Cytoplasmic Inheritance of Oligomycin Resistance in Chinese Hamster Ovary Cells

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ABSTRACT Oligomycin-resistant clones were isolated from Chinese hamster ovary cells by treatment of cells with ethidium bromide, followed by mutagenesis with ethylmethane sulfonate and selection in oligomycin. One clone (Oli^r 8.1) was chosen for further study. Oli^r 8.1 cells grow with a doubling time similar to that of wild-type cells, whether grown in the presence or absence of drug (doubling time of 13–14 h). In plating efficiency experiments, Oli^r 8.1 cells are ~100-fold more resistant to oligomycin than are wild-type cells. There is approximately a 32-fold increase in the resistance to inhibition by oligomycin of the mitochondrial ATPase from Oli^r 8.1 cells. The electron transport chain is functional in Oli^r 8.1 cells. Oligomycin resistance is stable in the absence of selective pressure. There is little or no cross-resistance of Oli^r 8.1 cells to venturicidin and dicyclohexylcarbodiimide, other inhibitors of the mitochondrial ATPase, or to chloramphenicol, an inhibitor of mitochondrial protein synthesis. Oligomycin resistance is dominant in hybrids between Oli^r 8.1 cells and wild-type cells. Fusions of enucleated Oli^r 8.1 cells with sensitive cells and characterization of the resulting "cybrid" clones indicates that oligomycin resistance in Oli^r 8.1 cells is cytoplasmically inherited.

Mitochondria are formed by the close cooperation of the nuclear and mitochondrial genetic systems. Several components of the mitochondrial oxidative phosphorylation systems (e.g., the ATPase, cytochrome *c* oxidase, and coenzyme QH_2–cytochrome *c* reductase) require the synthesis and assembly of gene products coded by both the nuclear and mitochondrial genomes. In lower eukaryotes, the isolation and characterization of mutants in both these systems has contributed to our understanding of the synthesis and assembly of these gene products and has permitted mapping the genes for several of the products (for reviews, see references 4, 38, 49). These mutants have also been used to study the transmission, recombination, and segregation of genes coded by the mitochondrial DNA (mtDNA) (for reviews, see references 3, 19).

Mutants with alterations in the ATPase have been isolated and extensively characterized in *Saccharomyces cerevisiae* and *Neurospora crassa.* These include mutants that are resistant to inhibitors of the ATPase, such as oligomycin (2, 28, 33, 41–43, 47), and mutants that have lost oligomycin-sensitive ATPase activity (10, 17, 48). An analysis of these mutants has established conclusively that subunit 9 of the ATPase (also known as the dicyclohexylcarbodiimide [DCCD]-binding protein) is a product of a mitochondrial gene in *S. cerevisiae* (42) and a product of a nuclear gene in *N. crassa* (41). These studies confirm earlier work on the biogenesis of the ATPase in these organisms (23, 49, 50). There are conflicting reports on the site of biosynthesis of the DCCD-binding protein in animal cells (1, 12, 13, 25).

Studies on the biogenesis and genetics of the mammalian mitochondria have been limited by the lack of suitable mutants. Recently, however, mammalian mutants with alterations in mitochondrial functions have been isolated and characterized. Cytoplasmic inheritance can be demonstrated in cells in culture by transferring the resistant phenotype to sensitive recipient cells by fusion with enucleated resistant cells (8, 51). Cytoplasmic (most likely mitochondrial) mutations that have been isolated include mutants with resistance to chloramphenicol (8, 34, 51), antimycin (20), rutamycin (29), erythromycin (16), and oligomycin (5, 22) and mutants with defects in mitochondrial protein synthesis (53). Nuclear mutations affecting mitochondrial functions have also been isolated (6, 7, 11, 14, 15, 27, 35, 44, 45).

In this paper we describe the isolation of Chinese hamster ovary (CHO) cells that are resistant to oligomycin. Genetic and biochemical characterization of one such mutant (Oli^r 8.1) indicates that there is an increased resistance of the mitochondrial ATPase to oligomycin and that oligomycin resistance is cytoplasmically inherited. Many of the properties of this mutant differ from the oligomycin-resistant Chinese hamster and mouse cells that have been isolated in other laboratories (22,

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26, 27, 29). A preliminary account of this work has been published (5).

MATERIALS AND METHODS

Cells

Wild-type CHO Pro$^-$ cells (CHO Toronto) were obtained from Dr. R. Draper, University of California, San Diego, California. CHO Pro$^-$ Tg^r Oua^r Abr^r cells were obtainnd from D. Chin, University of California, San Diego, California, and are resistant to thioguanine (5 μg/ml), ouabain (2 mM), and abrin (10^{-9} M).

Cell Culture

Cells were routinely grown as monolayers in plastic tissue-culture dishes (Falcon Labware, Div. Becton, Dickinson & Co., Oxnard, Calif., or Lux Scientific Corp., Newbury Park, Calif.) or plastic roller bottles (Corning Glass Works, Science Products Div., Corning, N. Y.) in Dulbecco's modified Eagle's medium (DMEM; Irvine Scientific, Irvine, Calif.) containing 1 mg/ml glucose, 0.11 mg/ml sodium pyruvate, and supplemented with nonessential amino acids (Grand Island Biological Co., Grand Island, N. Y.), 10% fetal calf serum (Irvine Scientific), and antibiotics (growth medium). Cells were grown at 37°C in a humidified atmosphere of 90% air: 10% CO_2. In one set of experiments, DMEM without glucose (GIBCO) was supplemented with galactose (1 mg/ml), nonessential amino acids, 10% fetal calf serum, and antibiotics. In another set of experiments, DMEM without glucose and sodium pyruvate (Irvine Scientific) was supplemented with glucose (1 mg/ml), nonessential amino acids, 10% fetal calf serum and antibiotics.

Plating Efficiency

10^2–10^5 cells were plated overnight in complete medium in a 100-mm tissue culture dish. The following day the medium was replaced with medium containing various concentrations of antibiotics or inhibitors. 7–10 d later the cultures were fixed and stained with 0.5% crystal violet, and clones containing 50 or more cells were counted.

Growth Curves

1–2 × 10^4 cells were plated into a 60-mm tissue culture dish containing either growth medium or growth medium with oligomycin. Cells were harvested from duplicate plates at daily intervals and counted in a Coulter counter (Coulter Electronics, Inc., Hialeah, Fla.).

Enucleation

Cells were enucleated by cytochalasin B treatment in Ficoll gradients, using the method of Wigler and Weinstein (52).

Cell Fusion

The product of the fusion of an enucleated cell (or cytoplast) and a whole cell will be referred to as a cybrid, whereas the fusion of two nucleated cells will be referred to as a hybrid.

Hybrids were obtained by plating 1 × 10^6 CHO Tg^r Oua^r Abr^r cells and 1 × 10^6 CHO Oli^r 8.1 cells in a 60-mm tissue culture dish. 24 h later the medium was removed and the cells were treated with 44% polyethylene glycol 6000 (PEG) in DMEM containing 10% dimethyl sulfoxide (DMSO) for 30 s as described (36). After recovery overnight, the cells were trypsinized and replated into DMEM. The following day the medium was replaced with selective medium containing ouabain (1 mM) plus HMT (hypoxanthine, 10^{-4} M; methotrexate, 5.5 × 10^{-7}; thymidine, 10^{-4} M) or ouabain (1 mM) plus oligomycin (0.001 μg/ml). Clones were isolated or counted ~2 wk later.

To obtain cybrids, we fused cytoplasts (enucleated Oli^r 8.1 cells) with CHO Tg^r Oua^r Abr^r cells in suspension, using 44% PEG in DMEM with DMSO as described (18). The cells were plated at densities of 1 × 10^4 to 1 × 10^5 per 100-mm tissue culture dish, and after 24 h selective medium containing oligomycin (0.001 μg/ml) plus thioguanine (5 μg/ml) was added. Cybrid clones were picked or counted 14–20 d later.

Isolation of Mitochondria and Submitochondrial Particles

Mitochondria were isolated from cells in late-log phase by differential centrifugation as described (15). Submitochondrial particles were prepared as described (37).

ATPase Assays

ATPase in mitochondria and submitochondrial particles was assayed in the presence of an ATP-regenerating system as described (21) except that the pH of the reaction mixture was 8.0.

Oxygen Consumption of Isolated Mitochondria

Oxygen consumption of isolated mitochondria was assayed at 30°C as described (15).

Inhibitors and Antibiotics

Stock solutions of oligomycin, rutamycin, and antimycin were prepared in 95% ethanol and added at various concentrations. The maximum final concentration of ethanol was 1% (vol/vol). This concentration of ethanol had no effect on cell growth, plating efficiency, or ATPase activity. Stock solutions of the other drugs (thioguanine, ouabain, and chloramphenicol) were prepared in DMEM.

Protein Determination

Protein determination was by the method of Lowry et al. (32), using bovine serum albumin as a standard.

Chemicals

The following chemicals were from Sigma Chemical Co., St. Louis, Mo.: thioguanine, adenosine triphosphate, pyruvate kinase, bovine serum albumin, and Fiske-Subbarow reducer. The following chemicals were obtained from Calbiochem-Behring Corp., American Hoechst Corp., La Jolla, Calif.: oligomycin, antimycin A, ouabain, abrin toxin, chloramphenicol, and ethidium bromide. Ethylmethane sulfonate was from Eastman Kodak Rochester, N. Y., phosphoenolpyruvate and cytochalasin B were from Aldrich Chemical Co., Inc., Milwaukee, Wis. and colcemid was from GIBCO. Venturicidin and PEG were from BDH Chemicals Ltd. Poole, England. Rutamycin was a generous gift of Eli Lilly Co., Indianapolis, Ind.

RESULTS

Selection of Oligomycin-resistant CHO Cells

The concentration of oligomycin (and other inhibitors of mitochondrial function) required to kill cells varies greatly depending on the cell line and on the energy source of the cell. For example, when galactose (1 mg/ml) is substituted for the normal concentration of glucose in the medium (1 mg/ml), less oligomycin is required to kill wild-type CHO cells (Fig. 1).

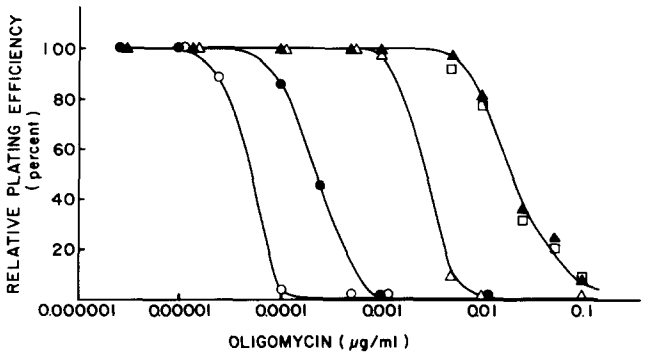

FIGURE 1 Relative plating efficiency of wild-type and mutant cells in the presence of oligomycin in medium containing glucose or galactose. Relative plating efficiency is the number of clones appearing in cultures with oligomycin relative to the number of clones in cultures without oligomycin, expressed as a percent. Control cultures received the equivalent amount of ethanol. CHO cells in DMEM containing 1 mg/ml glucose (●); CHO cells in DMEM containing 1 mg/ml galactose (○); Oli^r 8.1 cells in DMEM containing 1 mg/ml glucose (▲); Oli^r 8.1 cells in DMEM containing 1 mg/ml galactose (△). Oli^r 8.1 cells were grown for over 100 generations in the absence of oligomycin, and their plating efficiency was retested in DMEM containing 1 mg/ml glucose (□).

Similar effects of the energy source on the sensitivity of mouse cells (22, 35) and Chinese hamster V79 cells (54) to mitochondrial inhibitors have been reported.

The oligomycin-resistant CHO cells described in this paper were selected in DMEM containing 1 mg/ml glucose. CHO cells were plated at a density of 2×10^5 cells per 100-mm dish and allowed to attach overnight. The cells were then treated with ethidium bromide (2.5 μM) for 24 h in growth medium and subsequently mutagenized with ethylmethane sulfonate for 24 h. The mutagen was removed and the cells were allowed to recover in complete medium for 3 d. Oligomycin (0.001 or 0.005 μg/ml) was then added to the cultures and the medium was changed every 2 d. After the addition of oligomycin, the cells continued to grow for ~1 wk, whereupon the majority of the cells stopped dividing and detached from the surface of the dish. Oligomycin-resistant clones appeared after 2–3 wk, with most of the dishes containing at least one clone. Clones were isolated and then subcloned. All of the mutants showed a modal chromosome number ($2n = 20$–22) similar to that of the parental line. One subclone (Oli^r 8.1) was chosen for further study.

Growth Characteristics, Plating Efficiency, and Stability of Oli^r 8.1 Cells

The growth characteristics of the mutant Oli^r 8.1 cells, in the presence and absence of oligomycin, were compared to that of the wild-type CHO cells. As shown in Fig. 2, the growth of CHO cells is inhibited by oligomycin (0.001 μg/ml), whereas the growth of Oli^r 8.1 cells is not. Mutant Oli^r 8.1 cells grow with a doubling time similar to that of wild-type CHO cells, both in the presence and absence of oligomycin (doubling time of 13–14 h).

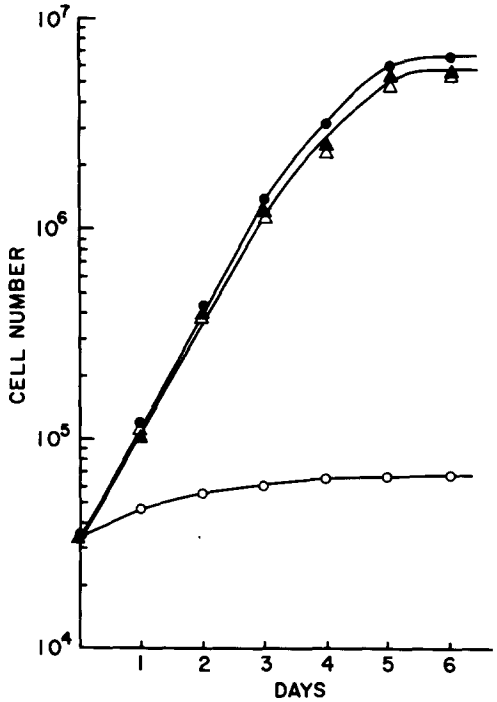

FIGURE 2 Growth curves of wild-type and oligomycin-resistant cells in the presence and absence of oligomycin. Wild-type CHO cells in growth medium without oligomycin (●) and in medium containing 0.001 μg/ml oligomycin (○). Oli^r 8.1 cells in medium without oligomycin (▲) and in medium containing 0.001 μg/ml oligomycin (△).

The dose-response curves of mutant and wild-type cells to oligomycin, in medium containing glucose or galactose as the energy source, are shown in Fig. 1. Mutant Oli^r 8.1 cells are ~100-fold more resistant to oligomycin than are wild-type cells (as judged by the concentration of oligomycin required for 50% inhibition). Lower concentrations of oligomycin are required to kill both wild-type and mutant cells when galactose is substituted for glucose in the medium (Fig. 1). The dose-response curves of mutant and wild-type cells to oligomycin are similar in medium with or without sodium pyruvate.

To determine the stability of the resistant phenotype, Oli^r 8.1 cells were grown for over 100 generations in the absence of selective pressure, and the resistance of the cells was retested. As shown in Fig. 1, oligomycin resistance in Oli^r 8.1 cells is stable in the absence of drug.

Mutant Oli^r 8.1 Cells Are Not Cross-resistant to Other Inhibitors of Mitochondrial Function

To determine the specificity of the resistance to oligomycin, Oli^r 8.1 cells were tested for cross-resistance to other inhibitors of the ATPase and of mitochondrial function. Oli^r 8.1 cells are cross-resistant to the closely related drug, rutamycin (oligomycin D). However, Oli^r 8.1 cells are not cross-resistant to N,N′-DCCD, which (like oligomycin) inhibits the mitochondrial ATPase by binding to subunit 9 of the enzyme (9, 39, 40, 46), or to venturicidin, which inhibits the mitochondrial ATPase at a site different from oligomycin (31). Oli^r 8.1 Cells are not resistant to ouabain, an inhibitor of the Na^+,K^+-dependent ATPase of the plasma membrane. Similarly, Oli^r 8.1 cells are not resistant to chloramphenicol, an inhibitor of mitochondrial protein synthesis, at 50 μg/ml. The mutant cells are slightly more resistant to antimycin (approximately two-to-fivefold), which inhibits the transfer of electrons between cytochrome *b* and c_1. Oli^r 8.1 cells are also not cross-resistant to colchicine. This drug was tested because CHO cells selected for resistance to colchicine display pleiotrophic cross-resistance to a wide range of drugs as a result of a modification of the plasma membrane of these cells (24).

Mitochondrial ATPase from Mutant Cells Is Resistant to Oligomycin

The ability of Oli^r 8.1 cells to grow in the presence of oligomycin could result from several possible alterations in the cell including a change in the permeability of the plasma membrane or the mitochondrial membrane or to an increased resistance of the mitochondrial ATPase to inhibition by oligomycin. To examine these possibilities, we isolated mitochondria and submitochondrial particles from mutant and wild-type cells and determined ATPase activities as a function of the oligomycin concentration. Wild-type and mutant mitochondria had comparable total ATPase activities. However, the ATPase activities of mitochondria (Fig. 3) and of submitochondrial particles isolated from Oli^r 8.1 cells were more resistant to oligomycin than was the ATPase activity of CHO cells. 50% inhibition of ATPase activity was obtained with 0.25 μg of oligomycin per milligram of mitochondrial protein from wild-type cells and 8 μg of oligomycin per milligram of mitochondrial protein from Oli^r 8.1 cells, indicating that the ATPase activity from mutant mitochondria is ~32-fold more resistant to oligomycin. Identical inhibition curves were obtained for ATPase activity, using mitochondria of Oli^r 8.1 cells grown for over 100 generations in the absence of oligomycin. These

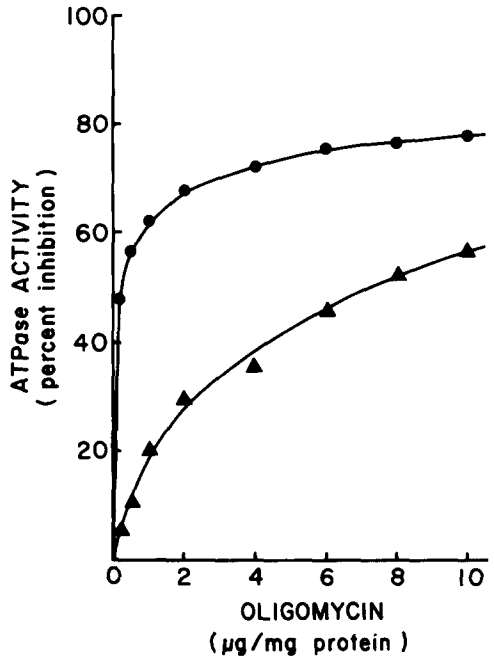

FIGURE 3 Inhibition of the ATPase activity of mitochondria by oligomycin. ATPase activity of mitochondria from wild-type CHO cells (●) and from Oli^r 8.1 cells (▲). The points represent the average of the ATPase assays from four experiments. Total ATPase activities averaged 0.23 μmol/min per mg for CHO cells and 0.22 μmol/min per mg for Oli^r 8.1 cells.

results indicate that resistance to oligomycin in mutant Oli^r 8.1 cells results from an increased resistance of the mitochondrial ATPase to oligomycin. There is no evidence for a change in the permeability of the plasma membrane or the mitochondrial membrane to oligomycin.

The Electron Transport Chain Is Functional in Oli^r 8.1 Cells

Because ATP synthesis is coupled to electron transfer, we examined the electron transport chain in Oli^r 8.1 cells. Oxygen consumption by isolated mitochondria from wild-type and Oli^r 8.1 cells was compared by use of various substrates and inhibitors of the electron transport chain. The addition of pyruvate plus malate stimulated oxygen consumption in both mutant and wild-type mitochondria. This oxygen consumption was almost totally inhibited by rotenone or antimycin. Similarly, the addition of succinate of α-glycerol phosphate stimulated oxygen consumption in both mutant and wild-type mitochondria. The succinate-dependent oxygen consumption was completely blocked by malonate and the α-glycerol phosphate-dependent oxygen consumption was totally blocked by antimycin. These results indicate that the electron transport chain is functional in the Oli^r 8.1 cells.

Oligomycin Resistance Is Dominant in Hybrids

We next examined whether oligomycin resistance behaves dominantly or recessively in somatic cell hybrids. Hybrids were made between Oli^r 8.1 cells and CHO Tg^r Oua^r Abr^r cells by selection in medium containing hypoxanthine, methotrexate, thymidine, and ouabain to avoid selective pressure on the oligomycin-resistant marker. Hybrid clones were isolated, grown up, and tested for plating efficiency. As shown in Fig. 4, the oligomycin resistance of the hybrids is similar to that of the mutant cells indicating that the mutation is dominant. Similar results were obtained when hybrids were selected directly in oligomycin plus ouabain. The hybrid nature of the clones was confirmed by karyotype analysis, which indicated that the clones had a near tetraploid modal chromosome number (Table I), and by plating efficiency experiments, which indicated that the clones were thioguanine sensitive, ouabain resistant, and abrin sensitive (Table I).

Cytoplasmic Transfer of Oligomycin Resistance

Experiments were next done to investigate the inheritance of oligomycin resistance. Oli^r 8.1 cells were enucleated and the resulting cytoplasts were fused with oligomycin-sensitive CHO Tg^r Oua^r Abr^r cells. The fusion mixture was placed in medium containing oligomycin and thioguanine to select against nucleated Oli^r 8.1 cells, unfused CHO Tg^r Oua^r Abr^r cells, and any hybrids resulting from the fusion of nucleated Oli^r 8.1 and CHO Tg^r Oua^r Abr^r cells. As shown in Table II, thioguanine- and oligomycin-resistant colonies appeared in these plates at a high frequency relative to the control plates, indicating that resistance to oligomycin can be transferred by fusion of enucleated Oli^r 8.1 cells with sensitive cells. No colonies appeared in the control plates containing only Oli^r 8.1 cells. A significant number of colonies did appear in the control plates containing CHO Tg^r Oua^r Abr^r cells alone or fusion mixtures of nucleated Oli^r 8.1 cells and CHO Tg^r Oua^r Abr^r cells but at a much lower frequency than that found by enucleated Oli^r 8.1 cells with CHO Tg^r Oua^r Abr^r cells.

Properties of Cybrids

Cybrids formed by the fusion of CHO Tg^r Oua^r Abr^r cells and enucleated Oli^r 8.1 cells should have the following properties if oligomycin resistance is cytoplasmically inherited: (*a*) oligomycin resistance similar to that of mutant Oli^r 8.1 cells; (*b*) chromosome complement similar that of to CHO Tg^r Oua^r Abr^r cells; and (*c*) abrin, ouabain, and thioguanine resistances similar to those of CHO Tg^r Oua^r Abr^r cells. To examine this, we isolated several presumptive cybrid clones and studied their properties. As shown in Fig. 5, the oligomycin resistance of these clones was similar to that of the mutant Oli^r 8.1 cells when tested in plating efficiency experiments. The clones also had the nuclear markers (ouabain, abrin, and thioguanine resistance) and the chromosome complement (modal chromo-

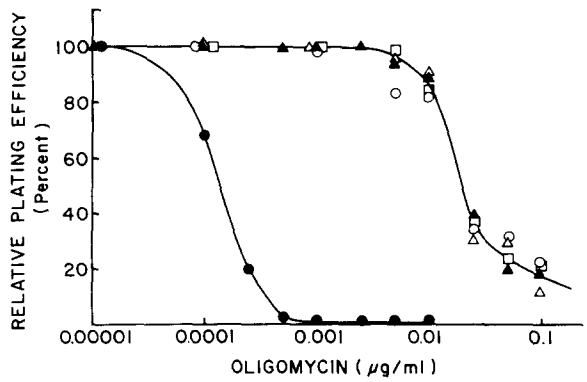

FIGURE 4 Relative plating efficiency of wild-type, mutant, and hybrid cells. Wild-type CHO Tg^r Oua^r Abr^r cells (●); Oli^r 8.1 cells (▲); and hybrids formed between CHO Tg^r Oua^r Abr^r cells and Oli^r 8.1 cells (○, △, □).

TABLE I

Properties of Parental Lines, Hybrids, and Cybrids

Strains	Model chromosome number	Drug resistance			
		Oligomycin*	Thioguanine‡	Ouabain§	Abrin‖
Parental lines					
Oli^r 8.1	22	R	S	S	S
CHO Tg^r Oua^r Abr^r	21	S	R	R	R
Hybrids (Oli^r 8.1 × CHO Tg^r Oua^r Abr^r)					
H101	40	R	S	R	S
H109	42	R	S	R	S
H111	38	R	S	R	S
H115	41	R	S	R	S
Cybrids (en Oli^r 8.1 × CHO Tg^r Oua^r Abr^r)					
Cy2	20	R	R	R	R
Cy3	20	R	R	R	R
Cy4	24	R	R	R	R
Cy5	23	R	R	R	R
Cy6	21	R	R	R	R
Cy12	22	R	R	R	R
Cu13	22	R	R	R	R
Cy14	21	R	R	R	R

* Sensitivity (*S*) to oligomycin is defined as the inability of cells to grow in the presence of 0.001 μg of drug per ml of medium. Resistant cells (*R*) are unaffected by this concentration of oligomycin.

‡ Sensitivity (*S*) to thioguanine is defined as the inability of cells to grow in the presence of 5 μg of drug per ml of medium. Resistant cells (*R*) are unaffected by this concentration of thioguanine.

§ Sensitivity (*S*) to ouabain is defined as the inability of cells to grow in the presence of 1 mM of drug. Resistant cells (*R*) are unaffected by this concentration of ouabain.

‖ Sensitivity (*S*) to abrin toxin is defined as the inability of cells to grow in the presence of 10^{-9} M drug. Resistant cells (*R*) are unaffected by this concentration of abrin toxin.

TABLE II

Cytoplasmic Transfer of Oligomycin Resistance

Cells plated	Ratio of parents	Average number of colonies per 10^6 sensitive cells
en Oli^r 8.1 × CHO Tg^r Oua^r Abr^r	1:1	1,260
	5:1	2,970
Oli^r 8.1 × CHO Tg^r Oua^r Abr^r	1:1	22
	5:1	31
Oli^r 8.1	—	0
CHO Tg^r Oua^r Abr^r	—	46

some number 20–24) of the oligomycin-sensitive CHO Tg^r Oua^r Abr^r parent (Table I). Thus, oligomycin resistance in Oli^r 8.1 cells appears to be cytoplasmically inherited.

DISCUSSION

This paper describes a CHO cell line (Oli^r 8.1) that is resistant to oligomycin, an inhibitor of the mitochondrial ATPase. The general properties of this mutant are: (*a*) there is an ~100-fold increase in the resistance of whole cells to oligomycin as determined by plating efficiency experiments, (*b*) oligomycin resistance is stable after many cell divisions in the absence of drug, (*c*) the resistance is relatively specific for oligomycin and rutamycin with little or no cross-resistance to other inhibitors of the mitochondrial ATPase (such as DCCD or venturicidin) or an inhibitor of mitochondrial protein synthesis (chloramphenicol), (*d*) there is an ~32-fold increase in the resistance of the mitochondrial ATPase to inhibition by oligomycin with little change in the total ATPase activity, (*e*) the electron transport chain is functional, (*f*) oligomycin resistance is dominant in hybrids with wild-type cells, (*g*) oligomycin resistance is cytoplasmically inherited.

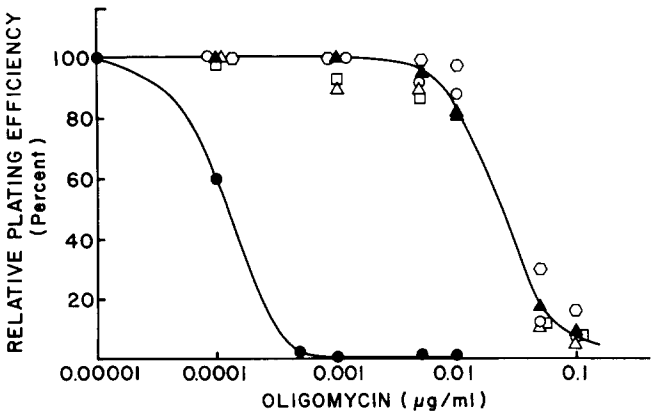

FIGURE 5 Relative plating efficiency of wild-type, mutant, and cybrid cells. Wild-type CHO Tg^r Oua^r Abr^r cells (●); Oli^r 8.1 cells (▲); and cybrids made between enucleated Oli^r 8.1 cells and CHO Tg^r Oua^r Abr^r cells (○, △, □, ○).

The concentration of oligomycin (and other inhibitors of mitochondrial function) required to kill cells depends on the cell line[1] and on the energy source of the cell. Cells in culture derive energy from both glycolysis and from oxidative phosphorylation. When cells are exposed to an inhibitor of oxidative phosphorylation, such as oligomycin, energy must be derived exclusively from glycolysis. Because the rate of glycolysis is slower when galactose is used as a carbon source than when glucose is used, cells are more sensitive to these drugs in medium containing galactose. However, the relative resistance of wild-type and mutant cells to inhibition by oligomycin is expressed independently of the energy source of the cell, unless the mutation alters the rate of glycolysis or oxidative phosphorylation.

The reduced sensitivity of the mitochondrial ATPase to oligomycin indicates that a constituent of the ATPase complex has been modified in the mutant cells. It is possible that a specific binding site for oligomycin has been altered by the mutation. Experiments are in progress to determine the molecular nature of the alteration of the ATPase in mutant Oli^r 8.1 cells.

The oligomycin-resistant mutant described here was obtained after treatment of CHO cells with ethidium bromide, followed by mutagenesis with ethylmethane sulfonate in an effort to reduce the number of mitochondrial genomes per cell before mutagenesis. Various attempts by others to increase the frequency of isolation of mitochondrial mutants have been described (16, 26, 29, 43, 53). However, it has not yet been demonstrated conclusively that any of these treatments enhance the frequency of obtaining mitochondrial DNA mutants, and more controlled experiments will have to be done to evaluate the effect of these agents.

[1] We have observed that different Chinese hamster cell lines vary greatly in their sensitivity to oligomycin.

Several other laboratories have recently isolated oligomycin-resistant mouse and hamster cell lines with quite different properties than the oligomycin-resistant Chinese hamster cell line described here. For example, the oligomycin-resistant CHO cells isolated by Lagarde and Siminovitch (27) have a recessive phenotype and are probably the result of a nuclear mutation(s). These mutants are cross-resistant to a number of drugs affecting mitochondrial functions, including rutamycin, peliomycin, ossamycin, venturicidin, efrapeptin, aurovertin, antimycin, and chloramphenicol. These mutants cannot grow when galactose (0.5%) is substituted for glucose in the medium. No direct examination of the ATPase was carried out and the biochemical basis for these oligomycin-resistant mutants is not clear. On the other hand, rutamycin resistance in the mouse fibroblasts isolated by Lichtor and Getz (29) appears to be cytoplasmically inherited. The mutant cells have a decreased level of mitochondrial ATPase activity and are cross-resistant to oligomycin, peliomycin, ossamycin, efrapeptin and leucinostatin. The mutant cells are also respiration-deficient and unable to oxidize substrates that generate NADH (30). In this regard they resemble a group of respiration-deficient Chinese hamster fibroblasts with defects in complex I of the electron transport chain (6, 11). The latter mutants, however, are not oligomycin resistant (G. A. M. Breen, unpublished observations). Oligomycin-resistant mouse fibroblasts having an increased resistance of the mitochondrial ATPase to inhibition by oligomycin, DCCD, and venturicidin have been isolated by Kuhns and Eisenstadt (26). Both stable and unstable mutants were isolated. However, the localization of the gene(s) conferring oligomycin resistance in their mutants has not been reported. Oligomycin-resistant mouse fibroblasts have also been isoated by Howell and Sager (22). No direct examination of the ATPase was carried out. Direct transmission of oligomycin resistance by cytoplast fusions could not be detected with their mutants, although incomplete cotransmission with chloramphenicol resistance was observed. Thus, oligomycin resistance in mammalian cells can be coded by either nuclear or mitochondrial genes, with several phenotypes being expressed. Additional biochemical experiments will be necessary to associate each of these mutations with specific altered proteins.

The oligomycin-resistant mutant described here should provide a tool to explore the role of the mammalian mitochondrial DNA in the biosynthesis of the ATPase. In addition, this cytoplasmic mutant, together with other cytoplasmic mutants (e.g., chloramphenicol resistant [8, 34, 51, 54], antimycin resistant [20] and erythromycin resistant [16]) will allow us to study recombination and segregation in mammalian mitochondria.

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