MITOCHONDRIAL MUTANTS OF THE YEAST *SACCHAROMYCES CEREVISIAE* SHOWING RESISTANCE *IN VITRO* TO CHLORAMPHENICOL INHIBITION OF MITOCHONDRIAL PROTEIN SYNTHESIS

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Received March 5, 1973

Summary: Two cytoplasmic genetic mutants of yeast, genetically separable by recombination, displaying high levels of chloramphenicol resistance have been isolated. Protein synthesis in isolated mitochondria of mutant [cap 2-r] is almost completely resistant to chloramphenicol inhibition while that in mitochondria of mutant [cap 1-r] is partially resistant. Biochemical differences between the two mutants were confirmed by studies of chloramphenicol inhibition of aerobic adaptation of anaerobically grown cells. The mutants appear to contain altered mitochondrial ribosomes.

A number of antibacterial antibiotics such as chloramphenicol (CAP), erythromycin (ERY) and spiramycin (SPI) are known to inhibit yeast mitochondrial, but not cytoplasmic protein synthesis while having no effect on the cytoplasmic ribosomal system (1). Mutants resistant to these antibiotics have been isolated in a number of laboratories and have been demonstrated to be due to mutations in the mitochondrial DNA (2-10). It has been shown for such mutants that the mutations to resistance are due either to a change in the mitochondrial ribosome (3,10,11) or to an alteration in the mitochondrial membrane such that the antibiotic is denied access to the sensitive ribosomes (9,10,12). Chloramphenicol resistant mutants previously isolated in our laboratory have all been cross-resistant to a variety of antibiotics, such as

mikamycin, carbomycin, lincomycin and oligomycin, in no instance have they been shown to result in a change in the mitochondrial ribosome (12,13). This report presents results of experiments with two new CAP resistant mutants isolated in this laboratory; the mutations are cytoplasmic, they show no cross-resistance to other antibiotics and they appear to be ribosomal rather than membrane mutants.

METHODS AND MATERIALS

The isolation and genetic characterization of the two mitochondrial mutants used in this study will be reported in detail elsewhere. Briefly, several spontaneously-arising CAP resistant mutants were isolated from a CAP sensitive diploid strain (H4094-1). Tetrad analysis and other genetic tests have established the mitochondrial location of the mutations and, furthermore, showed that these mutants represented two different mitochondrial genetic loci separable by recombination and denoted [cap1-r] and [cap2-r]. The mutants showed no cross-resistance to erythromycin, spiramycin, mikamycin, or oligomycin. Representative diploid isolates from the two loci [cap 1-16r] and [cap 2-21r] were used for the studies reported here.

Media, culture procedures, isolation of mitochondria, assay of mitochondrial protein synthesis, assay methods for cytochrome spectra and respiration and aerobic adaptation of anaerobically grown cells have been described elsewhere (1,10,12,13).

RESULTS AND DISCUSSION

The growth of the parent strain H4094-1 on ethanol as substrate is inhibited by 0.5 mg CAP/ml of medium. The mean generation time on ethanol of mutant [cap 2-21r] (3.4 hours in the absence of CAP) is only slightly affected by 4 mg CAP/ml (3.8 hours) while that of mutant [cap1-16r] shows a more substantial effect, being 3.3

hours in the absence of CAP and 4.3 hours in the presence of 4 mg/ml. Experiments were then performed to establish the biochemical basis of CAP resistance in these mutants.

In earlier studies we have found it possible to distinguish *in vivo* a mutation affecting the mitochondrial membrane from one resulting in a change in the mitochondrial ribosome (10,12,13). Anaerobic growth of yeast results in a loss of mitochondrial cytochromes and respiratory activity, with a concomitant change in the molecular organization of the membranes and an alteration of their permeability. On aeration of such cells, respiratory activity is restored but only if the mitochondrial protein synthesizing system is active. The development of cytochromes and respiration on aeration of anaerobically grown mutant cells containing antibiotic resistant mitochondrial ribosomes is unaffected by antibiotic (9,13). On the other hand, mutants have been isolated in which resistance is conferred by membrane permeability changes which result in exclusion of the antibiotic under normal aerobic growth conditions. However, anaerobic growth of such mutants also results in a membrane reorganization which removes the antibiotic sensitivity of aerobic induction (10,12).

The data in Table 1 are the results obtained when anaerobically grown cultures of mutants [*cap1-16r*], [*cap2-21r*] and the parent strain (H4094-1) are aerated in the presence and absence of CAP. Respiration and synthesis of cytochromes *b*, $a+a_3$ (but not cytochrome *c* synthesized on the cytoplasmic ribosome) are almost completely inhibited if CAP is present during aerobic induction of the parent strain. CAP had no significant effect on growth or respiration of the mutant [*cap2-r*] and only a slight effect on cytochrome synthesis. This is the phenotypic behaviour of mutants whose resistance is most probably due to altered mitoribosomes. On

TABLE 1

EFFECT OF CAP ON AEROBIC INDUCTION OF ANAEROBICALLY-GROWN CELLS

Strain	Additions[a]	Growth[b] mg/ml	Respiration	Cytochromes	
				c	b, $a+a_3$
H4094-1	None	3.6	140	+++	+++
	CAP	1.6	2.5	+++	-
[cap 1-r]	None	3.8	140	+++	+++
	CAP	2.7	76	+++	+
[cap 2-r]	None	3.5	176	+++	+++
	CAP	3.2	175	+++	++

(a) CAP was added at a final concentration of 4 mg/ml.

(b) Cells at 0.5 mg/ml were inoculated into 1% yeast extract-2% peptone-0.5% glucose and aerated for 24 hours at 28°. Respiration is expressed as ng atoms O_2/min/mg cells.

the other hand, mutant [capl-r] showed only a partial resistance to the effects of CAP, so that based on this data, it does not fall simply into either the class of a ribosome or membrane change. This partial resistance is more completely understood on consideration of the protein synthetic activity of isolated mitochondria.

The effect of CAP on amino acid incorporation into protein by mitochondria isolated from the three strains is shown in Figure 1. Protein synthesis by mitochondria isolated from the sensitive parental strain was inhibited 50% at 10 μM CAP and this inhibition increased through 85% at 100 μM to 98% at 1000 μM CAP. Mitochondria isolated from the two mutants were inhibited to a much smaller extent, additionally the data also indicate that the two mutants differ greatly in their in vitro CAP resistance. While amino acid incorporation into mitochondrial protein of both mutants was not inhibited by low CAP concentrations (10 and 20 μM), at higher CAP levels, protein synthesis by organelles isolated from mutant

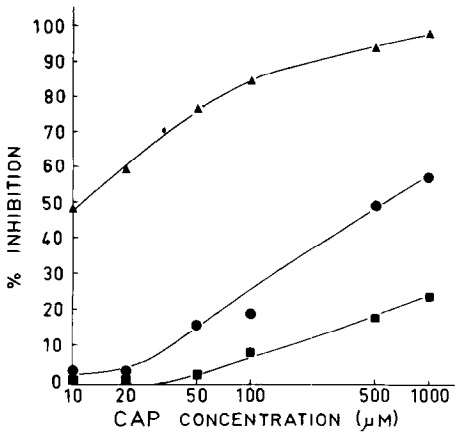

Figure 1. Inhibition by CAP of mitochondrial protein synthesis *in vitro* in H4094-1 (▲), [*cap 1-16r*] (●) and [*cap 2-21r*] (■). The data represent the mean of three or four experiments. The absolute level of activity (pmoles C^{14} - leucine incorporated into TCA-insoluble material /mg protein/20 min) ranged over values of 6-40 (H4094-1) 25-90 [*cap 1-16r*] and 22-87 [*cap 2-21r*]. Omission of exogenous ATP inhibited incorporation by 95% or more and cycloheximide inclusion had a negligible effect (0-10% inhibition); these results rule against significant contamination by bacteria or yeast cytoplasmic ribosomes, respectively.

[*cap2-r*] was more resistant that that of mitochondria from the [*cap1-r*] mutant (Figure 1).

Under our conditions of assay of amino acid incorporation into protein (i.e., low osmolarity), we have found that the mitochondria swell and partially disrupt, so that simple membrane permeability barriers are removed. The results presented may therefore be interpreted to indicate that for both CAP resistant mutants [*cap1-r*] and [*cap2-r*], resistance probably results from some alteration in the mitochondrial ribosome. Presumably, the alteration could involve either the mitochondrial ribosomal RNA or protein (9). The data from studies on CAP inhibition of growth rates, aerobic induction and mitochondrial protein synthesis *in vitro* all suggest that mutant [*cap2-r*] contains mitoribosomes that are almost completely resistant to CAP while the mitoribosomes of mutant [*cap1-r*] are only partially CAP resistant.

To the authors' knowledge, this is the first report for any organism with direct evidence that a CAP resistance mutation results from an alteration of the protein synthesis system (see ref. 14 for a review).

We thank Miss V. Mack for excellent technical assistance.

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