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EVIDENCE FOR THE EXISTENCE OF AN EXPRESSED MINOR VARIANT $tRNA^{Phe}$ IN YEAST

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Two expressed brewer's yeast $tRNAs^{Phe}$, a major and a minor one, have been purified and sequenced. The major $tRNA^{Phe}$ corresponds to the already known $tRNA^{Phe}$, whereas the minor one differs from the former in the substitution of T_6-A_{67} by C_6-G_{67} base pair in the "acceptor stem". The minor $tRNA^{Phe}$ contaminates all preparations of yeast $tRNA^{Phe}$ except those prepared by polyacrylamide gel electrophoresis.

Among all known tRNAs, the most studied one is probably yeast $tRNA^{Phe}$. Indeed, since its primary structure was determined (1), this macromolecule has been extensively studied in many fields, such as chemical modification in relation to its interaction with enzymes, NMR and Xray cristallography, the latter leading to the first tertiary model of a tRNA (2,3). Recently a variant $tRNA^{Phe}$ has been reported (4) which differs from the sequenced $tRNA^{Phe}$ by the substitution of T_6-A_{67} base pair by C_6-G_{67} base pair in the "acceptor stem". In this paper we report the existence of a minor $tRNA^{Phe}$ in yeast, corresponding to this variant $tRNA^{Phe}$ gene (4). It contaminates all studied preparations of $tRNA^{Phe}$, except those prepared by 10% polyacrylamide gel electrophoresis (PAGE).

MATERIAL AND METHODS

Purified brewer's yeast $tRNA^{Phe}$: batch n° 10050320-39, July 1986 and total brewer's yeast tRNA were from Boehringer-Mannheim (GFR). Counter-current distribution, to purify the $tRNAs^{Phe}$ out of total tRNA as well as the aminoacylation tests, were performed according to Dirheimer and Ebel (5). BD-cellulose column chromatography (6) and 10% polyacrylamide gel electrophoresis (PAGE) (7) were used for further purification of counter-current $tRNA^{Phe}$ fractions. tRNAs were eluted from gels as previously reported (8). The primary structure was determined by [^{32}P] postlabelling techniques as already published (8).

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RESULTS AND DISCUSSION

Minor and major tRNAs^{Phe} were found on 10% polyacrylamide gel electrophoresis performed on the most hydrophobic fraction obtained by counter- current distribution of total brewer's yeast tRNA (Fig. 1A and 1B). The minor tRNA^{Phe} was found on 10% PAGE in a band migrating much faster than the major tRNA^{Phe}. As shown in Fig. 2, tRNA^{Phe} prepared by counter-current distribution followed by BD-cellulose column chromatography, contains this minor species. This tRNA^{Phe} mixture as well as pure minor or major tRNAs^{Phe} were found to be aminoacylated to 1600 picomoles phenylalanine per A_{260} unit tRNA, meaning that both species are equally well aminoacylated. Among all used purification techniques, only 10% PAGE permits to separate the two isoacceptor tRNAs^{Phe}. Therefore both tRNAs^{Phe} were probably present in samples used for primary structure determination of tRNA^{Phe} (1) and its precursors (9). This contamination by the minor species could well account for the minor peaks and bands or low molar ratios of several fragments observed by most authors. But

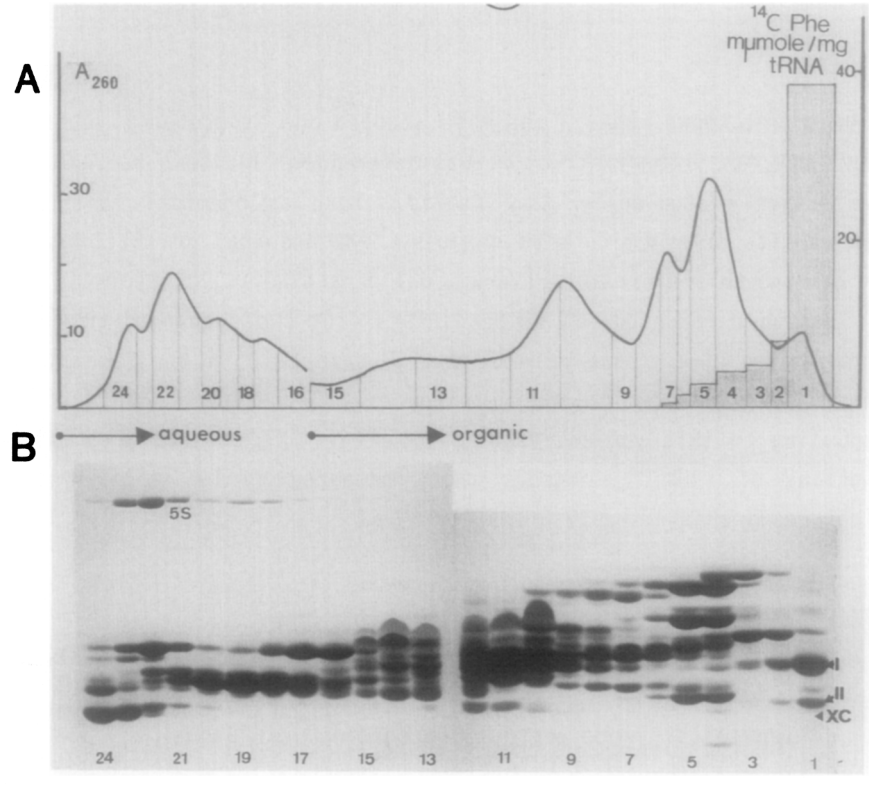

Figure 1.
A. Counter-current distribution pattern of brewer's yeast total tRNA. Gray boxes : [^{14}C]-Phe accepting activity.
B. Purification on 10% PAGE of counter-current distribution fractions 1 to 24. Fraction 1 contain two bands of tRNA^{Phe} : I : major tRNA^{Phe} species, II ; minor tRNA^{Phe} species.
XC : xylene cyanol blue dye.

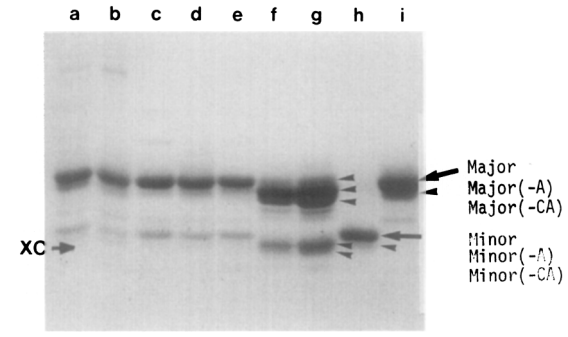

Figure 2. Electrophoretic pattern on 10% PAGE of tRNAs^{Phe} fractions
a and **b** : Counter-current distribution fraction of hydrophobic peak$_1$ (see figure 1).
c,**d**,**e** : tRNA^{Phe} purified by counter-current distribution followed by BD-cellulose column chromatography
f and **g** : Commercial tRNA^{Phe} (see text) : **g** contains twice as much tRNA^{Phe} as **f**. These samples were overloaded : The upper band of each major and minor tRNA^{Phe} belong to tRNA^{Phe} minus A and the lower one to tRNA^{Phe} minus CA. Only less than 5% of intact tRNA^{Phe} is present in that sample.
h : 10% PAGE purified minor tRNA^{Phe}
i : 10% PAGE purified major tRNA^{Phe}. This sample is overloaded. A lower band of tRNA^{Phe} minus A, less than 5% of the total, contaminates this pure sample. tRNA^{Phe} minus A is also present in small amounts in **a**, **b**, **c**, **d** and **e**, showing a slight hydrolysis of 3' end nucleotides. This situation seems to be extreme in the commercial sample.
Other bands than those indicated correspond to contaminating tRNAs or other degradation fragments of tRNA^{Phe}.
XC : xylene cyanol blue dye.

because this minor tRNA was present in low amounts it was not detected given the lack of accuracy of the sequencing techniques. Commercial tRNA^{Phe} also contains both species (Fig. 2), but they migrate faster on 10% PAGE than the counter-current distribution ones. As will be mentioned below, these tRNAs lack one or two nucleotides at their 3' end.

The major tRNA^{Phe} as well as the minor one contain the same minor nucleosides as found on cellulose thin layer plates in Rogg's et al. system (10) (not shown). Figure 3 shows electrophoresis-homochromatographies of partially hydrolysed 5' and 3' [^{32}P] labelled minor and major tRNAs^{Phe}. The differences between the two tRNAs^{Phe} are located at positions 6 and 67, as reported by Venegas **et al.** (4) in tRNA^{Phe} genes. In order to check whether these are the only differences or whether others exist elsewhere in the molecule, we performed enzymatic read-off gel analyses (11). These confirmed the above results and no other differences could be detected (not shown). We performed the same analyses on the two commercial tRNAs^{Phe}. These were found to be shorter at their 3' ends : the terminal Adenosine $_{76}$ was absent in all molecules, whereas 30% of them lacked Cytidine $_{75}$. Identical results were

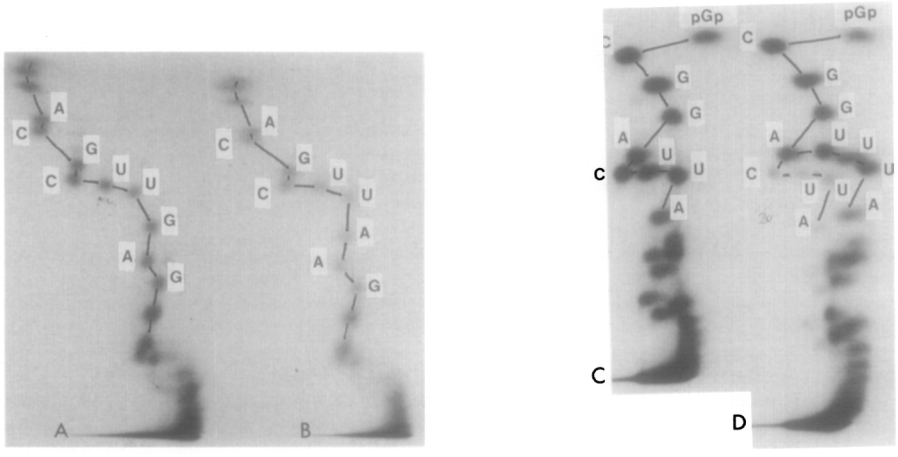

Figure 3. Wandering spot analyses of 3' and 5' [^{32}P] end labelled minor and major tRNAs^{Phe}.
A : 3' [^{32}P] minor tRNA^{Phe}
B : 3' [^{32}P] major tRNA^{Phe}
C : 5' [^{32}P] minor tRNA^{Phe}
D : 5' [^{32}P] "native" tRNA^{Phe} (minor and major)

obtained by aminoacylation : only 70% tRNA^{Phe} could be charged by phenylalanyl-tRNA synthetase even when a crude enzymatic extract, containing tRNA nucleotidyl transferase, was used. Moreover the addition to the enzymatic medium of CTP in addition to the already present ATP permitted to obtain the expected value of 1600 picomoles phenylalanine per mg tRNA^{Phe}.

The average amounts of minor and major tRNAs^{Phe} were determined by UV spectroscopy after elution from PAGE and by [^{32}P] quantitation on 3' or 5' end labelled tRNAs^{Phe} separated on 10% PAGE : minor tRNA^{Phe} represents 15-20% of total tRNA^{Phe}. This result leads us to think that among the 10 tRNA^{Phe} genes found by Venegas **et al.** (4), one or two could belong to the minor variant tRNA^{Phe}.

Because commercial tRNA^{Phe} as well as tRNA^{Phe} purified by BD-cellulose column chromatography contain both species (fig. 2), it is probable that all studies performed so far on brewer's yeast tRNA^{Phe} used the mixture of the two tRNAs^{Phe}. How did this tRNA^{Phe} mixture cristallise ? Did only the major tRNA^{Phe} cristallise out of the cristallization medium or did both tRNAs^{Phe} co-cristallise in the same cristal giving rise to a "unique" tertiary structure, despite the change of A-U into G-C : a purine-pyrimidine base pair in both cases. The fact that the minor tRNA^{Phe} migrates faster than the major one on gel might indicate a tighter structure at +4°C and in presence of urea 4 M (10% PAGE conditions), probably due to the G-C bond replaced by the A-U one. Thus some of the parameters of tertiary model building should be reinvestigated. Such a remark could also be made for NMR studies, but probably

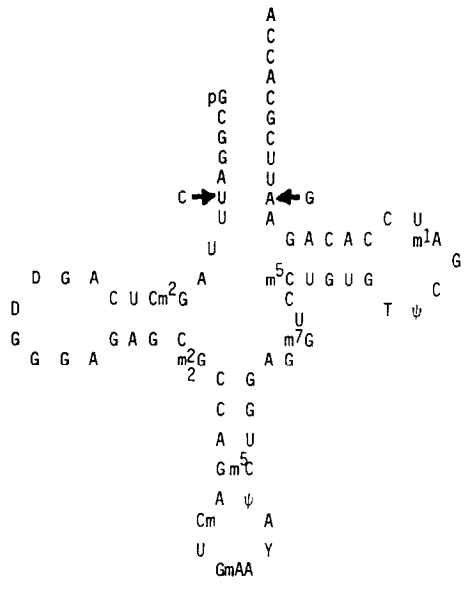

Figure 4. Cloverleaf model of major tRNA^{Phe} : arrows show the differences observed in minor tRNA^{Phe}.

not for enzymatic ones, because both tRNAs^{Phe} are aminoacylated to the same extent (Results not shown).

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REFERENCES

1. RajBhandary, U.L., Chang, S.H., Stuart, A., Faulkner, R.D., Hoskinson, R.P. and Khorana, H.G. (1967). Proc. Natl. Acad. Sci. US 57, 751-758.
2. Kim, S.H., Suddath, F.L., Quickley, G.J., McPherson, A., Sussman, J.L., Wang, A.H.J., Seeman, N.C. and Rich, A. (1974). Science 185, 435-440.
3. Robertus, J.D., Ladner, J.E., Finch, J.T., Rhodes, D., Brown, R.S., Clark, B.F.C. and Klug, A. (1974). Nature 250, 546-555.
4. Venegas, A., Bello, A., Villanueva, J., Zaldivar, J., Quiroga, M., Gomez, I., Bull, P. and Valenzuela, P. (1979). In : Siddiqui M.A.Q., Krauskopf M., Weissbach H. (eds), Molecular Approaches, gene expression, protein structure. Acad. Press, New York, pp. 33-59.
5. Dirheimer, G. and Ebel, J.P. (1967). Bull. Soc. Chim. Biol. 49, 1679-1687.
6. Gillam, I., Millward, S., Blew, D., Von Tigerstrom, M., Wimmer, E. and Tener, G.M. (1967). Biochemistry 10, 3043-3056.
7. Fradin, A., Gruhl, H. and Feldmann, H. (1975). FEBS Lett. 50, 185-189.
8. Keith, G., Pixa, G., Fix G. and Dirheimer, G. (1983). Biochimie 65, 661-672.
9. Knapp, G., Beckmann, J.S., Johnson, P.F., Fuhrman, S.A. and Abelson, J. (1978) Cell 14, 221-236.
10. Rogg, H., Brambilla, R., Keith, G. and Staehelin, M. (1976). Nucl. Acids Res. 3, 285-295.
11. Peattie, D.A. (1979). Proc. Natl. Acad. Sci. US 76, 1760-1764.
12. Ikemura, T. (1985). Mol. Biol. Evol. 2, 13-34.