Two Isoaccepting Seryl tRNAs Coded by Separate Mitochondrial Genes in Yeast

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Summary. In S. cerevisiae four isoacceptor mitochondrial tRNAs for serine have been separated by reversed phase chromatography. At least two of these species are products of different genes. In this work the deletion mapping technique has been used to locate two genes for tRNA\textsuperscript{ser}. The gene for tRNA\textsuperscript{ser} previously localized in the oliI region of the mitochondrial genome has been found to code for tRNA\textsuperscript{ser}, and another gene coding for tRNA\textsuperscript{ser} has been detected in the region where most of other tRNA genes are found. Results of fine mapping experiments allowed to localize this gene in the proximity of the gene for tRNA\textsuperscript{arg}.

Introduction

The number of genes for tRNA on mitochondrial DNA (mtDNA) from Saccharomyces cerevisiae has not been ascertained. While tRNA genes for all the twenty aminoacids have now been found (Wesolowski and Fukuhara, 1979), the existence of isoacceptors coded by different genes has been reported in some cases (Martin N. and Rabinowitz, 1978; Macino and Tzagoloff, 1979a; Martin R. et al., 1976) and is indicated by sequence studies of the oligomycin I gene (Macino and Tzagoloff, 1979b).

If isoacceptors are coded by distinct genes having different map positions, this should produce ambiguous results in the deletion mapping performed by the use of unfractionated tRNA. Moreover the proportion of each isoaccepting species can be different as a function of cellular state (Baldacci et al., 1979) and this stresses the necessity of more detailed knowledge about isoacceptor structure and physiology to identify and map new tRNA genes.

Materials and Methods

Preparation of Mitochondrial DNA and tRNA
Preparation of mitochondria was performed by the method reported by Faye et al. (1974).

For the preparation of mitochondrial tRNA and ligase preparations. For these preparations cells were grown in aerated flasks to the late exponential phase on a yeast extract peptone medium containing 2% glucose or galactose.

Chromatography and Annealing Conditions

The reversed phase chromatography (RPC-5) technique was essentially as described by Pearson et al. (1971). For analytical columns

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about 100μg [3H] seryl tRNA were applied to a 0.2 cm² x 50 cm column and developed with a linear gradient from 0.48 to 0.65 M. Approximately 60 fractions of 2 ml each were collected and TCA (trichloroacetic acid) precipitable radioactivity determined. For the preparative separation of isoacceptors about 400 μg [3H] seryl tRNA were loaded onto a 0.4 cm² x 80 cm column. TCA precipitable radioactivity was determined on 0.2 ml aliquots and the fractions corresponding to the peaks were pooled, dialysed against 2 mM ammonium acetate and lyophilized.

Separated aminoacyl tRNAs were dissolved in 50 mM ammonium acetate and used for hybridization experiments.

The hybridization procedure was as previously reported (We-sołowski and Fukuhara, 1979).

Hybridization on mtDNA from the mutants was considered positive when it reached at least 30% of the hybridization obtained with the mtDNA from the w.t.

Results

The isoacceptor pattern of mitochondrial seryl tRNA, as well as that of other aminoacyl tRNAs, exhibits specific variations as a function of growth conditions. Fig. 1 shows the elution profiles from RPC-5 columns of mitochondrial seryl tRNA. Cells (strain DM) grown to late exponential phase on glucose and on galactose were compared. In addition to the main component m2, three minor peaks were reproducibly found; m1 and m3 in galactose-grown cells, and m1 and m4 in glucose-grown cells. The peak m1 characteristically was higher in cells grown on glucose. Similar profiles have been obtained for seryl tRNA from the strain MH41-7B which had been used in most of tRNA mapping studies.

Annealing experiments were routinely performed with seryl-tRNA from cells grown to late exponential phase on 2% glucose and the results of the hybridization of this unfractionated seryl-tRNA to mtDNA from a series of rho- mutants of known deletion structure are reported in Table 1.

Our recent study (Baldacci et al., 1979) has indicated that m1 and m2 represent two distinct RNA sequences as shown by their additive hybridization to mtDNA. These species were isolated from glucose-grown cells in which the m1 is enriched. The pooled fractions of each species, were then annealed to the mtDNA from various rho- mutants of known deletion structure. Results are reported in Table 2.

The later eluting species m3 and m4 are in low amounts and we do not know whether they are different in primary structure.

The mtDNA of O13 mutant hybridized exclusively with tRNA1\textsuperscript{ser} (peak m2) while the DNA from strain CEP2 hybridized only with tRNA2\textsuperscript{ser} (peak m1). This fact suffices to conclude that the two isoacceptors have different nucleotide sequences corresponding to separate genes, since the two mtDNAs do not share...