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Identification of two erythromycin resistance mutations in the mitochondrial gene coding for the large ribosomal RNA in yeast

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ABSTRACT

Two independent erythromycin resistance mutations, E^R_{514} and E^R_{221}, have been identified in the mitochondrial gene coding for the 21S ribosomal RNA. The two mutations were found to be identical, corresponding to a A to G transition at the nucleotide position 1951 of the ribosomal RNA gene. In the secondary structure model of the ribosomal RNA, the E^R resistance site is found at the proximity of the chloramphenicol resistance sites located about 500 bases downstream.

INTRODUCTION

The presence of mitochondria is one of the major characteristics of eukaryotic organisms. The mitochondria show however several properties reminescent of the bacterial cells (cf. 1). The protein synthesis in mitochondria is sensitive to antibacterial drugs such as chloramphenicol and erythromycin (2). In yeast, many mitochondrial mutations are known which confer resistance to these drugs. By genetic and restriction enzyme analyses, chloramphenicol and erythromycin resistance mutations have been localized in the gene coding for the large ribosomal RNA (21S rRNA) of the mitochondrial ribosome (cf. 3). We show here that two independently isolated erythromycin resistance mutations correspond to a A to G transition of a single nucleotide in the 21S rRNA gene.

MATERIALS AND METHODS

Strains. The standard strain carrying E^R_{514} mutation is IL8-8C (α trp_1 his_1, rho^+ ω^+ C^R_{321} E^R_{514}), previously described by Netter et al. (4). For deletion mapping, the following rho$^-$ derivatives were used : F11 (C^R_{321} E^R_{514}) and E41, E1, E2, E3, E4, E5, E6 and E8 (all C^o E^R_{514}). For characterization of E^R_{221}, a rho$^-$ strain TR3-15A/E11 (C^o E^R_{221}) was isolated from the strain TR3-15A (a trp_2 his_1, rho^+ ω^- C^R_{321} E^R_{221} O^R_1 P^R_{454}). Erythromycin sensitive allele E^S was isolated from the strain FF1210-6C/C21/10 derived from FF1210-6C (α ura, rho^+ ω^- C^S E^S). Some properties of these rho$^-$ strains have been described previously (5). Genetic techniques and the method of construction of rho$^-$ mutants can be found in Fukuhara and Rabinowitz (6).

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RESULTS AND DISCUSSION

Fine localization of E^R_{514} mutation by rho⁻ deletion mapping

The mtDNA of Saccharomyces cerevisiae has an unusually high frequency of deletion mutation. This is known as "cytoplasmic petite colony mutation" or "rho-" mutation (reviewed in ref. 9). The deletion is large, and can occur at any part of the genome. The retained mtDNA represents only a small segment of the wild type mtDNA. The rho- mutation has been therefore a convenient tool for deletion mapping of any mitochondrial mutations (10). Furthermore, the segment of mtDNA retained in rho- mutants is usually amplified into a large repetitive DNA, so that the genes carried by this segment can be isolated in large amounts and used for direct sequence analysis without further amplification.

The erythromycin resistance mutation E^R_{514} is one of the most commonly used mitochondrial genetic markers. From a strain carrying this mutation (IL8-8C), we have isolated a series of rho- mutants which retained this genetic marker. Restriction enzyme analysis showed that the mtDNAs of these mutants contained, as expected, several restriction fragments in common. These fragments were shown to hybridize to the unique region of the wild type mtDNA coding for the 21S rRNA. We have established a detailed restriction map of this region, and the sequences represented by the rho- E^R mtDNAs have been localized on the map. This is shown in Figure 1. The mtDNA of the rho- strain E1 contained a large part of the MboI-480 base pairs (bp) fragment (actually 463 bp) and no sequence to the left of it ; another rho- strain, E3, contained also a part of the MboI fragment but nothing to the right of it. Since the two

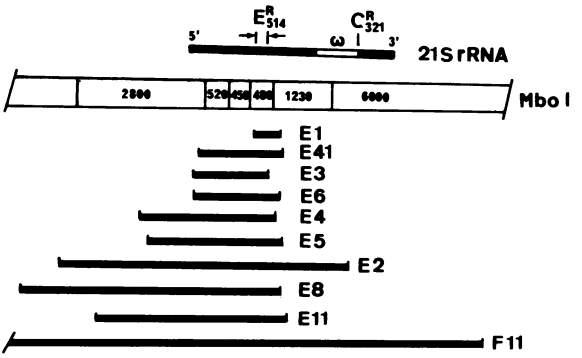

Figure 1 : Deletion mapping of the erythromycin resistance mutation E^R_{514}. 21S rRNA structural gene is shown by a thick line interrupted by the intron ω. Other thick lines represent the mtDNA sequences carried by various rho- mutants. These sequences were analyzed by many restriction enzymes and positioned on the wild type restriction map. Only MboI sites are shown. From this diagram, the position of the E^R mutation was deduced to be within the MboI-480 bp fragment.

strains have the E^R mutation in common, it should lie within the MboI-480 fragment. Therefore, we have isolated this fragment from the rho⁻ strain F11 which carried, in addition to E^R_{514}, the whole region coding for the 21S rRNA. The DNA sequence of the fragment was determined, and its orientation defined with respect to the rRNA gene.

Comparison between E^R_{514} and E^S alleles

The erythromycin sensitive allele E^S was examined on the corresponding MboI fragment isolated from a rho⁻ mutant carrying this allele. This mutant (strain C21/10) was derived from the erythromycin-sensitive strain FF1210-6C. Although the E^S allele does not allow a positive selection of such rho⁻ clones, it was possible to select rho⁻ clones carrying the 21S rRNA gene : these clones can restore, by recombination, respiratory growth of several tester mutants defective in the 21S rRNA gene (5).

The mtDNA isolated from the E^S rho⁻ mutant contained the three MboI fragments, 520, 480 and 420, characteristic of this region. The Mbo-480 fragment was again entirely sequenced and compared to the corresponding fragment carrying the E^R_{514} mutation. We found a single base substitution. The E^R_{514} corresponded to A (E^S) to G (E^R) transition on the non-coding (RNA-like) strand (Figure 2).

Identification of E^R_{221} mutation

In the same way, we examined another erythromycin resistance mutation E^R_{221}. E^R_{514} and E^R_{221} are two independent mutations, since they have been initially isolated from two different strains. The two mutations show however a very similar resistance phenotype, and can not be distinguished by recombination analysis. They are mapped at a single genetic locus called rib III (4). From the strain TR3-15A carrying the E^R_{221} marker, a rho⁻ strain, E11, was isolated which retained it. Again, MboI-480 fragment was isolated from this rho⁻ mtDNA and sequenced completely. We found that the

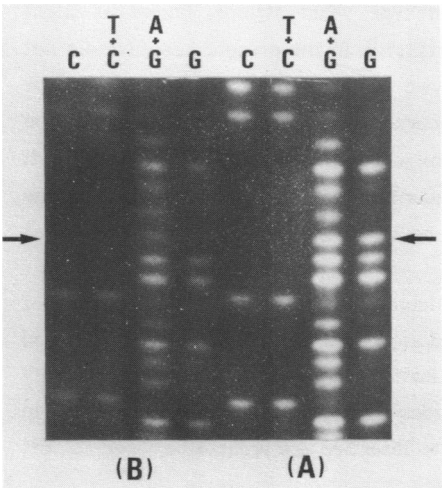

Figure 2 : Nucleotide change corresponding to the E^R_{514} mutation. A Maxam-Gilbert sequencing gel is shown. The difference between the MboI-480 fragments from the E^R strain F11 (A) and from the E^S strain C21/10 (B) is indicated by arrows.

Table 1 : Comparison of the erythromycin sensitivity region in various mtDNA sequences.

Organism	Sequence	Position	Erythromycin
E. coli	GACGG A AAGAC	2058	sensitive (cf. 18)
Tobacco chloroplast (25)	GACAG A AAGAC	2067	sensitive (cf. 21)
S. cerevisiae mitochondria (E^S)	GACGG A AAGAC	1951	sensitive
id. (E^R)	GACGG G AAGAC	1951	resistant
Mouse mitochondria (26)	GACGA G AAGAC	1065	?
A. nidulans mitochondria (24)	AACGA G AAGAC	ca. 1790	resistant ? (22)
S. cerevisiae cytoplasm (27)	GGAAA G AAGAC	2398	resistant (23)

The erythromycin region is one of the most constant sequences in various mtDNAs. Although erythromycin resistance in different systems is not always clear in the literature, there may be a correlation between the resistance and the A to G change (underlined).

sequence was exactly the same as the MboI-480 fragment carrying E^R_{514}. It is therefore concluded that E^R_{514} and E^R_{221} are identical.

A formal identification of E^R mutations needs a direct comparison between the mutant E^R DNA and its original isomitochondrial E^S DNA, because there may exist some nucleotide changes among different wild type yeast strains, known as silent polymorphism of the mitochondrial rRNA genes (11,12). In the present case, we did not use such an isomitochondrial series. However, we found that all the strains had the same restriction fragment patterns in the concerned region of the mtDNA, and the deletion mapping could localize the E^R mutations within the single MboI-480 fragment. Since the whole sequence of the fragment was identical for all strains, except for one nucleotide, the erythromycin mutations could be unambiguously identified with that nucleotide.

In a separate work (in preparation), we have determined the total sequence of the 21S rRNA gene. The E^R mutation is localized at the nucleotide 1951 from the 5' end of the gene. The chloramphenicol resistance mutation C^R_{321} which has been previously identified (11) lies approximately at 500 nucleotides downstream in the mature RNA. In the strains called ω^+, a 1143 bp intron and a 66 bp insertion are present between the E^R and C^R_{321} loci.

The region of E^R mutation shows a highly conservative sequence of the rRNA gene in various organisms. In Table 1, we compared the equivalent sequences of several systems. The data, though limited, suggest that an A to G change may be correlated with the erythromycin resistant systems.

Position of E^R mutation on the secondary structure model of 21S rRNA

The secondary structure of yeast mitochondrial 21S rRNA, deduced from the DNA sequence, fits in well with the general secondary structure models proposed for the large ribosomal RNA of various organisms (13,14,15). In particular, the central part, the domain VI according to Branlant et al. (13), shows many stretches of sequences homologous to E. coli large rRNA, as shown in Figure 3. The non-paired sequences are particularly well conserved, suggesting their functional importance in the ribosome. The erythromycin mutation is found in one of such unpaired regions. Interestingly, in the folded structure of the rRNA, the erythromycin resistance site and the chloramphenicol sites are brought very close to each other, although they are more than 600 bases apart on the primary sequence. This may be correlated with the fact that erythromycin

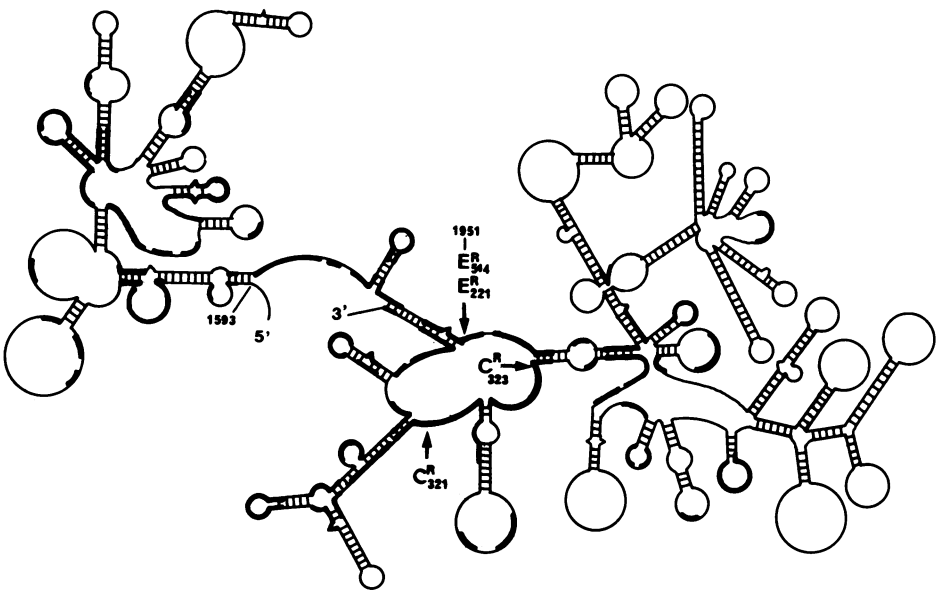

Figure 3 : Positions of erythromycin and chloramphenicol resistance sites on a secondary structure model of the large ribosomal RNA. The domain V-VI of the 21S rRNA is shown. The sequence was folded into a paired structure according to the general model of Branlant et al. (11). The chloramphenicol resistance mutations were taken from the data of Dujon (10). Thick lines indicate the sequences homologous to E. coli large rRNA. The structure at the extreme right part is a preliminary form, as the sequences of this AT rich region contain uncertainties.

prevents chloramphenicol binding, and vice versa (16,17). In bacteria, erythromycin and chloramphenicol seem to interfere with the peptide elongation cycle through interaction with the large ribosomal subunit (18). In yeast, these drugs have been shown to inhibit in vitro protein synthesis of the mitochondrial ribosomal system (19).

By the present study, we have identified a major site of erythromycin resistance on the large ribosomal RNA of yeast mitochondria. It should be noted however that other regions of the rRNA also seem to be involved in the response to erythromycin, since there exist, in the 21S rRNA gene, several other mutations which affect the sensitivity of the mitochondrial system to erythromycin (4,5). Also, some nuclear mutations are known to modify the erythromycin-related phenotype. For example, E^{R}_{514} mutation can be suppressed by nuclear mutations (20). Such suppressors should help to identify the genes coding for the mitochondrial ribosomal proteins, since most of these proteins are coded by the nuclear DNA. Thus any identified mutations on the rRNA gene are a useful tool for dissecting the functional assembly of the mitochondrial ribosome.

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