THE PRIMARY STRUCTURE OF $tRNA^{Phe}$ FROM *BACILLUS STEAROTHERMOPHILUS*

C. GUERRIER-TAKADA, G. DIRHEIMER*, H. GROSJEAN and G. KEITH*

*Laboratoire de Chimie Biologique, Université libre de Bruxelles 67 rue des Chevaux, B. 1640 Rhode St. Genese Belgium, and *Institut de Biologie Moléculaire et Cellulaire du C.N.R.S., rue René Descartes, 67 000 Strasbourg, France*

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1. Introduction

The comparison of the sequences of all the tRNAs from different organisms that are recognised by the same aminoacyl-tRNA ligase, constitutes one possible approach to investigate the molecular basis of the tRNA: aminoacyl-tRNA ligase recognition process. It can also help determine the relationship between evolution and primary structure of these molecules. In this work we have determined the primary structure of $tRNA^{Phe}$ from a thermophilic microorganism (*Bacillus stearothermophilus*, strain NCA 1518). This tRNA is fully charged by yeast and *E. coli* phenylalanine-tRNA ligases [1]. It was therefore of great interest to check whether or not it contained the composite nucleotide sequence involved in the aminoacylation process of these enzymes as suggested by Dudock et al. [2] and Kern et al. [3].

2. Materials and methods

The bulk [^{32}P] tRNA from *Bacillus stearothermophilus* strain NCA 1518 grown in minimal medium containing [^{32}P] orthophosphate was isolated by phenol treatment and DEAE-cellulose column chromatography eluted with NaCl M. In order to obtain highly purified [^{32}P] $tRNA^{Phe}$, this bulk [^{32}P] tRNA was submitted to three further column chromatographic purification steps [4]. (i) tRNA was first enriched by chromatography on a BD cellulose column. Due to the presence of a Y-like base, $tRNA^{Phe}_{B.stearo.}$ strongly bound to the exchanger and could only be eluted in the hydrophobic fraction using 1 M NaCl and 20% ethanol. (ii) In the second step we used the affinity property of tRNAs with complementary anticodons to stick together, as described by Eisinger et al. [5] and Grosjean et al. [6]. This fractionation was performed on Biogel P200 Hy-$tRNA_2^{Glu}$ **(*E. coli*) (whose anticodon is complementary to that of $tRNA^{Phe}$ and which was commercially available [6]. (iii) Finally pure [^{32}P] $tRNA^{Phe}$ could be obtained by chromatography on a RPC 2 column. Details concerning these purification procedures will be published elsewhere [4].

The structural investigations for the determination of [^{32}P] tRNA primary sequence and the conditions for complete and partial hydrolyses with either T_1 or pancreatic RNases and chemical recurrent stepwise degradation were as previously described [7–9].

3. Results

Fig.1 shows the oligonucleotide overlaps obtained with both total and partial enzymatic hydrolyses and the derivatisation of the primary structure.

Fig.2 shows this nucleotide sequence drawn as a cloverleaf. Similarities between this $tRNA^{Phe}$ and the corresponding $tRNAs^{Phe}$ from prokaryotes (*E. coli* and mycoplasma) [10,11] and eukaryotes (rabbit, calf, wheat germ and yeast) [12–14] are depicted in fig.2A, 2B and 2C.

$tRNA^{Phe}_{B.stearo}$ contains 76 nucleotides including 7 minor ones whereas $tRNA^{Phe}$ from mycoplasma [11], *E. coli* [10], wheat germ [13], yeast (*Saccharomyces cerevisiae*) [14] and mammals [12] contain respectively 5/10/14/14 and 17 minor nucleo-

** *E. coli* $tRNA_2^{Glu}$ chemically coupled by its periodate oxidized 3' end to hydrazine-treated Bio-Gel P-200.

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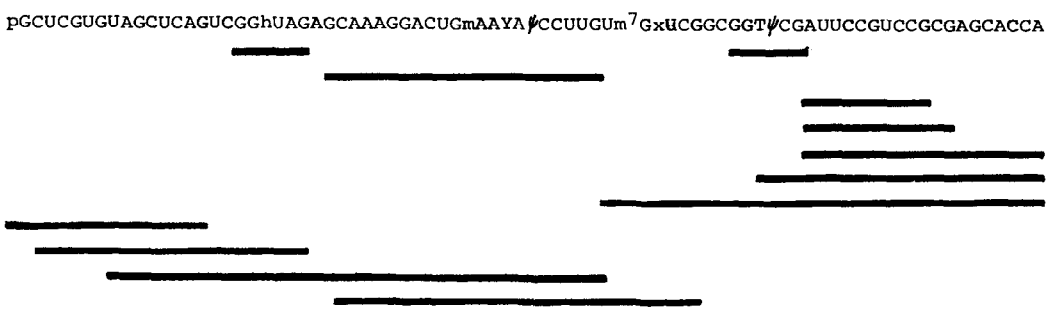

Fig.1. Primary structure of *B.stearothermophilus* $tRNA^{Phe}$. Summary of overlapping fragments from exhaustive T_1 and pancreatic RNase digestions and partial T_1 digestion.

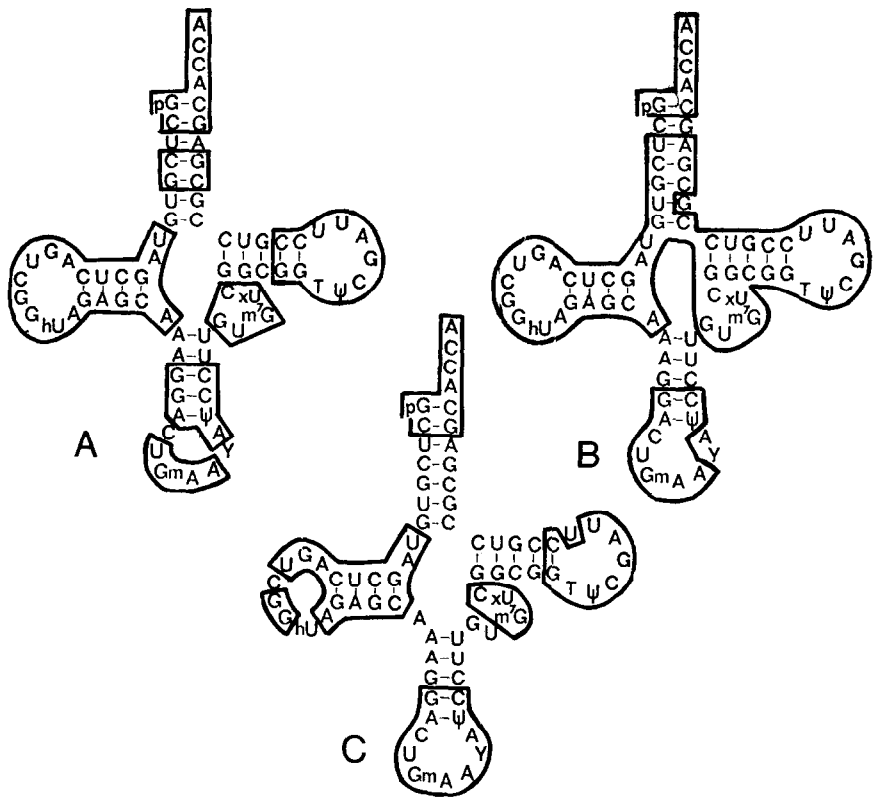

Fig.2. Comparison of the cloverleaf model of $tRNA^{Phe}_{B.stearo.}$ to other $tRNAs^{Phe}$ of known structure. In the boxes; (A) Common sequences with *E. coli* $tRNA^{Phe}$ [11] (B) Common sequences with *Mycoplasma* $tRNA^{Phe}$ [10] (C) Common sequences with eukaryotic $tRNAs^{Phe}$ [12–14] Nucleotide modifications are disregarded in these comparisons.

tides. It is interesting to point out that its extent of modification permits to situate *Bacillus stearothermophilus* $tRNA^{Phe}$ between $tRNA^{Phe}$ from *Mycoplasma sp* (Kid), a primitive prokaryotic organism, and that of *E. coli*, whereas eukaryotic $tRNAs^{Phe}$ are much more modified. The G–C pairs, (14 for 21 pairs) are not above the average yield found in non-thermophilic organisms. It must also be emphasized that *Bacillus stearothermophilus* $tRNA^{Phe}$ looks more like *Mycoplasma* $tRNA^{Phe}$ than *E. coli* $tRNA^{Phe}$.

In contrast to other known prokaryotic $tRNAs^{Phe}$, *Bacillus stearothermophilus* $tRNA^{Phe}$ has a Y-like base and an *O'*-methylated G(Gm) in the anticodon loop. Worth mentioning is that both Y base and Gm have been found, so far, only in eukaryotic $tRNAs^{Phe}$. This Y base of *B. stearothermophilus* ($Y_{B.s.}$) is easily excised by mild acidic treatment (phosphate buffer at pH 2.9) as described by Blobstein et al. [15], but it differs from Y and peroxy-Y in its chromatographic properties: it migrates much faster than Y and peroxy-Y on silica gel t.l.c. developed with the upper phase of the solvent: ethylacetate/1-propanol/water (4:1:2). Fluorescence spectras of both the free $Y_{B.s.}$ base and $tRNA^{Phe}_{B.stearo.}$ and studies on the influence of the presence or absence of the Y base on biological properties of tRNAs will be published elsewhere (19). Further experiments using synthetic or natural Y from *Torula* yeast $tRNA^{Phe}$ ($Y_{T.}$) in order to check whether $Y_{B.s.}$ is identical to $Y_{T.}$ or not, are under way.

Modification of the U in the extra loop, called xU, is not yet sure and could be an artefact. At first we thought that this U was modified because analyses of U-m^7G-xU-C-G have a spot migrating as pUp in our analytical system. Surprisingly only the first analyses gave us this spot and finally the composition of that oligonucleotide was found to be 2 U/1 C/1 m^7G and 1 G. Indeed the xUp surely corresponds to pUp and not to a modified U. It could arise from partial degradation of m^7G by action of triethylammonium carbonate, pH 10, followed by the excision of the modified derivative, finally giving pUp upon alkaline or enzymic hydrolysis.

$tRNA^{Phe}$ from *Bacillus stearothermophilus* has the fourth base from the 3'C-C-A end and the hU stem identical with those of all known $tRNAs^{Phe}$. These bases may be involved in the aminoacylation process of yeast phenylalanine-tRNA ligase as suggested by Dudock et al. [2] and Kern et al. [3]. This composite sequence may explain the acylation of $tRNA^{Phe}_{B.stearo.}$ obtained with yeast phenylalanine-tRNA ligase. The same composite sequence has been found, so far, in nearly 25% of all sequenced tRNAs. Some of these tRNAs are misacylated under normal conditions or in the presence of organic solvents by phenylalanine tRNA ligases [1]. This structural property may have been maintained during evolution as an important site for the aminoacylation [16]. These observations finally lead us to suggest, like several other authors [17,18], that all these tRNAs might derive from a common ancestral gene as will be proposed elsewhere [19].

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