An antisuppressor mutant of *Saccharomyces cerevisiae* deficient in isopentenylated tRNA has reduced Δ^2-isopentenylpyrophosphate: tRNA-Δ^2-isopentenyl transferase activity

Howard M. Laten, Ruth M. Timmons and Sopiah Suid

Loyola University of Chicago, Biology Department, 6525 North Sheridan Road, Chicago, IL 60626, USA

Received 26 October 1984

We have previously reported the isolation and initial characterization of a mutation in *Saccharomyces cerevisiae*, designated *mod5-1*, that reduces the capacity of altered tyrosine tRNAs to suppress ochre nonsense mutations. The mutation results in the virtual elimination of the modified tRNA nucleoside, N^6-Δ^2-(isopentenyl)adenosine, normally found adjacent to the anticodons of certain tRNA species. We demonstrate here that *MOD5* codes for Δ^2-isopentenylpyrophosphate: tRNA-Δ^2-isopentenyl transferase, or a protein that regulates its synthesis.

tRNA isopentenyltransferase *tRNA modification* N^6-Δ^2-*(Isopentenyl)adenosine*
Saccharomyces cerevisiae *tRNA antisuppression*

1. INTRODUCTION

Nucleoside modification is a conspicuous feature of tRNA, and the functional role of tRNA modification has been the focus of many studies [1,2]. Assigning functions to specific nucleosides, however, has been less than straightforward, and the absence of distinguishable phenotypes in mutants lacking certain modifications has led to the suggestion that some modified nucleosides are dispensible [2].

Mutants lacking the hypermodified derivative, N^6-Δ^2-(isopentenyl)adenosine (i^6Ado), normally found adjacent to the 3′-end of the anticodons of many tRNAs that decode mRNA triplets beginning with Urd, have been isolated from *Escherichia coli* [3,4], and the yeasts *S. cerevisiae* [5] and *Sch. pombe* [6,7].

Prior to the characterization of these mutants, the function of i^6Ado and its 2-methylthiolated derivative had been the subject of several in vitro investigations [8–10]. The results of these studies strongly suggested that isopentenylation greatly enhances translational efficiency [8–10]. The more recent mutant studies suggested that the effects are far more subtle [3–7].

We have determined that the product of the *MOD5* gene in *S. cerevisiae* is the last enzyme in the pathway leading to the isopentenylation of tRNA, namely Δ^2-isopentenylpyrophosphate:tRNA-Δ^2-isopentenyl transferase, or possibly a molecule that regulates its expression. The analogous gene in *Sch. pombe*, designated *SIN1*, also codes for the transferase or a product responsible for its regulation (R. Fluri, personal communication). This enzyme has been partly purified and characterized from *S. cerevisiae* [11], *E. coli* [12,13], *L. acidophilus* [14], and *Zea mays* [15].

2. MATERIALS AND METHODS

The following related strains of *S. cerevisiae* served as sources of tRNA or enzymes or both: HL370 and HL431 (*MOD5*$^+$), HL430 and HL1320 (*mod5-1*). HL431 is a revertant of HL430; HL430

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/85/$3.30

and HL1320 differ in that the former carries the ochre suppressor, *SUP7-o*. The *mod5-1* mutation results in greater than 90% loss of i^{6}Ado from unfractionated tRNA [5,16].

Cells were grown to mid-logarithmic phase in YPD medium [17], and harvested by centrifugation. Cells were disrupted by agitation in buffer-saturated phenol, and the tRNA was isolated by phenol extraction, ethanol precipitation, and DEAE-cellulose chromatography as in [5].

For the detection of Δ^2-isopentenylpyrophosphate:tRNA-Δ^2-isopentenyl transferase activity, cells were grown and harvested as above, and mechanically disrupted by three 1-min pulses in a Bead Beater (Biospec Products) at 4°C. A cell-free lysate was prepared as in [11], and the protein precipitating between 20 and 80% ammonium sulfate saturation was collected, dialyzed, and assayed for transferase activity by the method of Kline et al. [11], using i^{6}Ado-deficient tRNA isolated from the *mod5-1* mutant rather than chemically treated tRNA.

To verify the incorporation of radiolabel into i^{6}Ado, the RNA was digested to nucleosides with snake venom phosphodiesterase, ribonuclease A, and bacterial alkaline phosphatase as in [5]. The digest was loaded on a Sep-Pak cartridge (Waters Associates), and the cartridge was washed with 10 ml water. The cartridge was then washed with 5 ml methanol, and the methanol wash was evaporated to dryness under a stream of nitrogen. The sample was redissolved in 50 μl of 50% methanol containing nucleoside standards, and loaded on a 3.9 mm × 30 cm μBondapak C_{18} HPLC column (Waters Associates). The sample was eluted with 50% methanol, and radiolabeled fractions were detected by liquid scintillation.

3. RESULTS

Fig.1 summarizes the transfer of ^{14}C-label from Δ^3-isopentenylpyrophosphate to tRNA. The Δ^3-isomer is not a substrate for the enzyme that catalyzes the transfer of the isopentenyl group to the appropriate adenosine in the anticodon loops of those tRNAs subject to isopentenylation [11–14]. The actual substrate is the Δ^2-isomer which is synthesized in the reaction by pig liver isopentenylpyrophosphate isomerase. Transfer of the isopentenyl group to tRNA was maximal when

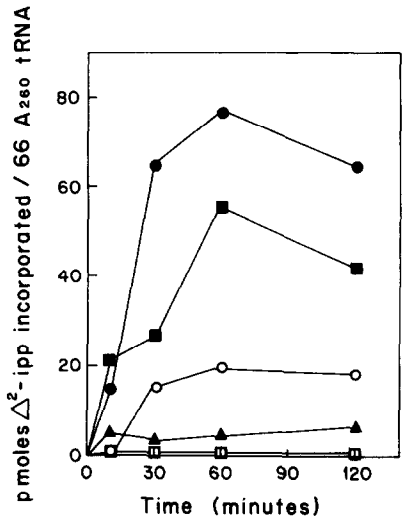

Fig.1. Transfer of ^{14}C-label from Δ^2-isopentenylpyrophosphate to tRNA. The reaction mixture contained: 2.5 μCi Δ^3-isopentenylpyrophosphate (57 mCi/mmol), 66 A_{260} units tRNA (from HL430 unless otherwise noted), 3 mg pig liver isomerase (0.9 units/mg), and 5 mg of the 20–80% ammonium sulfate fraction (HL431, ●—●; HL370, ■—■; HL430, ○—○; HL370, tRNA from HL370, ▲—▲; HL370, boiled control, □—□), in a total volume of 2.0 ml of 0.05 M Tris–HCl, 0.005 M $MgCl_2$, 0.02 M β-mercaptoethanol (pH 7.5). Aliquots were removed and the tRNA was isolated as in [11].

the sources of enzyme were the *MOD5*$^+$ strains HL370 and HL431, and the tRNA was derived from the *mod5* strain, HL430, and therefore deficient in i^{6}Ado. Enzyme from the former strain catalyzed the transfer of 56 pmol substrate to 66 A_{260} units of unfractionated i^{6}Ado-deficient tRNA after 60 min, and enzyme from the latter catalyzed the transfer of 77 pmol. In addition, the enzyme preparation from HL370 catalyzed the transfer of 5 pmol isopentenylpyrophosphate when tRNA from strain HL370 was used instead of the i^{6}Ado-deficient tRNA. Enzyme derived from HL430 catalyzed the transfer of 20 pmol substrate to the isopentenyladenosine-deficient tRNA after 60 min.

In order to confirm that the product being monitored was isopentenylated tRNA, the radiolabelled material monitored in fig.1 was digested to nucleosides and fractionated as described in section 2. Fig.2 illustrates the results of

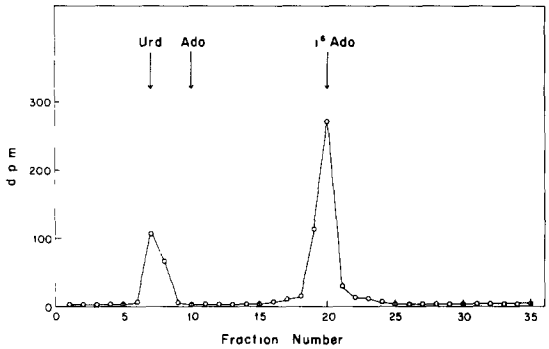

Fig.2. Reversed-phase HPLC elution profile of a ^{14}C-isopentenylated tRNA digest. A 50-μl sample containing 1000 dpm of ^{14}C-labelled products and 1 A_{254} each of Urd, Ado, and i^{6}Ado, was loaded on a 3.9 mm × 30 cm μBondapak C_{18} HPLC column and eluted with 50% methanol at a flow rate of 1.0 ml/min; 30-s fractions were collected directly into scintillation cocktail and counted.

the HPLC analysis. Most of the radiolabel comigrated with authentic i^{6}Ado; this peak did not appear in undigested controls (not shown). The first peak in fig.2 was the result of residual levels of Δ^3-[^{14}C]isopentenylpyrophosphate that remain bound to DEAE-cellulose at moderate salt concentrations.

4. DISCUSSION

Our results confirm that a yeast mutant characterized by a deficiency in isopentenylated tRNA has a reduced capacity to transfer an isopentenyl group from Δ^2-isopentenylpyrophosphate to a specific adenosine residue in certain tRNAs. Whether this reduction is due to a structural defect in Δ^2-isopentenylpyrophosphate: tRNA-Δ^2-isopentenyl transferase or to a decrease in the level of this enzyme cannot be resolved by this study.

The reduction in enzyme activity in the mutants is clearly incomplete, and this correlates with our reports that tRNAs from mutant cultures contained between 2 and 9% of the wild-type i^{6}Ado levels [5,16]. In addition, an independently isolated antisuppressor mutation, *asu2-2* [18], is allelic to *mod5-1*, and contains 44% of the wild-type level of tRNA-associated i^{6}Ado (F. Webb, personal communication). Transfer RNA from an analogous mutant of *Sch. pombe* (*sin1*) contains 1% of the wild-type i^{6}Ado levels [16]. Since significant loss of the i^{6}Ado modification from tRNA appears to have little effect on cell growth in yeast [5–7], the apparent discrimination against tight mutants that completely lack isopentenylated tRNA is curious. The absence of tight mutants suggests that a second gene product is responsible for isopentenylation of tRNA in these mutants.

The possibility that mitochondria serve as the source of the residual isopentenylated tRNA and transferase activity obtained from mutant cultures has been eliminated by Martin and Hopper who demonstrated that the two mitochondrial tRNAs normally containing i^{6}Ado lack this modification in a *mod5* genetic background [19]. Their findings also suggest that the nuclear encoded transferase functions in both the cytoplasm and mitochondria.

A second possibility is that another cytoplasmic enzyme can isopentenylate tRNA, albeit not as effectively as the *MOD5* gene product. A good candidate for this role is Δ^2-isopentenylpyrophosphate:AMP-Δ^2-isopentenyl transferase. The existence of this enzyme, which catalyzes the transfer of the isopentenyl group to 5′-AMP, has been explicitly demonstrated in tobacco [20,21] and slime mold [22,23], and its presence in *S. cerevisiae* and *Sch. pombe* has been strongly implicated [16].

ACKNOWLEDGEMENTS

This work was funded by NIH grant GM31023 and by a Sigma Xi Grant-in-Aid (awarded to S.S.).

REFERENCES

[1] Nishimura, S. (1979) in: Transfer RNA: Structure, Properties, and Recognition (Schimmel, P.R., Soll, D. and Abelson, J.N. eds) pp.59–79, Cold Spring Harbor Laboratory, New York.
[2] Bjork, G.R. (1983) in: Processing of RNA (Apirion, D. ed.) pp.291–330, CRC Press, Cleveland, OH.
[3] Yanofsky, C. and Soll, L. (1977) J. Mol. Biol. 113, 663–677.
[4] Eisenberg, S.P., Soll, L. and Yarus, M. (1979) J. Mol. Biol. 135, 111–126.
[5] Laten, H.M., Gorman, J.W. and Bock, R.M. (1978) Nucleic Acids Res. 5, 4329–4342.
[6] Janner, F., Vogeli, G. and Fluri, R. (1980) J. Mol. Biol. 139, 207–217.
[7] Thuriaux, P., Minet, M., Hofer, F. and Leupold, U. (1975) Mol. Gen. Genet. 142, 251–261.

[8] Fittler, F. and Hall, R.H. (1966) Biochem. Biophys. Res. Commun. 25, 441–446.
[9] Gefter, M.L. and Russell, R.L. (1969) J. Mol. Biol. 39, 145–157.
[10] Furuichi, Y., Wataya, Y., Hayatsu, H. and Ukita, T. (1970) Biochem. Biophys. Res. Commun. 41, 1185–1191.
[11] Kline, L.K., Fittler, F. and Hall, R.H. (1969) Biochemistry 11, 4361–4371.
[12] Bartz, J.K. and Soll, D. (1972) Biochimie 54, 31–39.
[13] Rosenbaum, N. and Gefter, M.L. (1972) J. Biol. Chem. 247, 5675–5680.
[14] Holtz, J. and Klambt, D. (1975) Hoppe-Seyler's Z. Physiol. Chem. 356, 1459–1464.
[15] Holtz, J. and Klambt, D. (1978) Hoppe Seyler's Z. Physiol. Chem. 359, 89–101.
[16] Laten, H.M. and Zahareas-Doktor, S. (1984) Proc. Natl. Acad. Sci. USA, in press.
[17] Sherman, F., Fink, G.R. and Lawrence, C.L. (1978) Methods in Yeast Genetics, p.61, Cold Spring Harbor Laboratory, New York.
[18] McCready, S.J. and Cox, B.S. (1973) Mol. Gen. Genet. 124, 305–320.
[19] Martin, N.C. and Hopper, A.K. (1982) J. Biol. Chem. 257, 10562–10565.
[20] Chen, C.-M. and Melitz, D.K. (1979) FEBS Lett. 107, 15–20.
[21] Chen, C.-M. (1982) in: Plant Growth Substances (Wareing, P.F. ed.) pp.155–163, Academic Press, New York.
[22] Taya, K., Tanaka, Y. and Nishimura, S. (1978) Nature 271, 545–546.
[23] Ihara, M., Taya, Y., Nishimura, S. and Tanaka, Y. (1984) Arch. Biochem. Biophys. 230, 652–660.