ORIGIN OF THE NUCLEOSIDE Y IN YEAST $tRNA^{Phe}$

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1. Introduction

A modified A has been found adjacent to the 3'-end of the anticodon in most of the tRNA's and in a few tRNA's an unmodified A or a modified G occupies this position. In yeast $tRNA^{Phe}$ the modified nucleoside Y was found next to the 3'-end of the anticodon [1]. Y has the structure of a hypermodified nucleoside [2, 3]. Its biosynthesis has not yet been elucidated, and it was even uncertain from which normal nucleoside Y is biosynthetically derived.

In this communication we report studies on the question if Y is a modified A or a modified G. Yeast strains auxotroph for purine bases were grown on radioactive adenine or radioactive guanine, the tRNA's from these yeasts were prepared and the base Y^+ was isolated. The specific radioactivity of Y^+ was compared with the specific radioactivity of A and G isolated from the same tRNA's. Y^+ had the same specific radioactivity as G, and this was clearly different from the specific radioactivity of A. From these results we may conclude that the biosynthesis of Y proceeds by the modification of a G residue.

2. Materials and methods

[8-^{3}H]adenine and [8-^{3}H]guanine were from Amersham Buchler. Haploid yeast strains were used, in experiment 1 the red adenine requirer D 286–2A (a ade 1 his 1) with a genetic block some steps before IMP, and in experiment 2 strain gua2 (source: W.T.E. Gardner, University of Sheffield) with a block just before GMP. The growth medium contained 1 litre distilled water: 6.7 g yeast nitrogen base without amino acids from Difco and 20 g Glucose. Growth was performed aerobically at 30°C in a volume of 10 litre. Strain D 286–2A was grown in the presence of 40 mg histidine, 10 mg labelled adenine (0.25 mCi), and to minimize the incorporation of label into guanine 10 mg each of xanthine and gunaine per litre. Strain gua2 was grown in the presence of 10 mg labelled guanine (0.1 mCi). tRNA's were isolated similarly to refs. [4, 5]. The base Y^+ was isolated as described in [6]. Paper chromatography was carried out in the upper phase of the system: ethyl acetate, 1-propanol, water, 4:1:2 (by volume).

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3. Results and discussion

After the base Y^+ had been isolated by treatment with acid and extraction with chloroform [6] tRNA was split with T2 RNAase and the products were separated first by paper electrophoresis at pH 3.5. The material from the spots containing Ap, Gp, Up, and Cp was then treated with alkaline phosphatase and rechromatographed on paper. Y^+ was also purified by chromatography in the same system.

The purity of the obtained products was controlled by their ultraviolet spectra. The treatment and the storage of the material during the whole experiment were essentially the same for all the products. So we may assume that the slow ^{3}H-exchange at C–8 of the purine bases had been equal in G, A, and Y^+. The results of two experiments with different yeast strains and reversed label are presented in table 1. The values

Table 1
Specific radioactivity (cpm/μmole) of Y^+ and the common purine nucleosides.

Experiment	Y^+	G	A
1	188 000	183 000	570 000
2	760 000	739 000	1 000

In experiment 1 strain D 286–2A was grown in the presence of [^{3}H] adenine and non-radioactive guanine and xanthine. In experiment 2 strain gua2 was grown in the presence of [^{3}H] guanine.

for Y^+ were calculated with an ϵ_{237nm} of 3×10^4 $M^{-1} \cdot cm^{-1}$ [6]. In both experiments no significant radioactivity was found in U and C.

The very good coincidence between the specific radioactivity of Y^+ and G, in contrast to that of A makes it clear that Y is biosynthetically derived from G.

The majority of the results published here were already presented at the EMBO workshop on tRNA Structure and Function in Gothenburg (June, 1973). At the same time, similar results were reported by Grunberger et al. [7].

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