POST-TRANSCRIPTIONAL MODIFICATION OF TYROSINE tRNA AS A FUNCTION OF GROWTH IN *BACILLUS SUBTILIS*

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1. Introduction

Several authors have described modifications of the chromatographic patterns of isoaccepting tRNA species of *B. subtilis* during growth and sporulation [1–6]. In particular an important change in the ratio of the two major species of $tRNA^{Tyr}$ was reported, associated with stages of development, species I being predominant in vegetative cells and species II in stationary phase cells and spores. A reversal in their relative amounts has been shown to occur soon after the germination of spores and during the transition from the exponential to the stationary phase of growth [6–8].

Such changes could result from differential transcription of tRNA genes, post-transcriptional modification of the same species or conformational changes. In an attempt to define the nature of the difference(s) between the two $tRNA^{Tyr}$ species, we have purified them and as a first step total enzymatic hydrolysis was performed and the resulting nucleosides were analysed. From this study, we can conclude that the difference between the two species involves a modification of an A residue. We also give the composition of rare nucleosides in *B. subtilis* $tRNA^{Tyr}$.

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2. Materials and methods

B. subtilis strain W168 was grown in a 2X nutrient growth medium [9]. The major species, $tRNA^{Tyr}_{I}$ and $tRNA^{Tyr}_{II}$ were isolated from cells harvested respectively in exponential growth phase and in early stationary phase. $tRNA^{Tyr}$ species were purified by three chromatographic runs on benzoylated DEAE cellulose [10]. Further purification was achieved by chromatography on RPC 5 column [11]. The tyrosine acceptance activity of purified $tRNA^{Tyr}_{I}$ and $tRNA^{Tyr}_{II}$ was respectively 1700 and 1830 pmol per A_{260} unit. The acceptance activity for contaminant tRNAs expressed as a percentage of that for tyrosine was 5.6 for $tRNA^{Tyr}_{I}$ and 0.6 for $tRNA^{Tyr}_{II}$.

In order to determine their nucleoside composition, both $tRNA^{Tyr}_{I}$ and $tRNA^{Tyr}_{II}$ have been analysed according to Rogg et al. [12]. Approximately 8 A_{260} units of each tRNA were hydrolysed overnight at 37°C in 0.2 ml 0.05.M Tris–HCl pH 7.5, 5.10^{-4} M $MgCl_2$ containing 50 μg of pancreatic RNase (Worthington RAF), 50 μg of alkaline phosphatase (Worthington BAPF) and 60 μg of snake venom phosphodiesterase (Worthington VPH). Subsequent treatment with 20 units of RNase T_1 during 2 h at 37°C was performed in order to open and further digest oligonucleotides terminated with guanosine 2′-3′ cyclic phosphate which could have been formed by traces of guanyloribonucleases present in the

digestion mixture. The digest was finally analysed on cellulose t.l.c. plates (aluminium foil 20 X 50 cm - Merk 5563/0001) using the solvents A: *n*-butanol, isobutyric acid, NH_4OH 25%, H_2O 75/37,5/2,5/25 v/v/v (24 h, descending) and B: $(NH_4)_2SO_4$ saturated water, isopropanol, 0.1 M sodium acetate pH 6, 79/2/19 v/v/v (4 h, ascending). Most nucleosides were separated under these conditions. Each spot was carefully scraped from the t.l.c. and the nucleoside eluted in 0.5 ml water. Spectra at pH 1 and pH 13 were drawn in order to determine their relative quantities.

Study of u.v. spectrum of $tRNA^{Tyr}$ between 300 and 400 nm was used for search of s^4U. As reported by Lippsett et al. [13] tRNAs which contain s^4U have u.v. absorption maximum at 336 nm (ϵM at that wavelength is 15 000). Estimation of dihydrouridine was done by measuring the time dependant absorption loss at $A_{230\ nm}$ in 0.1 M KOH of $tRNA^{Tyr}$ (a decrease of 8220 $A_{230\ nm}$ corresponds to a molar solution of hU) [14].

3. Results

The change in the relative amounts between the two major species (peaks I and II) of $tRNA^{Tyr}$ shown in fig.1 is identical to that already reported [5–7]. The two first peaks have important shoulders. Vold [5] reported a similar phenomenon for peak II. The presence of similar shoulders has previously been described by Keller et al. [16] who have shown that they corresponded to tRNA dimers.

The $tRNAs^{Tyr}$ from peaks I and II have been analysed as described under Materials and methods whereas peak III $tRNA^{Tyr}$ could not be purified in sufficient amounts and was not analysed.

As shown in figs.2 and 3, the two major isoacceptor $tRNAs^{Tyr}$ species differ only by one nucleoside. In fact the u.v. visualisation on t.l.c. of $tRNA^{Tyr}_{II}$ digest showed a light blue fluorescent fast moving spot at the place where the $tRNA^{Tyr}_{I}$ digest showed a dark blue spot. The latter one corresponds to 6-(Δ^2-isopentenyl) adenosine (i^6A) as shown by its spectra at pH 13 (fig.3). Furthermore i^6A from enriched yeast $tRNA^{Ser}$ fraction digest migrates at the same place (not shown here), whereas the corresponding spot in $tRNA^{Tyr}_{II}$ corresponds to 2-methylthio-6-(Δ^2-isopentenyl)

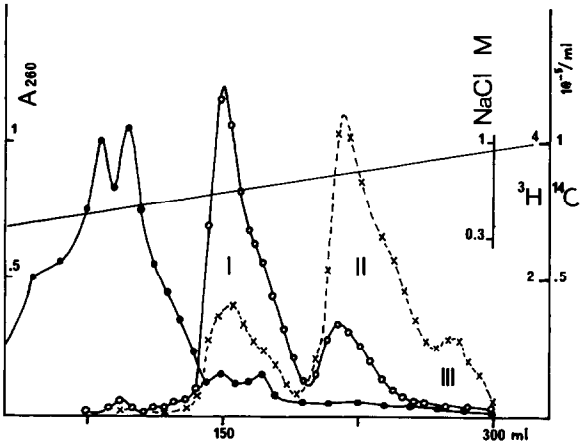

Fig.1. Cochromatography of *Bacillus subtilis* tRNA from vegetative and early stationary phase cells on RCP 5 columns [11]. Open circles: [^{3}H]tyrosyl $tRNA^{Tyr}$ from vegetative cells (20 A_{260} units). Crosses: [^{14}C]tyrosyl $tRNA^{Tyr}$ from stationary cells (57 A_{260} units). Closed circles: absorption at 260 nm. Aminoacylation of $tRNA^{Tyr}$ was carried out during 30 min at 37°C. One ml reaction mixture contained: Tris–HCl (pH 7.2) 100 μmol; $MgCl_2$ 15 μmol; ATP 5 μmol; [^{14}C]L-tyrosine (S.A.: 180 mCi/mmol) 0.05 μmol or [^{3}H]L-tyrosine (S.A.: 43 Ci/mmol) 0.01 μmol; unlabelled L-tyrosine 0.04 μmol and 0.05 μmol of all other unlabelled L-amino acids; 30–40 A_{260} units of tRNA; crude aminoacyl tRNA ligase corresponding to 180 μg of protein, extracted from exponential phase cells according to the method of Yamane and Sueoka [15].

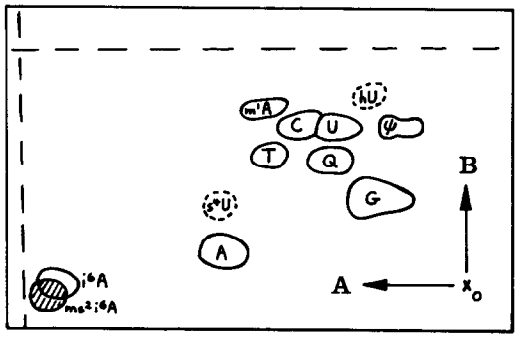

Fig.2. Chromatography on thin layer plates of the nucleosides from *B. subtilis* $tRNAs^{Tyr}$. Hydrolyses, solvents and analysis conditions are as described under Materials and methods. All spots except i^6A and ms^2i^6A were found at equivalent levels in both $tRNAs^{Tyr}$. The nucleosides s^4U and hU whose positions are drawn with dotted lines [12] have not been analysed here like the other ones. Their presence and relative quantities have been determined directly as mentioned under Materials and methods. Both $tRNAs^{Tyr}$ contain equivalent amounts of: G = 23–25, A = 14–16, C = 21–23, U = 12–14, ψ = 2, hU = 1, m^1A = 1, T = 1, s^4U = 1, Q = 1, whereas one residue of i^6A is present in $tRNA^{Tyr}_{I}$ only and one residue of ms^2i^6A in $tRNA^{Tyr}_{II}$ only.

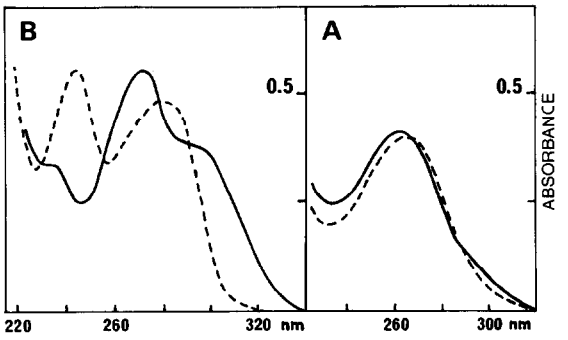

Fig.3. Spectra of i^6A and ms^2i^6A at pH 1 (full lines) and pH 13 (dotted lines). A: i^6A found in $tRNA^{Tyr}_{I}$ - B: ms^2i^6A found in $tRNA^{Tyr}_{II}$.

adenosine (ms^2i^6A) [16]. Fig.3 shows the spectra of i^6A and ms^2i^6A as determined after elution of the t.l.c. spots. No other difference between the two $tRNAs^{Tyr}$ could be detected with this method.

Both $tRNAs^{Tyr}$ from *Bacillus subtilis* contain the base Q, whose spectra (not shown here) are identical to those described by Harada and Nishimura [18]. They also contain one ribothymine, one 1-methyladenosine, two pseudouridine, probably one dihydrouridine as determined by the decrease in the u.v. absorption at 230 nm in 0.1 M KOH (0.025 A_{230} for 2 A_{260} solution) and one s^4U as shown by the u.v. spectrum between 300 and 400 nm (fig.4).

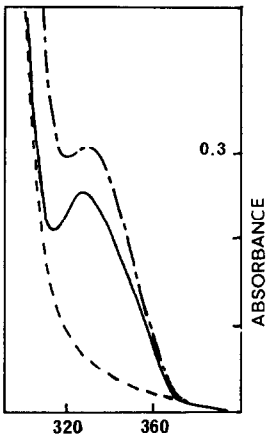

Fig.4. Determination of s^4U. Absorption spectra performed in distilled water. Concentration of 15 A_{260} per ml. *B. subtilis* $tRNAs^{Tyr}$ (both give identical spectras): (–.–.–) *E. coli* total tRNA (approx. one s^4U per molecule tRNA): (——). Yeast total tRNA (contains no s^4U): (– – – – – –).

4. Discussion

These results indicate that $tRNA^{Tyr}_{I}$ and $tRNA^{Tyr}_{II}$ species from *B. subtilis* only differ in the extent of modification of an adenosine, $tRNA^{Tyr}_{I}$ having the partially modified 6-(Δ^2-isopentenyl)adenosine and $tRNA^{Tyr}_{II}$ the fully modified 2-methylthio-6-(Δ^2-isopentenyl)adenosine. The latter could correspond to the unidentified methylated nucleotide which was found by Kersten [19] to be characteristic for tRNA populations from *B. subtilis* stationary phase cells. However for conclusive evidence, more information should be obtained from primary sequence studies in order to demonstrate that this is the only difference between the two species; this aspect is being currently investigated. Similar results concerning the modification of the adenosine adjacent to the 3′ of the anticodon have also been found in $tRNA^{Tyr}$ from *E. coli* infected with the defective transducing bacteriophage $\phi 80\ dsu^+_{III}$. In that case three forms of suppressor tyrosine tRNAs have been found, differing only by the degree of modification of that adenosine. Either A, or i^6A or ms^2i^6A where found in that position [20]. Therefore we suggest that i^6A in *B. subtilis* $tRNA^{Tyr}_{I}$ and ms^2i^6A in *B. subtilis* $tRNA^{Tyr}_{II}$ could occupy the same position adjacent to the anticodon as is the case in *E. coli* $tRNA^{Tyr}$.

The modification of $tRNA^{Tyr}$ which occurs when cells enter the sporulation period could play a role in regulating translation during this stage. This possibility led us to further investigations, currently in progress, such as functional comparisons of the two $tRNA^{Tyr}$ species, study of the occurrence of the modification in parallel with metabolic events and levels of modifying enzyme as a function of the growth stage.

Acknowledgements

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