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Single Nuclear Gene Inherited Cross Resistance and Collateral Sensitivity to 17 Inhibitors of Mitochondrial Function in *S. cerevisiae*

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Summary. Previous tetrad analyses defined a yeast strain (332-7c) as containing a single nuclear gene (11.8 map units from the centromere) conferring resistance to oligomycin. Resistance to 18 additional inhibitors of mitochondrial function (Table 1) was determined on (i) ascospore isolates from tetrads segregating 2 resistant: 2 sensitive for oligomycin (Table 2) and (ii), spontaneously derived sensitive isolates of the oligomycin resistant strain (Tables 3 and 4). The observed pattern of resistance suggests that the gene for resistance to oligomycin also results in (i) cross resistance to rutamycin, venturicidin, triethyltin bromide, antimycin A, carbonylcyanide m-chlorophenylhydrazone, tetra-N-butylammonium bromide, dibenzyldimethylammonium chloride, triphenylmethylphosphonium bromide, chloramphenicol, carbomycin and tetracycline and (ii), collateral sensitivity to paromomycin, neomycin, dequalinium chloride, ethidium bromide and acriflavin.

Introduction

Many nuclear mutations affecting specific mitochondrial functions in yeast have been indentified. Beck *et al* (1971) have organized 23 classes of mitochondrial genes into eight major phenotypic groups. In general the effect of these mutations on one mitochondrial function is studied. In view of the known dependence of mitochondrial function on the precise three-dimensional orientiation of molecular components (Hackenbrock, 1972) it would be invaluable to study single nuclear mutations that influence many diverse mitochondrial functions.

We have isolated a strain with a well defined single nuclear gene inherited resistance to oligomycin and chloramphenicol (Rank, 1973). The different mode of action (Table 1) and molecular structure of oligomycin and chloramphenicol indicated that the double resistance was due to the alteration of some fundamental component of the inner mitochondrial membrane rather than a detoxification process. We have studied the effect of the oligomycin resistant gene on 19 inhibitors of diverse mitochondrial functions such as protein and DNA synthesis, respiration and energy conservation; resistance to 17 of these inhibitors was influenced by a single nuclear gene.

Materials and Methods

Genetic strains. Strains used were somatic isolates of 332-7c or GR317, and haploid ascospore isolates derived from sporulated diploid cells of 332-7c by GR317. The genotype of 332-7c is *a his6 met trp1 oli^{PR1}; [ery^{R1}rho+]*. The genotype of GR317 is *α ade2 ura1 oli ±; [ery^{s}rho+]*. Markers enclosed by brackets ([]) refer to cytoplasmically inherited resistance to erythromycin ([ery^{R1}]) and to the cytoplasmically inherited determinant for respiratory

Table 1. Concentration and mode of actions of inhibitors used

Inhibitor	Amount added to disk	Mode of action	References
Oligomycin	20.0 μg	inhibits oxidative phosphorylation	Walter *et al.* (1967)
Rutamycin	50.0 μg	inhibits oxidative phosphorylation	Walter *et al.* (1967)
Venturicidin	2.5 mg	inhibits oxidative phosphorylation	Walter *et al.* (1967)
Triethyltin bromide (TET)	0.05 μl	inhibits oxidative phosphorylation	Aldridge *et al.* (1971)
Antimycin A	0.05 μg	inhibits electron transport	—
Carbonylcyanide, m-chlorophenyl hydrazone (CCCP)	10.0 μg	uncouples energy conservation	Heytler *et al.* (1962)
Tetra-N-butylammonium bromide (TBA)	15.0 mg	cationic penetrating agent	Bakeeva *et al.* (1970)
Dibenzyldimethylammonium chloride (DDA)	12.5 mg	cationic penetrating agent	Bakeeva *et al.* (1970)
Triphenylmethylphosphonium bromide (TPMP)	10.0 mg	cationic penetrating agent	Bakeeva *et al.* (1970)
Chloramphenicol	1.2 mg	inhibits mitochondrial protein synthesis	Grivell *et al.* (1971)
Erythromycin	2.5 mg	inhibits mitochondrial protein synthesis	Grivell *et al.* (1971)
Carbomycin	2.5 mg	inhibits function of 50S ribosomal subunit	Pestka (1971)
Tetracycline	2.5 mg	inhibits function of 30S ribosomal subunit	Pestka (1971)
Paromomycin	5.0 mg	inhibits mitochondrial and cytoplasmic protein synthesis	Davey *et al.* (1970)
Neomycin	2.5 mg	inhibits mitochondrial and cytoplasmic protein synthesis	Davey *et al.* (1970)
Dequalinium chloride	0.25 mg	—[a]	—[a]
Ethidium bromide	5.0 μg	inhibits synthesis of mitochondrial DNA	Goldring *et al.* (1970)
Acriflavin	5.0 μg	inhibits mitochondrial RNA synthesis and induces petites	Fukuhara *et al.* (1970)
Cetyltrimethylammonium bromide (CTAB)	2.5 mg	reversible uncoupler of terminal cytochrome	Wiseman (1971)

[a] The mechanism of interference with mitochondrial function by dequalinium chloride is unknown; however, Hugo and Frier (1970) indicate that in bacteria nucleic acids are the prime targets of dequalinium acetate.

sufficiency ([*rho*+]). The symbol oli^{PR1} is used to denote a centromere-linked (Rank, 1973) pleiotropic nuclear gene that results in cross resistance or collateral sensitivity to a number of different agents that interfere with mitochondrial function; oligomycin (*oli*) resistance is arbitrarily used as the primary definition of the phenotype.

Sixty-three tetrads dissected from the 332-7c by GR317 hybrid (Rank, 1973) were parental ditype for resistance to chloramphenicol and oligomycin; thus it was assumed that the double resistance was inherited by a single nuclear gene (oli^{PR1}). We have determined the cross resistance and collateral sensitivity to inhibitors of mitochondrial function by (i) the 24 ascospore isolates of 6 complete tetrads from the 332-7c by GR317 hybrid, (ii) five spontaneously derived chloramphenicol and oligomycin sensitive isolates of 332-7c and (iii), seven spontaneously derived isolates of 332-7c that have reduced resistance to chloramphenical.

Inhibition of Mitochondrial Function. In the absence of mitochondrial function, *S. cerevisiae* can derive sufficient energy for growth by fermentation of glucose. However, mitochondrial function is required when energy is obtained from a nonfermentable energy source such as glycerol. Thus the area of growth inhibition on YEPG (1% yeast extract, 2% bacto-peptone, 4% glycerol, 2% agar) in excess of growth inhibition on YEPD (1% yeast extract, 2% bacto-peptone, 2% glucose, 2% agar) was taken as a quantitative measure of the inhibition of mitochondrial function. Chemicals were applied to 12.7 mm antibiotic disks (Schleicher and Schuell Inc.) placed on the surface of YEPD and YEPG plates that had been previously spread with 0.2 ml of culture grown for 48 hours in liquid YEPG. Plates were incubated for 3 days at 28° C and the area of inhibition (mm^2) surrounding the disk was determined.

Chemicals. The amount of chemical applied to antibiotic disks and their mode of action is recorded in Table 1. Solvents used were: 95% ethanol for oligomycin, rutamycin, venturicidin, antimycin A, carbonylcyanide m-chlorophenylhydrazone (CCCP), chloramphenicol, carbomycin, erythromycin, ethidium bromide and acriflavin; H_2O for triethyltin bromide (TET), tetra-N-butylammonium bromide (TBA), dibenzyldimethylammonium chloride (DDA), triphenylmethylphosphonium bromide (TPMP), paromomycin, cetyltrimethylammonium bromide (CTAB); 0.1 N HCl for tetracycline; 0.1 M phosphate buffer at pH 7.9 for neomycin; and 50% ethanol for dequalinium chloride. Sources of the various inhibitors were: K and K Laboratories for TET, TBA, DDA, and TPMP; Eastman Kodak for CTAB; Calbiochem for antimycin A, CCCP, and tetracycline; Sigma for oligomycin, neomycin and acriflavin; Dr. H. Machamer, Park Davis and Co. for chloramphenicol and paromomycin; Dr. D. C. Hankinson and Dr. J. R. Speare of the Eli Lilly Co. for rutamycin and erythromycin; Dr. J. Mattoon, Johns Hopkins Univ. for venturicidin; Dr. D. MacLaren, Glaxo Canada Ltd. for dequalinium chloride; Dr. G. Woolfe, Boots Pure Drug Co. for ethidium bromide; and Dr. N. Belcher, Pfizer Inc. for carbomycin.

Results

The 2:2 Segregation for Resistance (R): Sensitivity (s) with 18 Inhibitors of Mitochondrial Function

Six tetrads were selected from the cross of 332-7c by GR317 that segregated 2:2 for (i) mating type, (ii) all 5 auxotrophic markers and (iii), resistance to chloramphenical and oligomycin. The two sensitive and two resistant isolates from each tetrad were treated with antibiotics by the disk method and the area of growth inhibition with different inhibitors was calculated. The effect of the gene (oli^{PR1}) responsible for chloramphenicol and oligomycin resistance on the resistance and sensitivity to other inhibitors was evaluated by calculating the mean inhibition of different inhibitors on the 12 oligomycin resistant and 12 oligomycin sensitive isolates from the 6 tetrads (Table 2).

a) General Observations on the Inhibition of Mitochondrial Function. Four of the 18 compounds used (Table 2) inhibited growth when glucose (YEPD) was used as an enery source. Erythromycin did not have an effect on YEPG because

Table 2. Cross resistance and collateral sensitivity of oli^{PR1} and *oli* ± isolates segregating 2:2 in 6 tetrads from the cross 332–7c × GR317

Treatment	Area of inhibition in mm² on YEPG[a]		Area of inhibition in mm² on *YEPD*[b]	
	Oligomycin sensitive isolates	Oligomycin resistant isolates	Oligomycin sensitive isolates	Oligomycin resistant isolates
Oligomycin	255.1 ± 31.5	0	0	0
Rutamycin	386.6 ± 38.4	0	0	0
Venturicidin	288.0 ± 37.5	0	0	0
TET	701.7 ± 102.5	18.4 ± 14.8	0	0
Antimycin A	491.7 ± 136.6	0	0	0
CCCP	600.3 ± 103.8	238.9 ± 67.8	0	0
TBA	267.4 ± 38.1	0	0	0
DDA	500.9 ± 47.0	67.0 ± 34.3	0	0
TPMP	544.4 ± 44.5	0	0	0
Tetracycline	492.5 ± 66.4	0	0	0
Chloramphenicol	981.6 ± 122.0	0	0	0
Erythromycin	0	0	0	0
Paromomycin	194.3 ± 65.9	629.4 ± 71.2	0	336.2 ± 49.9
Neomycin sulfate	65.2 ± 19.0	365.5 ± 101.4	0	142.3 ± 33.7
Dequalinium chloride	107.7 ± 12.2	363.9 ± 32.5	0	107.1 ± 32.6
Ethidium bromide	154.9 ± 22.3	476.0 ± 135.4	0	0
Acriflavin	628.5 ± 56.3	956.0 ± 106.3	0	0
CTAB	467.9 ± 137.8	631.4 ± 173.4	159.6 ± 52.8	282.0 ± 67.7

[a] The values entered are the mean of 5 observations ± 95% confidence limits of the mean except for treatment with ethidium bromide and acriflavin; data on one of the oligomycin resistance isolates is not included since there was an unusual inhibition on YEPD.
[b] Only one replicate was obtained for each ascospore isolate. The values entered are the mean ± 95% confidence limits.

of the presence of a cytoplasmically inherited marker for erythromycin that segregated 4R:0s (Rank, 1973). Inhibition of growth on YEPD by paromomycin, neomycin, dequalinium chloride and CTAB indicates that at the concentrations used they can affect nonmitochondrial functions. However, all four inhibitors (paromomycin, neomycin, dequalinium chloride and CTAB) could be shown to interfere with mitochondrial function since the area of inhibition on YEPG was greater than that on YEPD. Inhibition of both mitochondrial and non-mitochondrial functions by paromomycin and neomycin was also observed by Davey, Haslam and Linnane (1970). An exception to the general pattern of no inhibition of growth on YEPD by acriflavin and ethidium bromide was also observed since one of the 12 oligomycin resistant isolates was sensitive to these agents on YEPD; interference with mitochondrial function was also apparent since the area of inhibition on YEPG was approximately twice that on YEPD.

b) Cross Resistance of Oligomycin Resistant Ascospores. Chloramphenicol, rutamycin, venturicidin, tetracycline and antimycin A gave identical segregations to oligomycin in all 6 tetrads; that is, all 12 oligomycin resistant isolates were not inhibited on YEPG whereas all 12 oligomycin sensitive isolates on YEPG gave

Table 3. Area of inhibition (mm^2) of different strains when different chemicals were applied to antibiotic disks on YEPG

Chemical applied to disk	Sensitive isolates of 332–7c						
	110[a]	92	108	267	326	332-7c[a]	GR317[a]
Oligomycin	131.6 ± 15.0	188.1	188.1	188.1	188.1	0.0	461.3 ± 24.3
Rutamycin	211.6 ± 30.8	289.4	289.4	289.4	254.1	0.0	647.72 ± 31.6
Venturicidin	149.1 ± 17.5	128.4	100.9	128.4	128.4	0.0	325.7 ± 45.8
TET	236.8 ± 50.9	220.3	289.4	157.5	188.1	126.2 ± 9.2	531.6 ± 33.7
Antimycin A	231.1 ± 25.7	220.3	188.1	188.1	220.3	0.0	570.0 ± 34.1
CCCP	227.7 ± 24.7	580.8	628.7	489.7	446.5	29.6 ± 22.5	683.6 ± 42.1
TBA	0.0	0.0	0.0	0.0	100.9	0.0	254.1 ± 22.7
DDA	0.0	628.7	628.7	534.5	534.5	0.0	388.8 ± 27.4
TPMP	769.5 ± 142.3	1008.1	1130.6	891.8	891.8	0.0	516.6 ± 33.6
Tetracycline	656.1 ± 71.4	446.5	580.8	446.5	678.2	0.0	181.2 ± 21.8
Chloramphenicol	586.4 ± 37.0	220.3	364.8	364.8	364.8	0.0	792.1 ± 45.2
Erythromycin	0.0	0.0	0.0	0.0	0.0	0.0	402.7 ± 33.1
Paromycin	179.4 ± 20.9	128.4	100.9	100.9	128.4	455.1 ± 24.0	308.1 ± 18.7
Neomycin	0.0	0.0	75.0	100.9	0.0	347.9 ± 127.5	117.1 ± 11.9
Dequalinium chloride	115.1 ± 19.3	128.4	128.4	128.4	100.9	264.9 ± 105.5	107.9 ± 10.7
Ethidium bromide	173.1 ± 18.6	289.4	254.1	254.1	289.4	415.4 ± 95.3	296.9 ± 32.6
Acriflavin	746.0 ± 47.3	729.2	836.1	678.2	729.2	793.0 ± 55.7	650.2 ± 39

[a] Values for cultures 332–7c–110, 332–7c and GR317 are the mean and 95% confidence limits of the mean of 10 replicates.

a zone of inhibition with chloramphenicol, tetracycline, venturicidin, rutamycin and antimycin A. Cross resistance of oligomycin resistant isolates to TET, CCCP, TBA, DDA and TPMP was also observed when the reaction to these agents was calculated (Table 2). As seen in Table 2 the area of inhibition of the oligomycin resistant isolates was statistically less than the area of inhibition of oligomycin sensitive isolates. Compounds TET and CCCP were not expected to show 2R:2s segregations since both parents (332-7c and GR317) are sensitive to these agents (Table 3). Nevertheless strain 332-7c is clearly less sensitive to TET and CCCP than GR317; therefore the greater resistance of oligomycin resistant isolates to TET and CCCP is interpreted as being inherited by the same pleiotropic gene (oli^{PR1}). The cationic penetrating agents (TBA, DDA, TPMP) did not give 2R:2s segregations for all 6 tetrads; one tetrad segregated 1R:3s for TBA, two tetrads segregated 3R:1s for DDA and one tetrad segregated 4R:0s for TPMP. The variable expressivity in these tetrads is similar to that observed for chloramphenicol resistance in certain crosses (Rank, 1973); however, it does not obscure the statistical cross resistance to TBA, DDA and TPMP of oligomycin resistance isolates (Table 2).

The 4R:0s segregation for $[ery^{R1}]$ in the above 6 tetrads could mask cross resistance to oli^{PR1} isolates by macrolides other than erythromycin. The $[ery^{R1}]$ marker was lost by asymmetrical distribution (Rank and Bech-Hansen, 1972) in one diploid isolate of the 332-7c by GR317 cross. Ascospore isolates of three complete tetrads were tested for sensitivity to chloramphenicol, paromomycin, neomycin, erythromycin, and carbomycin. The expected pattern of collateral

sensitivity to the aminoglycosides (paromomycin and neomycin) by chloramphenicol resistant ascospores was observed. Of the two macrolides tested only carbomycin showed cross resistance to chloramphenicol resistant ascospores. Chloramphenicol resistant ascospores were not inhibited on YEPG by carbomycin whereas chloramphenicol sensitive ascospores had a zone of inhibition of 392.2 mm^2 when carbomycin was added to disks on YEPG; chloramphenical resistant and sensitive ascospores were inhibited to the same extent by erythromycin.

c) Collateral Sensitivity of Oligomycin Resistant Ascospores. The oligomycin resistant strain 332-7c was observed to be statistically more sensitive than GR317 to paromomycin, neomycin, dequalinium chloride, ethidium bromide and acriflavin (Table 3). The inheritance of the enhanced sensitivity of 332-7c to these agents appears to be due to the oligomycin resistance gene since there is a statistically greater sensitivity to these inhibitors on YEPG with ascospore isolates containing the oligomycin resistance allele (Table 2). Paromomycin, neomycin and dequalinium chloride also inhibited the growth of the oligomycin resistant isolates on YEPD but had no effect on the oligomycin sensitive isolates. Thus the oligomycin resistant isolates show collateral sensitivity to paromomycin, neomycin, dequalinium chloride, ethidium bromide and acriflavin on YEPG, and collateral sensitivity to paromomycin, neomycin and dequalinium chloride on YEPD.

d) Lack of Cross Resistance and Collateral Sensitivity to CTAB. The membrane active agent CTAB inhibited growth of oligomycin resistant and sensitive isolates on YEPG to the same extent; similarly the inhibition on YEPD was not statistically different for the oligomycin resistant and sensitive isolates. The inheritance of CTAB indicates that mitochondrial inhibitors need not show cross resistance or collateral sensitivity to the oligomycin resistant isolates.

Cross Resistance and Collateral Sensitivity of Chloramphenicol and Oligomycin Sensitive Isolates of 332-7c

A routine check of several thousand clones of strain 332-7c revealed five strains (332-7c-92, 332-7c-108, 332-7c-110, 332-7c-267 and 332-7c-326) that were sensitive to chloramphenicol and oligomycin. If the pleiotropic phenotype was inherited by a single gene then it could be expected that resistance to other inhibitors may also be altered in these clonal isolates. As seen in Table 3 the chloramphenicol and oligomycin sensitive isolates of 332-7c have also simultaneously become clearly sensitive to other inhibitors (rutamycin, venturicidin, antimycin A, CCCP, DDA, TPMP, and tetracycline). However, TET resistance of the sensitive clones is intermediate between the two parents and TBA resistance is maintained by all strains except 332-7c-326. The enhanced sensitivity of 332-7c to paromomycin, neomycin, dequalinium chloride and ethidium bromide was reduced in the chloramphenicol and oligomycin sensitive isolates of 332-7c since the area of inhibition was less for the sensitive clones (Table 3). The tolerance to ethidium bromide of 332-7c and the five clonal isolates was also tested by determining the concentration of ethidium bromide required to inhibit growth on YEPG. Strain 332-7c was clearly more sensitive to ethidium bromide since growth was inhibited by 0.5 μg/ml whereas the five clonal isolates grew at concentrations of up to 2.0 μg/ml of ethidium bromide. The simultaneous alteration in

Table 4. Area of inhibition (mm^2) of 7 partially chloramphenicol-sensitive isolates of 332–7c when different chemicals were applied to antiobiotic disks on YEPG

Chemical applied to disk	Partial chloramphenicol-sensitive isolates of 332–7c						
	1	2	3	4	5	40	44
Oligomycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rutamycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Venturicidin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TET	489.7	489.7	404.9	580.8	446.5	446.5	364.8
Antimycin A	0.0	0.0	0.0	0.0	0.0	0.0	0.0
CCCP	0.0	0.0	0.0	0.0	100.9	0.0	0.0
TBA	364.8	364.8	364.8	289.4	404.9	446.5	404.9
DDA	364.8	404.9	404.9	404.9	364.8	364.8	446.5
TPMP	891.8	836.1	781.9	729.2	729.2	678.2	836.1
Tetracycline	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chloramphenicol	100.9	0.0	0.0	0.0	50.6	0.0	0.0
Erythromycin	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Paromomycin	326.3	289.4	289.4	326.3	580.8	628.7	449.7
Neomycin	157.5	128.4	75.0	100.9	289.5	326.5	289.4
Dequalinium chloride	157.5	128.4	128.4	188.1	157.5	188.1	188.1
Ethidium bromide	326.3	364.8	326.3	446.5	364.8	364.8	404.9
Acriflavin	781.9	781.9	729.2	836.1	678.2	729.2	891.8

multiple collateral sensitivity is also consistent with the notion that the pleiotropic phenotype is inherited by a single gene.

Cross Resistance and Collateral Sensitivity of Oligomycin Resistant Isolates of 332-7c that were Partially Chloramphenicol Sensitive

Spontaneously occurring isolates of 332-7c were observed to have a reduced ability to tolerate chloramphenicol while still retaining their resistance to oligomycin. When replicated from YEPD to YEPG containing 3 mg/ml of chloramphenicol, the altered strains produced approximately 1/20 of the number of cells observed for 332-7c. Seven of these partially chloramphenicol sensitive isolates were tested for sensitivity to inhibitors by the disk method (Table 4). Only two of the seven isolates (332-7c-1, 332-7c-5) were scored as chloramphenicol sensitive by the disk method since the partial growth on chloramphenicol was sufficient to eliminate the zone of inhibition. However, all seven strains showed a remarkably enhanced sensitivity to TET and the cationic penetrating agents (TBA, DDA, TPMP); cross resistance and collateral sensitivity to other inhibitors were unchanged.

Discussion

Fifteen of the 17 inhibitors of mitochondrial function were observed to give a statistically different inhibition with oligomycin sensitive and resistant isolates (Table 2). In addition oligomycin resistant isolates were cross resistant to carbomycin when the [ery^{R1}] marker was eliminated by asymmetrical distribution. A further indication of the positive correlation of multiple cross resistance and collateral sensitivity with the oligomycin phenotype was found in an analysis of oligomycin sensitive isolates of 332-7c. Oligomycin sensitive isolates were

sensitive to most inhibitors to which 332-7c was formerly resistant (rutamycin, venturicidin, antimycin A, CCCP, DDA, TPMP, tetracycline and chloramphenicol) and conversely they became more resistant to four inhibitors (paromomycin, neomycin, dequalinium chloride and ethidium bromide) of 332-7c (Table 3). Thus the inheritance of the multiple cross resistance and collateral sensitivity by a single pleiotropic nuclear gene *oli*^{PR1} is indicated by (i) the association of the pleiotropic phenotype with oligomycin resistant ascospores in tetrads segregating 2:2 for oligomycin resistance: sensitivity (Table 2), and (ii) the loss of the pleiotropic phenotype in oligomycin sensitive isolates of 332-7c (Table 3). The multiple cross resistance of *oli*^{PR1} is similar to that observed by Avner and Griffiths (1973a) for their "Class I" mutants; it is of interest to know if their strains would show collateral sensitivity to paromomycin, neomycin, dequalinium chloride, ethidium bromide and acriflavin. The true relationship between the multiple cross resistance of *oli*^{PR1} and "Class I" mutants requires a more precise understanding of the mode of inheritance of "Class I" mutants (Avner and Griffiths, 1973b).

The molecular modification underlying the *oli*^{PR1} phenotype is unknown but is believed to result in an alteration of membrane structure. Alteration of the mitochondrial inner membrane by *oli*^{PR1} is suggested by the resistance to agents known to interfere with membrane functions. Of the inhibitors of oxidative phosphorylation, rutamycin sensitivity of ATPase has been shown to be dependent upon the presence of components of the mitochondrial inner membrane (Tzagoloff and Meagher, 1972). Similarly, energy conservation and electron transport are two processes known to occur in the inner mitochondrial membrane; resistance to CCCP (an uncoupler of energy conservation) and antimycin A (an inhibitor of electron transport) were also observed for *oli*^{PR1}. Synthetic cation (TBA, DDA, TPMP) transport is an energy-dependent process. Although the precise mode of action of these inhibitors is unknown the inhibition is at the level of the mitochondrial membrane (Bakeeva *et al.*, 1970); *oli*^{PR1} also resulted in cross resistance to these compounds. Ethidium bromide is believed to interfere with the synthesis of mitochondrial DNA by producing distortions in an inner mitochondrial-membrane attachment site (Perlman and Mahler, 1971; Bech-Hansen and Rank, 1972). Enhanced sensitivity to ethidium bromide by *oli*^{PR1} is indicative of an alteration of the mitochondrial inner membrane resulting in enhanced sensitivity to ethidium bromide. The mechanism of collateral sensitivity to dequalinium chloride may be very similar to that of ethidium bromide because of the proposed interaction of dequalinium chloride with nucleic acids (Hugo and Frier, 1970). Collateral sensitivity of concanavalin A resistant mutants to various agents has also been reported and was suggested as being consistent with the "fluid mosaic" model of the membrane (Till *et al.*, 1973).

Inhibitors of protein synthesis (chloramphenicol, carbomycin, tetracycline, paromomycin and neomycin) are believed to have their effect at the level of ribosomes (Pestka, 1971; Grivell *et al.*, 1971). Resistance to these antibiotics could be the result of an alteration of the mitochondrial membrane (inner or outer) such that antibiotic transport into the mitochondrial matrix (presumed site of mitochondrial protein synthesis) does not occur. Certainly the diverse structure (Pestka, 1971) and mode of action (Table 1) of these antibiotics suggests

that oli^{PR1} does not result in detoxification. Some evidence for the interference with carbomycin transport is found in the observations that (i) *oli* ± strains lacking [ery^{R1}] were carbomycin sensitive, (ii) *oli* ± strains containing [ery^{R1}] were carbomycin resistant and (iii) oli^{PR1} strains without [ery^{R1}] were carbomycin resistant. Presumably the carbomycin resistance of *oli* ± [ery^{R1}] was conferred by an erythromycin resistance ribosome (Grivell *et al.*, 1971) coded for by [ery^{R1}]. Since the mode of cytoplasmically inherited erythromycin resistance is at the level of the mitochondrial ribosome (Grivell *et al.*, 1971), cross resistance to carbomycin implies carbomycin transport into the mitochondrial matrix. Hence resistance by oli^{PR1} strains lacking [ery^{R1}] suggests a lack of transport of carbomycin.

Loss of cross-resistance to TET and the cationic penetrating agents by partially chloramphenicol-sensitive isolates of 332-7c (Table 4) was accompanied by maintenance of the rest of the pleiotropic phenotype. These altered strains have a high heritability since the back mutation rate is less than $1/10^6$ (authors, unpublished observations). Our current hypothesis is that these strains are the result of a heritable membrane component that alters the expression of the oli^{PR1} product.

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