ON THE NATURE OF THE CONTROL OF THE TRANSMISSION AND EXPRESSION OF MITOCHONDRIAL ANTIBIOTIC RESISTANCE FACTORS IN SACCHAROMYCES CEREVISIAE

by

MICHAEL F. WAXMAN

A dissertation submitted to the
Graduate Faculty in Biology in partial
fulfillment of the requirements for
the degree of Doctor of Philosophy,
The City University of New York.

1974

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

May 1, 1974
Date

N. R. Eaton
Chairman of Examining Committee
Professor N. Eaton, Brooklyn College

May 15, 1974
Date

Louis G. Morber
Executive Officer

C. Beam
Professor C. Beam

Brooklyn College
Institution

D. Wilkie
Professor D. Wilkie

Brooklyn College/University College, London
Institution

D. Beattie
Professor D. Beattie

Mt. Sinai Medical School
Institution

J. Marmur
Professor J. Marmur

Albert Einstein College of Medicine
Institution

L. Skogerson
Professor L. Skogerson

Columbia College of P & S
Institution

The City University of New York

ABSTRACT

On the nature of the control of the transmission and the expression of mitochondrial antibiotic resistance factors in Saccharomyces cerevisiae.

by Michael F. Waxman

Advisor: Prof. Norman R. Eaton

The results of this study clearly demonstrated that nuclear factors can an do control the distribution of mitochondria from zygotes to their daughter cells. All methods of analysis used; random diploids, zygote clone and zygote cell lineage analysis attested to this fact. It is also evident that the inhibition of nuclear RNA transcription and/or cytoplasmic protein synthesis in young zygotes for 90 minutes consistently altered the preferential transmission of mitochondrial types to zygote daughter cells, observed in untreated zygotes.

Studies in which mitochondrial protein synthesis was inhibited with the antibacterial antibiotics indicated that mitochondrial protein synthesis played little or no part in the events leading to the recombination of M-DNA and subsequent transmission of mitochondrial resistance factors to the descendants of the zygotes.

The analysis of the derepression and repression of mitochondrial function prior to mating by growth on glycerol or 10% glucose respectively indicated that there might be some form of selection

favoring the transmission of functional mitochondria (derepressed) to zygote daughter cells. However, these results were inconsistent and the inconsistencies might be attributed to the varying degrees of repression or derepression of the cells prior to and during mating.

The genetic analysis on the cellular location of the control function showed it to reside in the nuclear gemone, since the control function showed a Mendelian segregation pattern (2:2) at meiosis. These results were consistent with the results of cycloheximide and thiolutin treatment, which indicated that nuclear RNA transcription and subsequent translation of this RNA into proteins was necessary for the manifestation of the control function.

The analysis of suppressive petite mutants in crosses to strains carrying nuclear control genes has shown that these control genes could alter the suppressiveness of the suppressive petite mutants to levels approaching that of the neutral petite phenotype.

Genetic analysis has indicated that suppressiveness and the control function responsible for the asymmetrical distribution of mitochondrial resistance factors were not manifestations of a common molecular mechanism, but two independent mechanisms which could be additive in their effects.

The analysis of the omega factor and its effects in crosses to strains possessing the nuclear control determinants indicated that the omega factor (ω^+ or ω^-) did not modify or alter the

asymmetrical inheritance pattern of strains carrying nuclear control genes.

ACKNOWLEDGEMENT

I am deeply indebted to my advisor Dr. Norman Eaton for his constant advise and guidance in regard to both my research and in the preparation of this manuscript.

I would also like to thank Dr. David Wilkie for his interest in my research and for his helpful suggestions. Dr. Wilkie also participated as a reader of this dissertation, along with Drs. Julius Marmur, Diana Beattie, Carl Beam and Lawrence Skogerson. I am grateful to all of them for their help in improving this manuscript.

I also wish to thank Mrs. Bea Spates for her help in the preparation of the media used in this study and Miss Lois Liebman for her artistic talent which is evident in all the illustrations presented in this manuscript.

And not least, I take pleasure in expressing my deepest thanks to my wife, Mrs. Barbara Waxman, whose constant understanding and encouragement made the completion of this study possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

CHAPTER I

INTRODUCTION

The phenomenon of cytoplasmic inheritance first reported by Correns in 1909 (Correns, 1909) has been interpreted (Ephrussi, 1953; Roodyn and Wilkie, 1968; Sager, 1960) to mean that cytoplasmic organelles might possess an independent genetic system. Mutants with abnormalities of chloroplast or mitochondrial function, studied by classical genetic techniques, showed anomalous behavior. Instead of following the pattern of simple Mendelian segregation the mutant characteristic was transmitted by a mechanism that appeared to depend on the transfer of cytoplasm. This behavior strongly suggested the presence of a cytoplasmic genetic determinant.

It is now generally recognized that the mitochondrion contains DNA (M-DNA) which is distinct from nuclear DNA, as well as the enzymatic machinery necessary to transcribe and to translate genetic information into protein products (Ashwell and Work, 1970; Schatz, 1970; Rabinowitz and Swift, 1970; Borst and Kroon, 1969).

Our current knowledge of the informational content of M-DNA is still poor. Recently it has been reported (Hollenberg et at., 1969) that in yeast M-DNA is found as closed covalent circles, about 26μm long (equivalent to about 5×10^7 daltons). In most studies investigators have found that M-DNA comprises between 15% and 23% of the total DNA

of the yeast cell. The proportion of M-DNA in the total DNA of haploid cells is about the same as diploid cells. The diploid cell contains about 0.009pg (5.4×10^9 daltons) of M-DNA and a haploid cell about 0.005pg (3×10^9 daltons) (Williamson, 1970). If the contour length of M-DNA is 26μm, then the haploid cell contains about 50 and the diploid cell 100 M-DNA molecules. On the basis of the estimate by Avers et al., (1967) that a diploid cell contains 40 to 50 mitochondria, there are about two copies of M-DNA molecules per mitochondrion. Recently, Hoffman and Avers, (1973) have reported that in an electron microscopy study of yeast there appears to be only one giant mitochondrion per cell. However, in view of the massive evidence to the contrary, (Damsky et al, 1969; Osumi and Sando, 1969; Plattner et al, 1970; Plattner and Schatz, 1969; Mahler et al, 1970) we must wait for more information bearing on this point before we can reach any conclusions on the number of mitochondria per yeast cell.

Moleculear hybridization studies have shown that mitochondrial ribosomal RNA is almost certainly derived from M-DNA. In Neurospora (Wood and Luck, 1969), yeast (Fukuhara, 1967; Wintersberger, 1968), and Tetrahymena (Suyama, 1967), mitochondrial ribosomal RNA hybridizes to M-DNA but not nuclear DNA. Saturation levels up to 10% are obtained. In Neurospora (Schäfer and Küntzel, 1972) M-DNA with a molecular weight of 6 to 7×10^7 daltons is believed to contain

one cistron for each ribosomal RNA. M-DNA does not hybridize with cytoplasmic ribosomal RNA demomstrating that the cytoplasmic and mitochondrial ribosomal RNA are transcribed from different sources. The mitochondrion also contains some species of tRNA which are specified by M-DNA in yeast (Casey et al, 1969; Faye et al, 1973) and in rat liver (Nass and Buck, 1969). Recent work by Dawid (1970) strongly suggests that in Xenopus laevis egg mitochondria the specification of stable mitochondrial RNA components occupies a large fraction of the available genetic information. Estimates of combined molecular weights of the two ribosomal RNA's of Xenopus mitochondria are between 1.1 and 1.3 x 10^6 daltons. Since the molecular weight of Xenopus M-DNA is about 10 x 10^6 daltons one expects to find a hybridization level of 12% for ribosomal RNA, if it is coded by the M-DNA and is one copy of each of the ribosomal cistrons is present per M-DNA molecule. This is exactly what Dawid found. In addition, Dawid observed that 4S RNA hybridized with 3% of the M-DNA. Therefore, a total of 2 x 15% = 30% of the mitochondrial DNA is concerned with the elaboration of stable RNA components. A realistic estimate might be 40% of the mitochondrial genome coding for stable RNA components. The remaining 60% is equivalent to approximately 3,000 amino acids or 20 proteins.

Studies of protein synthesis in intact cells and isolated mitochondria have suggested that the principal, if not the only, proteins

synthesized are insoluble lipoproteins of the inner membrane (Groot et al, 1972). The fact that mitochondria synthesize only a small number of membrane associated proteins has been shown by a comparison of normal and "petite" (respiratory-deficient) yeast grown on two different radioisotopes. When the mitochondria which were labeled with different isotopes were mixed and separated on polyacrylamide gels only a few proteins were found to be missing in the "petite" mutant (Groot et al, 1972; Weislogel and Butow, 1971). Groot et al, (1972) have found that the missing proteins correspond to the mitochondrial products observed in the membranes membranes labeled in the presence of chloramphenicol. It is apparent that the mitochondrion synthesizes relatively few proteins which at most account for a very small percentage of the inner membrane.

A number of drugs have been found to inhibit selectively either mitochondrial or cytoplasmic protein synthesis. Among the most commonly employed inhibitors of mitochondrial protein synthesis are the antibacterial antibiotics chloramphenicol and erythromycin (Lamb et al, 1968). The synthesis of proteins in the cytosol is inhibited by cycloheximide (So and Davies, 1963). The utilization of these inhibitors has given further insight into the relative contribution of the two protein synthetic systems to the total pool of mitochondrial proteins. When yeast is incubated in a medium containing cycloheximide and radioactive amine acids, cytoplasmic

synthesis is effectively blocked, and virtually all of the labeled protein products are found in close association with the inner membrane of the mitochondrion (Ashwell and Work, 1970). Similar results have been reported in other organisms, suggesting that the bulk of soluble matrix proteins and proteins of the inner membrane and outer membrane of the mitochondrion are synthesized in the cytosol.

Schatz (1968) and Kovac and Weissova (1968) have reported that "petite" mutants of yeast synthesize a cold labile ATPase, which is indistinguishable from F_1 of respiratory sufficient cells on the basis of its physical and catalytic properties. This observation suggests a cytoplasmic origin of the enzyme. This observation is supported by inhibitor studies (Tzagoloff, 1969; Tzafoloff et al, 1972). When glucose-repressed yeast are incubated in a derepressing medium containing chloramphenicol, the ATPase activity of the mitochondrial fraction remains constant but a significant amount of new ATPase appears in the soluble cytoplasmic fraction. This accumulation of ATPase in the cytoplasm is not seen when the incubation is carried out in the presence of cycloheximide. Under these conditions neither the mitochondrial nor the cytoplasmic fractions show any increase in ATPase activity. In order to determine whether all the subunits of the F_1 are synthesized on cytoplasmic ribosomes glucose-repressed cells

were drepressed in the presence of chloramphenicol and C^{14}-leucine to label all the cytoplasmic products. The ATPase was isolated and the enzyme was purified by precipitation with F_1 antiserum. The purified enzyme was then analyzed by sodium dodecyl sulfate acrylamide gel electrophoresis. The results showed that all five subunits of the F_1 were labeled in the isolated enzyme. Similar results were found when the ATPase was purified from a "petite" mutant derived from the same strain of yeast. On the basis of these experiments it can be concluded that all five known subunits of F_1 are also translated and assembled into a functional ATPase independently of mitochondrial protein synthesis. Similar studies (Tzagoloff, 1970) have shown that oligomycin sensitivity conferring protein (OSCP) is also synthesized on cytoplasmic ribosomes.

Another mitochondrial enzyme which has received much attention is cytochrome oxidase. This enzyme is found within the mitochondrion bound to its inner membrane. The biosynthesis of cytochrome oxidase requires products of both mitochondrial and cytoplasmic protein synthesis. It has been shown (Clark-Walker and Linnane, 1966; Clark-Walker and Linnane, 1967) that the appearance of cytochrome oxidase in yeast during aerobic adaptation is inhibited by both chloramphenicol and cycloheximide. Chen and Charalampous (1969) reported evidence for the presence of both cytoplasmic and mitochondrial precursors of cytochrome oxidase

in yeast on the basis of studies with inhibitors of protein synthesis.

Recent studies on the biosynthesis of cytochrome oxidase in yeast (Mason and Schatz et al, 1972; Mason et al, 1973) has shown that cytochrome oxidase consists of seven polypeptides. The three largest of these are synthesized on mitochondrial ribosomes and the four smaller ones on cytoplasmic ribosomes.

These studies indicate that the formation of a functional mitochondrion involves a close interaction between cytoplasmic and mitochondrial protein synthetic systems.

The concept of dependence of mitochondrial development on information derived from both nuclear and mitochondrial DNA is strongly supported by the existence of both chromosomal and cytoplasmic mutations affecting mitochondrial functional integrity. Respiratory-deficient mutants of yeast have been studied in many laboratories (Ephrussi, 1953; Sherman and Slonimski, 1964). Yeast are suitable for study because severe mitochondrial abnormality, even complete absence of oxidative ability, is compatible with continued viability since Saccharomyces cerevisiae can grow well anaerobically using glycolysis exclusively. There are at least 63 nuclear genes which have been shown to be involved in mitochondrial function (Beck et al, 1971) and mutations in any one of these genes severely restricts mitochondrial activity. The most exhaustively investigated nuclear gene involved in mitochondrial

function is the gene which specifies iso-1-cytochrome c (Sherman et al, 1966).

Mutations in M-DNA which cause the "petite" condition can be divided into two categories based on "dominance" in diploids derived from crosses between mutant strains and respiratory-sufficient strains ("grande"). Neutral "petites" are cytoplasmic mutants which, when crossed to a respiratory-sufficient strain, segregate only grande descendant diploid cells from the zygotes which are themselves "grande." The "petite" condition is never seen again even when the diploid is sporulated and the meiotic products analyzed. Suppressive "petites" are cytoplasmic mutants which suppress normal respiratory behavior in crosses to "grande" strains so that in some cases, as many as 99 percent of the diploid cells derived from zygotes are "petite." The mechanism of this suppressive behavior is not known, but suggestions have been made that a competition of replication rates between normal and suppressive M-DNA is involved (Carnevali et al, 1969) or that massive non-reciprocal recombination between "petite" M-DNA and "grande" M-DNA may be responsible for the suppressive characters (Deutsch et al, 1974).

Another class of cytoplasmically inherited mutations are mutations to drug resistance. The mitochondrial protein synthetic system is sensitive to the antibacterial antibiotics chloramphenicol,

erythromycin, paromomycin, spiramycin, lincomycin, mikamycin, etc. Since yeasts are facultative anaerobes, sensitivity to these antibiotics is only manifest when these organisms are grown on a nonfermentable substrate, such as, glycerol, lactate or ethanol. Mutants resistant to these drugs occur spontaneously at a low frequency and can be induced by mutagenic agents. It has been shown (Thomas and Wilkie, 1968a; Thomas and Wilkie, 1968b; Linnane et al, 1968) that these resistance mutants can arise from modification of either nuclear DNA or M-DNA. Nuclear mutants are resistant to these drugs presumably because of a permeability change in the cellular membranes or mitochondrial membrane, whereas cytoplasmically inherited resistance is considered to be due to a modification of mitochondrial ribosomal protein(s) or ribosomal RNA (Pestka, 1971; Smith et al, 1969; Otaka et al, 1970; Lai and Weisblum, 1971; Kuntzel, 1969).

The location of the resistance mutation can be deduced from the presence or absence of meiotic segregation; nuclear mutations show 2:2 meiotic segregation for resistance and sensitivity, while extrachromosomal resistance mutations do not show meiotic segregation and at meiosis yield 4:0 or 0:4 ratios for resistance and sensitivity.

The change to "petite" can be selectively induced with acriflavin which causes a cytoplasmic inherited respiratory deficiency

frequently accompanied by gross alteration or apparent total loss of M-DNA (Borst, 1972). In either case it appears that most or all of the genetic information carried in the DNA is lost. Since it can also be shown that cytoplasmically inherited resistance factors are sometimes lost in "petites" derived from cytoplasmic resistance mutants (i. e. resistance is not transmitted in crosses to drug sensitive "grande" strains). One concludes that the resistance factors are located on the mitochondrial chromosome (Thomas and Wilkie, 1968; Linnane et al, 1968).

The analysis of crosses involving strains carrying different resistance factors has shown (Thomas and Wilkie, 1968) that diploid cells from zygotes give rise, mitotically, to parental and recombinant types. The mechanism of the recombination and the control of the segregation of mitochondrial markers to diploid cells are subjects of intensive study in several laboratories.

Physical demonstration of recombination has been reported from several laboratories. Shannon et al, (1972) mass mated two populations, differing in both the density of M-DNA and mitochondrial markers (i. e. wild type crossed with a suppressive "petite" of lower M-DNA buoyant density), and showed that within two generations a significant fraction of cells in the population appeared intermediate in both characteristics. In another study Michaelis et al, (1973) crossed two cytoplasmic "petite" mutants one carrying a gene conferring

resistance to chloramphenicol the other a gene conferring resistance to erythromycin. The two parents also differed in the M-DNA buoyant densities. Diploid "petite" recombinants were observed which carried both genes and contained not a mixture of the two parental DNA's but a new species of M-DNA of intermediate buoyant density. It was also observed that new degrees of suppressiveness, different from those of the parentals, sometimes resulted from the recombination of the M-DNA.

The control of the transmission of mitochondrial markers (parental and recombinant) to descendant diploids cells from a zygote is also under investigation. Early reports (Saunders et al, 1970; Rank and Bech-Hansen, 1972) suggested that the asymeterical distribution (polarity) of recombinant classes to daughter cells of the zygote was closely linked to or controlled by the mating type locus itself. Coen et al, (1970) and Bolotin et al, (1971), using resistance to erythromycin (E) and chloramphenicol (C) as mitochondrial markers, concluded that the preferential transmission of markers (polarity of transmission) is a feature of certain strains which possess a hereditary factor ω, that manifests its effects only when crossed to ω^- strains ("heterosexual crosses"). Approximately symmetrical distributions of reciprocal mitochondrial types were observed only in "homosexual" crosses between strains carrying the same ω factor.

These results were obtained by sampling random diploids from many zygotes resulting from mass matings. Bolotin et al, (1971)

also showed that the frequency of recombinants could be increased by ultraviolet irradiation of the ω^+ parent prior to mass mating, but irradiating the ω^- parent had little or no effect. Based on these results the investigators proposed that mitochondria can be classified as male or female analogous to bacteria: ω^+ mitochondria can be compared to Hfr or F^+ bacteria and ω^- to F^-. It was further proposed that the genetic material is transferred from one mitochondrion into a recipient mitochondrion and that this transfer is under the direct control of the omega factor, which is regarded as cytoplasmic and presumably mitochondrial. In zygotes from heterosexual crosses, the frequency of transmission of mitochondrial genes would be determined by the distance of the genes from the point of injection of DNA from the ω^+ into the ω^- mitochondrion. Here injection is regarded as unidirectional but in homosexual crosses presumably any mitochondrion can act as either donor or recipient.

More recently, the segregation of mitochondrial markers in zygotes and zygote daughter cells was investigated by Wilkie and Thomas (1973) by micromanipulation of parental cells. The results indicated that multiple recombinational events were occurring in zygotes and that the asymmetry of at least parental types was a non-random process.

Recent electron microscopy studies indicated that mitochondria

may lose their structural integrity in zygotes (Smith et al, 1972). The authors suggested that the degeneration of mitochondria in newly formed zygotes can result in the release of their DNA molecules which could then proceed to undergo multiple recombinational events, analogous to those in phage infected bacteria. The products would then be available for transmission to the buds for a limited period of time. This period may coincide with the time during which mitochondria are disorganized. After reorganization of the mitochondria there is a "stabilization" of the mitochondrial types leading to preferential distribution to subsequent buds.

Lukins et al, (1973) concluded that the bulk of the recombinational events take place rapidly in zygotes and those recombinants are then segregated along with parental genomes in a random process.

An analysis (Howell et al, 1973) was recently made of a series of crosses between sensitive yeasts from widely different sources and strains carrying two mitochondrial markers in the cis configuration. The results suggest that the nature of the sensitive strain markedly affects the inheritance of any pair of mitochondrial markers. This variability was observed for three parameters: the polarity of recombination, recombination frequency and the frequency of transmission of each of the markers. The observations were not compatible with polarity being determined by a simple mitochondrial sex factor and suggest several different interactions one of which may be

nuclear.

In view of our ignorance of the fate of mitochondria and their chromosomes in zygotes and of the mechanism of inheritance of mitochondria and/or mitochondrial genomes by zygote daughter cells (zygote buds), any theory of mitochondrial behavior based on segregation data alone must be speculative, particularly where the segregants are analyzed many cell divisions after the recombinational events. To provide further information on these points and to elucidate possible control mechanisms in the recombination and transmission of mitochondrial genomes an extensive analysis of random diploids, whole zygote clones and zygote cell lineages were undertaken. This dissertation describes and discusses the results of these studies.

CHAPTER II

MATERIALS AND METHODS

MEDIA: The media used were YEPD (1% yeast extract, 2% peptone, 2% glucose); YEPG (1% yeast extract, 2% peptone, 3% glycerol); YEPG-ERY (consisted of YEPG with 2mg erythromycin/ml); YEPG-CAP (YEPG with 2mg chloramphenicol/ml); YEPG-ERY+CAP (YEPG supplemented with 2mg erythromycin/ml and 2mg chloramphenicol/ml); minimal medium (0.67% Difco yeast nitrogen base without amino acids, 2% glucose); sporulation medium (0.05% glucose, 0.01% yeast extract, 1% potassium acetate). Two percent agar was added to make solid medium.

STRAINS: Table 1 lists all strains employed in this study.

ISOLATION OF ANTIBIOTIC RESISTANT MUTANTS: The sensitive strains used were unable to grow on a nonfermentable substrate, such as glycerol, in the presence of 0.5mg/ml chloramphenicol or 0.1mg/ml erythromycin, while resistant strains could grow on concentrations as high as 4mg/ml. Spontaneously arising resistance mutants were obtained by plating approximately 10^7-10^8 cells on a petri dish containing glycerol and 4mg/ml of antibiotic. Colonies which appeared were purified and tested for the location (mitochondrial or nuclear) of the resistance factors by mating mutants of opposite mating type possessing different resistance factors, sporulation of diploids and tetrad analysis.

TABLE 1

YEAST STRAINS

Strain	Nuclear Genotype	Mitochondrial Genotype	Origin
D587-4B	α his1	$C^S E^S$	F. Sherman
D587-4B-2	α his1	$C^S E^R$	Spontaneous Mutant of D587-4B
D587-4B-4	α his1	$C^R E^S$	" " "
D587-4B-7	α his1	$C^R E^S$	" " "
D587-4B-9	α his1	$C^R E^S$	" " "
E290	a his4 trp1	$C^S E^S$	G. Fink
E290-1	a his4 trp1	$C^S E^R$	Spontaneous Mutant of E290
E290-3	a his4 trp1	$C^R E^S$	" " "
D585-11C	a lys1	$C^S E^S$	F. Sherman
D585-11C-1	a lys1	$C^S E^R$	Spontaneous Mutant of D585-11C
D585-11C-2	a lys1	$C^S E^R$	" " "

TABLE 1 (cont.)

YEAST STRAINS

Strain	Nuclear	Mitochondrial Genotype	Origin
D585-11C-3	a lys1	$C^{R}E^{S}$	Spontaneous Mutant of D585-11C
1315-2C	α ade2 his1 ura	$C^{S}E^{S}$	D. Hawthorne
1315-2C-1	α ade2 his1 ura	$C^{S}E^{R}$	Spontaneous Mutant of 1315-2C
1315-2C-2	α ade2 his1 ura	$C^{R}E^{S}$	" " "
1381-17C	a ade8 trp1 ura1 gal	$C^{S}E^{S}$	D. Hawthorne
1381-17C-1	a ade8 trp1 ura1 gal	$C^{S}E^{R}$	Spontaneous Mutant of 1381-17C
N11C	α ade3 his lys ura	$C^{S}E^{S}$	N. Eaton
N11C-1	α ade3 his lys ura	$C^{S}E^{R}$	Spontaneous Mutant of N11C

TABLE 1 (cont.)

YEAST STRAINS

Strain	Nuclear Genotype	Mitochondrial Genotype	Origin
62-25A	a his lys	$C^{R}E^{S}$	D. Wilkie
ade7	α ade7	$C^{S}E^{S}$	H. Roman
M-6C	α ade2 ura3 leu	$C^{S}E^{S}$	" "
X2181-1A	a ade1 trp1 his2	$C^{S}E^{S}$	R. Mortimer
S1795A	a ade6 his4 trp5 ura1	$C^{S}E^{S}$	" "
D545-4A	α arg4-17 his5-2	$C^{S}E^{S}$	F. Sherman
D609-12A	α arg4-17 lys1-1	$C^{S}E^{S}$	" "
ade 5	α ade5 ura	$C^{S}E^{S}$	H. Roman
1323-1B	α lys1 ura	$C^{S}E^{S}$	D. Hawthorne

TABLE 1 (cont.)

YEAST STRAINS

Strain	Nuclear Genotype	Mitochondrial Genotype	Origin
MR-1A	∝ lys1	$C^{R}E^{S}$	Meiosis of D587-4B-4 X D585-11C-2
MR-1B	a lys1	$C^{R}E^{S}$	" " "
MR-1C	∝ his1	$C^{R}E^{S}$	" " "
MR-1D	a his1	$C^{R}E^{S}$	" " "
MR-17A	∝ his trp1	$C^{S}E^{R}$	Meiosis of D587-4B-2 X E290-3
MR-17B	a his	$C^{S}E^{R}$	" " "
MR-17C	∝ trp1	$C^{S}E^{R}$	" " "
MR-17D	a his	$C^{S}E^{R}$	" " "
MR-18A	∝ his trp1	$C^{S}E^{R}$	" " "
MR-18B	a his	$C^{S}E^{R}$	" " "

TABLE 1 (cont.)

YEAST STRAINS

Strain	Nuclear Genotype	Mitochondrial Genotype	Origin
MR-18C	a his	$C^{S}E^{R}$	Meiosis of D587-4B-2 X E290-3
MR-18D	∝ trp1	$C^{S}E^{R}$	" " "
MR-19A	a his trp1	$C^{S}E^{R}$	" " "
MR-19B	∝ his trp1	$C^{S}E^{R}$	" " "
MR-19C	∝ his	$C^{S}E^{R}$	" " "
MR-19D	a his	$C^{S}E^{R}$	" " "
Neutral Petite Mutants			
D587-4B-2np	∝ his1	$C^{S}E^{O}$	Ethidium Bromide Induced Mutant
E290-3np	a his4 trp1	$C^{O}E^{S}$	" " "

TABLE 1 (cont.)

YEAST STRAINS

Strain	Nuclear Genotype	Mitochondrial Genotype	Origin
Suppressive Petite Mutants			
D587-4B-2sp$_1$	α his1	$C^S E^O$	Spontaneous Mutant of D587-4B-2
D587-4B-2sp$_2$	α his1	$C^S E^O$	" " "
D587-4B-2sp$_3$	α his1	$C^S E^O$	" " "
D587-4B-2sp$_4$	α his1	$C^S E^O$	" " "
D587-4B-2sp$_5$	α his1	$C^S E^O$	" " "
E290-3sp$_2$	a his4 trp1	$C^O E^S$	Spontaneous Mutant of E290-3
E290-3sp$_3$	a his4 trp1	$C^O E^S$	" " "
E290-3sp$_4$	a his4 trp1	$C^O E^S$	" " "
E290-3sp$_5$	a his4 trp1	$C^O E^S$	" " "
E290-3sp$_6$	a his4 trp1	$C^O E^S$	" " "

TABLE 1 (cont.)

YEAST STRAINS

Strain	Nuclear Genotype	Mitochondrial Genotype	Origin
MR-19Asp_1	a his trp1	$C^S E^O$	UV Induced Mutant of MR-19A
MR-19Bsp_2	α his trp1	$C^S E^O$	" " " "
MR-19Csp_8	α his	$C^S E^O$	" " " "
MR-19Dsp_4	a his	$C^S E^O$	" " " "
SPT-2A	α his trp1	$C^S E^O$	Meiosis of D587-4B-2sp_2 X E290-3
SPT-2B	a his	$C^S E^O$	" " "
SPT-2C	α his trp1	$C^S E^O$	" " "
SPT-2D	a his	$C^S E^O$	" " "
Omega Factor Bearing Strains			
D22	a ade2	$C^S E^S \omega^+$	D. Wilkie
D22-1	a ade 2	$C^S E^R \omega^+$	Spontaneous Mutant of D22

TABLE 1 (cont.)

YEAST STRAINS

Strain	Nuclear Genotype	Mitochondrial Genotype	Origin
Omega Factor Bearing Strains (cont.)			
D6	α arg met	$C^{S}E^{S}\omega^{+}$	D. Wilkie
D6-2	α arg met	$C^{S}E^{R}\omega^{+}$	Spontaneous Mutant of D6
D6-3	α arg met	$C^{R}E^{S}\omega^{+}$	" " "
55R5-3C	a ura	$C^{S}E^{R}221\omega^{-}$	P. P. Slonimski
IL16-10B	his	$C^{R}_{321}E^{R}\omega^{-}$	" "

All mutants isolated were stable with respect to their antibiotic resistance. Standard techniques of crossing, sporulation and tetrad analysis were used (Mortimer & Hawthorne; 1969).

ANALYSIS OF RANDOM DIPLOIDS: To obtain zygotes, cells of each strain were grown for 16 hours in YEPD with aeration at 30^0C. Samples of each mating pair were mixed in fresh YEPD, pelleted by centrifugation and allowed to mate for 5 hours. The supernatant was was decanted and the cells washed with sterile distilled water. Samples of the mating mixture were inoculated into a minimal medium to select for diploids. The cultures were then incubated for 24 hours, after which they were plated on solid minimal medium and again incubated for 48 hours. Colonies that grew were scored for drug resistance or sensitivity by replicating onto plates containing the antibiotics.

ZYGOTE AND ZYGOTE LINEAGE ANALYSIS: Samples of cultures in midlog phase were transferred to opposite ends of an agar slab containing 0.1% yeast extract, 0.2% peptone and 0.2% glucose. Haploid cells of each parent were micromanipulated so that they touched each other. For zygote lineage analysis buds were removed from the zygote. After micromanipulation the agar slab was placed on a solid synthetic minimal medium so that only diploid cells would grow. After 48 hours, clones arising from zygotes and their buds were suspended, counted, plated on synthetic minimal medium and incubated for 48 hours. The

plates were replicated to drug containing media to assay for resistance and/or sensitivity of the clones. In cases where clones showed complete sensitivity to a drug or drugs, the original colony was further tested by plating the remaining cells (between 10^5 and 10^6 cells) on plates containing the drug; even a small proportion of resistant cells would be detected by the second test.

TREATMENT OF ZYGOTES WITH ANTIBIOTICS: Young zygotes were transferred by micromanipulation to media containing cycloheximide 1μg/ml) or thiolutin (20μg/ml) for varying times and then removed with a micromanipulator. Cells treated with the antibacterial antibiotics erythromycin, chloramphenicol or paromomycin, were grown, mated and cloned in the presence of the antibiotic(s).

ISOLATION OF SUPPRESSIVE PETITE MUTANTS AND DETERMINATION OF THEIR SUPPRESSIVENESS: Approximately 200 cells were spread over the surface of YEPD plates. After 3 days the respiratory capacity of the colonies was determined using the tetrazolium-agar overlay method of Ogur et al, (1957). This straining method distinguishes three colony types: respiratory sufficient, (grande), respiratory deficient (petite) and sectored colonies consisting of both grande and petite cells. Colonies exhibiting the petite phenotype were purified. The suppressiveness of these petites was determined either by mass mating tests to grande strains or by zygote clone analysis in which petite cells and grande cells were paired by micromanipulation, zygotes

isolated and cloned and the diploid progeny analyzed as discussed above (Zygote and Zygote lineage Analysis). The percent suppressiveness (%S) of a petite strain is:

$$\%S = \frac{X - Y}{1 - Y} \times 100$$

where X is the fraction of petite colonies produced by the zygote(s) and Y is the fraction of petite cells in the normal haploid tester strain.

ISOLATION OF NEUTRAL PETITE MUTANTS: Cells were grown overnight in YEPD containing 10μg/ml ethidium bromide. The treated cells were then spread on the surface of YEPD plates, cloned and analyzed by use of the tetrazolium staining method. The neutrality of the petite mutants was determined by the same methods employed in determining the suppressiveness of suppressive petites discussed above.

ANTIBIOTICS: Concentrated stock solutions of chloramphenicol (Park Davis), erythromycin (Eli Lilly), paromomycin (Park Davis), cycloheximide (Calbiochem) and thiolutin (Pfizer) in ethanol were diluted in media as required, the final ethanol concentration never exceeding 3% $^{v}/_{v}$.

CHAPTER III

RANDOM DIPLOID, WHOLE ZYGOTE CLONE AND LINEAGE ANALYSIS

Crosses were made between various strains carrying the mitochondrial resistance factors, E^R (erythromycin resistance) or C^R (chloramphenicol resistance). Newly formed zygotes were isolated and cloned on solid minimal medium, and the individual diploid clones were sampled and plated. The resulting colonies were scored for mitochondrial phenotype as described in the section on methods. The results are shown in Table 2. In 5 of the crosses there is a consistent pattern of inheritance of mitochondrial types. These crosses all involved mitochondrial mutants isolated from the parental strain D587-4B. Whole zygote clones showed a preponderance of cells which inherited the D587-4B mitochondrial phenotype. This asymmetrical distribution was also seen when random diploids were sampled from mass mating mixtures (Table 3). It is not clear from these results whether there is also an asymmetrical distribution of recombinant types, which in any case, appear at a low frequency. In the three crosses not involving the D587-4B strain no consistent pattern of inheritance is observed.

The asymmetrical distribution of parental and/or recombinant classes is not a new finding, as already discussed in the introduction. The problem is to elucidate the possible mechanisms controlling the

TABLE 2

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples				Colonies Scored
		C^RE^S	C^SE^R	C^RE^R	C^SE^S	
D587-4B-2(C^SE^R) X E290-3(C^RE^S)	1		1.00			633
	2		1.00			473
	3		1.00			425
	4	.182	.747	.071		673
	5	.118	.862	.020		473
	6	.100	.900			732
	7	.170	.716	.110	.004	522
	8	.020	.904	.076		393
	9		1.00			399
	10	.242	.734	.020	.004	760
	Mean	.083	.886	.030	.001	5,513
D587-4B-4(C^RE^S) X E290-1(C^SE^R)	1	.793	.163		.044	540
	2	.974	.024		.002	587
	3	.920	.080			724
	Mean	.896	.089		.015	1,851

TABLE 2 (continued)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples				
		C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
D587-4B-4(C^RE^S) X 1381-17C-1(C^SE^R)	1	.693	.302		.005	191
	2	.672	.136	.149	.043	235
	3	.851	.121	.018	.010	322
	Mean	.739	.186	.056	.019	748
D587-4B-4(C^RE^S) X D585-11C-2(C^SE^R)	1	.734	.188	.031	.047	128
	2	1.00				330
	3	.867	.044	.035	.054	203
	Mean	.867	.077	.022	.034	661
D587-4B-9(C^RE^S) X E290-1(C^SE^R)	1	.820	.136	.040	.004	543
	2	1.00				740
	3	.802	.190	.005	.003	407
	Mean	.874	.109	.015	.002	1,690

TABLE 2 (continued)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples					
		C^RE^S	C^SE^R	C^RE^R	C^SE^S	Petite	Colonies Scored
1315-2C-2(C^RE^S) X D585-11C-2(C^SE^R)	1	.087	.891	.015	.007		275
	2	.534	.466				554
	3		1.00				382
	Mean	.207	.786	.005	.002		1,211
1323-1B(C^SE^S) X D585-11C-2(C^SE^R)	1		.609		.391		432
	2		.453		.547		377
	3		.595		.405		394
	Mean		.552		.448		1,203
1315-2C-2(C^RE^S) X 1381-17C-1(C^SE^R)	1	.246	.150	.041	.120	.443	440
	2	.401	.151	.002	.006	.440	655
	3	.955				.045	467
	Mean	.534	.100	.014	.042	.309	1,562

TABLE 3

DISTRIBUTION OF MITOCHONDRIAL TYPES IN RANDOM DIPLOIDS

Cross	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Petite	Colonies Scored
D587-4B-2(C^SE^R) X E290-3(C^RE^S)	.117	.855	.020	.010		955
D587-4B-4(C^RE^S) X 1381-17C-1(C^SE^R)	.971	.027	.002			453
D587-4B-4(C^RE^S) X D585-11C-2(C^SE^R)	1.00					
D587-4B-9(C^RE^S) X E290-1(C^SE^R)	.883	.074	.019	.024		674
1315-2C-2(C^RE^S) X D585-11C-2(C^SE^R)	.353	.617	.012	.018		170
1323-1B(C^SE^S) X D585-11C-2(C^SE^R)		.641		.359		427
1315-2C-2(C^RE^S) X 1381-17C-1(C^SE^R)	.284	.423	.019	.036	.238	507

transmission of mitochondria. For this purpose zygote lineage analysis was undertaken of the crosses which showed a consistent pattern of inheritance. Table 4 shows the results of the lineage analysis of zygotes and zygote daughter cells from the cross D587-4B-2 X E290-3. A striking and repeating pattern of transmission of mitochondrial factors to zygote daughter cells is seen. In the six zygote lineages analyzed, the first bud from each zygote inherited more or less exclusively the E^R marker contributed by the D587-4B-2 parent. The second bud from each zygote inherited the other parental mitochondrial type (C^R). Bud 3 and subsequent buds tended to inherit predominantly E^R. Three zygote lineages showed a tendency for the proportion of recombinants to increase in the later buds, while the three other lineages analyzed this tendency was not evident. The preponderance of E^R can be attributed to the transmission of this mitochondrial type almost exclusively to the first and third buds. In zygote lineage experiments, zygotes were formed following the placing of the two parental cells together by micromanipulation. In almost all zygotes analyzed the first bud arose from a central position as shown in figure 1, although in rare instances the first bud arose from a terminal position (see fig. 1D). The second bud always arose from a terminal position, which could however be derived from either the a or α parent. In some cases, therefore, the second bud inherited the C^R marker from that part of the zygote contributed by the E^R parental cell. The third and subsequent buds

TABLE 4

DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE CELL LINEAGED FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Clone Source	Frequencies of Mitochondrial Types in Cell Samples				Colonies Scored
	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	
ZYGOTE 1 (Fig. 1A)					
Bud 1		1.00			464
Bud 2	.9997	.0003			772
Bud 3		1.00			507
Bud 4		1.00			1,437
Bud 5		.596	.359	.045	1,460
Bud 6 and subsequent	.635	.008	.032	.325	379
ZYGOTE 2 (Fig. 1B)					
Bud 1		1.00			579
Bud 2	.685	.015	.256	.044	529
Bud 3		1.00			508
Bud 4		1.00			578
Bud 5		1.00			630
Bud 6 and subsequent		1.00			495

TABLE 4 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE CELL LINEAGES FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Clone Source	Frequencies of Mitochondrial Types in Cell Samples				Colonies Scored
	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	
ZYGOTE 3 (Fig. 1C)					
Bud 1	.176	.733	.064	.027	408
Bud 2	1.00				488
Bud 3		.996	.034		490
Bud 4		1.00			532
Bud 5		1.00			478
Bud 6 and Subsequent	.00005	.99995			1,296
ZYGOTE 4					
Bud 1	.004	.996			466
Bud 2	.984	.016			504
Bud 3		1.00			533
Bud 4 and subsequent	.005	.922	.068	.005	366

TABLE 4 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE CELL LINEAGES FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Clone Source	Frequencies of Mitochondrial Types in Cell Samples				Colonies Scored
	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	
ZYGOTE 5 (Fig. 1D)					
Bud 1		1.00			694
Bud *					
Bud 3	.004	.996			684
Bud 4	.077	.542	.377	.004	273
Bud 5		1.00			518
Bud 6 and subsequent	.189	.691	.120		535
ZYGOTE 6 (Fig. 1E)					
Bud 1		1.00			652
Bud 2	1.00				613
Bud 3		1.00			441
Bud 4		1.00			751
Bud 5		1.00			557
Bud 6 and subsequent		1.00			479

*Bud died

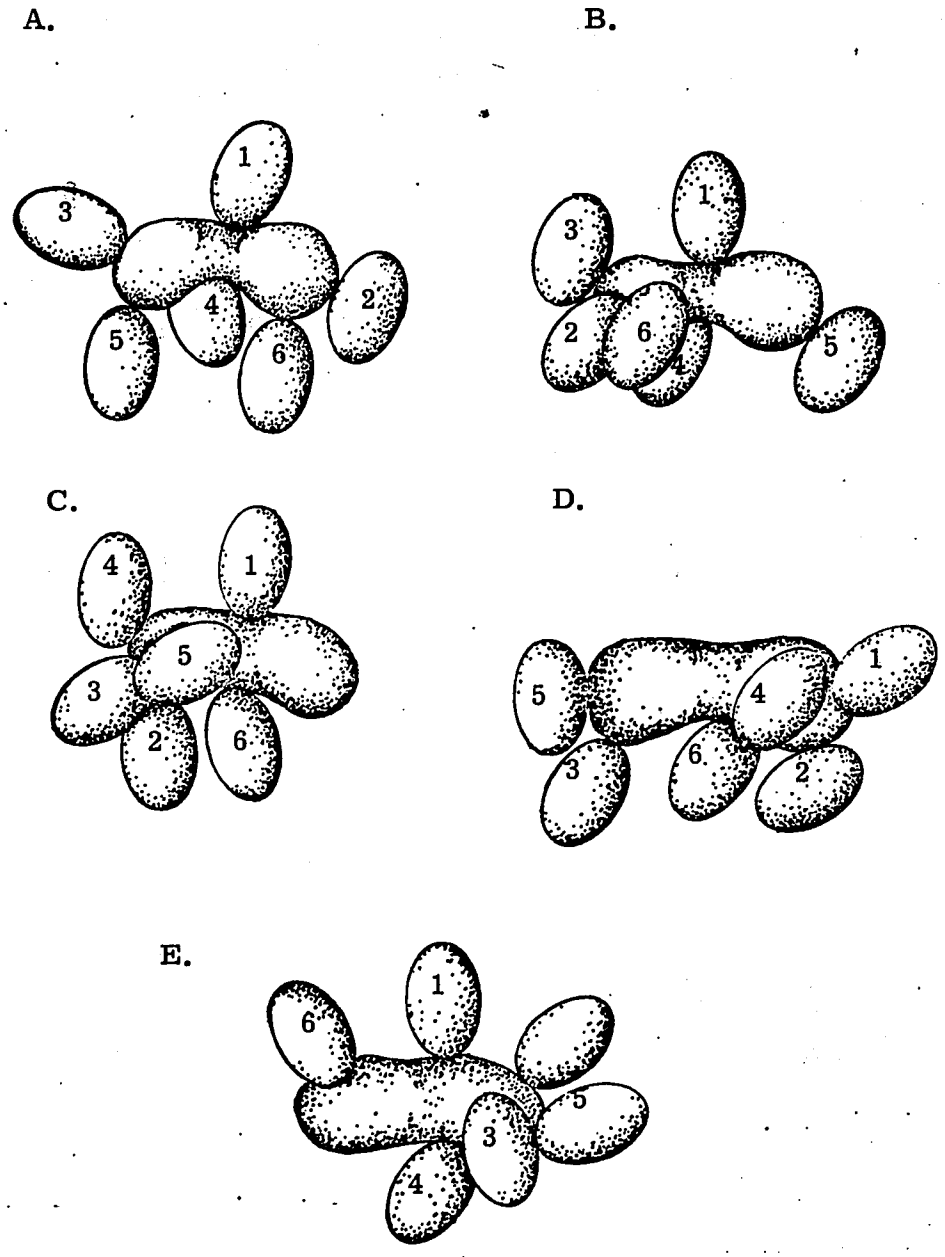

Fig. 1.--Untreated zygotes and zygote daughter cells from the cross D587-4B-2 ($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$).

arise in almost any position, and in some instances the third and subsequent buds arise from the same end of the zygote (see fig. 1C).

Figure 2 represents an extensive lineage analysis of the cross D587-4B-2 X E290-3, the data from which are given in Table 5. The lineage of Bud 1 showed an almost exclusive inheritance of E^R; only the progeny of Bud 1I revealed a different inheritance. Bud 3 and its progeny inherited exclusively E^R. The lineage analysis of Bud 4 showed a heterogeneous pattern of inheritance. Bud 4 and its decendent buds (excluding Buds 4A through 4F) inherited only C^SE^S, but the lineage analysis indicated that Bud 4 must have inherited both parental types from the zygote. Apparently the parental mitochondrial types were preferentially segregated to the earlier buds of Bud 4, while the double sensitive recombinant class was preferentially retained by Bud 4. Of Buds 4D, 4E and 4F, only Bud 4E is not petite and could be analyzed with respect to its mitochondrial inheritance. The progeny of Bud 4E are composed of E^R and C^RE^R, indicating that Bud 4A must have been initially heterogeneous with respect to its mitochondrial population, i.e., C^RE^R or C^R. Bud 5 and its descendants are also heterogeneous, since all mitochondrial classes are observed. Bud 6 and its progeny are exclusively E^R and C^RE^R, while Bud 7 and its progeny inherited all classes of mitochondrial types except E^R. Bud 8 and its progeny inherited predominantly C^SE^S and E^R mitochondrial types. Bud 9 and subsequent buds (zygote and

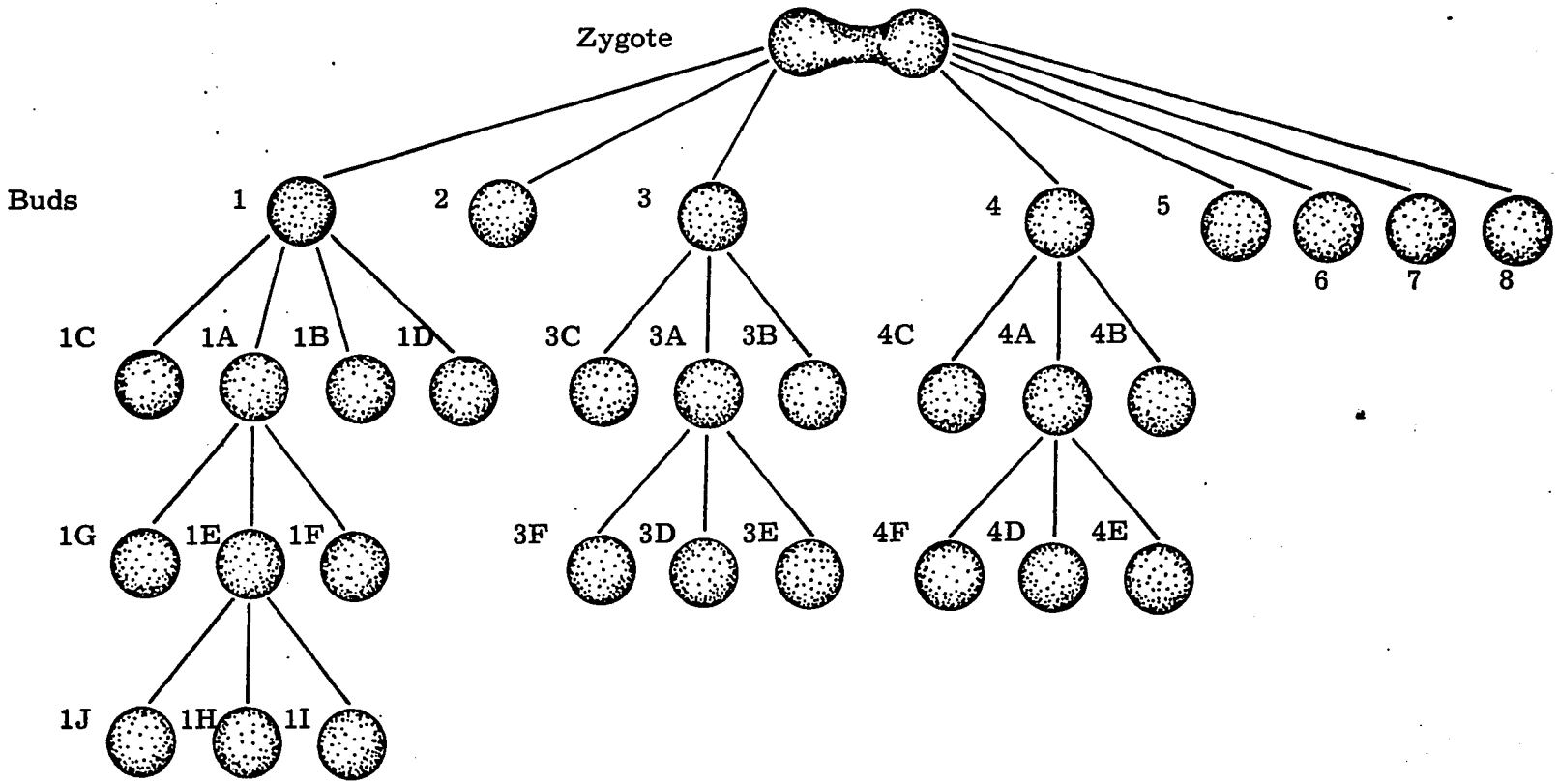

Figure 2. An extensive lineage analysis of the cross D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

TABLE 5

DISTRIBUTION OF MITOCHONDRIAL TYPES IN AN EXTENSIVE ZYGOTE LINEAGE ANALYSIS OF THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Frequencies of Mitochondrial Types in Cell Samples						
Clone Source	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Petite	Colonies Scored
Bud 1		1.00				429
Bud 1A		1.00				494
Bud 1B		1.00				397
Bud 1C		1.00				398
Bud 1D		1.00				537
Bud 1E					1.00	529
Bud 1F		1.00				443
Bud 1G		1.00				286
Bud 1H					1.00	630
Bud 1I	.030			.970		278
Bud 1J					1.00	417
Bud 2*						
Bud 3		1.00				538
Bud 3A		1.00				1,326
Bud 3B		1.00				507
Bud 3C*						
Bud 3D		1.00				645
Bud 3E		1.00				627
Bud 3F		1.00				627

*Bud died

TABLE 5 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN AN EXTENSIVE ZYGOTE LINEAGE ANALYSIS OF THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Clone Source	Frequencies of Mitochondrial Types in Cell Samples					
	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Petite	Colonies Scored
Bud 4				1.00		622
Bud 4A		1.00				374
Bud 4B	.268	.238		.494		459
Bud 4C	.051			.949		355
Bud 4D					1.00	408
Bud 4E		.897	.103			350
Bud 4F					1.00	541
Bud 5	.480	.099	.55	.266		394
Bud 6		.656	.344			546
Bud 7	.318		.121	.561		478
Bud 8		.171		.829		473
Bud 9 and subsequent	.068	.013	.221	.698		542

ninth and subsequent buds) inherited all classes of mitochondrial types.

This analysis is consistent with the limited analysis shown in Table 4. Bud 1 and Bud 3 apparently inherited predominantly the E^R parental type. The progeny of Bud 4 and Bud 5 are heterogeneous (Table 4). Since the progeny of the first 3 buds of the zygote account for 87.5 percent of the clone the inheritance of these buds is very significant. It can be seen from this lineage that parental mitochondrial types are segregated to the earlier buds, while the later buds received a heterogeneous population of mitochondrial genomes. There is also a trend toward an increase in the number of recombinant genomes segregated to the later buds.

CHAPTER IV

THE EFFECTS OF DRUGS ON THE TRANSMISSION OF MITOCHONDRIAL FACTORS

To check the possible involvement of cytoplasmic protein synthesis with the control mechanism responsible for this consistent asymmetrical distribution, zygotes were treated with cycloheximide for various times after which the mitochondrial types of their progeny were determined. It is clear (Table 6) that the duration of treatment with CHI is critical. Treatment of zygotes for 30, 60, 120 and 150 minutes did not significantly alter the pattern of inheritance in this cross. Whereas, 90 minutes exposure significantly and consistently altered this pattern i. e. the otherwise preferential transmission of E^R was abolished, as well as the normally asymmetrical distribution of recombinant classes. Assuming that CHI inhibits protein synthesis on cytoplasmic ribosomes, the conclusion that protein (s) normally synthesized in the cytoplasm is responsible for the asymmetrical distribution of mitochondrial types seems unavoidable. To gain more information about the time and sequence of events within zygotes leading to the control of the transmission of mitochondrial types to buds, zygotes were allowed to mature for various times prior to exposure to CHI. The results (Table 7) show that 30 minutes maturation prior to 60 minutes of CHI consistently altered the mitochondrial inheritance of zygote daughter cells, whereas, 60 or 90 minutes maturation prior to CHI treatment caused no significant alteration. These results suggest

TABLE 6

THE EFFECTS OF CYCLOHEXIMIDE TREATMENT ON THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D587-4B-2 (C^SE^R) X E290-3(C^RE^S)

Frequencies of Mitochondrial Types in Cell Samples						
Time (min.)	Zygote	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
30	1		1.00			468
30	2	.252	.733	.005	.010	1,103
30	3	.294	.703		.003	605
Mean		.182	.812	.002	.004	2,176
60	1	.120	.856	.018	.006	619
60	2		1.00			662
60	3	.220	.720		.060	642
Mean		.113	.859	.006	.022	1,923
90	1	.800	.040	.005	.155	640
90	2	.273	.445	.191	.091	596
90	3	.323	.676	.001		648
90	4	.600	.380	.001	.019	791
Mean		.499	.385	.050	.066	2,675
120	1		.909	.005	.086	635
120	2		1.00			563
120	3		1.00			521
120	4		1.00			531
Mean			.977	.001	.022	2,250
150	1	.052	.940	.003	.005	629
150	2		1.00			633
150	3	.064	.849	.076	.011	738
Mean		.039	.930	.026	.005	2,000
Mean values for untreated zygotes (c.f. Table 3)		.083	.886	.030	.001	5,513

TABLE 7

THE EFFECTS OF VARYING THE MATURATION TIME OF YOUNG ZYGOTES PRIOR TO TREATMENT WITH CYCLOHEXIMIDE FOR 60 MIN. ON THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS

D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Maturation Time (min.)	Zygote	Frequencies of Mitochondrial Types in Cell Samples $C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
30	1	.401	.403	.102	.094	362
30	2	.394	.584	.002	.020	409
30	3	.514	.484		.002	449
Mean		.436	.490	.035	.039	1,220
60	1	.009	.977	.014		441
60	2	.191	.788	.005	.016	434
Mean		.100	.882	.010	.008	875
90	1		.989	.011		351
90	2	.170	.759	.066	.005	423
90	3		1.00			365
Mean		.057	.915	.026	.002	1,139
Mean values for untreated zygotes (c.f. Table 3)		.083	.886	.030	.001	5,513

that sometime between 30 and 60 minutes after mating cytoplasmic protein synthesis becomes necessary for the control mechanism responsible for the asymmetrical distribution. The effects of inhibiting protein synthesis during this time are apparently reversible since zygotes treated for 60 minutes with CHI show no change in the inheritance pattern. Therefore the events occurring in the zygote between 30 and 60 minutes may be necessary for the initiation of the events which occur between 60 and 90 minutes, these events appear to be irreversibly blocked by CHI. A visible effect of CHI treatment is the change in the position of the first bud. The first bud is usually initiated centrally on the fusion bridge between the two mated cells; in CHI treated zygotes the first bud forms only at the a or α ends of the zygotes; subsequent buds can initiate at any point on CHI treated zygotes, as shown in figure 3.

To answer the question of the mechanism by which CHI affects the inheritance of the zygote and its buds, zygote lineage analysis was undertaken. There are two alternatives; 1) CHI randomizes the distribution of mitochondrial types present in the zygote or 2) CHI specifically alters the normal asymmetry. The results are shown in Table 8. These results indicate that the second possibility is correct: there is no randomization of mitochondrial types distributed to buds, but, instead, a change in the inheritance of the first bud. After CHI treatment the first bud now inherits exclusively C^R.

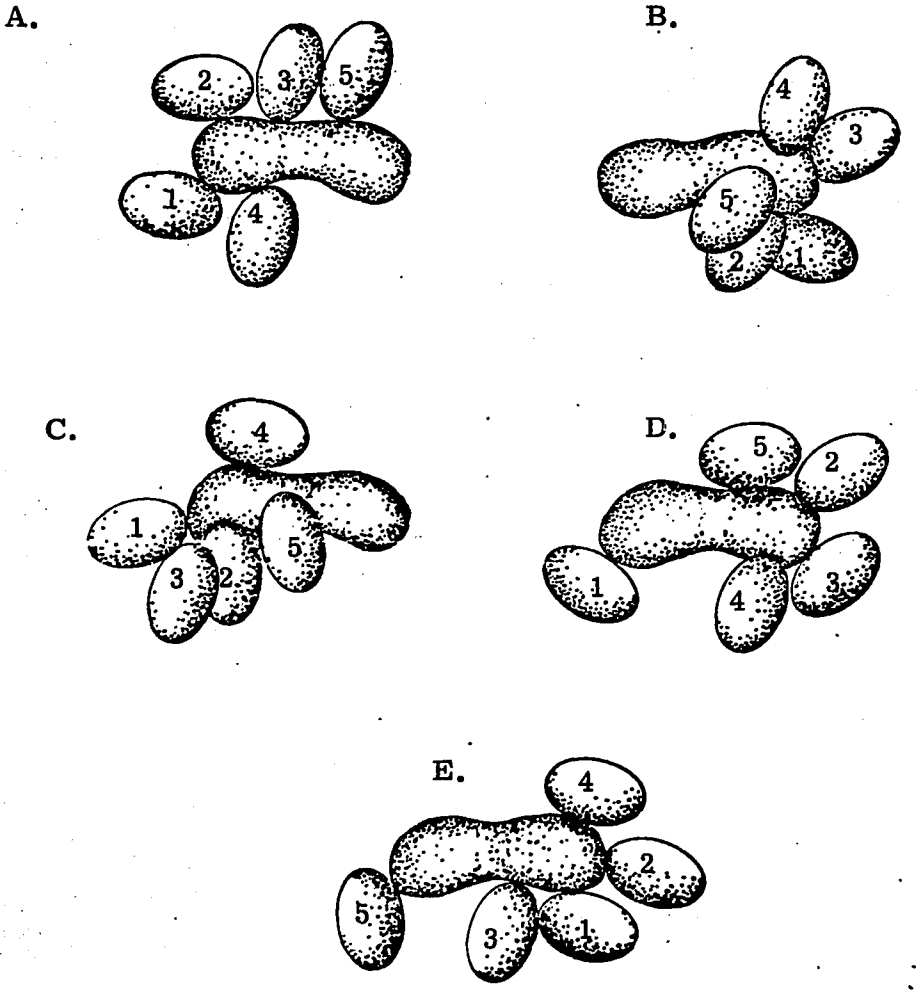

Fig. 3. Cycloheximide treated zygotes and zygote daughter cells from the cross D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$).

TABLE 8

DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE CELL LINEAGES AFTER 90 MIN. TREATMENT OF YOUNG ZYGOTES WITH CYCLOHEXIMIDE (D587-4B-2(C^SE^R) X E290-3(C^RE^S)

	Frequencies of Mitochondrial Types in Cell Samples					
Clone Source	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Petite	Colonies Scored
ZYGOTE 1						
Bud 1	1.00					593
Bud 2	.942	.002	.054	.002		589
Bud 3		1.00				586
Bud 4		1.00				644
Bud 5		1.00				692
Bud 6 and subsequent		1.00				577
ZYGOTE 2						
Bud 1	1.00					689
Bud 2	.880	.036	.002	.082		503
Bud 3	.098	.004	.002	.304	.592	552
Bud 4		.776			.224	496
Bud 5		1.00				453
Bud 6 and subsequent		1.00				637
ZYGOTE 3						
Bud 1	1.00					621
Bud 2		1.00				642
Bud 3		.992	.008			611
Bud 4 and subsequent	.091	.830	.079			971

In two of the lineages analyzed (Zygote 1 and Zygote 2) Bud 2 received a preponderance of C^R, while Bud 2 of the lineage of Zygote 3 received exclusively E^R. In the lineage of Zygote 2, the inheritance of Bud 3 has changed in comparison to untreated zygote lineages. The majority of the "grande" descendants of Bud 3 are double sensitive (C^SE^S), although the majority of the clone is "petite." In the lineages analyzed the later buds (Buds 4 and 5) show a distribution of mitochondrial types similar to the later buds of untreated zygotes.

The change in the inheritance of the mitochondrial phenotype of Bud 1 appears to be responsible to a large extent for the apparent randomized pattern of inheritance observed in the whole zygote clones treated with CHI for 90 minutes. Therefore an interference with cytoplasmic protein synthesis for 90 minutes after mating appears to alter the pattern of inheritance through its effect on Bud 1.

The drug thiolutin has been shown to inhibit the activity of nuclear RNA polymerases in yeast (Jimenez et al, 1973) and to arrest DNA transcription at a concentration as low as 2μg/ml. In the strains employed in this study thiolutin stopped growth in liquid medium at a concentration of 20μg/ml. Presumably, this is the concentration required to block transcription in our strains. When newly formed zygotes were transferred to a medium containing 20μg/ml of thiolutin, budding was prevented, similar to CHI treatment. The results obtained with thiolutin (Table 9) were strikingly similar

TABLE 9

EFFECTS OF DURATION OF THIOLUTIN TREATMENT ON THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D587-4B-2(C^SE^R) X E290-3(C^RE^S)

Frequencies of Mitochondrial Types in Cell Samples						
Time (min.)	Zygote	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
60	1	.136	.815	.049		617
90	1	.764	.148	.041	.047	492
90	2	.312	.585	.010	.093	497
90	3	1.00				873
Mean		.692	.244	.017	.047	1,862
120	1	.036	.950	.011	.003	727
150	1		.993	.007		431
Mean values for untreated zygotes (c.f. Table 3)		.083	.886	.030	.001	5,513

to those CHI. It can be seen that a 60 minutes treatment did not alter the inheritance of mitochondrial types in the zygote clones, while treatment for 90 minutes significantly altered this pattern: the majority of the descendants of the three zygotes analyzed showed an increase in the inheritance of C^R. As with zygotes treated with CHI, 120 or 150 minutes treatment with thiolutin had no effect on the asymmetry observed in untreated zygotes. The effects of the drug may be seen only with 90 minutes treatment and increasing the duration of treatment does not alter the pattern of asymmetry. Ninety minutes treatment may be critical in the sense that this is the time after zygote inception that the first bud is normally initiated under our condition. Somehow interfering with the initiation of the first bud at this time and changing its position may be in part responsible for the alteration of the pattern of inheritance. Therefore blocking either transcription (thiolutin) or translation (CHI) of nuclear products alters the sequence of events which normally results in an asymmetrical distribution of mitochondrial types.

The possible effects of inhibiting mitochondrial protein synthesis on mitochondrial inheritance were also investigated. D587-4B-2 and E290-3 were grown in liquid YEPD containing 200µg/ml paromomycin, an antibiotic which specifically blocks protein synthesis in yeast mitochondria (Wilkie, 1970). A concentration of 200µg/ml of this antibiotic totally inhibits growth of both strains on a non-fermentable substrate.

After growth on YEPD containing paromomycin for 18 hours the cells were mated and the zygotes were isolated and cloned. All operations were carried out on YEPD paromomycin. The distribution of mitochondrial factors are shown in Table 10. Inhibiting mitochondrial protein synthesis prior to mating, during mating and subsequent to mating, has apparently no effect on the asymmetrical distribution of mitochondria.

The effects of inhibiting selectively the protein synthetic capacity of the mitochondria of one of the mating pairs was investigated. Both strains were grown, mated and the diploids cloned in the presence of 4mg/ml of either erythromycin or chloramphenicol in YEPD one of which inhibits each of the parents. The results are shown in Tables 11 and 12. Table 11 shows that the asymmetry of mitochondrial types is accentuated; there is an increase in the number of diploid decendants inheriting the E^R factor and a concomitant decrease in the cells inheriting the C^R factor. This effect is expected since 4mg/ml erythromycin severly restricts mitochondrial protein synthesis in sensitive cells (E290-3). Therefore sensitive mitochondria would be non-functional in both energy metabolism and the synthesis of protein. Cells inheriting these mitochondria would not grow (after the glucose was exhausted) while cells receiving E^R mitochondria would increase in numbers and the frequency of E^R cells should increase in the zygote clone. The effects of chloramphenicol in such an experiment (Table 12)

TABLE 10

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES CROWN, MATED AND CLONED IN THE PRESENCE OF PAROMOMYCIN (200μg/ml) (D587-4B-2 X E290-3)

	Frequencies of Mitochondrial Types in Cell Samples				
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1		.998	.002		612
2	.283	.682	.020	.015	594
3	.038	.899	.063		682
Mean	.107	.860	.028	.005	1,888
Mean values for untreated zygotes (c.f. Table 3)	.083	.886	.030	.001	5,513

TABLE 11

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES GROWN, MATED AND CLONED IN THE PRESENCE OF OF ERYTHROMYCIN (4mg/ml) (D587-4B-2 X E290-3)

	Frequencies of Mitochondrial Types in Cell Samples				
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1		.959	.041		681
2	.002	.991	.005	.002	552
3		.982	.018		707
Mean	.001	.977	.021	.001	1,940
Mean values for untreated zygotes (c.f. Table 3)	.083	.886	.030	.001	5,513

TABLE 12

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES GROWN, MATED AND CLONED IN THE PRESENCE OF CHLORAMPHENICOL (4mg/ml) (D587-4B-2 X E290-3)

	Frequencies of Mitochondrial Types in Cell Samples				
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1	.522	.056	.416	.006	356
2	.004	.121	.875		536
3	.876	.113	.011		557
Mean	.467	.097	.434	.002	1,449
Mean values for untreated zygotes (c.f. Table 3)	.083	.886	.030	.001	5,513

show that, as in the presence of erythromycin, cells possessing a resistant phenotype (C^R) would be able to proliferate after release from glucose repression, while cells not possessing a resistant genome could not respire and would not grow. Therefore there would be an increase in the frequency of C^R cells in the clone.

The effects of both erythromycin and chloramphenicol on the distribution of mitochondrial types to zygote daughter cells was investigated. The results in Table 13 show that when both mitochondrial populations (C^RE^S and C^SE^R) are inhibited and there is no selection for a mitochondrial type, the asymmetrical distribution observed in untreated zygote clones is seen. The results of the paromomycin and the erythromycin + chloramphenicol experiments show that mitochondrial protein synthesis apparently plays little or no part in the transmission of mitochondrial types and probably in the recombination of M-DNA in strains employed in this study.

TABLE 13

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES GROWN, MATED AND CLONED IN THE PRESENCE OF BOTH ERYTHROMYCIN AND CHLORAMPHENICOL (4mg/ml) (D587-4B-2 X E290-3)

	Frequencies of Mitochondrial Types in Cell Samples					
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Petite	Colonies Scored
1	.018	.946	.003		.033	958
2	.029	.889	.003	.012	.067	646
3		.953			.047	617
Mean	.016	.929	.002	.004	.049	2,221
Mean values for untreated zygotes (c.f. Table 3)	.083	.886	.030	.001		5,513

CHAPTER V

THE EFFECTS OF REPRESSION AND DEREPRESSION ON THE TRANSMISSION OF MITOCHONDRIA

It is well documented that mitochondrial function is subject to repression. When cells are grown anaerobically or in a high concentration of glucose, they develop little respiratory activity and contain greatly reduced amounts of mitochondrial enzymes. An attempt to ascertain the effects of glucose repression on mitochondrial activity and on the distribution of mitochondria to zygote daughter cells was undertaken. Strain D587-4B-2 was grown in YEP-10% glucose (a repressing medium) prior to mating, whereas, strain E290-3 was grown in YEPG (a non-repressing medium) prior to mating. The cells of both strains were micromanipulated, mated and zygotes isolated and cloned. The results of such an analysis are shown in Table 14. It is clear from the data that growth in a repressing medium prior to mating does alter the distribution of mitochondrial types, but that this alteration is not as consistent or as dramatic as treatment of zygotes with CHI. Zygote 1 and Zygote 4 show frequencies of mitochondrial types similar to untreated zygotes. To obtain more information on the effects of glucose repression, zygote lineage analysis was studied. It is clear from the results shown in Table 15, that the selective repression and derepression of mating partners prior to mating does have effects on the distribution of

TABLE 14

THE EFFECTS OF GLUCOSE REPRESSION AND DEREPRESSION ON THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D587-4B-2(GROWN ON 10% GLUCOSE) X E290-3 (GROWN ON GLYCEROL)

Frequencies of Mitochondrial Types in Cell Samples					
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1	.109	.757	.007	.127	614
2	.369	.550	.081		409
3	.392	.561	.047		556
4	.150	.828		.022	559
Mean	.255	.674	.034	.037	2,138
Mean values for untreated zygotes (c.f. Table 3)	.083	.886	.030	.001	5,513

TABLE 15

THE EFFECTS OF GLUCOSE REPRESSION AND DEREPRESSION ON THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE CELL LINEAGES FROM THE CROSS D587-4B-2(GROWN ON 10% GLUCOSE) X E290-3 (GROWN ON GLYCEROL)

	Frequencies of Mitochondrial Types in Cell Samples				
Clone Source	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
ZYGOTE 1					
Bud 1	.030	.660	.270	.040	431
Bud 2	1.00				881
Bud 3	.720	.020	.050	.210	369
Bud 4	.997			.003	361
Bud 5	.620	.005	.002	.371	440
Bud 6 and subsequent		1.00			536
ZYGOTE 2					
Bud 1				1.00	592
Bud 2				1.00	643
Bud 3				1.00	525
Bud 4				1.00	483
Bud 5				1.00	571
Bud 6 and subsequent				1.00	536

TABLE 15 (cont.)

THE EFFECTS OF GLUCOSE REPRESSION AND DEREPRESSION ON THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE CELL LINEAGES FROM THE CROSS D587-4B-2(GROWN ON 10% GLUCOSE) X E290-3 (GROWN ON GLYCEROL)

Clone Source	Frequencies of Mitochondrial Types in Cell Samples				
	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
ZYGOTE 3					
Bud 1		.975	.025		408
Bud 2	.621	.295	.002	.082	454
Bud 3	.002	.989	.009		453
Bud 4	.043	.946		.011	535
Bud 5		1.00			496
Bud 6 and subsequent		1.00			566
ZYGOTE 4					
Bud 1	.071	.836	.093		657
Bud 2	.918	.038		.044	607
Bud 3	.475	.005	.520		592
Bud 4		1.00			781
Bud 5		.992	.008		649
Bud 6 and subsequent		1.00			791

TABLE 15 (cont.)

THE EFFECTS OF GLUCOSE REPRESSION AND DEREPRESSION ON THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE CELL LINEAGES FROM THE CROSS D587-4B-2(GROWN ON 10% GLUCOSE) X E290-3 (GROWN ON GLYCEROL)

Clone Source	Frequencies of Mitochondrial Types in Cell Samples				
	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
ZYGOTE 5					
Bud 1		1.00			517
Bud 2	.882	.004	.002	.112	529
Bud 3	.159	.751	.086	.004	579
Bud 4		1.00			513
Bud 5		1.00			659
Bud 6 subsequent		1.00			578

mitochondrial types to zygote daughter cells, but these effects are variable. The lineage of Zygote 1 shows a clear distortion of the asymmetry observed in untreated lineages. Buds 3, 4 and 5 have inherited a high frequency of C^R, whereas, the first and sixth and subsequent buds still inherit a preponderance of E^R. The frequency of the recombinant classes has also increased to levels not seen in untreated lineages. The lineage of Zygote 2 is not easily explained, but we can speculate on the possible reasons behind the result. Massive recombinational events may have occurred early after zygote inception and these recombinational events are reflected in the frequencies of mitochondrial types inherited by the diploid descendants of the zygote. Another possibility is that within the zygote a selection for the double sensitive recombinant class was established and this class was preferentially segregated to zygote daughter cells. The only similarity observed in the lineages of Zygotes 1 and 2 is the increase in the frequencies of the recombinant classes. The lineages of Zygote 3 and Zygote 5 show no apparent alteration in the asymmetry observed in untreated lineages. Whereas, the lineage of Zygote 4 shows an alteration in the inheritance pattern, Bud 3 and its descendants inherited a preponderance of C^R types, which are not seen in untreated lineages.

The variability observed in whole zygote clones and zygote lineages might be attributed to the varying degrees of repression and

derepression of cells prior to and during mating. The repression and derepression of individual mitochondrial populations within a zygote might have established a selective pressure favoring the segregation of derepressed mitochondrial genomes to zygote daughter cells. The nature of the selective mechanism is obscure at present.

If, indeed, growth of mating partners prior to mating in different carbohydrate sources could alter the asymmetrical distribution of mitochondria, then either the control mechanism is subject directly to glucose repression or, more likely, there is a selection for functional mitochondria to be segregated to zygote daughter cells.

CHAPTER VI

THE GENETIC ANALYSIS OF THE CONTROL DETERMINANT

The results of glucose and glycerol growth studies prompted the question; does the zygote control the transmission of mitochondria or does the mitochondrion control its own transmission? To answer this question the analysis of tetrads derived from crosses involving D587-4B-2 was undertaken. If the information necessary for the control of the transmission of mitochondria segregates at meiosis then it is encoded in the nuclear genome, but if no meiotic segregation is observed then it is encoded on the mitochondrial chromosome. Tables 16 and 17 describe the results of such an analysis. Three tetrads from the cross D587-4B-2 X E290-3 and one tetrad from the cross D587-4B-4 X D585-11C-2 were tested. The ascospore cultures were crossed to strains which showed no control over the transmission of mitochondria. Whole zygote clones from these crosses were examined for a consistent and repeatable asymmetry of transmission of mitochondrial resistance factors. Three of the four tetrads showed a 2:2 segregation for the controlling function, showing that the preferential transmission of mitochondria in these crosses is controlled by a nuclear gene(s). These results are consistent with the results of CHI and thiolutin treatment, which indicated that nuclear transcription and subsequent translation

TABLE 16

DISTRIBUTION OF MITOCHONDRIAL TYPES IN CROSSES INVOLVING SISTER ASCOSPORE CULTURES DERIVED FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples				
		$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
MR-17A($C^{S}E^{R}$)	1	.649	.315	.031	.005	550
X	2	.174	.639	.047	.140	551
E290-3($C^{R}E^{S}$)	3	.075	.858	.029	.038	716
	Mean	.299	.604	.036	.061	1,817
MR-17B($C^{S}E^{R}$)	1	.972	.004		.024	546
X	2		1.00			514
1315-2($C^{R}E^{S}$)	3	.002	.998			568
	Mean	.325	.667		.008	1,628
MR-17C($C^{S}E^{R}$)	1	.125	.813	.003	.059	738
X	2	.199	.748	.009	.044	699
E290-3($C^{R}E^{S}$)	3	.260	.724	.011	.005	635
	Mean	.194	.762	.008	.036	2,072
MR-17D($C^{S}E^{R}$)	1	.004	.893	.012	.091	572
X	2		1.00			665
1315-2C-2($C^{R}E^{S}$)	3	.246	.754			556
	Mean	.083	.883	.004	.030	1,793

TABLE 16 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN CROSSES INVOLVING SISTER ASCOSPORE CULTURES DERIVED FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

		Frequencies of Mitochondrial Types in Cell Samples				
Cross	Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
MR-18A($C^{S}E^{R}$)	1	.158	.776	.050	.016	443
X	2	.611	.338	.045	.006	486
E290-3($C^{R}E^{S}$)	3	.216	.730	.037	.017	541
	Mean	.328	.615	.044	.013	1,470
MR-18B($C^{S}E^{R}$)	1		1.00			593
X	2	.327	.673			603
1315-2C-2($C^{R}E^{S}$)	3	.405	.584	.010	.001	785
	Mean	.245	.752	.003		1,981
MR-18C($C^{S}E^{R}$)	1	.112	.888			511
X	2		1.00			657
1315-2C-2($C^{R}E^{S}$)	3		1.00			592
	Mean	.037	.963			1,760
MR-18D($C^{S}E^{R}$)	1	.060	.911	.011	.018	666
X	2	.485	.511		.004	789
E290-3($C^{R}E^{S}$)	3	.258	.627	.072	.043	511
	Mean	.268	.682	.028	.022	1,966

TABLE 16 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN CROSSES INVOLVING SISTER ASCOSPORE CULTURES DERIVED FROM THE CROSS D587-4B-2(C^SE^R) X E290-3(C^RE^S)

		Frequencies of Mitochondrial Types in Cell Samples				
Cross	Zygote	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
MR-19A(C^SE^R)	1	.331	.665	.002	.002	507
X	2	.375	.595	.002	.028	502
1315-2C-2(C^RE^S)	3	.695	.303		.002	442
	Mean	.467	.521	.001	.011	1,451
MR-19B(C^SE^R)	1	.204	.735	.050	.011	686
X	2	1.00				611
E290-3(C^RE^S)	3	.301	.595	.074	.030	570
	Mean	.502	.443	.041	.014	1,867
MR-19C(C^SE^R)	1		1.00			757
X	2	.082	.784	.003	.131	575
E290-3(C^RE^S)	3		1.00			626
	Mean	.027	.928	.001	.044	1,958
MR-19D(C^SE^R)	1		1.00			943
X	2	.284	.668	.022	.026	696
1315-2C-2(C^RE^S)	3		1.00			515
	Mean	.095	.889	.007	.009	2,154

TABLE 17

DISTRIBUTION OF MITOCHONDRIAL TYPES IN CROSSES INVOLVING SISTER ASCOSPORE CULTURES DERIVED FROM THE CROSS D587-4B-4($C^{R}E^{S}$) X D585-11C-2($C^{S}E^{R}$)

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples				
		$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
MR-1A($C^{R}E^{S}$)	1	.506	.450		.044	631
X	2	1.00				492
1381-17C-1($C^{S}E^{R}$)	3	1.00				501
	Mean	.835	.150		.015	1,624
MR-1B($C^{R}E^{S}$)	1	.804	.081	.003	.112	384
X	2	.995			.005	408
1315-2C-1($C^{S}E^{R}$)	3	.802	.177	.006	.015	334
	Mean	.867	.086	.003	.044	1,126
MR-1C($C^{R}E^{S}$)	1	.802	.165	.011	.022	545
X	2	.806	.170	.008	.016	513
E290-1($C^{S}E^{R}$)	3	.948	.035		.017	401
	Mean	.853	.123	.006	.018	1,459
MR-1D($C^{R}E^{S}$)	1	.276	.702		.022	464
X	2	.153	.687		.160	518
Z1-2D-1($C^{S}E^{R}$)	3	.817	.145		.038	477
	Mean	.415	.512		.073	1,459

of this information into proteins was necessary for the control to be manifested.

Another observation from Tables 16 and 17 is that control of mitochondrial transmission segregates independently from mating type. Therefore, the mating type locus is not linked to or responsible for the control function.

CHAPTER VII

THE ANALYSIS OF THE TRANSMISSION OF RECOMBINANT GENOMES

Recent reports (Wilkie and Thomas, 1973; Lukins et al, 1973) have shown that in some crosses massive recombinational events occur in zygotes, our lineage analysis has shown that the early buds received predominantly parental type mitochondria. This type of analysis, however, did not distinguish between actual deferred mitochondrial (DNA) recombination and the alternative possibility that recombination occurs early and recombinants are randomly distributed to zygote daughter but somehow not expressed.

In an attempt to answer this question the following study was undertaken. Parental cells were micromanipulated together, zygotes formed and buds removed and transferred to YEPG supplemented with erythromycin and chloramphenicol (4mg/ml). After the fifth bud was transferred the zygote was then micromanipulated onto the antibiotic medium. The zygotes and their buds were observed and diagrammed at the time of transfer (Time 0) and at 24, 48, and 72 hours. After 72 hours the agar slab was transferred to YEPD to allow growth. Table 18 shows the results of this study. The lineage of Zygote 1 is diagrammed in figure 4. The uppermost diagram shows the zygote and the position of initiation of its buds. The lower figure shows the zygote and its buds at the times indicated. The lineage

TABLE 18

DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE CELL LINEAGES FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$) AFTER TREATMENT FOR 72 HOURS WITH 4mg/ml OF CHLORAMPHENICOL AND ERYTHROMYCIN

	Frequencies of Mitochondrial Types in Cell Samples				
Clone source	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
ZYGOTE 1					
Bud 1	.002	.031	.967		448
Bud 2		1.00			737
Bud 3	.177	.139	.681	.003	621
Bud 4	1.00				654
Bud 5		.659	.341		715
Bud 6 and subsequent	1.00				706
ZYGOTE 2					
Bud 1	.994		.006		501
Bud 2		1.00			526
Bud 3	1.00				622
Bud 4	1.00				522
Bud 5	1.00				531
Bud 6 and subsequent	1.00				
ZYGOTE 3					
Bud 1		.770		.230	405
Bud 2	1.00				352
Bud 3	1.00				601
Bud 4*					
Bud 5	1.00				620
Bud 6 and* subsequent					

*Bud died

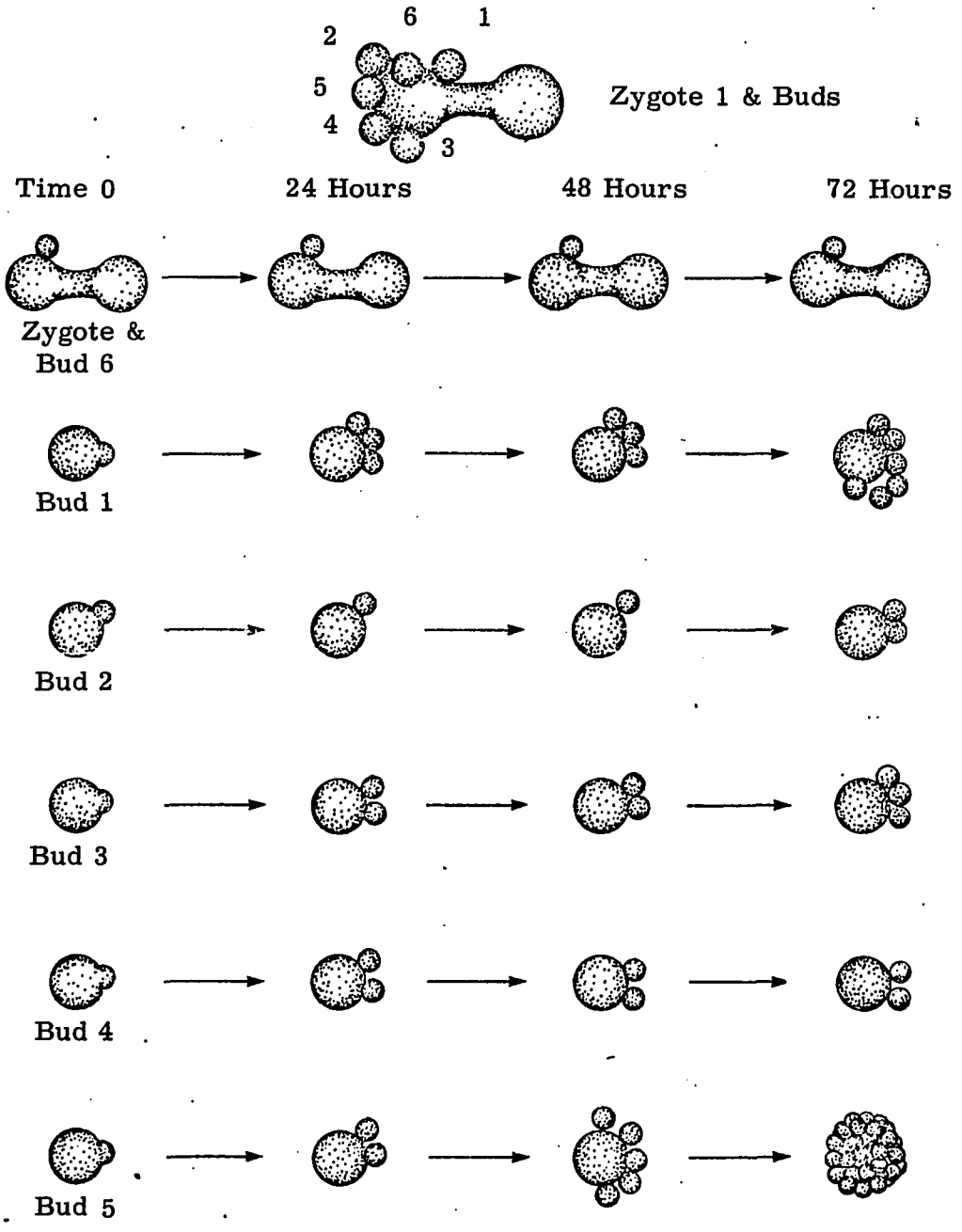

Figure 4. The analysis of the transmission and expression of recombinant genomes ($C^{R}E^{R}$) in zygotes and zygote daughter cells in the cross D587-4B-2 ($C^{S}E^{R}$) X E290-3 ($C^{R}E^{S}$).

of Zygote 1 shows that the descendants of Bud 1 are predominantly the double resistant type. As diagrammed in figure 4, Bud 1 initiated six small buds (all inviable) in the presence of the antibiotics indicating that Bud 1 contained at least one doubly resistant mitochondrion. In studies involving sensitive parental strains no bud initiation was observed once the cells were transferred to the drug medium. In the presence of the antibiotics only the double resistant mitochondrial genome is functional and this genome must therefore be selectively replicated causing an increase in recombinant genomes in the bud progeny (Table 18). Recombinant genomes were not transmitted to the progeny buds of Bud 1 while it was exposed to the antibiotics, since these buds apparently did not bud. As seen in the extensive lineage analysis (Table 5) Bud 1 normally receives and predominantly transmits the E^R to its progeny. Therefore, the small buds may have inherited the E^R parental type and the predominant type left in Bud 1 might have been the recombinant class (C^RE^R).

Bud 2 and its daughter cells are exclusively E^R, although figure 4 shows that Bud 2 initiated two small buds. In untreated lineages Bud 2 was always predominantly C^R. These results indicate either that most or all C^R genomes are segregated to the small buds or that they may have been selectively lost by some unknown mechanism. Bud 3 shows an increase in the

frequency of daughter cells which inherited the C^RE^R. Bud 4 and its progeny are exclusively C^R. In untreated lineages of this cross the progeny of Bud 4 are predominantly E^R. This result can be attributed to the selective transmission of E^R genomes to the early buds which were inviable. Bud 5 and its progeny cells were E^R and C^RE^R. Figure 4 shows that Bud 5 had proliferated enough to form a small colony of approximately 30 cells. This indicates that some buds of Bud 5 must have received predominantly the C^RE^R genomes. The inheritance of predominantly C^RE^R genomes would allow a faster growth rate and some progeny buds might have received exclusively the double resistant genome. The descendants of the Sixth and subsequent buds were exclusively C^R as observed with the progeny of Bud 4.

The lineage of Zygote 2 is diagrammed in figure 5. Bud 1 was predominantly C^R. As discussed earlier, in untreated lineages Bud 1 is predominantly E^R. This change in the inheritance of Bud 1 may be attributed to the selective segregation of E^R genomes into inviable buds or possibly a selective replication of C^R genomes or a selective loss of E^R genomes. The descendant diploid cells of Bud 2 are E^R and not C^R as observed in untreated lineages. Since the small buds of Bud 2 (see fig. 5) were inviable, genomes segregated to them were lost and these lost genomes may have been C^R. This kind of speculation could apply also to Buds 3,

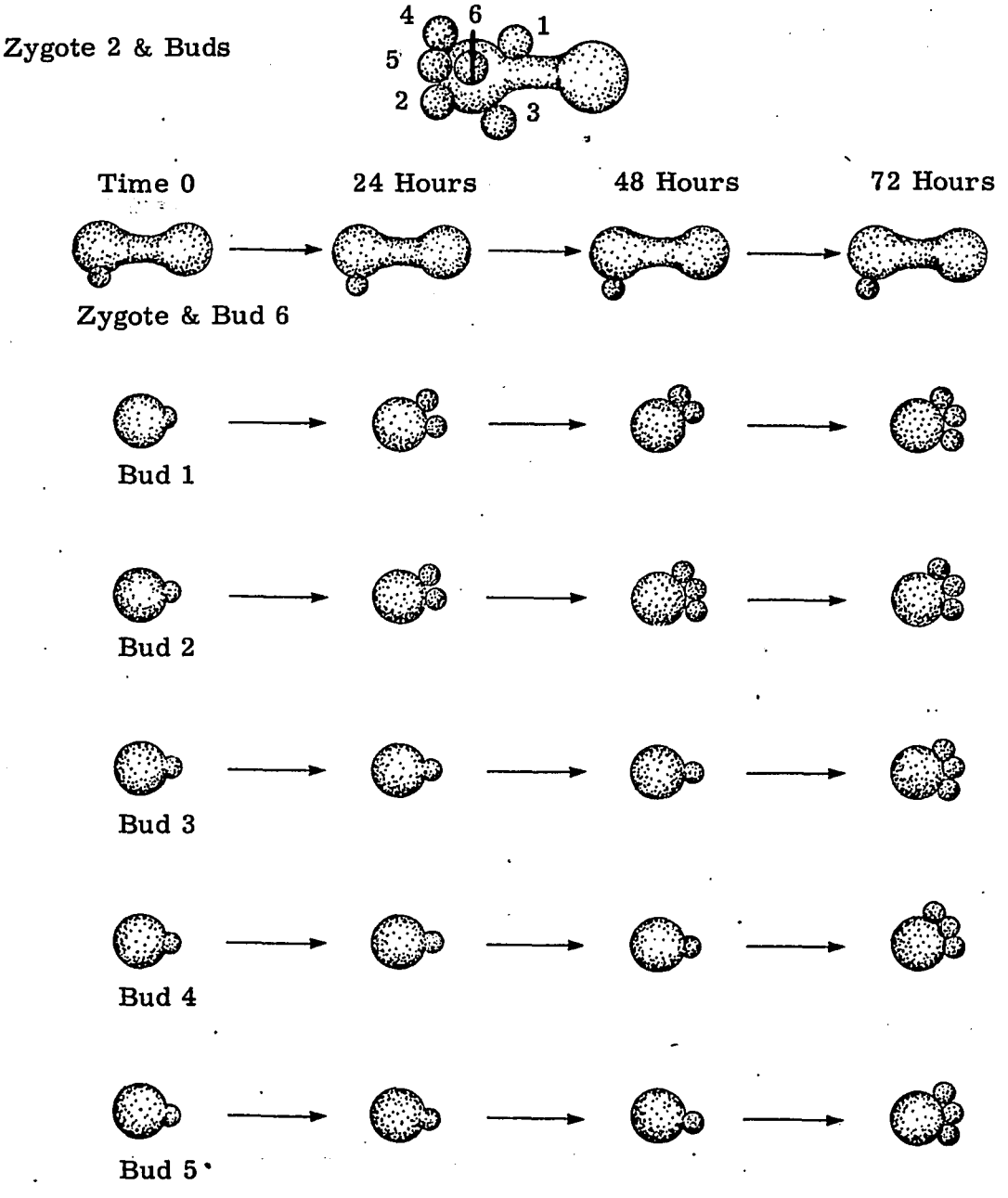

Figure 5. The analysis of the transmission and expression of recombinant genomes ($C^{R}E^{R}$) in zygotes and zygote daughter cells in the cross D587-4B-2 ($C^{S}E^{R}$) X E290-3 ($C^{R}E^{S}$).

4, 5, 6 and subsequent buds, all of which have inherited predominantly C^R. All zygote daughter cells (buds) initiated buds which were inviable and the genomes segregated to these buds were therefore lost. These genomes should have been predominantly E^R on the basis of comparison with untreated zygote lineages.

Zygote 3 and its progeny buds are diagrammed in figure 6, and Table 18 shows the results of this lineage study. Bud 1 and daughter cells inherited predominantly E^R as in untreated lineages. The initiation of only one bud could be responsible since this would maintain a relatively large population of E^R mitochondria within Bud 1 which could be transmitted to its buds after removal from the drug medium. The descendants of Bud 2 are exclusively C^R as observed in untreated lineages. This result could be explaines by the incomplete segregation of C^R genomes to inviable buds. Bud 3 and Bud 5 were exclusively C^R. This change in inheritance may be attributed to some selection for the C^R genomes. Bud 4 died.

This study reveals several important points: 1) massive recombinational events probably do not occur in zygotes of this cross; 2) either C^RE^R genomes are not randomly distributed to the buds, or the frequency of recombination in the buds is not equal, since not all buds initiated the same number of small buds; 3) zygotes after transfer to drug medium initiated no new buds, indicating the apparent absence of C^RE^R genomes in the zygote at the time of

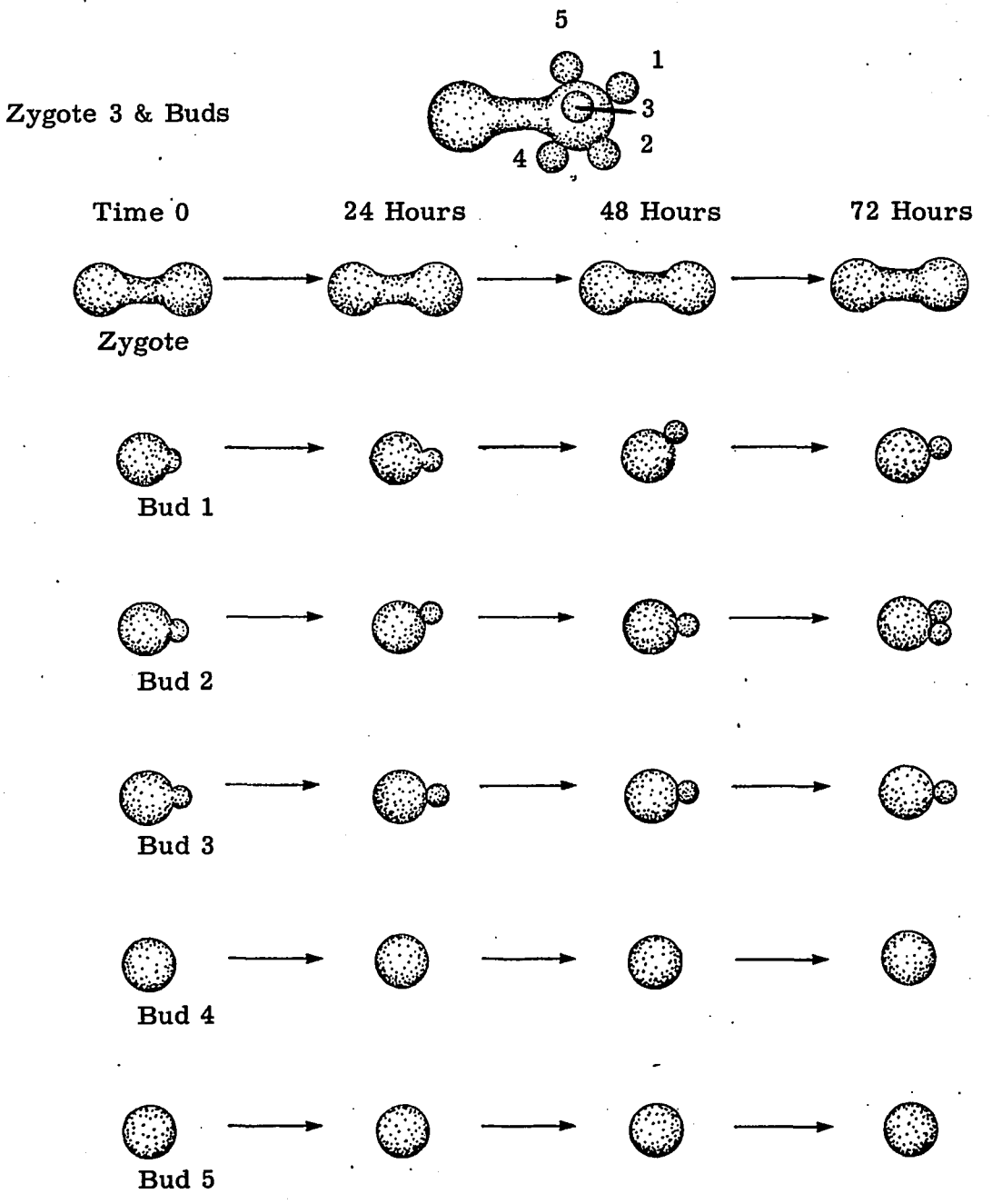

Figure 6. The analysis of the transmission and expression of recombinant genomes ($C^{R}E^{R}$) in zygotes and zygote daughter cells in the cross D587-4B-2 ($C^{S}E^{R}$) X E290-3 ($C^{R}E^{S}$).

transfer to the drug medium; 4) genomes could be selected for within buds and this selection is seen in the progeny of these buds: 5) Treatment of cells for 72 hours with 4mg/ml of both erythromycin and chloramphenicol did not induce the "petite" phenotype.

It must be reemphasized that in control lineages under the same conditions, involving sensitive parental strains, no aborted buds formed on the drug medium.

CHAPTER VIII

THE EFFECTS OF THE PETITE MUTATION ON THE TRANSMISSION OF MITOCHONDRIA

In an attempt to obtain more information on the nature of the control of the distribution of mitochondria to zygote daughter cells a study on the effect of the petite mutation on the distribution of mitochondria was undertaken. It was assumed that the petite phenotype could be used as a mitochondrial marker and its transmission to zygote daughter cells could be followed in a manner similar to the antibiotic resistance markers. Table 19 shows the results of the effects of the neutral petite mutation on the distribution of mitochondrial types. As expected, the neutral petite phenotype is not inherited (or never reappears) in the diploid progeny of the zygote. The neutral petite mutant derived from D587-4B-2 shows no preferential transmission of its petite phenotype to zygote daughter cells. Therefore, the neutral petite mutation might be accompanied by the loss of mitochondria or the mitochondria may be present but the loss or extensive degradation of its DNA might prevent its expression. Recent reports (Mehrotra and Mahler, 1968; Carnevali et al., 1969; Saunders et al., 1970) have indicated that extensive alteration or even loss of M-DNA could occur concomitantly with the appearance of the neutral petite phenotype.

Recent reports have also indicated that some suppressive petite mutants retain mitochondrial antibiotic resistance factors

TABLE 19

THE EFFECT OF THE NEUTRAL PETITE MUTATION ON THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples $C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
D587-4B-2	1		1.00			515
X	2		1.00			558
E290-3np*	3		1.00			704
D587-4B-2np*	1	1.00				644
X	2	1.00				489
E290-3	3	1.00				436

*Independent Isolated Neutral Petites from the Parental Strains D587-4B-2 and E290-3

(Michaelis et al, 1973; Faye et al, 1973) and information for the elaboration of mitochondrial t-RNAs (Faye et al, 1973). Although suppressive petite mutants have lost genetic information and the M-DNA may be substantially modified, they possess some functional mitochondrial genes. To study the possible influence of these genes on the distribution of mitochondrial factors, crosses employing mutants of this type were analyzed. Table 20 shows the results of a random diploid analysis of the distribution of mitochondrial types to zygote daughter cells in crosses involving five suppressive petite mutants derived from D587-4B-2. All mutants were highly suppressive, and the grande descendants of the zygote inherited exclusively the mitochondrial phenotype of the "grande" parental strain. Table 21 shows the results of crosses between these five suppressive petite mutants and E290-3 and Table 22 shows the results of zygote clone analysis of similar crosses. These results indicate that random diploid analysis underestimates the suppressiveness of the mutants. This error might be attributed to the slower growth of petites as compared to "grande" cells and to the changing culture conditions. As the culture ages, the glucose concentration diminishes and non-fermentable end products accumulate, producing an environment increasingly selective for "grande" cells. This selection could be expected to increase their frequency.

The results of a random diploid analysis involving the suppressive

TABLE 20

DISTRIBUTION OF MITOCHONDRIAL TYPES IN RANDOM DIPLOIDS

FROM THE CROSSES

D587-4B-2sp$_n^*$ X 62-25A(C^RE^S)

Cross	Frequencies of Mitochondrial Types in Cell Samples						
	Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
D587-4B-2sp$_1$ X 62-25A	.948	.052	.052				384
D587-4B-2sp$_2$ X 62-25A	.929	.071	.071				960
D587-4B-2sp$_3$ X 62-25A	.925	.075	.075				522
D587-4B-2sp$_4$ X 62-25A	.965	.035	.035				254
D587-4B-2sp$_5$ X 62-25A	.890	.110	.110				400

*Independently Isolated Suppressive Petite Mutants of

D587-4B-2(C^SE^R)

TABLE 21

DISTRIBUTION OF MITOCHONDRIAL TYPES IN RANDOM DIPLOIDS FROM THE CROSSES

D587-4B-2sp$_n^*$ X E290-3(C^RE^S)

Cross	Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
	Frequencies of Mitochondrial Types in Cell Samples						
D587-4B-2sp$_1$ X E290-3	.706	.294	.294				357
D587-4B-2sp$_2$ X E290-3	.911	.089	.089				892
D587-4B-2sp$_3$ X E290-3	.721	.279	.279				1,448
D587-4B-2sp$_4$ X E290-3	.705	.295	.295				735
D587-4B-2sp$_5$ X E290-3	.864	.136	.136				881

*Independently Isolated Suppressive Petite Mutants of D587-4B-2(C^SE^R)

TABLE 22

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSSES D587-4B-2sp$_n^*$ X E290-3($C^R E^S$)

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples: Petite	Grande	Colonies Scored
D587-4B-2sp$_1$ X E290-3	1	1.00		369
	2	1.00		510
	3	1.00		410
D587-4B-2sp$_2$ X E290-3	1	1.00		610
	2	1.00		545
	3	1.00		852
	4	1.00		806
	5	1.00		844
D587-4B-2sp$_3$ X E290-3	1	1.00		526
	2	1.00		390
	3	1.00		532
D587-4B-2sp$_4$ X E290-3	1	1.00		522
	2	1.00		733
	3	1.00		811
D587-4B-2sp$_5$ X E290-3	1	1.00		693
	2	1.00		667
	3	1.00		560

*Independently Isolated Suppressive Petite Mutants of D587-4B-2

petite mutant D587-4B-2sp$_2$ crossed to strains from widely different sources is shown in Table 23. It is evident from the table that the suppressive mutant is highly suppressive in all crosses in this study. The results of zygote clone analysis of such crosses is shown in Table 24. These results substantiate those from random diploid analysis that show that this suppressive mutant is highly suppressive in all crosses studied. Lineage analysis of one of the crosses, D587-4B-2sp$_2$ X E290-3, was studied. Table 25 describes the results of this analysis. The lineages of the three zygotes studied revealed that the "grande" phenotype was never inherited by the diploid progeny of the zygotes.

Crosses involving five suppressive petite mutants derived from E290-3 are shown in Table 26. These suppressive mutants were moderately to highly suppressive in these crosses. Table 27 shows the results of crosses involving the suppressive petite mutant E290-3sp$_3$ and strains from various sources. The mutant is highly suppressive in all crosses and the "grande" progeny of the zygotes inherited the mitochondrial phenotype of the "grande" parental strain. The analysis of whole zygote clones involving the suppressive mutant (E290-3sp$_3$) and strains from various sources (Table 28) substantiate the results of the random diploid analysis of these crosses.

TABLE 23

DISTRIBUTION OF MITOCHONDRIAL TYPES IN RANDOM DIPLOIDS

FROM THE CROSSES BETWEEN

D587-4B-2sp$_2$ AND OTHER STRAINS

Cross	Frequencies of Mitochondrial Types in Cell Samples						
	Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
D587-4B-2sp$_2$ X D22	.987	.013				.013	780
D587-4B-2sp$_2$ X X2181-1A	.861	.139				.139	671
D587-4B-2sp$_2$ X D585-11C-3	1.00						868
D587-4B-2sp$_2$ X S1795A	.990	.010				.010	591

TABLE 24

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSSES BETWEEN D587-4B-2sp$_2$ AND OTHER STRAINS

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples: Petite	Grande	Colonies Scored
D587-4B-2sp$_2$	1	.986	.014	729
X	2	.990	.010	839
D22	3	.990	.010	893
D587-4B-2sp$_2$	1	1.00		1,060
X	2	1.00		796
X2181-1A	3	1.00		713
D587-4B-2sp$_2$	1	1.00		840
X	2	1.00		750
D585-11C-3	3	1.00		748
D587-4B-2sp$_2$	1	1.00		707
X	2	1.00		600
S1795A	3	1.00		610
D587-4B-2sp$_2$	1	1.00		1,595
X	2	1.00		1,202
62-25A	3	1.00		1,528

TABLE 25

DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE LINEAGES FROM THE CROSS D587-4B-2sp$_2$ X E290-3

Clone Source	Frequencies of Mitochondrial Types in Cell Samples					
	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Petite	Colonies Scored
Zygote 1						
Bud 1					1.00	486
Bud 2					1.00	530
Bud 3					1.00	531
Bud 4					1.00	451
Bud 5					1.00	544
Bud 6 and subsequent					1.00	575
Zygote 2						
Bud 1					1.00	528
Bud 2					1.00	559
Bud 3					1.00	495
Bud 4					1.00	606
Bud 5					1.00	577
Bud 6 and subsequent					1.00	472
Zygote 3						
Bud 1					1.00	584
Bud 2					1.00	486
Bud 3					1.00	580
Bud 4					1.00	424
Bud 5					1.00	835
Bud 6 and subsequent					1.00	430

TABLE 26

DISTRIBUTION OF MITOCHONDRIAL TYPES IN RANDOM DIPLOIDS FROM THE CROSSES

ade7 X E290-3sp$_n$ *

Cross	Frequencies of Mitochondrial Types in Cell Samples						
	Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
ade7 X E290-3sp$_2$	.724	.276				.276	692
ade7 X E290-3sp$_3$	.698	.302				.302	898
ade7 X E290-3sp$_4$	.673	.327				.327	355
ade7 X E290-3sp$_5$	.999	.001				.001	928
ade7 X E290-3sp$_6$	.663	.337				.337	710

*Independently Isolated Suppressive Petite Mutants of E290-3

TABLE 27

DISTRIBUTION OF MITOCHONDRIAL TYPES IN RANDOM DIPLOIDS FROM THE CROSSES BETWEEN E290-3sp$_3$* AND OTHER STRAINS

Cross	Frequencies of Mitochondrial Types in Cell Samples						
	Petite	Grande	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
D545-4A X E290-3sp$_3$	.978	.022				.022	893
D609-12A X E290-3sp$_3$	.925	.075				.075	704
M-6C X E290-3sp$_3$	.922	.078				.078	618
N11C-1 X E290-3sp$_3$	.977	.023		.023			664

*Independently Isolated Suppressive Petite Mutant of E290-3

TABLE 28

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSSES BETWEEN E290-3sp$_3$ AND OTHER STRAINS

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples: Petite	Grande	Colonies Scored
D545-4A	1	1.00		507
X	2	1.00		560
E290-3sp$_3$	3	1.00		861
D609-12A	1	1.00		889
X	2	1.00		836
E290-3sp$_3$	3	1.00		846
M-6C	1	.973	.027	486
X	2	.981	.019	592
E290-3sp$_3$	3	.982	.018	544
N11C-1	1	1.00		674
X	2	1.00		789
E290-3sp$_3$	3	1.00		766

Table 29 describes the results of a random diploid analysis of crosses involving D587-4B-2 and the suppressive mutants derived from E290-3. In these crosses the suppressivity of the mutants is no longer observable and the suppressive mutants behave as neutral petite (or petites with low suppressiveness), which is expected since D587-4B-2 possesses a nuclear determinant(s) responsible for the asymmetrical transmission of its mitochondrial type. The results of zygote clone analysis shown in Table 30 substantiate the results of random diploid analysis. It is clearly evident from these results that nuclear factors could alter or modify the suppressiveness of suppressive petites. Therefore, care must be exercised in determining the suppressiveness of petites, since the suppressiveness changes in crosses to different strains. Since this neutral petite possesses mitochondrial structures which were visualized by tetrazolium straining, the results also indicate that the suppressive petites derived from D587-4B-2 retain some function which is absent in the neutral petite, (derived from D587-4B-2), which allows the control function to operate.

Table 31 describes the results of a lineage analysis of the cross E290-3sp$_3$ X D587-4B-2. It is clear from the results of the three lineages analyzed that all of the buds of the zygotes receive predominantly the mitochondrial type of the D587-4B-2 parent. In only one bud (Bud 1, Zygote 2) was an appreciable suppressiveness

TABLE 29

DISTRIBUTION OF MITOCHONDRIAL TYPES IN RANDON DIPLOIDS FROM THE CROSSES D587-4B-2 X E290-3sp$_n$*

Cross	Frequencies of Mitochondrial Types in Cell Samples						
	Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
D587-4B-2 X E290-3sp$_2$	.010	.990		.990			572
D587-4B-2 X E290-3sp$_3$	.013	.987		.987			632
D587-4B-2 X E290-3sp$_4$	.007	.993		.993			721
D587-4B-2 X E290-3sp$_5$	.003	.997		.997			513
D587-4B-2 X E290-3sp$_6$	.033	.967		.967			559

*Independently Isolated Suppressive Petite Mutants

TABLE 30

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES

FROM THE CROSSES

D587-4B-2($C^{S}E^{R}$) X E290-3sp_n *

Cross	Zygote	Petite	Grande	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
		Frequencies of Mitochondrial Types in Cell Samples						
D587-4B-2 X E290-3sp_2	1	.008	.992		.992			907
	2	.018	.982		.982			653
	3	.008	.992		.992			728
D587-4B-2 X E290-3sp_3	1	.021	.979		.979			487
	2	.039	.961		.961			584
	3	.032	.968		.968			650
	4	.010	.990		.990			389
	5	.018	.982		.982			490
	6	.007	.993		.993			416
	7	.014	.986		.986			290

* Independently Isolated Suppressive Petite Mutants from the Parental strain E290-3

TABLE 30 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES

FROM THE CROSSES

D587-4B-2(C^SE^R) X E290-3sp_n*

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples: Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
D587-4B-2 X D290-3sp_4	1	.093	.907		.907			666
	2	.049	.951		.951			772
	3	.070	.930		.930			762
D587-4B-2 X E290-3sp_5	1	.023	.977		.977			587
	2	.009	.991		.991			714
	3	.011	.989		.989			646

*Independently Isolated Suppressive Petite Mutants of the Parental Strain E290-3

TABLE 30 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES

FROM THE CROSSES

D587-4B-2($C^{S}E^{R}$) X E290-3sp_{n}*

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples						
		Petite	Grande	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
D587-4B-2 X E290-3sp_{6}	1	.044	.956		.956			596
	2	.024	.976		.976			553
	3	.023	.977		.977			830

* Independently Isolated Suppressive Petite Mutants from the Parental Strain E290-3

TABLE 31

DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTE LINEAGES FROM THE CROSS D587-4B-2 X E290-3sp$_3$*

Clone Source	Frequencies of Mitochondrial Types in Cell Samples						
	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Petite	Grande	Colonies Scored
Zygote 1							
Bud 1		.974			.026	.974	724
Bud 2		.983			.017	.983	521
Bud 3		.990			.010	.990	310
Bud 4		.979			.021	.979	535
Bud 5		.976			.024	.976	582
Bud 6 and subsequent		.990			.010	.990	873
Zygote 2							
Bud 1		.800			.200	.800	459
Bud 2		.981			.019	.981	524
Bud 3		.975			.025	.975	551
Bud 4		.989			.011	.989	647
Bud 5		.981			.019	.981	685
Bud 6 and subsequent		.984			.016	.984	506
Zygote 3							
Bud 1		.989			.011	.989	628
Bud 2		.975			.025	.975	714
Bud 3		.979			.021	.979	431
Bud 4		.969			.031	.969	873
Bud 5		.990			.010	.990	382
Bud 6 and subsequent		.995			.005	.995	756

*Suppressive Petite Mutant from the Parental strain E290-3

demonstrated. Again, it is apparent that nuclear factors modify the apparent suppressiveness of suppressive petite. In a recent report (Bech-Hansen and Rank, 1973) it has been independently shown that nuclear genes could modify the suppressiveness of suppressive petite mutants. These authors proposed that the nuclear factor was necessary for the maintenance of the "grande" phenotype and that mutation of this factor decreased the stability of the grande phenotype, leading to increased suppressiveness. The nuclear factor in D587-4B-2 is, however, clearly involved in the transmission of mitochondrial types to zygote daughter cells and not with the stability of the grande phenotype, since our neclear factor operates in crosses not involving any petite mutation. Futhermore the spontaneous frequency is much too low to make undetected spontaneous petites the basis for our findings(See Table 32).

Table 33 summarizes the suppressiveness of all suppressive petite mutants employed in this study. The suppressive mutants derived from D587-4B-2 were highly suppressive in all crosses. The suppressive petite mutants derived from E290-3 were highly suppressive in all crosses except when crosses to strains derived from D587-4B-2.

Table 34 presents the results of an analysis of the kinetics of suppressiveness and the distribution of mitochondrial types in zygotes from the cross D587-4B-2sp_2 X E290-3. This study was undertaken to

TABLE 32

LIST OF THE SPONTANEOUS PETITE FREQUENCY OF STRAINS USED IN THE STUDY OF NEUTRAL AND SUPPRESSIVE PETITES

Strain	Spontaneous Petite Frequency (%)	Colonies Scored
D587-4B-2	0.70	451
E290-3	0.60	465
D609-12A	7.00	589
S1795A	3.00	556
M-6C	5.80	180
X2181-1A	1.90	305
D585-11C-3	4.80	495
D22	1.10	991
ade7	2.40	941
N11C-1	1.10	640
62-25A	1.20	723
MR-19B	3.90	1,558
D545-4A	0.90	913

TABLE 33

LIST OF THE DEGREE OF SUPPRESSIVENESS OF SUPPRESSIVE PETITE STRAINS USED IN THIS STUDY (FROM RANDOM DIPLOID ANALYSIS)

Cross	Suppressiveness (%)
D587-4B-2sp$_1$ X 62-25A	94.7
D587-4B-2sp$_2$ X 62-25A	92.8
D587-4B-2sp$_3$ X 62-25A	92.4
D587-4B-2sp$_4$ X 62-25A	96.5
D587-4B-2sp$_5$ X 62-25A	88.9
D587-4B-2sp$_1$ X E290-3	70.4
D587-4B-2sp$_2$ X E290-3	99.1
D587-4B-2sp$_3$ X E290-3	71.9
D587-4B-2sp$_4$ X E290-3	70.3
D587-4B-2sp$_5$ X E290-3	86.3
D587-4B-2sp$_2$ X D22	98.7
D587-4B-2sp$_2$ X X2181-1A	85.8
D587-4B-2sp$_2$ X D585-11C-3	100
D587-4B-2sp$_2$ X S1795A	99.0

TABLE 33 (continued)

LIST OF THE DEGREE OF SUPPRESSIVENESS OF SUPPRESSIVE PETITE STRAINS USED IN THIS STUDY (FROM RANDOM DIPLOID ANALYSIS)

Cross	Suppressiveness (%)
E290-3sp$_2$ X ade7	71.7
E290-3sp$_3$ X ade7	69.1
E290-3sp$_4$ X ade7	66.5
E290-3sp$_5$ X ade7	99.9
E290-3sp$_6$ X ade7	65.5
E290-3sp$_3$ X D545-4A	97.8
E290-3sp$_3$ X D609-12A	91.9
E290-3sp$_3$ X M-6C	91.7
E290-3sp$_3$ X N11C-1	97.7
E290-3sp$_2$ X D587-4B-2	0.30
E290-3sp$_3$ X D587-4B-2	0.60
E290-3sp$_4$ X D587-4B-2	0.00
E290-3sp$_5$ X D587-4B-2	0.00
E290-3sp$_6$ X D587-4B-2	2.61

TABLE 34

ANALYSIS OF THE KINETICS OF SUPPRESSIVENESS AND THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTES FROM THE CROSS D587-4B-2sp_2 X E290-3

Maturation Time	Zygote	Frequencies of Mitochondrial Types in Cell Samples						Colonies Scored
		Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	
0 Min.	1	1.00						880
0 Min.	2	1.00						472
0 Min.	3	1.00						867
0 Min.	4	1.00						824
15 Min.	1	1.00						685
15 Min.	2	1.00						766
30 Min.	1	1.00						860
30 Min.	2	1.00						932
30 Min.	3	1.00						620
30 Min.	4	1.00						792
30 Min.	5	1.00						844
30 Min.	6	1.00						731
30 Min.	7	1.00						430

TABLE 34 (continued)

ANALYSIS OF THE KINETICS OF SUPPRESSIVENESS AND THE DISTRIBUTION OF MITOCHONDRIAL TYPES IN ZYGOTES FROM THE CROSS D587-4B-2sp$_2$ X E290-3

Maturation Time	Zygote	Frequencies of Mitochondrial Types in Cell Samples						Colonies Scored
		Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	
60 Min.	1	1.00						742
60 Min.	2	1.00						701
60 Min.	3	1.00						700
60 Min.	4	1.00						852
60 Min.	5	1.00						745
60 Min.	6	1.00						781
90 Min.	1	1.00						669
90 Min.	2	1.00						811
90 Min.	3	1.00						152
90 Min.	4	1.00						707
90 Min.	5	1.00						304
120 Min.	1	1.00						744
120 Min.	2	1.00						523

determine whether the suppressiveness of D587-4B-2sp$_2$ could be modified by transferring young zygotes after varying maturation times to a non-fermentable medium (YEPG). After 72 hours the agar slab was transferred to YEPD to allow further growth of the zygotes and their daughter cells. It was hoped that selective pressures were established in the zygotes and their progeny to retain or maintain the grande phenotype on the nonfermentable medium. Observations during and after 72 hours of the zygotes and their progeny revealed a heterogeneous response to the selection. Most of the zygotes produced colonies on glycerol of approximately 1mm in diameter, but some produced colonies in 72 hours, as large as 3mm in diameter or as small as 100 cells. The results indicated that selection had been established, but few daughter cells received grande genomes while the majority of the daughter cells received petite genomes as evident from the reduced growth rate of the colonies. Those daughter cells which inherited only petite genomes could not grow, while cells which received grande genomes or a mixture of genomes could grow. Transfer to YEPD eliminated the selective pressures and apparently the grande phenotype was then lost in all the zygote progeny, since when the remaining cells of each zygote clone were spread over the surface of a petri dish containing YEPG, no growth was observed; entire zygote clones had become petite. The time of transfer to YEPG

had apparently no effect on this result. Even though selective pressures were established earlier for some zygotes and later for others, no differences in the range of colony size was observed for each time class. Apparently, the suppressive character is expressed soon after zygote inception, since all zygotes were petite. If the zygotes which were transferred early had retained the grande phenotype and those transferred later did not, one could attribute the results to dealyed expression of the suppressive phenotype, but this was not observed.

If the control function responsible for the asymmetrical distribution of mitochondrial types was also responsible for the suppressiveness of the suppressive petite mutants derived from D587-4B-2, one would expect that suppressive petite mutants derived from ascospore sister cultures from the cross D587-4B-2 X E290-3 would show a Mendelian segregation of the suppressive phenotype in crosses to strains lacking the control function. It would be expected that the segregation of the suppressive phenotype correspond to the segregation of the control function. Table 35 shows the results of this study, which employed ultra violet induced suppressive petite mutants of the tetrad MR-19 sister ascospores. The control function was shown to have been segregated to MR-19C and MR-19D ascospore sister cultures (see Table 17). Table 35 shows that no segregation of the suppressive phenotype was observed and that all of the suppressive petite

TABLE 35

DISTRIBUTION OF MITOCHONDRIAL TYPES IN CROSSES INVOLVING SUPPRESSIVE PETITES DERIVED FROM SISTER ASCOSPORES FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$).

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples: Petite	Grande	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
MR-19Asp$_1$ X MR-19B($C^{S}E^{R}$)	1	1.00						575
	2	1.00						342
	3	1.00						869
	4	.997	.003		.003			745
	5	1.00						865
MR-19Bsp$_2$ X E290-3($C^{R}E^{S}$)	1	1.00						226
	2	1.00						650
	3	1.00						473
	4	1.00						676
	5	1.00						560

TABLE 35 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN CROSSES INVOLVING SUPPRESSIVE PETITES DERIVED FROM SISTER ASCOSPORES FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples						
		Petite	Grande	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
MR-19Csp$_{8}$ X E290-3($C^{R}E^{S}$)	1	1.00						295
	2	1.00						327
	3	1.00						343
	4	1.00						427
	5	1.00						244
	6	1.00						466

TABLE 35 (cont.)

DISTRIBUTION OF MITOCHONDRIAL TYPES IN CROSSES INVOLVING SUPPRESSIVE PETITES DERIVED FROM SISTER ASCOSPORES FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X E290-3($C^{R}E^{S}$)

Cross	Zygote	Frequencies of Mitochondrial Types in Cell Samples						
		Petite	Grande	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
MR-19Dsp$_4$ X MR-19B($C^{S}E^{R}$)	1	1.00						976
	2	1.00						687
	3	.999	.001		.001			808
	4	1.00						740
	5	.998	.002		.002			581
	6	.998	.002		.002			556
	7	.984	.016		.016			618

ascospore cultures were highly suppressive in the crosses shown. Therefore, the control function is not responsible for the suppressiveness of these mutants, and both suppressiveness and the control function operate independently. Strains possessing the nuclear determinant(s) can, however, modify the suppressiveness of suppressive petites when crossed to each other (Table 29).

In crosses involving a suppressive petite mutant the "grande" diploid descendants upon sporulation give rise in rare instances to tetrads in which the four ascospores were petite. A random diploid analysis of crosses involving one such tetrad is presented in Table 36. No segregation of the suppressive petite phenotype was observed and all the ascospore cultures were suppressive in the crosses analyzed. These results concur with the results of the analysis of the suppressive petite mutants isolated from the MR-19 tetrad (see Table 35). Therefore, as stated previously, the nuclear control function responsible for the asymmetrical pattern of inheritance and the suppressiveness of petite mutants are not manifestations of a common mechanism.

TABLE 36

DISTRIBUTION OF MITOCHONDRIAL TYPES IN A RANDOM DIPLOID ANALYSIS OF PETITE ASCOSPORE SISTER CELLS DERIVED FROM THE CROSS D587-4B-2sp_2 X E290-3

Cross	Frequencies of Mitochondrial Types in Cell Samples						
	Petite	Grande	C^RE^S	C^SE^R	C^RE^R	C^SE^S	Colonies Scored
SPT-2A* X D585-11C-1	.905	.095		.095			560
SPT-2B* X ade 7	.999	.001				.001	681
SPT-2C* X D585-11C-1	.752	.248		.248			626
SPT-2D* X ade 7	.987	.013				.013	993

* Petite ascospore sister cell

CHAPTER IX

STUDIES INVOLVING STRAINS CARRYING THE OMEGA FACTOR

Theories concerning mitochondrial inheritance have been briefly presented in the introduction, among these the most controversial is the proposal of mitochondrial sex. The proponents of this theory visualize a factor (omega) which may exist in two forms ω^+ and ω^-, and which control the transmission of recombinant classes as well as parental classes. This factor is also involved in the control of the asymmetry observed for parental and recombinant classes. To obtain information on the possible existence of a hierarchy of controlling factors, crosses were made between strains carrying ω^+ or ω^- and strains which carried the nuclear determinant(s) responsible for the asymmetrical transmission of mitochondrial type. The results of a random diploid analysis of these crosses are described in Table 37. The first three crosses involve mutants derived from the strain D587-4B, which were shown to carry a nuclear determinant responsible for the preferential transmission of mitochondrial type. The results show that a preferential transmission of the mitochondrial type carried by mutants derived from D587-4B is consistently observed. Cross 1 and Cross 2 involved ω^+ strains, while Cross 3 involved a ω^- strain. In Cross 3 the asymmetrical transmission of parental types was reduced and an increase in the frequency of the inheritance of the double resistant mitochondrial

TABLE 37

DISTRIBUTION OF MITOCHONDRIAL TYPES IN RANDOM DIPLOIDS

Cross	Frequencies of Mitochondrial Types in Cell Samples				
	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
(1) D587-4B-2($C^{S}E^{R}$) X D22($C^{S}E^{S}\omega^{+}$)		.788		.212	916
(2) D587-4B-4($C^{R}E^{S}$) X D22-1($C^{S}E^{R}\omega^{+}$)	.824	.133	.014	.029	924
(3) D587-4B-4($C^{R}E^{S}$) X 55R5-3C($C^{S}E^{R}\omega^{-}$)	.539	.295	.166		835
(4) D6-3($C^{R}E^{S}\omega^{+}$) X D22($C^{S}E^{S}\omega^{+}$)	.419			.581	415
(5) D6-3($C^{R}E^{S}\omega^{+}$) X D22-1($C^{S}E^{R}\omega^{+}$)	.470	.432	.054	.035	685
(6) D6($C^{S}E^{S}\omega^{+}$) X D22-1($C^{S}E^{R}\omega^{+}$)		.259		.741	506
(7) D6-2($C^{S}E^{R}\omega^{+}$) X E290-3($C^{R}E^{S}$)	.539	.382	.067	.012	319
(8) D6-3($C^{R}E^{S}\omega^{+}$) X 55R5-3C($C^{S}E^{R}\omega^{-}$)	.288	.355	.357		726
(9) IL16-10B($C^{R}E^{S}\omega^{-}$) X 55R5-3C($C^{S}E^{R}\omega^{-}$)	.853	.077		.070	762
(10) IL16-10B($C^{R}E^{S}\omega^{-}$) X D22-1($C^{S}E^{R}\omega^{+}$)	.506	.471	.001	.022	1,248

type was observed, while the double sensitive recombinant class was not observed. Crosses 4 and 5 are homosexual crosses ($\omega^+ \times \omega^+$) and no significant polarity was observed. Cross 6 involved the parental strain D6 from which the antibiotic resistance mutants (D6-2 and D6-3) were derived. Parental strains and mutants derived from them were crossed to determine if any resistance marker had an effect on the distribution of mitochondria. The results of Cross 6 show a polarity of transmission of the D6 mitochondrial type. Since no polarity was observed in crosses involving D6-2 or D6-3 this polarity could be attributed to a possible selection for the wild type mitochondrial genome. Cross 7 involves an unclassified strain (with respect to omega type) E290-3. There is a slight preferential transmission of the parental mitochondrial type contributed by the E290-3 strain and a polarity of transmission of the double resistant mitochondrial type. Cross 8 is a heterosexual cross ($\omega^+ \times \omega^-$). Low polarity of parental mitochondrial types was observed and an extreme polarity of transmission of the double resistant type is seen. Cross 9 is a homosexual cross, but a high polarity was observed for both parental and recombinant classes. These results are not in accord with the definition of a homosexual cross. Bolotin et al, (1971) observed no polarity in homosexual crosses. Cross 10 is a heterosexual cross, no polarity was observed for parental types, but polarity was observed in the recombinant

classes. Since the strain IL16-10B showed an extremely low frequency of zygote formation, zygote clone analysis will not be presented for Cross 9 and 10. In the analysis of three crosses involving the ω^- strain 55R5-3C one of the recombinant classes was not observed.

Tables 38, 39 and 40 describe the results of zygote clone analysis of Crosses 1, 2 and 3. In all zygotes analyzed the mitochondrial type of the mutants derived from D587-4B are preferentially transmitted to the diploid descendants of the zygotes. These results concur with the results of the random diploid analysis of these crosses. The first three crosses indicated that either alternative form of the omega factor did not alter or modify the control function responsible for the preferential transmission of the mitochondrial type contributed by mutants of D587-4B.

Tables 41 and 42 show the results of homosexual crosses. Individual zygote clones exhibit polarity of parental types. The mean values of the classes of mitochondrial types however, show no polarity of transmission and concurs with the results of random diploid analysis.

The results of Cross 6 is described in Table 43. This zygote clone analysis shows a repeated asymmetrical transmission of the mitochondrial type contributed by the D22-1 strain. These results are not consistent with the results of random diploid analysis. The results could be attributed to the small sample of zygotes analyzed which might have skewed the results.

TABLE 38

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D587-4B-2($C^{S}E^{R}$) X D22($C^{S}E^{S}\omega^{+}$)

Frequencies of Mitochondrial Types in Cell Samples					
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1		.962		.038	719
2		1.00			831
3		.827		.173	884
4		.994		.006	660
5		1.00			811
6		.703		.297	751
Mean		.914		.086	4,656

TABLE 39

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D587-4B-4($C^{R}E^{S}$) X D22-1($C^{S}E^{R}\omega^{+}$)

	Frequencies of Mitochondrial Types in Cell Samples				
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1	.864	.117	.011	.008	766
2	.804	.196			632
3	.873	.092	.020	.015	473
4	.912	.051	.014	.023	672
5	1.00				843
Mean	.891	.094	.007	.008	3,386

TABLE 40

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D587-4B-4($C^{R}E^{S}$) X 55R5-3C($C^{S}E^{R}\omega^{-}$)

Frequencies of Mitochondrial Types in Cell Samples					
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1	1.00				360
2	1.00				668
3	.844	.156			726
4	.900		.100		588
5	.508	.113	.379		551
Mean	.850	.054	.096		2,893

TABLE 41

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D6-3($C^{R}E^{S}\omega^{+}$) X D22($C^{S}E^{S}\omega^{+}$)

Frequencies of Mitochondrial Types in Cell Samples					
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1				1.00	608
2				1.00	676
3	.728			.272	647
4	1.00				672
5	.470			.530	661
Mean	.440			.560	3,264

TABLE 42

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D6-3($C^{R}E^{S}\omega^{+}$) X D22-1($C^{S}E^{R}\omega^{+}$)

Frequencies of Mitochondrial Types in Cell Samples					
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1	.029	.960	.011		692
2	.648	.333	.015	.004	724
3	1.00				611
4	.873	.109	.005	.013	724
5		1.00			521
6	.352	.279	.357	.012	606
Mean	.484	.447	.065	.005	3,761

TABLE 43

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D6($C^{S}E^{S}\omega^{+}$) X D22-1($C^{S}E^{R}\omega^{+}$)

	Frequencies of Mitochondrial Types in Cell Samples				
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1		.736		.264	580
2		1.00			715
3		1.00			1,056
4		.993		.003	668
5		.948		.052	631
Mean		.936		.064	3,650

The results of zygote clone analysis of Cross 7 are shown in Table 44. These results agree with the results of the analysis of random diploids. Individual zygotes show polarity, but the mean values of parental types exhibit low polarity. The recombinant classes show the polarity that was observed in random diploids.

Table 45 shows the results of a heterosexual cross not involving mutants of D587-4B. Individual clones show polarity for the transmission of parental and/or recombinant types, but the mean values of the frequencies of the transmission of mito-types agrees with the results of the analysis of random diploids. No polarity was observed in the transmission of parental classes, but it is clear that an extreme polarity was observed in the transmission of the recombinant classes. The $C^{S}E^{S}$ mitochondrial type was never observed in the diploid descendants of the zygotes analyzed. In all crosses involving the 55R5-3C strain the $C^{S}E^{S}$ or $C^{R}E^{R}$ mitochondrial types are never observed in the diploid progeny.

TABLE 44

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D6-2($C^{S}E^{R}\omega^{+}$) X E290-3($C^{R}E^{S}$)

Frequencies of Mitochondrial Types in Cell Samples					
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1	.428	.496	.041	.035	633
2	.334	.149	.480	.037	356
3	.715	.283	.002		431
4		1.00			427
5	1.00				559
Mean	.495	.386	.105	.014	2,406

TABLE 45

DISTRIBUTION OF MITOCHONDRIAL TYPES IN WHOLE ZYGOTE CLONES FROM THE CROSS D6-3($C^{R}E^{S}\omega^{+}$) ✕ 55R5-3C($C^{S}E^{R}\omega^{-}$)

Frequencies of Mitochondrial Types in Cell Samples					
Zygote	$C^{R}E^{S}$	$C^{S}E^{R}$	$C^{R}E^{R}$	$C^{S}E^{S}$	Colonies Scored
1	.189		.811		916
2	.513	.004	.483		1,218
3		1.00			1,045
4	.016	.342	.642		584
5	.428	.246	.326		602
6	.188	.032	.780		436
Mean	.222	.271	.507		4,801

CHAPTER X

DISCUSSION

The study of mitochondrial inheritance has been impeded by three problems which have not been fully resolved; (1) the recombination of mitochondrial DNA molecules has been physically demonstrated (Michaelis et al, 1973; Shannon et al, 1973), but the processes responsible for bringing together, pairing and recombination of membrane bound mitochondrial DNA molecules are unknown; (2) how is the transmission of mitochondrial factors controlled, it at all; (3) is the inheritance of mitochondrial factors not a problem of transmission but one of the control of expression of mitochondrial genomes. The frequency of recombination has been shown by vary greatly and to depend on the mating partners used in the cross. In most crosses studied the frequencies of recombination were low. However, in crosses involving an ω^- strain the frequencies of recombination were much higher. It has been proposed (Bolotin et al, 1971; Avner et al, 1973; Wolf et al, 1973) that the ω factor is involved in the recombinational process and is also responsible for the transmission of recombinant and parental genomes to the progeny of the zygotes. A survey of the strains classified ω^- by these authors has indicated that most if not all of these strains were derived from one or two parental strains, raising

the possibility that the increase in the frequency of recombinant types observed in crosses involving these strains may reflect a unique characteristic possessed by these strains and not necessarily a mitochondrial sex factor.

The second unresolved problem is the transmission of mitochondrial genomes. Lukins et al, (1973) recently proposed that the segregation of mitochondrial genotypes, both parental and recombinant, is a random process associated with the transmission of mitochondria to buds. This dissertation clearly demonstrates the existence of nuclear factors which could profoundly effect the transmission of mitochondrial markers to the progeny of zygotes. Since as we have shown the transmission of mitochondria is not always a random process, then the frequency of recombinants observed in crosses may not reflect only the frequency of recombination, but may wholly or in substantial part reflect the frequency of their transmission to the zygote progeny. Ours is not the first report of nuclear factors effecting the transmission of mitochondria, Howell et al, (1973) have reported several different "interactions", one possibly nuclear.

Another aspect of transmission is the transmission of the petite phenotype by suppressive petite mutants. It has been shown (Ephrussi et al, 1955) that the suppressive character is the property of the mitochondrial genome. In a recent report (Bech-Hansen

and Rank, 1973) it was shown that nuclear mutations could effect the suppressiveness of suppressive petite mutants. We have also shown that nuclear factors sometimes alter or modify the suppressiveness of petite mutants.

The last unresolved problem concerns the expression of the mitochondrial genome. It is still unknown whether or not all of the mitochondrial genomes within a mitochondrion or cell are expressed and no detailed study has dealt with this problem. The extensive lineage analysis described in Table 5 showed that all the progeny of Bud 1 inherited exclusively E^R except Bud 1I. The C^R and $C^S E^S$ containing genomes inherited by Bud 1I must have been transmitted to Bud 1 and some of its progeny but these genomes were only expressed by Bud 1I and its progeny. The lineage of Bud 1 strongly suggests that either a genome may remain silent or that the manifestation of a genome may require more than one representative per cell. Another point that must be made is that transmission and expression cannot at present be separated; detection presently requires expression.

LITERATURE CITED

Ashwell, M., and T. S. Work 1970. The biogenesis of mitochondria. Ann. Rev. Biochem. 39: 251-290.

Avers, C. J., Rancourt, M. W., and F. H. Lin 1964. Mitochondrial phenotypes in normal and respiratory-deficient baker's yeast cell. J. Cell Biol. 23: 7A.

Avner, P. R., Coen, D., Dujon, B., and P. P. Slonimski 1973. Mitochondrial genetics. IV. Allelism and mapping studies of oligomycin resistant mutants in S. cerevisiae. Molec. gen. Genet. 125: 9-52.

Bech-Hansen, N. T., and G. H. Rank 1973. Suppressiveness and asymmetrical distribution of cytoplasmically inherited markers in Saccharomyces cerevisiae. (abstract) Genetics 74: S18 supplement.

Beck, J. C., Parker, J. H., Balcavage, W. X., and J. R. Mattoon 1971. Mendelian genes affecting development and function of yeast mitochondria. In Autonomy and Biogenesis of Mitochondria and Chloroplasts (Boardman, N. K., Linnane, A. W., and Smillie, R. M., eds) pp. 194-204, American Elsevier Publishing Co., New York.

Bolotin, M. Coen, D. Deutsh, J., Dujon, B., Netter, P., Petrochilio, E., and P. P. Slonimski 1971. La recombinaison des mitochondries chez Saccharomyces cerevisiae. Bull Inst. Pasteur 69: 215-239.

Borst, P., and A. M. Kroon 1969. Mitochondrial DNA: Physicochemical properties, replication and genetic function. Int. Rev. Cytol. 26: 107-190.

Borst, P. 1972. Mitochondrial nucleic acids. Ann. Rev. Biochem. 41: 333-376.

Carnevali, F., Morpurgo, G., and G. Tecce 1969. The cytoplasmic DNA from "petite" colonies of Saccharomyces cerevisiae and a hypothesis on the nature of the mutation. Science 163: 1331-1333.

Casey, J., Fukuhara, H., Getz, G. S. and M. Rabinowitz 1969. Hybridization of mitochondrial ^{3}H-leucyl-tRNA with

mitochondrial DNA of grande and petite yeast. J. Cell Biol. 43: 18A.

Chen, W. L., and F. C. Charalampous 1969. Mechanism of induction of cytochrome oxidase in yeast. I. Kinetics of induction and evidence for accumulation of cytoplasmic and mitochondrial precursors. J. Biol. Chem. 244: 2767-2776.

Clark-Walker, G. D., and A. W. Linnane 1966. In vivo differentiation of yeast cytoplasmic and mitochondrial protein synthesis with antibiotics. Biochem. Biophys. Res. Commun. 25: 8-14.

_____ 1967. The biogenesis of mitochondria in Saccharomyces cerevisiae. J. Cell Biol. 34: 1-14.

Coen, D., Deutsch, J., Netter, P., Petrochilo, E., and P. P. Slonimski 1970. Mitochondrial genetics I. Methodology and phenomenology. Soc. Exptl. Biol. Symp., 24: 449-496.

Correns, C. 1909. Vererbungsversuche mit blass (gelb) grünen und bunt blättrigen Sippen bei Mirabilis, Urtica, und Lunaria. Zeit. Induk. Abst. u. Vererbund., 1: 291-329.

Damsky, C. H., Nelson, W. M., and A. Claude 1969. Mitochondria in anaerobically grown, lipid limited brewers yeast. J. Cell Biol. 43: 174-179.

Dawid, J. B. 1970. The nature of mitochondrial RNA in oocytes of Xenopus laevis and its relation to mitochondrial DNA. In: The Development and Interrelationships of Cell Organelles (P. L. Miller ed) pp. 227-246, Cambridge University Press, Cambridge, London.

Ephrussi, B. 1953. Nucleo-Cytoplasmic Relations in Microorganisms. Oxford University Press, New York.

Ephrussi, B., Hottinguer, H., and H. Roman 1955. Suppressiveness: A new factor in the genetic determination of the synthesis of respiratory enzymes in yeast. Proc. Natl. Acad. Sci. U.S. 41: 1065-1071.

Ephrussi, B., Jakob, H., and H. J. S. Grandchamp 1966. Etudes

sur la suppressivite des mutants a deficience respiratoire de la levure. II. Etapes de la mutation grande en petite provoquee par le facteur suppressif. Genetics 54: 1-29.

Fay, G., Fukuhara, H., Grandchamp, C., Lazowska, J., Michel, F., Casey, J., Getz, G. S., Locker, J., Rabinowitz, M., Bolotin-Fukuhara, M., Coen, D., Deutsch, J., Dujon, B., Netter, P., and P. P. Slonimski 1973. Mitochondrial nucleic acids in the petite colonie mutants: Deletions and repetitions of genes. Biochimie 55: 779-792.

Fukuhara, H. 1967. Informational role of mitochondrial DNA studied by hybridization with different classes of RNA in yeast. Proc. Natl. Acad. Sci. U.S. 58: 1065-1072.

Groot, G. S. P., Rouslin, W., and G. Schatz 1972. Promitochondria of anaerobically grown yeast IV. Effect of oxygen on promitochondrial protein synthesis. J. Biol. Chem. 247: 1735-1742.

Hoffman, H. P., and C. J. Avers 1973. Mitochondrion of yeast: Ultrastructural evidence for one giant, branched organelle per cell. Science 181: 749-751.

Hollenberg, C.P., Borst, P., Thuring, R. W. J., and E. F. J. Van Bruggen 1969. Size, structure and genetic complexity of yeast mitochondrial DNA. Biochim. Biphys. Acta 186: 417-419.

Howell, N., Trembath, M. K., Linnane, A. W., and H. B. Lukins 1973. Biogenesis of Mitochondria. 30: An analysis of polarity of mitochondrial gene recombination and transmission. Molec. gen. Genet. 122: 37-51.

Jimenez, A., Tipper, D. D., and J. Davies 1973. On the mode of action of thiolutin an inhibitor of macromolecular synthesis in Saccharomyces cerevisiae. Antimicrobial Agents and Chemotheraphy 3: 729-738.

Kovac, L., and K. Weissova 1968. Oxidative phosphorylation in yeast. III: ATPase activity of the mitochondrial fraction from a cytoplasmic respiratory-deficient mutant. Biochim. Biophys. Acta 153: 55-59.

Kuntzel, H. 1969. Proteins of mitochondrial and cytoplasmic ribosomes from Neurospora crassa. Nature 222: 142-146.

Lai, G. J., and B. Weisblum 1971. Altered methylation of ribosomal RNA in an erythromycin-resistant strain of Staphylococcus aureus. Proc. Natl. Acad. Sci. U.S. 68: 856-860.

Lamb, A. J., Clark-Walker, G. D., and A. W. Linnane 1968. The biogenesis of mitochondria, 4. The differentiation of mitochondrial and cytoplasmic protein synthesis in vitro by antibiotics. Biochim. Biophys. Acta 161: 415-427.

Linnane, A. A., Saunders, G. W., Gingold, E. B., and H. B. Lukins 1968. The biogenesis of mitochondria, 5. Cytoplasmic inheritance of erythromycin resistance in Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. U.S. 59: 903-910.

Lukins, H. B., Tate, J. R., Saunders, G. W., and A. W. Linnane 1973. The biogenesis of mitochondria, 26. Mitochondrial recombination: the segregation of parental and recombinant mitochondrial genotypes during vegetative division of yeast. Molec. gen. Genet. 120: 17-25.

Mahler, H. R. Pearlman, P. S., and D. B. Metrotra 1971. Mitochondrial specification of the respiratory chain. In Autonomy and Biogenesis of Mitochondria and Chloroplasts (Boardman, N. K., Linnane, A. W., and Smillie, R. M., eds) pp. 492-511, American Elsevier Publishing Co., New York.

Mason, T. L., and G. Schatz 1973. Cytrochrome c oxidase of bakers yeast. II. Site of the translation of the protein components. J. Biol. Chem. 248: 1355-1360.

Mason, Y. L., Poyton, R. O., Warton, D. C., and G. Schatz 1973. Cytochrome c oxidase from bakers yeast. I. Isolation and properties. J. Biol. Chem. 248: 1346-1354.

Mehrotra, B. D., and H. R. Mahler 1968. Characterization of some unusual DNAs from the mitochondria from certain "petite" strains of Saccharomyces cerevisiae. Arch. Biochem. Biophys. 128: 685-703.

Michaelis, G., Petrochilo, E., and P. P. Slonimski 1973. Mitochondrial genetics III. Recombined molecules of mitochondrial DNA obtained from crosses between cytoplasmic "petite" mutants of Saccharomyces cerevisiae: Physical and genetic characterization. Molec. gen. Genet. 123: 51-65.

Mortimer, R. K., and D. C. Hawthorne 1969. Yeast genetics. In The Yeasts (Rose, A. H., and J. S. Harrison eds) vol. 1: pp. 385-460, Academic Press, New York.

Nass, M. M. K., and C. A. Buck 1969. Comparative hybridization of mitochondrial and cytoplasmic aminoacyl transfer RNA with mitochondrial DNA from rat liver. Proc. Natl. Acad. Sci. U.S. 62: 506-513.

Ogur, M., St. John, R., and S. Nagai 1957. Tetrazolium overlay technique for population studies of respiratory deficiency in yeast. Science 125: 928-929.

Osumi, M., and N. Sando 1969. Division of yeast mitochondria in synchronous culture. J. Electron Microsc. 18: 47-56.

Otaka, E., Teraoka, H., Tamaki, M., Tanaka, K., and S. Osawa 1970. Ribosomes from erythromycin-resistant mutant of Escherichia coli Q13. J. Mol. Biol. 48: 499-510.

Peska, S. 1971. Inhibitors of ribosomal function. Ann. Rev. Biochem. 40: 697-710.

Plattner, H., and G. Schatz 1969. Promitochondria of anaerobically grown yeast. III. Morphology. Biochemistry 8: 339-343.

Rabinowitz, M., and H. Swift 1970. Mitochondrial nucleic acids and their relation to the biogenesis of mitochondria. Physiol. Rev. 50: 376-427.

Rank, G. H., and N. T. Bech-Hansen 1972. Somatic segregation, recombination, asymmetrical distribution and complementation tests of cytoplasmically-inherited antibiotic-resistance mitochondrial markers in S. cerevisiae. Genetics 72: 1-15.

Roodyn, D. B., and D. Wilkie 1968. The Biogenesis of Mitochondria. London: Methuen.

Sager, R. 1960. Genetic systems in Chlamydomonas. Science 132: 1459-1465.

Saunders, G. W., Gingold, E. B., Trembath, M. K., Lukins, H. B. and A. W. Linnane 1970. Mitochondrial genetics in yeast: Segregation of a cytoplasmic determinant in crosses and its loss or retention in the petite. In Autonomy and Biogenesis of Mitochondria and Chloroplasts (Boardman, N. K., Linnane,

A. W., and Smillie, R. M., eds) pp. 251-314, American Elsevier Publishing Co., New York.

Schafer, K. P., and H. Kuntzel 1972. Mitochondrial genes in Neurospora: A single cistron for ribosomal RNA. Biochem. Biophys. Res. Commun. 46: 1312-1318.

Schatz, G. 1970. Biogenesis of mitochondria. In Membranes of Mitochondria and Chloroplasts (Racker, E., ed) pp. 251-314, Van Nostrand Reinhold, New York.

_____. 1968. Impaired binding of mitochondrial adenosine triphosphatase in the cytoplasmic petite mutant of Saccharomyces cerevisiae. J. Biol. Chem. 243: 2192-2199.

Shannon, G., Rao, A., Douglass, S., and R. S. Criddle 1972. Recombination in yeast mitochondrial DNA. J. Supramolecular Structure 1: 145-151.

Sherman, F., and P. P. Slonimski 1964. Respiratory-deficient mutants of yeast. II. Biochemistry. Biochem. Biophys. Acta 90: 1-15.

Sherman, F., Stewart, J. W., Margoliash, E., Parker, J., and W. Campbell 1966. The structural gene for yeast cytochrome c. Proc. Natl. Acad. Sci. U. S. 55: 1498-1504.

Smith, I., Goldthwaite, C., and D. Dubnau 1969. The genetics of ribosomes in Bacillus subtillis. Cold Spring Harbor Symp. Quant. Biol. 34: 85-89.

Smith, D. G., Scrivastava, K. C., and D. Wilkie 1972. Ultra-structural changes in mitochondria of zygotes in Saccharomyces cerevisiae. Microbios 6: 231-238.

So, A. G., and E. W., Davie 1963. Incorporation of amino acids into protein in a cell-free system from yeast. Biochemistry 2: 132-136.

Suyama, Y. 1967. The origins of ribonucleic acids in Tetrahymena pyriformis. Biochemistry 6: 2829-2839.

Thomas, D. Y., and D. Wilkie 1968a. Inhibition of mitochondrial synthesis in yeast by erythromycin: cytoplasmic and nuclear factors controlling resistance. Genetic Res. 11: 33-42.

Tzagoloff, A. 1969. Assembly of the mitochondrial membrane system. II. Synthesis of the mitochondrial adenosine triphosphatase, F. J. Biol. Chem. 244: 5027-5033.

_____. 1972. A model of membrane biogenesis. J. Bioenerg. 3: 39-45.

_____. 1970. Assembly of the mitochondrial membrane system. J. Biol. Chem. 245: 1545-1551.

Weislogel, P. O., and R. A. Butow 1971. Control of the mitochondrial genome in Saccharomyces cerevisiae. J. Biol. Chem. 246: 5113-5119.

Wilkie, D., and D. Y. Thomas 1973. Mitochondrial genetic analysis by zygote cell lineages in Saccharomyces cerevisiae. Genetics 73: 367-377.

Williamson, D. H. 1970. The effect of environmental and genetic factors on the replication of mitochondrial DNA in yeast. In Control of Organelle Development (Miller, P. L., ed) Soc. Expt. Biol. Symp. 24: 247-276. Academic Press, New York.

Wintersberger, E. and G. Viehhauser, 1968. Function of Mitochondrial DNA in yeast. Nature 220: 699-702.

Wolf, K., Dujon, B., and P. P. Slonimski 1973. Mitochondrial genetics. V. Multifactorial mitochondrial crosses involving a mutation conferring paromomycin-resistance in S. cerevisiae. Molec. gen. Genet. 125: 53-90.