THE STRUCTURAL GENE FOR RIBOFLAVIN SYNTHETASE IN *SACCHAROMYCES CEREVISIAE*

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1. Introduction

The properties of riboflavin synthetase from yeast have been studied by Plaut and his co-workers [1–3]. Enzymatic investigations concerning the earlier steps of the riboflavin biosynthetic chain have not met with success so far.

Genetic experiments with riboflavin-deficient mutants of *Saccharomyces cerevisiae* have demonstrated the involvement of 6 genes in the biosynthesis of the vitamin (rib_1, rib_2, rib_3, rib_4, rib_5, and rib_7) [4–6]. Accumulation studies performed with these mutants have yielded several compounds which are involved in the biosynthetic process [7–9].

Accumulation of 6,7-dimethyl-8-ribityllumazine by rib_5 mutants was taken as evidence suggesting that the gene rib_5 codes for riboflavin synthetase [4, 7]. This hypothesis is confirmed by direct enzyme studies which are described in this paper.

2. Materials and methods

6,7-Dimethyl-8-ribityllumazine was synthesized [10].

The riboflavin-deficient mutants of *S. cerevisiae* have been described [4, 11]. The diploid strains D 4 and D 5 were constructed by the mating of strains HK 873-4A × HK 950-30A and HK 873-4A × HK 878, respectively.

The culture medium contained (per l) yeast extract (Oxoid), 10 g; peptone, Oxoid, 10 g; sucrose, 20 g; riboflavin, 20 mg. Cells were grown in a 10 l laboratory fermentor at 30° without aeration. They were harvested by centrifugation, washed with saline, and stored at −25°.

Cells were autolyzed at 20° according to the procedure of Harvey and Plaut [1]. The resulting suspension was centrifuged at 15,000 *g* for 20 min. The supernatant was dialyzed for 6 hr against a solution containing 0.05 M phosphate buffer pH 7 and 0.01 M Na_2SO_3.

Activity of riboflavin synthetase was determined essentially as described by Plaut et al. [2]. Protein was determined by the procedure of Warburg and Christian [12].

3. Results and discussion

Specific activities of riboflavin synthetase in mutants of *S. cerevisiae* are shown in table 1. Data were collected from a minimum number of 3 independent experiments. The variation of repeat controls did not exceed 15% in any case.

Haploid strains defective in either of the genes rib_1, rib_2, rib_3, rib_4, and rib_7 have levels of riboflavin synthetase which are similar to that of the wild strain. Not even trace amounts of riboflavin synthetase were found in haploid rib_5 mutants. Furthermore, no enzyme activity was found after ammonium sulfate fractionation of cell extract from these mutants. Previous experiments have shown that rib_5 mutants excrete the substrate of riboflavin synthetase, 6,7-dimethyl-8-ribityllumazine, into the culture medium [7]. These data demonstrate that rib_5 is the structural gene coding for riboflavin synthetase. Speculations that yet another gene, rib_6, might be involved in the

Table 1
Specific activity of riboflavin synthetase in mutants of *S. cerevisiae.*

Strain	Genes relevant to this work	Specific activity (nmoles/mg hr)
S 288 C	wild type	4.3
HK 843	rib_{1-2}	2.3
HK 859 (ATCC 22091)	rib_{2-3}	4.1
HK 645	rib_{3-1}	1.9
HK 829	rib_{4-1}	2.6
HK 873-4A	rib_{5-3}	< 0.04
HK 950-30A	rib_{5-7}	< 0.04
HK 878	rib_{5-4}	< 0.04
N 179	rib_{7-2}	5.8
D 4	$\frac{rib_{5-3}}{rib_{5-7}}$	0.3
D 5	$\frac{rib_{5-3}}{rib_{5-4}}$	< 0.04

last step of riboflavin biosynthesis were shown to be incorrect by recent studies of Oltmanns [4, 5].

Enzyme studies were also performed with two diploid strains D 4 and D 5 with the genotypes rib_{5-3}/rib_{5-7} and rib_{5-3}/rib_{5-4}, respectively. These strains do not require riboflavin for growth. Their protrophic character is due to intragenic complementation of the respective rib_5 alleles [4]. Specific activity of riboflavin synthetase in strain D 4 is 0.3 nmoles/mg hr corresponding to some 10% of the wild type level. This confirms that the function of riboflavin synthetase is partially restored by intragenic complementation of the alleles rib_{5-3} and rib_{5-7}. No riboflavin synthetase activity can be detected in the protrophic diploid strain D 5. We suppose that in this case intragenic complementation leads to the formation of an enzyme species which is unstable under the experimental conditions.

The hypothesis that intragenic complementation is due to interaction of enzyme subunits exhibiting different structural defects has found wide acceptance (for review see [13]). On this basis it may be supposed that riboflavin synthetase consists of two or more identical subunits. Direct studies of the subunit structure of the enzyme are required to test this hypothesis.

The genes rib_2, rib_3, rib_4 and rib_7 could be assigned to different steps in the biosynthesis on the basis of accumulation studies [4, 6]. No specific accumulation product was found in rib_1 mutants, however [9]. Oltmanns and Bacher have suggested that this gene codes for the first enzyme of the riboflavin pathway but the other possibility that the gene might exert a regulatory function was not ruled out [6]. The present results demonstrate that the activity of the last enzyme in the pathway, riboflavin synthetase, is not affected by mutation in gene rib_1. This confirms the hypothesis that rib_1 is not a regulatory gene but rather a structural gene coding for the first enzyme of the pathway as suggested by Oltmanns and Bacher.

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