyme has been isolated and sequenced (2). Nonsense mutations in the CCA gene of *E. coli* do not impair viability, so it is clear that all essential tRNA genes of *E. coli* encode the CCA sequence (3). The observation that mutants in the *E. coli* cca gene do display a slower growth rate implicates the enzyme in tRNA repair (4). In eukaryotes, however, tRNA genes do not have the CCA sequence found in mature tRNAs and it must be added post-transcriptionally (1). Although there is little direct evidence linking the enzyme to repair activity in eukaryotes, it presumably fulfills this role as well.

Genetic and biochemical studies with the *E. coli* enzyme have enabled the isolation of the gene encoding the enzyme (2), the purification of the enzyme (6), and its continued characterization (7). Genetic and biochemical studies with the *E. coli* nucleotidyltransferase gene and in the accompanying paper (7) on biochemical characterization of the *Saccharomyces cerevisiae* tRNA nucleotidyltransferase gene and in the accompanying paper (7) on biochemical characterization of the *Saccharomyces cerevisiae* tRNA nucleotidyltransferase gene and in the accompanying paper (7) on biochemical characterization of the *Saccharomyces cerevisiae* tRNA nucleotidyltransferase gene.

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**MATERIALS AND METHODS**

**Yeast Strains, Media, and Genetic Methods**—The ts352 mutant (MATα ts352 ade2-101 his3Δ200 ura3-52 tyr1) was isolated from a collection of temperature-sensitive mutants (8). Backcrossing this mutant to the strain SS328 (MATα ade2-101 his3Δ200 ura3-52 lys2) yielded the four segregants of tetrad 1, namely 352-1A (MATα ts352 ade2-101 his3Δ200 ura3-52 lys2), 352-1B (MATα ts352 ade2-101 his3Δ200 ura3-52 lys2), 352-1C (MATα ade2-101 his3Δ200 ura3-52 lys2 tyr1), and 352-1D (MATα ade2-101 his3Δ200 ura3-52 tyr1). These segregants were used for the biochemical studies. The 352 gene product was produced by transforming pJDB207-352 (see below) into W303-1B (MATα ade2-1, his3-11,15 leu2-3,112 ura3-1 trp1-1 can1-100). Media and standard genetic techniques are described by Sherman et al. (9).

**Identification and Characterization of the ts352 Mutant by Northern Analyses**—A collection of approximately 1000 temperature-sensitive...
TABLE I

| Segregants | 23 °C | 37 °C |
|------------|-------|-------|
|            | pmol CTP transferred/ nmol tRNA |       |
| A          | 0     | 4     |
| B          | 8     | 9     |
| C          | 91    | 145   |
| D          | 91    | 162   |

**TABLE II**

| Segregants | Picomoles ATP accepted/nmol tRNA |
|------------|---------------------------------|
| A          | 70                              |
| B          | 80                              |
| C          | 0                               |
| D          | 4                               |

**RESULTS**

**Identification of the ts352 Mutant**—The ts352 mutant was originally selected from a collection of temperature-sensitive mutants (8), because it showed increased steady-state levels of mRNAs produced by the poly(A)-binding protein gene (PAB) (11), the ribosomal protein gene TCM1 (10), and the STE2 gene (12) when shifted from the permissive (23 °C) to the nonpermissive (37 °C) temperature. To assure that the ts phenotype and the altered steady-state level of the mRNAs tested were caused by the same mutation, the original mutant (MATa ts352 ade2-101 his3Δ200 ura3-52 tyr1) was backcrossed to SS328 (MATa ade2-101 his3Δ200 ura3-52 lys2) and the resulting tetrads analyzed. All 49 tetrads examined showed a 2:2 segregation of the temperature-sensitive phenotype indicating that this phenotype was caused by a mutation in a single locus (data not shown). To determine if the temperature-sensitive phenotype and the mRNA accumulation are caused by the same mutation, RNA was analyzed from segregants of five tetrads grown at the permissive (23 °C) and nonpermissive (37 °C) temperatures. All five tetrads showed a cosegregation of the temperature-sensitive phenotype and elevated levels of PAB, TCM1, and STE2 mRNAs. The data from two tetrads is presented in Fig. 1. The STE2 gene, encoding the α-pheromone receptor, is normally only expressed in cells of the α mating type and that pattern is maintained in ts352 if it is grown at the permissive temperature. A shift of ts352 cells to the restrictive temperature results in an increase of the STE2 mRNA in α cells and an inappropriate expression of the STE2 mRNA in α cells (Fig. 1). Although there were only a few MATa segregants tested in this analysis, we assume that the STE2 expression in α cells under nonpermissive conditions is caused by the ts352 mutation.

**Isolation and Characterization of the Wild Type 352 Locus**—Our strategy for the isolation of the wild type 352 locus was to transform the temperature-sensitive strain 352-1B (MATα

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1 The abbreviation used is: kb, kilobase.
2 P. Herman, personal communication.
Yeast tRNA Nucleotidyltransferase Mutant

ts352 ade2-101 ura3-52 his3Δ200 with a yeast genomic library cloned into YCP50 (a gift from Scott Emr, California Institute of Technology). Transformants were selected for a Ura+ phenotype at 23 °C and subsequently tested for their ability to grow at 37 °C. Approximately 20,000 transformants were obtained and 15 were able to grow at 37 °C. All 15 transformants yielded the same plasmid which contained a 15 kb fragment in YCP50. Subsequent subcloning experiments located the complementing activity to a 4.8 kb ClaI-BamHI fragment (Fig. 2). Deletion mapping and sequence analysis revealed that the DNA required for temperature resistant growth con tains an open reading frame of 1638 nucleotides which could encode a protein of 546 amino acids.

A computer assisted homology search (Intelligenetics, XFASTP program, NBRF database in BIONET National Computer Resource for Molecular Biology) revealed that this hypothetical protein shows similarity to the ATP(CTP):tRNA nucleotidyltransferase of E. coli (EC 2.7.7.25). The protein contains an open reading frame of 1638 nucleotides which could have had its CCA ends removed by snake venom phosphodiesterase. As can be seen from the data in Table I, two of the four segregants have a reduced ability to carry out this reaction regardless of whether the assay was done at 23 or 37 °C. The two segregants with a deficiency in this enzyme activity are also the two that have the temperature-sensitive growth defect (data not shown).

Nucleotidyltransferase Activity in Wild Type and ts352—Extracts were prepared from the segregants of tetrad 1 and tested for their ability to add radiolabeled CTP to tRNAs that have had their CCA ends removed by snake venom phosphodiesterase. As can be seen from the data in Table I, two of the four segregants have a reduced ability to carry out this reaction regardless of whether the assay was done at 23 or 37 °C. The two segregants with a deficiency in this enzyme activity are also the two that have the temperature-sensitive growth defect (data not shown).

We next used Northern analysis to examine the effect of the ts352 mutation on tRNA maturation. Cultures of each segregant were grown at 23 °C, harvested, and suspended in media prewarmed to 23 or 37 °C. tRNAs were isolated immediately following suspension in fresh media (zero time) and at 1-h intervals and separated by gel electrophoresis. The RNAs were transferred to nylon membranes and probed with an oligonucleotide probe complementary to a tRNAAfε (13).

To determine whether the shorter tRNAs actually have an incomplete CCA sequence, we tested the ability of tRNAs in the 3-h 37 °C timepoints for their ability to accept ATP. CTP was included in all of the reactions so that tRNAs in complete CCA sequence, we tested the ability of tRNAs in the 3-h 37 °C timepoints for their ability to accept ATP. CTP was included in all of the reactions so that tRNAs in these strains than in the normal strains. We conclude from these experiments that the ts352 mutation results in a deficiency in tRNA nucleotidyltransferase activity.

The 352 Allele Codes for Yeast tRNA Nucleotidyltransferase—The sequence similarity discovered by computer comparisons lead us to hypothesize that the gene we had isolated coded for the yeast ATP (CTP):tRNA nucleotidyltransferase, and the experiments showing that the ts352 mutants are deficient in this activity are certainly consistent with this.
Yeast tRNA Nucleotidyltransferase Mutant

TABLE III

tRNA nucleotidyltransferase activity in wild type and overproducing cells

| Strain and plasmid | Nanomoles CTP transferred/min/mg |
|--------------------|---------------------------------|
| W3031B-JDB207     | 1.2                             |
| W3031B-JDB207-352 | 190.0                           |

DISCUSSION

We have described the identification and characterization of a novel temperature-sensitive yeast mutant ts352. Based on the characteristics of this mutant, we propose that it is defective in the tRNA processing enzyme tRNA nucleotidyltransferase. tRNAs with incomplete 3' termini are more abundant in the mutant than in the wild type, and these shorter tRNAs accumulate upon incubation at 37 °C. The nucleotidyltransferase activity is strongly affected in the mutant as indicated by the observation that extracts prepared from ts352 mutants do not contain significant transferase activity whether assays are done at 23 or 37 °C. In vivo temperature-sensitive phenotypes are not always mimicked in vitro. This lack of correlation is, for example, observed with some temperature-sensitive alleles of prp11 (19, 20).

The temperature-sensitive phenotype of the yeast mutant is in contrast to the phenotype of E. coli mutants with altered nucleotidyltransferase. In the latter, inactivation of tRNA nucleotidyltransferase results only in a decreased growth rate (3), presumably because all essential tRNAs in E. coli are coded by genes with a CCA sequence. In yeast, the CCA sequence must be added post-transcriptionally so the enzyme is essential for growth. The fact that a temperature-sensitive mutant was isolated indicates that there are not multiple nucleotidyltransferase genes in this organism.

As might be expected in a strain with a primary defect in tRNA biosynthesis, additional phenotypes are revealed in cells grown at the nonpermissive temperature. Originally ts352 was selected based on an increase in the steady-state levels of three different mRNAs. This suggests a link between an alteration in the translational machinery and the stability of mRNA. Degradation of mRNA may be influenced by several factors and is not understood completely (21). However, it is established that cycloheximide, an inhibitor of chain elongation in protein synthesis, leads to stabilization of many mRNAs (22-28). It seems reasonable that a depletion of functional tRNA molecules would lead to a decrease in the rate of chain elongation and thereby to a protection of mRNA from degradation.

Not only does the ts352 mutant display alterations in steady-state levels of mRNAs, the mutant has altered regulation of expression of the STE2 gene at the nonpermissive temperature. The STE2 gene, encoding the a-hormone receptor, is expressed only in cells with an a-specific receptor, is expressed only in cells with an a-specific receptor.
that the 352 locus codes for the yeast nucleotidyltransferase. First, if the sequences essential for complementation are present in multiple copies, the nucleotidyltransferase activity in extracts is elevated. Second, the protein predicted from the complementing DNA sequence shows significant homology to the sequence of the E. coli enzyme. Interestingly, the similarity is restricted to the amino terminal end of the protein. Cudny et al. (2) noted the presence of the sequence Gly-X-Gly-X-X-Gly beginning at amino acids 66 and 272 in the E. coli protein. This sequence had been implicated previously in nucleotide-binding sites of some nucleotide-binding proteins (28), and in subsequent experiments Zhu et al. (29) demonstrated that changing glycine 70 to an aspartic acid resulted in the loss of AMP-incorporating activity. The yeast enzyme does not have a comparable sequence. The longest open reading frame in the yeast gene would code a protein of 62 kDa, somewhat smaller than the molecular mass reported in the early literature (30) and slightly larger than the size we determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of enzyme purified as described in the accompanying manuscript (7). Further work with the mutant and the corresponding gene should allow further insight into the structure, biosynthesis, and biochemistry of nucleotidyltransferase in yeast and into the maturation of tRNA in eukaryotic cells.

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REFERENCES
1. Deutscher, M. P. (1982) in The Enzymes (Boyer, P. D., ed) Vol. XV, pp. 183–215, Academic Press, Orlando, FL
2. Cudny, H., Lupski, J. R., Godson, G. N., and Deutscher, M. P. (1986) J. Biol. Chem. 261, 6444–6449
3. Zhu, L. and Deutscher, M. P. (1987) EMBO J. 6, 2473–2477
4. Deutscher, M. F., Foulds, J., and McClain, W. H. (1974) J. Biol. Chem. 249, 6696–6699
5. Seidman, J. G., and McClain, W. H. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 1491–1495
6. Cudny, H., and Deutscher, M. P. (1986) J. Biol. Chem. 261, 6450–6453
7. Chen, J., Kirchner, G., Abei, M., and Martin, N. C. (1989) J. Biol. Chem. 265, 16221–16224
8. Vijayaraghavan, U., Company, M., and Abelson, J. (1989) Genes & Dev. 3, 1206–1216
9. Sherman, F., Fink, G. R., and Hicks, J. B. (1983) Methods in Yeast Genetics: Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
10. Schultz, L. D., and Friesen, J. D. (1983) J. Bacteriol. 155, 8–14
11. Sache, A. B., Bond, M. W., and Kornberg, R. D. (1986) Cell 45, 827–835
12. Rirkholder, A. R., and Hartwell, I. H. (1985) Nucleic Acids Res. 13, 8463–8475
13. Feinberg, A. P. and Vogelstein, B. (1984) Anal. Biochem. 137, 262–267
14. Najarian, D., Shu, H. H., and Martin, N. C. (1986) Nucleic Acids Res. 14, 9561–9578
15. Weissebach, J., Martin, R., Dirheimer, G. (1975) Eur. J. Biochem. 56, 527–532
16. Ito, H., Fukuda, Y., Murata, K., and Kimura, A. (1983) J. Bacteriol. 153, 163–168
17. Boeke, J. D., LaCroute, F., and Fink, G. R. (1984) Mol. & Gen. Genet. 197, 345–346
18. Beggs, J. D. (1981) in Genetic Engineering (Williams, R., ed) Vol. 2, pp. 175–203, Academic Press, Orlando, FL
19. Lustig, A. J., Lin, R.-J., and Abelson, J. (1986) Cell 47, 963–965
20. Liu, H.-J., Lustig, A. J., and Abelson, J. (1987) Genes & Dev. 1, 7–18
21. Brawerman, G. (1980) Cell 27, 9–10
22. Kelly, K., Cochran, D. H., Giles, C. D., and Leder, P. (1980) Cell 23, 603–610
23. Sive, H. L., Heintz, N., and Roeder, R. G. (1984) Mol. Cell. Biol. 4, 2723–2734
24. Kelly, R., Shaw, D. R., and Ennis, H. L. (1987) Mol. Cell. Biol. 7, 799–805
25. Mullner, E. W., and Kuhn, L. (1988) Cell 53, 815–825
26. Gay, D. A., Sisodia, S. S., and Cleveland, D. W. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5763–5767
27. Johnson, A., and Herskowitz, I. (1985) Cell 42, 237–247
28. Russo, M. W., Lukas, T. J., Cohen, S., and Staros, J. V. (1985) J. Biol. Chem. 260, 5205–5208
29. Zhu, L., Cudny, H., and Deutscher, M. P. (1986) J. Biol. Chem. 261, 14875–14877
30. Sternbach, H., von der Haar, F., Schlimme, E., Gaertner, E., and Cramer, F. (1971) Eur. J. Biochem. 22, 169–172
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