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CONFORMATIONAL CHANGES AT THE PEPTIDYL TRANSFERASE CENTER OF ANTIBIOTIC RESISTANT MUTANTS OF *Saccharomyces cerevisiae*

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SUMMARY

Ribosomes from *Saccharomyces cerevisiae* wild type (Y166) and trichodermin (TR1) and narciclasine (NAR2b) resistant strains were affinity labeled with p-nitrophenylcarbamyl-(^{3}H)Phe-tRNA. Resistant ribosomes were able to bind covalently two to five times more aminoacyl-tRNA derivative than control. The labeling pattern of the individual ribosomal proteins was also altered in the two mutants. These results evidence a change in the conformation of the peptidyl transferase center that brings the 3' end of the aminoacyl-tRNA closer to several ribosomal proteins.

INTRODUCTION

Mutant cells resistant to antibiotics that inhibit peptidyl transferase activity have been isolated in prokaryotic and eukaryotic species (1). In general, ribosomes of such mutants have been shown to present lower affinities for the antibiotics to which the cells are resistant though cases in which the binding capacity of the particles is not affected by the mutation have also been reported (2). In any case, changes in the structural conformation of the peptidyl transferase center due to mutation in some of the ribosomal components are frequently postulated and in some cases convincingly shown, though the proofs offered for such conformational alterations have been mainly indirect (3).

Reactive derivatives of aminoacyl-tRNAs have been extensively used for affinity labeling of ribosomal components of the peptidyl

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transferase center (4). A comparative study of these components labeled in wild type and mutant ribosomes could supply data confirming the possible conformational changes taking place in the resistant ribosomes.

p-Nitrophenylcarbamyl-Phe-tRNA has previously been used as an analog of peptidyl-tRNA to identify the ribosomal components near the peptidyl transferase in the ribosomes of Escherichia coli (5), rat liver (6) and Saccharomyces cerevisiae (7). In the present report we have used this derivative in an attempt to show conformational changes in ribosomes of two mutants of Saccharomyces cerevisiae resistant to trichodermin and narciclasine (8,9).

EXPERIMENTAL PROCEDURES

All the methods used in this report have been described previously (7). Ribosomes were prepared by sea sand grinding from S.cerevisiae wild type (Y166) and mutants resistant to trichodermin (TR1) and narciclasine (NAR2b) in YEP medium up to middle exponential phase. High salt washed ribosomes (8) were used throughout all the experimental work.

p-Nitrophenylcarbamyl-(^{3}H)Phe-tRNA (PNCP-(^{3}H)Phe-tRNA) was prepared from p-nitrophenylchloroformate (Sigma) and ^{3}H-Phenylalanyl-tRNA (77 Ci/mmol) according to Czernilofsky and Kuechler (5). Binding of PNCP-(^{3}H)Phe-tRNA was carried out in 20 mM magnesium acetate, 60 mM KCL, 10 mM NH_4Cl, 2 mM Phosphate buffer pH 7.3 and 6 mM B-mercaptoethanol, containing 250 uM 80S ribosomes, 45 uM PNCP-(^{3}H)Phe-tRNA and 0.15 mg/ml of poly uridylic acid at 30°C for 15 min. Binding tests were carried out in 50 ul samples by filtration through cellulose nitrate. Large scale treatments for electrophoretic analysis were performed in 6 ml samples.

Covalent binding was performed at 37°C for 1h. After incubation samples were treated with RNAse T1 (20μg/ml) and RNAse A(200 μg/ml) and then precipitated with trichloroacetic acid. In some test 20 mM EDTA was added to the samples before RNAse treatment in order to disassemble the ribosomes.

Extraction of ribosomal proteins with 67% acetic acid and electrophoresis in two dimensional gels was carried out by standard techniques (10).

RESULTS AND DISCUSSION

Ribosomes obtained from S.cerevisiae Y166 (wild type), TR1 (trichodermin resistant (8)) and NAR2b (narciclasine resistant (9)) were tested for poly U-dependent binding of PNCP-(^{3}H)Phe-tRNA at 20 mM Mg^{2+} concentration in the absence of supernatant factors. Aliquots of

TABLE 1. Total and covalent binding of PNCP-(^{3}H)-Phe-tRNA to wild type and antibiotic resistant ribosomes.

	Ribosomes from strain:					
	Y166		TR1		NAR2b	
Binding	pmols	%	pmols	%	pmols	%
Conditions 1						
Total	1.65	100	1.39	100	1.23	100
Covalent	0.06	3.6	0.10	7.2	0.28	22.7
Conditions 2						
Total	1.34	100	1.35	100	1.37	100
Covalent	0.05	3.7	0.105	7.6	0.29	21.2

The data listed under Conditions 1 represent the average of at least seven experiments while those under Conditions 2 (treatment with RNAse in the presence of EDTA) correspond to results of only one experiment.

the sample were also treated in conditions for covalent bond formation at 37°C for 1h. and after treatment with RNAse, precipitated with TCA. The results show (Table 1, Conditions A) that the binding capacity of the resistant ribosomes is slightly affected, being decreased to about 15% and 25% of the wild type particles in the case of Tr1 and NAR2b ribosomes respectively. The fraction of the bound PNCP-Phe-tRNA that became covalently, is however drastically increased in the mutant particles being doubled in TR1 and multiplied by five in NAR2b.

That the RNAse resistant label is actually covalently bound to the particles and not simply protected by the particle from RNAse attack, was shown by pretreating the particles, after incubation for covalent bond formation and prior to the RNAse attack, with EDTA in order to disassemble the ribosome (Table 1, conditions B).

The increase of covalent binding taking place in the mutant ribosomes is a clear indication of higher accesibility of reactive

amino groups of proteins located near the binding ribosomal site of the 3' end of the PNCP-Phe-tRNA molecule. The most straightforward explanation for this higher accesibility is the existance of a conformational change in that region of the resistant ribosomes.

Ribosomal components labeled in the particles. Two dimensional gel electrophoresis of proteins extracted from the treated particles of the three strains allowed us to identify those ribosomal components labeled by PNCP-Phe-tRNA. Fig. 1 summarizes the results of several experiments indicating significant differences in the pattern of protein labeling in the three strains. Except for proteins L34 and L2/3, the most heavily labeled, the labeling pattern is quantitatively and qualitatively different in the three cases. The extent of labeling changes for some proteins while other proteins are labeled in one strain but not in the others.

Since little is known about the structural features of the yeast ribosome, the significance of these labeling alterations with respect to the involvement of the affected proteins in the peptidyl transferase activity is difficult to evaluate at the present moment. However, the results clearly prove that the conformational change, responsible for the resistance to the antibiotics, alters the position of some of the proteins near the peptidyl-tRNA binding site of the peptidyl transferase center.

In general strain NAR2b differs more from the wild type than TR1. This tallies with the unusual charasteristics of this mutant, which, in addition to being cross-resistant to trichodermine, is resistant to the totally unrelated antibiotic anthelmicine (9).

Proteins from the small ribosomal subunit became labeled in the three strains, thus confirming the proximity of peptidyl transferase center to the interface region of the particle. It is interesting that strain NAR2b shows the highest labeling of small subunit proteins, mainly protein S33.

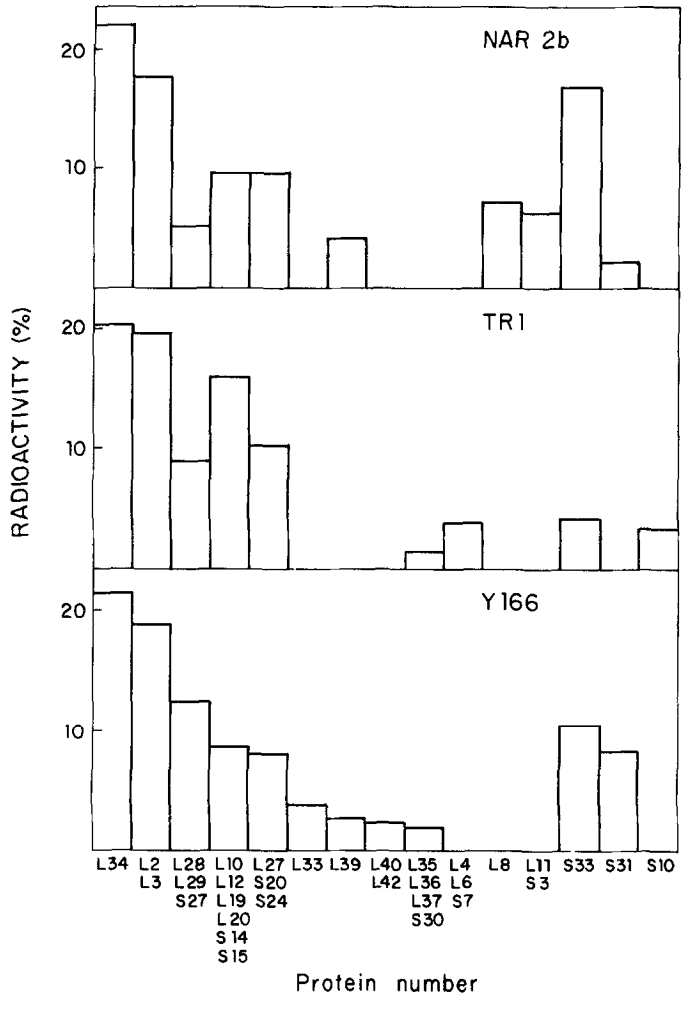

FIGURE 1. Proteins labeled by PNCP-(^{3}H)-Phe-tRNA.-

The results are the average of five and six experiments in the case of wild type and mutant strains respectively. The data have been normalized taking as 100% the sum of the radioactivity in the five most labeled proteins of each experiment.

In a previous report we studied by affinity labeling the proteins at the peptidyl transferase center of the yeast ribosome (7). The results obtained then show differences with those reported in this communication. However, notably different conditions availed in each case. In our previous work there was excess of PNCP-tRNA over ribosomes in the reaction mixtures in order to maximize the extent of labeling while in the work described here, were the possibilities of unspecific

binding had to be minimized, there was excess of ribosomes. In addition, the binding of PNCP-Phe-tRNA was previously carried out in the presence of supernatant fraction which was found to stimulate the binding to the particles. Moreover, the ribosomal subunits were separated after treatment, considerably improving the resolution of the two demensional gel electrophoresis. In the present work, perfect identification of the labeled proteins was thought not to be required since we were more interested in the comparative differences in labeling.

In any case, disregarding the problem of the proteins actually located at the aminoacyl-tRNA 3' terminal binding site, the results presented in this report directly evidence for the first time the existance of structural alteration near the peptidyl transferase active center in the antibiotic resistant mutants. The changes detected suggest that some proteins move nearer the 3' terminal of PNCP-Phe-tRNA in the mutant ribosomes, the amount of covalently bound label increasing noticeably. These results are also compatible with the idea that, in wild type strains, proteins are not abundant in the peptidyl transferase center, stressing the role that rRNA might play in the ribosomal active center.

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