

# BIOSYNTHESIS OF THE NUCLEOSIDE Y IN YEAST tRNA<sup>Phe</sup>: INCORPORATION OF THE 3-AMINO-3-CARBOXYPROPYL-GROUP FROM METHIONINE

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## 1. Introduction

The nucleoside Y in yeast tRNA<sup>Phe</sup> [1,2] and in the tRNA<sup>Phe</sup> of several other organisms [3–6] has one of the most complex structures of all modified nucleosides known. The biosynthesis of this nucleoside is not evident from its structure; we only know that it is derived from guanosine [7,8].

Nishimura et al. [9] found that the 3-amino-3-carboxypropyl-group can be transferred from *S*-adenosylmethionine onto uridine to form the odd nucleoside X in *E. coli* tRNA. The hypothesis for the present study was a similar pathway in the synthesis of Y. A part of the side chain, as represented by the large characters in fig. 1, might be derived from methionine.

A yeast strain auxotroph for methionine was grown on radioactive methionine with the marker in the carboxyl group. This radioactivity was shown to be incorporated into the nucleoside Y, without isotopic dilution. Practically no radioactivity was found in the

guanosine residues of the tRNA. We concluded that the 3-amino-3-carboxypropyl-group is transferred from methionine or a closely related product, such as *S*-adenosylmethionine, into Y.

## 2. Materials and methods

1-[<sup>14</sup>C]L-methionine was from Amersham Buchler. A yeast strain (D 6,  $\alpha$ ) auxotroph for methionine and uracil was used. The growth medium contained per 1 liter bidistilled water: 6.7 g yeast nitrogen base without amino acids from Difco, 20 g glucose, 1 g potassium dihydrogenphosphate, 20 mg uracil, 150 mg D,L-methionine and 50  $\mu$ Ci 1-[<sup>14</sup>C]L-methionine. Growth was performed aerobically at 28°C in a vol of 5 liter for 2 days. tRNA's were extracted with a mixture containing 2% triisopropyl-naphthalene sulfonate (TNS), 1% sodium chloride and 50 mM Tris pH = 7.6. 5 ml per g yeast were used. After 3–4 hr stirring at room temperature an equal vol of water-saturated phenol was added and stirring was continued for 2 days. After precipitation with ethanol the crude tRNA was subjected to DEAE cellulose column chromatography and purified with a gradient of 0.1–1 M sodium chloride and 0.05 M sodium acetate pH = 6. tRNA<sup>Phe</sup> was separated from the bulk of tRNAs by chromatography on a BD cellulose column according to [10,12]. The base Y<sup>+</sup> was isolated as described [2,7]. Paper chromatography was carried out either in system A: 1-butanol, acetic, water 4:1:2 (by vol) or in system B: ethylacetate, 1-propanol, water 4:1:2 (the upper phase).

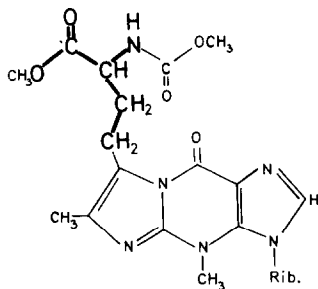


Fig. 1. Structure of the nucleoside Y in *S. cerevisiae*.

### 3. Results and discussion

The base  $Y^+$  was purified and identified by paper chromatography in system A. The fluorescent spot was eluted from the paper and further characterized by the UV absorption [10] and the fluorescence spectra [4,11]. The spectra were corrected against the paper blank.  $Y^+$  was then rechromatographed on paper and the radioactivity was measured by counting paper strips. The radioactivity coincided exactly with the fluorescent spot. Quantitative data are given in table 1. As a control, guanosine was isolated from the same tRNA by splitting with T2 RNAase, paper electrophoresis, treatment with alkaline phosphatase and chromatography in system B. The specific radioactivity of guanosine is also represented in table 1. The results show that the radioactivity from the carboxyl group of methionine was incorporated in the nucleoside Y without isotopic dilution. Nearly no radioactivity was found in the guanosine from which Y is derived. This indicates that methionine or one of its direct metabolites such as S-adenosyl-methionine provides in one step four carbon and one nitrogen atoms for the side chain of Y.

This result indicates that the biosynthesis of Y does not proceed via one relatively large molecule as donator for the imidazo-ring as well as the large part of the side chain, as proposed by Nakanishi et al. [1]. The theory of a stepwise condensation of smaller molecules with G is also in accordance with the structure of Y in *T. utilis* [5], where the ring system is normal, but the side chain is missing. A very simple

model for the biosynthesis of the imidazo guanosine ring system could be the condensation of pyruvate with guanosine of N-3-methyl-guanosine.

The results presented here have to be completed by in vitro studies. We are now trying to develop a system for this kind of investigation.

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Table 1  
Radioactivity of input methionine and isolated  $Y^+$   
and guanosine

	(mCi/mol)
L-Met (calculated)	100
$Y^+$ (found)	140
G (found)	0.17