TURNOVER OF THE 5′-END PHOSPHATE IN YEAST tRNA IN VIVO

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1. Introduction

It is generally accepted that 3′-terminal adenosine of the tRNA molecule slowly turns over in vivo [1]. The results of our experiments indicate that the 5′-terminal phosphate of tRNA shows similar metabolic turnover in the stationary yeast cells.

2. Materials and methods

2.1. *Yeast culture growth and labelling*

Yeast cells (*Saccharomyces cerevisiae*, strain I 22, obtained from the Institute of the Fermentation Industry, Warsaw) were grown at 37°C in aerated synthetic medium [2], containing 300 mg KH_2PO_4/l. Orthophosphate was exhausted from medium at a cell concentration of 2.7 g dry mass/l and a further growth occured at the zero concentration of orthophosphate. The culture reached the stationary phase at a cell concentration of 5.5 g dry mass/l. After 1–2 h (17–22 h after the culture inoculation) 5 mCi $KH_2{}^{32}PO_4$ (spec. act. 5 mCi/mg P, Isotope Production Center, Warsaw) were added and the culture was further incubated for 1 h. The culture (2.0 liter) was poured into 4 liter cold unlabelled culture of the same age and the total volume was immediately cooled to 0–2°C with dry ice/acetone mixture. The cells were collected by low speed centrifugation, washed with cold water and used for tRNA preparation.

2.2. *tRNA isolation and purification*

tRNA was isolated as in [3]. Of cell dry mass 25 g used for the preparation yielded about 100 mg tRNA. In 3 experiments tRNA was further separated from 5 S rRNA by Sephadex G-100 filtration as in [4]. The fractions corresponding to 4 S RNA were concentrated, precipitated with ethanol, solubilised and dialysed against water.

2.3. *Analysis of the tRNA hydrolysates*

tRNA was hydrolysed with 0.3 M KOH at 37°C for 24 h. The neutralised digest was then loaded on Dowex 1 × 2 (formate) column and eluted as in [5]. Mononucleotides: Cp, Ap, Up and nucleoside diphosphate (pGp) were further purified using one of the following procedures:

A. Each nucleotide was purified by charcoal (Barneby-Cheney, no. 1654, USA) adsorption/elution technique as in [6] and subjected to paper chromatography on Whatman no. 1 in solvent I (iso-propanol–water–ammonia–trichloroacetic acid [7].

B. The peak fractions obtained from Dowex were adsorbed on 1.8 × 3 cm columns as in section 2.3. The columns were then washed with 100 ml 0.25 M NaCl in 1 M HCl and finally with water [8]. The adsorbed nucleotides were eluted with ethanol–ammonia–water mixture [6]. The material after evaporation was subjected to paper chromatography in solvent I. pGp was further purified by two-dimensional chromatography in solvent I and II (ammonium sulphate–ammonium acetate–iso-propranol) [9]. The purified nucleotides were identified by ultraviolet spectra.

2.4. *Hydrolysis of pGp*

pGp (final product of purification) was hydrolysed with 1 M $HClO_4$ at 100°C for 30 min [10]. The in-

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organic phosphate was extracted with *n*-butanol as phosphomolybdate complex, whilst the esterified phosphate remained in the water phase. Alternatively pGp was treated with rye-grass 3′-nucleotidase, type III (Sigma, USA) as in [9]. The product (pG) was separated by paper chromatography in solvent II.

2.5. *Radioactivity and phosphate assays*

Nucleotide spots were cut out and counted on Tesla, model NZQ 611 (Czechoslovakia) with thin window Geiger-Müller counter. The solutions after acid hydrolysis (butanol phase and water phase) were applied to the filter paper disc (Whatman no. 1, 2 cm diam.) and counted. The phosphate estimations were performed after the digestion of the spots or discs with H_2SO_4 and $HClO_4$ as in [11].

Only the relative specific activities of the nucleotides between different experiments can be compared.

3. Results and discussion

Among the nucleoside 3′(2′)-phosphates isolated after the hydrolysis of tRNA only Cp exhibits a high specific activity (table 1). During the alkaline hydrolysis any nucleoside retains at its 3′(2′)-position a phosphate group originating from 5′-position of its neighbouring nucleoside. Therefore the high specific activity of Cp is due to an independent turnover of adenosine at amino acid accepting end of tRNA [12] or its post-transcriptional addition [13]. As shown in table 1, pGp from the 5′-end of tRNA exhibits higher specific activity than the 3 nucleotides obtained from internal positions of the polynucleotide chains. The higher specific activity of pGp was observed when both the procedures of its purification (see sections 2.3.A and 2.3.B) were used. The elution of the inorganic pyrophosphate, oligophosphate (sodium metaphosphate, $n\sim10$. International Enzymes Ltd) and polyphosphate (potassium metaphosphate, $n\sim1500$, Hopkin and Williams Ltd, Essex) from charcoal column by 0.25 M NaCl in 1 M HCl was checked by paper chromatography in solvent I. Traces (if any) of the inorganic orthophosphate can be removed by paper chromatography in each of the two solvents used. Therefore, it seems improbable that the high specific activity can be ascribed to the radiochemical impurities. Relatively high specific activity was observed regardless of the separation of 5 S rRNA from tRNA. It is worth mentioning that an independent turnover of the 5′-phosphate end of 5 S rRNA in rabbit reticulocytes was not shown in [14].

To distinguish between a high labelling of the phosphates at 3′(2′)- and 5′-position of pGp in 3 experiments the fraction was subjected to $HClO_4$ hydrolysis in the conditions which favours a splitting of 3′(2′)-phosphate [10]. About 45% total pGp phosphate was converted into inorganic form, while 87% and 6% 3′- and 5′-phosphate, respectively, were released. The fractions derived from acid hydrolysis are probably cross-contaminated by phosphates from 5′- and 3′(2′)-positions, respectively. Nevertheless a higher specific acitvity of unhydrolysed phosphate was observed (table 2). A similar result was obtained by treatment of pGp with 3′-nucleotidase. pG of high specific activity was obtained from the hydrolysate (table 2, expt. V). The both lines of experiments

Table 1
Specific activity of nucleotides after alkaline hydrolysis

Purification procedure	Expt. no.	Ap	Cp	Gp	Up	pGp
A	I	660	3450	475	770	4170
A	II	250	900	175	235	720
A	III	280	6150	230	650	14 500
B	IV	490	2440	305	390	1100
B	V	667	3004	239	347	1147

The specific activities of nucleotides are expressed in cpm/μmol nucleoside phosphate. tRNA was isolated as in [3] (expt. I, II) and subjected to further purification by Sephadex G-100 filtration (expt. III–V). The nucleotides were purified according to procedure A or B (see section 2) as indicated in the table

Table 2
Specific activity of the products of the limited hydrolysis of pGp

Expt. no.	pGp	3′-P[a]	5′-P[b]
II	720	245	1200
III	14 500	8000	21 000
IV	1100	295	1660
V	1147	–	2530

[a] 3′-P, inorganic phosphate after hydrolysis with $HClO_4$, predominantly from 3′(2′)-position of pGp
[b] 5′-P, organic phosphate after treatment with $HClO_4$, predominantly from 5′-position of pGp or pG after hydrolysis with 3′-nucleotidase (see section 2 for details and table 1 for the description of the pGp purification)

support our explanation that an independent exchange of the 5′-phosphate of tRNA does occur. The high specific specific activity of pGp was detected after exposure of the phosphate starved yeast cells to [^{32}P]orthophosphate in the stationary phase. The biological meaning of the phenomenon remains unclear. We guess that the exchange of the phosphate residue occurs when the precursors of tRNA are processed. Alternatively splitting and adding of the end phosphate may occur on the mature polynucleotide molecules and the phenomenon has some meaning for regulation. The possibility exists that the whole pG-end residue is actually exchanged although the event should be certainly a fairly complex process.

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