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Erythromycin and spiramycin resistance mutations of yeast mitochondria: nature of the *rib2* locus in the large ribosomal RNA gene

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ABSTRACT

Two linked genetic loci, rib 2 and rib 3, of yeast mitochondrial genome are the sites of mutations that confer resistance to erythromycin and/or spiramycin. We have examined two mutations at the rib 2 locus. Mutation E^R_{354} was found at the nucleotide position 3993 of the large ribosomal RNA gene ; it corresponded to a C to G transversion leading to a double resistance to erythromycin and spiramycin. Mutation S^R_{551} was found also at the same position, but the C was replaced by a T, conferring resistance to spiramycin only. Rib 2 and rib 3 are 836 base pairs apart on the gene sequence, but are very close to each other in the secondary structure of ribosomal RNA.

INTRODUCTION

We have previously identified a site of erythromycin resistance mutations in the gene coding for the large ribosomal RNA (21S rRNA) of yeast mitochondria (1). The site corresponds to the genetic locus rib 3 defined by Netter and collaborators (2). Since the nucleotide sequence of this region is well conserved in various organisms, an equivalent site in E. coli 23S rRNA can be unambiguously identified at the nucleotide position 2058. In the general secondary structure models of rRNA (3,4,5), this site is localized at the "peptidyl transferase loop". More recent reports (6,7) have shown that this precise position in bacterial RNA is indeed the site of erythromycin resistance, as revealed by a nucleotide change or by methylation. Clearly the rib 3 locus and its bacterial equivalent are major targets of macrolide group inhibitors. In the present work, we have identified another ribosomal locus responsible for the resistance to erythromycin and/or spiramycin. The locus, called rib 2 of the yeast mitochondrial genome, had been genetically mapped very close to the chloramphenicol resistancce locus (rib 1) (2). We have examined two mutations at the rib 2 locus : E^R_{354} which confers resistance to both erythromycin and spiramycin, and S^R_{551} which gives a spiramycin resistance only.

MATERIALS AND METHODS

Strains. The two mutations studied here have been isolated and genetically studied by Netter and collaborators (2). The respiratory sufficient strain Saccharomyces cerevisiae MH200-41 (a ural, rho$^+$ ω^+ CR_{321} ER_{354}) was mutagenized by ethidium bromide according to a standard procedure (8) and a rho$^-$ clone R30 (CR_{321} ER_{354}) was isolated. The mitochondrial DNA of this strain was used for preparation of the 21S RNA gene fragment carrying the ER_{354} mutation. The other rib 2 mutant strain, IL262-2B (a ural, rho$^+$ ω^+ CR_{321} SR_{551}) was also mutagenized and a rho$^-$ clone E3 (CR_{321} SR_{551}) was obtained. The mitochondrial DNA of this clone was used for identification of SR_{551}.

Sequence analysis of mutations. The two rho$^-$ clones have large deletions in their mitochondrial DNA, retaining only the 21S rRNA gene and flanking sequences. The mitochondrial DNA was purified according to Casey et al. (9). It was first digested with the restriction enzyme Sal I which makes a single cut in yeast mitochondrial DNA within the rRNA gene. The Sal I site happens to be only two nucleotide pairs away from the CR_{321} marker (rib 1) which is very close to the rib 2 locus. The digested DNA was dephosphorylated and 5' labelled with T4-polynucleotide kinase. The labelled DNA was recut at a Hpa II site about 520 base pairs downstream from the labelled site and subjected to sequence analysis according to Maxam and Gilbert (10).

RESULTS

Physical localization of rib 2 locus

The approximate position of rib 2 locus has been determined by several laboratories on the physical map of mitochondrial DNA (see review in 11). These studies concluded that rib 2 locus lies immediately to the right of rib 1 (Figure 1) at a distance of at most two hundred base pairs. The mitochondrial DNA of the two rho$^-$ clones carrying rib 2 mutations showed restriction patterns quite similar to those of our standard mitochondrial DNA (MH41-7B) for which we know the complete nucleotide sequence of the rRNA gene (12). Therefore we sequenced the 300 base pair long region

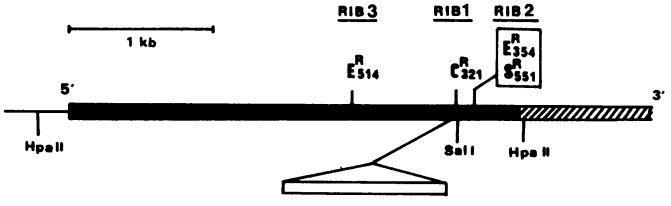

Figure 1. Map of rib loci on the mitochondrial large rRNA gene. Full bar corresponds to the mature rRNA sequence. Hatched bar codes for the 3' tail of the precursor rRNA. Open bar is the optional intron omega. The two mutations studied here are boxed.

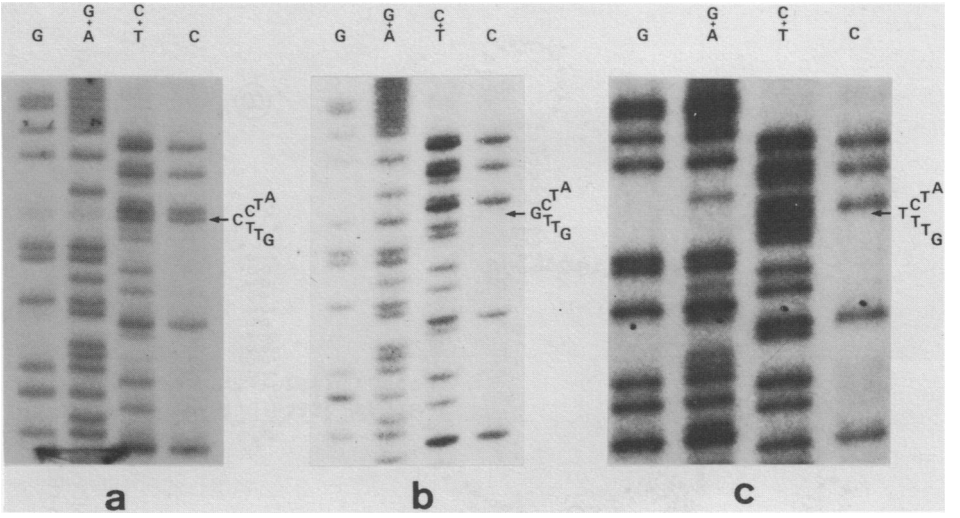

Figure 2. Single nucleotide changes in rib 2 locus mutations. DNA was 5' labelled at Sal I site (see Figure 1) and sequenced toward the 3' end of the rRNA gene.

to the right of the Sal I site and compared it to the standard DNA carrying the wild type allele of rib 2 locus.

Identification of E^R_{354} and S^R_{551}

The 250 nucleotides immediately to the right of the Sal I site in the R30 mitochondrial DNA showed the same sequence as the wild-type DNA, except one nucleotide at the position 3993 (our numbering system includes the 1142 base pairs of intron omega and the 66 base pairs mini-insert ; either one or both can be absent in certain strains). This site is 109 base pairs downstream from the C^R_{321} marker of the rib 1 locus. The normal nucleotide C was substituted by a G on the non-coding strand (Figure 2a and b). Since this is the only change in the expected region for the rib 2 locus, it is concluded that this transversion corresponds to E^R_{354} mutation which confers a double resistance to erythromycin and spiramycin.

When the clone E3 carrying S^R_{551} mutation was examined for the same region, we found a single nucleotide change precisely at the same position, 3993. C was changed to a T (Figure 2c). This transition therefore corresponds to the S^R_{551} mutation which confers resistance to spiramycin but not to erythromycin.

DISCUSSION

Relation between rib 2 and rib 3 loci

The two mutations E^R_{354} and S^R_{551} define a second locus for resistance to macrolide antibiotics in the mitochondrial large rRNA gene. In intron-less rRNA genes,

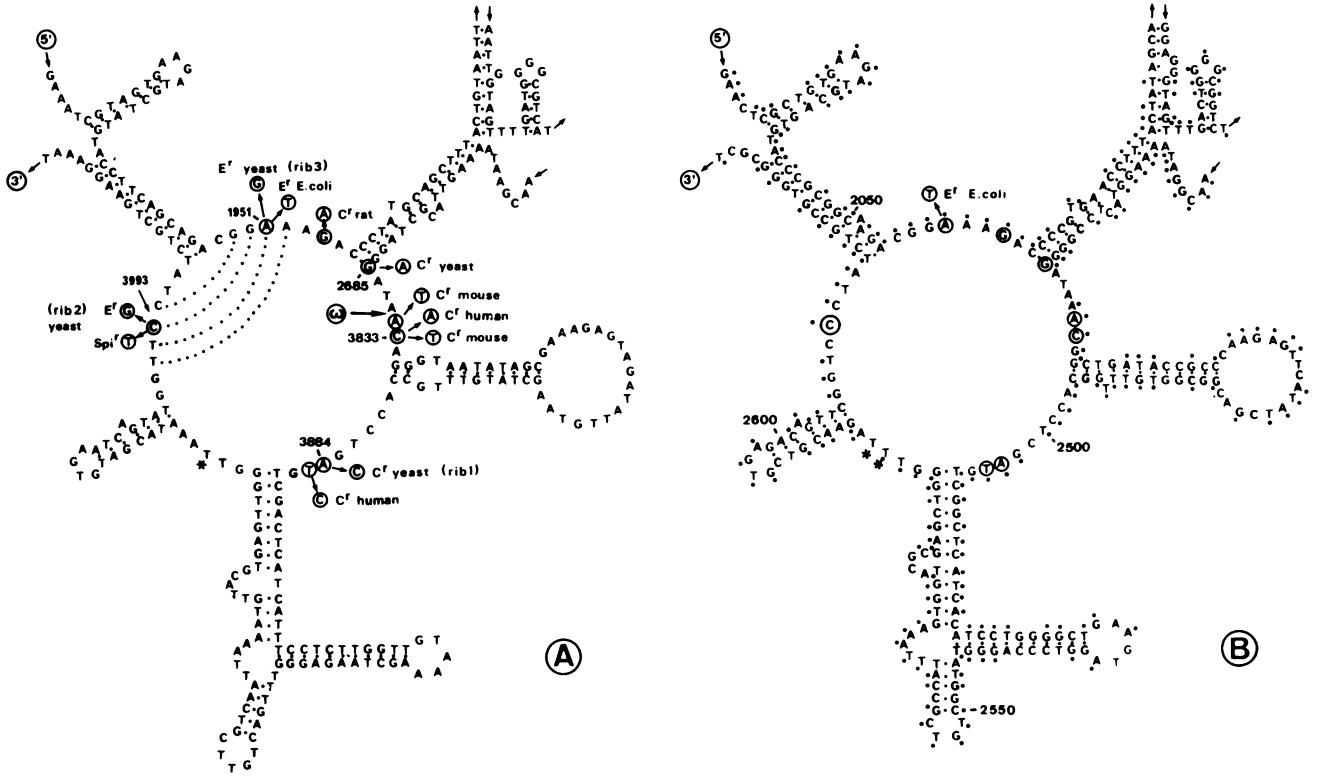

Figure 3. Positions of rib loci on the peptidyl transferase loop of rRNA. The folded structure follows the general model of Branlant et al. (3). T is read U in RNA. A : mitochondrial 21S rRNA ; B : E. coli 23S rRNA.

Table 1. Erythromycin/spiramycin resistance sites of the large ribosomal RNA gene.

Organism	rib 3	rib 2	Resistance to ery.	spi.	ref.
Yeast mitochondria	(1946)GACGG A AAGAC	.....GGTT C CTATCTGC(4001)	S	S	12,22
Escherichia coli	(2053)GACGG A AAGAC	.....GGTC C CTATCTGC(2619)	S	S	3,4,5,6
Mays chloroplast	(2147)GACGG A AAGAC	.....GGTC C ATATCTGG(2714)	?	?	14
Tobacco chloroplast	(2062)GACAG A AAGAC	.....GGTC C ATATCCGG(2631)	?	?	15
Paramecium mito.	(1553)GACGG A AAGAC	.....GGTC C CTATCTGT(2589)	S	S	16,25
Chlamydomonas mito.		.....GGTC C ATATCCGN ($)	S	S	24,25
Aspergillus nid. mito.	(1907)AACGA G AAGAC	.....GGTT T CTATCTTC (4299)	(R)	?	17,26,27
Human mitochondria	(1046)GACGA G AAGAC	.....GGTT T CTATCTAC(1436)	(R)	?	18
Mouse/rat mito.	(1060)GACGA G AA°AG	.....GGTT T CTATCTAT(1457)	R	?	19,21,28
Yeast cytoplasm	(2393)GGAAA G AAGAC	.....AGTT T TACCCTAC(2985)	R	R	20,22

$: Partial sequence ; ° : a chloramphenicol resistance site (28) ; R : resistance ; S : sensitivity ; (R) : probably resistant ; ? : unknown.

this site is 836 base pairs away from the previously identified erythromycin resistance mutation ER^{R}_{514} (rib 3). However, the two loci are brought very close to each other in the folded structure of the rRNA molecule (Figure 3). Interestingly, the short stretches of sequences carrying mitochondrial rib 2 and rib 3 are potentially capable of forming a paired structure in which they are adjacent base pairs. In fact, for this part of the loop Noller and coworkers (5) proposed a paired structure, while other groups (3,4) presented it in a non-paired form. The possible flexibility of such a pairing region may be related to the mechanism of macrolide resistance. In animal mitochondria (which generally seem to be resistant to erythromycin although published data are somewhat controvertial), this region may well be within a paired segment (3).

Netter and collaborators (2) have shown that there are strong interactions between rib 1, rib 2 and rib 3. Single and double resistance to erythromycin and spiramycin are known among the mutants of rib 2 as well as rib 3. Other resistance mutations are also known which are not allelic to the rib loci, but linked to them (13, cf. 11). Analysis of these mutations may help to define detailed functional sub-domains in this part of the rRNA molecule.

Sequences equivalent to rib 2 and rib 3 in other organisms

In Table 1, we have compared the regions equivalent to rib 2 and rib 3 loci in the rRNA gene from some typical ribosomal systems. It is apparent that the sequences of the two regions do not vary much. It is interesting to note that the different organisms may be divided into two classes by sequence criteria : one has an A at rib 3 and a C at rib 2, while the other class has a G at rib 3 and a T at rib 2. The first class includes erythromycin and/or spiramycin-sensitive ribosomal systems ; the second class seems to represent systems resistant to these drugs. If this rule can apply to many other organisms, it would mean that the natural resistance or sensitivity of a ribosomal system to macrolide antibiotics is primarily determined by the large rRNA gene sequence, rather than the genes for other components of the ribosomal system.

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