MITOCHONDRIAL GENETIC ANALYSIS BY ZYGOTE CELL LINEAGES IN *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Yeast strains were constructed carrying multiple mitochondrial markers conferring resistance to the inhibitors erythromycin, chloramphenicol, paromomycin and oligomycin. A pedigree analysis of two crosses was made by micromanipulating buds from zygotes. The first few daughter buds isolated from the zygotes sometimes gave rise to diploid clones which had a mixture of mitochondrial types. All possible classes of mitochondrial parental and recombinant types were found although they never appeared all together as the progeny from a single zygote. It was inferred that multiple recombination events took place in zygotes and in some of the buds derived from them. After removal of the first four or so daughter buds, subsequent buds from the zygote carried one mitochondrial type only. In cross I in which three markers were analyzed this was most frequently one of the parental types. In cross II (involving four mitochondrial markers) the later buds from the zygotes were frequently of recombinant mitochondrial type.

IT is no longer a point of contention that mitochondria possess an intrinsic genetic system. In the yeast *Saccharomyces cerevisiae* it is based on a mitochondrial DNA molecule of 26 μm contour length (see BORST 1970). The integrity of replication of this molecule is comparatively low and cells frequently arise in culture in which mitochondrial DNA may be grossly altered in base composition or lost entirely (see review of WILLIAMSON 1970). However extensive the lesion in the DNA, there is a common loss of some genetic information in all cases resulting in the irreversible respiratory mutant known as *petite*. It was this feature of genetic instability of the yeast mitochondrion that allowed us to locate genetic factors controlling resistance to certain antibiotics in mitochondrial DNA, by demonstrating the coincident loss of a resistance gene with the induction of the petite mutation (THOMAS and WILKIE 1968a; LINNANE *et al.* 1968). Having characterized a number of mutants resistant to different antibiotics in this way, it was then seen that these factors recombine or otherwise reassort in zygotes from crosses to give rise to segregant diploid cells during development of zygote clones (THOMAS and WILKIE 1968b). Segregation of resistance factors during vegetative growth of diploid clones from zygotes *per se* is indicative of extrachro-

mosomal control of resistance; but it was also shown that segregant diploid cells, when sporulated, gave non-Mendelian ratios of resistance to sensitivity.

In these earlier studies, cells from individual zygote clones were sampled and scored for inheritance of resistance factors. On zygote formation there is established a mixed population of mitochondria (D. G. SMITH, K. C. SRIVASTAVA and D. WILKIE 1972, in press) and operationally the problem can be likened to that of a bacteriophage cross with the potential for repeated interaction among multiple genomes, the products in this case being transmitted to daughter cells. A powerful tool of phage genetics, namely the ability to control the multiplicity of infection, is not available in mitochondrial genetics, as no method of varying the number of mitochondrial genomes is yet known. In general, each cell from a zygote clone when plated and allowed to develop into a colony was homogeneous for a single mitochondrial phenotype. In other words, segregation must have been completed early in the development of the zygote clone. The results (COEN *et al.* 1971; THOMAS and WILKIE 1968b) also showed that in a number of cases but not all, there was unequal segregation of reciprocal classes, both parental and recombinant, and it was apparent that the mechanism of the recombinational process and the transmission of mitochondrial genomes was complex. To obtain more detailed information on these processes, it was considered necessary to analyze the mitochondrial complement of individual daughter cells issuing from zygotes.

MATERIALS AND METHODS

Strains and cultural methods: Strains 41 (*ure, his,* α), 4a (*arg, a*) and D22 (ade_2. *a*) were used in this analysis. To obtain zygotes, cells of each strain in the cross were pre-grown for sixteen hours on yeast extract (1%), glucose (2%) shake culture, spun down and transferred as individual mass inocula onto an agar plate containing yeast extract and glycerol (4%). After four hours' incubation at 30°C, cells of each strain were mixed on the agar surface with the aid of a few drops of water and incubated for a further two hours. After this time, the mixture usually contained a high proportion of zygotes (about 20%).

Micromanipulation: A sample of the mating mixture was transferred to a yeast extract-glycerol agar block for microdissection. With the de Fonbrune micromanipulator, zygotes were isolated and daughter cells separated as they were budded off. Generation time of buds was about two hours. Frequently, buds from the daughter cells were also separated for analysis. It was possible to arrest development of buds by placing the preparation under refrigeration (4°C) overnight and continuing the microdissection the next day. Throughout this procedure, zygotes maintained their characteristic morphology. Variations in the procedure were made in some experiments as follows: (1) zygotes were obtained by pairing up individual cells of the two parents by micromanipulation; (2) microdissection was carried out on an agar block either supplemented with 1 mg/ml erythromycin or in which glucose replaced glycerol as the carbon source.

Antibiotics: Erythromycin (E) as the gluceptate was a gift from the Abbott Co.; chloramphenicol (C) and paromomycin (P) were obtained from Parke-Davis and oligomycin (O) from Sigma Chemical Co.

Resistant mutants: Techniques for the detection and isolation of mutants resistant to antimitochondrial drugs are described in THOMAS and WILKIE (1968a). Multiple resistant strains were obtained following independent mutational steps to resistance on each drug, starting usually with E. Mitochondrial location of the resistance factor was established after each step (THOMAS 1969; THOMAS 1972, in preparation), both by tetrad dissection and by showing loss of resistance in some ρ^- petites. The resistance to O of strain 4a was first picked up in strain D6. This was

crossed to D22, a resulting resistant diploid was sporulated and strain *4a* was obtained from one of the four resistant ascospores.

Analysis of clones: Dissected buds were incubated together with the original zygote until resultant colonies were comprised of about 10^6 cells. Each colony was taken up into suspension and several aliquots were spread on plates of yeast extract-glucose medium. Colonies that grew (on average about 500 in each case) were scored for drug resistance by velvet pad replica-plating onto plates containing drugs, singly and in combinations. In all cases where this replica test indicated sensitivity to a particular drug, the original colony was further tested by dropping-out the remaining cells (between 10^5 and 10^6) on plates containing the drug; even a small proportion of resistant cells would be detected by the second test. Stability of multiple-resistance segregants was tested in many cases by subculture from selective and non-selective media.

RESULTS

In vivo the strains *41* and *D22* are sensitive to all four inhibitors. That is to say in yeast extract-glycerol medium the cells of each strain failed to grow at the following concentrations: erythromycin—25 μg/ml; chloramphenicol—100 μg/ml; paromomycin-50 μg/ml; and oligomycin—0.5 μg/ml. Concentrations used to select resistant mutants were: erythromycin–; chloramphenicol–2; paromomycin–1 mg/ml; and oligomycin–2.5 μg/ml. These concentrations were also used to check resistance of segregants.

The results of zygote lineage analysis in cross I (between strain *41* of mitochondrial genotype $O^SC^R{}_9E^R{}_{76}$ and strain *4a*, $O^R{}_1C^SE^S$) are shown in Table 1. The main points are: (1) each cell from a bud clone when plated gave rise to a stable, apparently homogeneous colony as regards mitochondrial phenotypes; (2) recombinant classes arose from ten zygotes out of sixteen which were dissected (the term "recombinant" is used in a general sense to denote phenotypes other than parental, but see DISCUSSION); (3) all six possible recombinant types were seen, although never all together, from any one zygote; (4) only in two cases were reciprocal products of recombination observed among the progeny from a single zygote while both parental types appeared together in three cases; (5) in a few cases, the mitochondrial types and their frequencies transmitted by a zygote to different buds were strikingly similar (Table 1). In some zygotes (see Table 1 for example, zygotes G and H), although only three genotypes segregated, the buds and the final zygote clone had two of these genotypes represented in similar frequencies. In another example (see Table 1, zygotes D) different buds from the same zygote which segregated four mitochondrial types had these genotypes present in similar proportions. Thus a degree of synchony of segregation can sometimes be detected when the initial cell of a colony contains a mixture of mitochondrial types; (6) late buds, as exemplified by the final zygote clone, generally inherited a parental genome only, preferentially that of parent *4a* $O^R{}_1C^SE^S$. Second and subsequent generation daughter cells derived from the first generation of zygote buds frequently inherited a different mitochondrial complement from that of the latter (Table 1).

It is clear from these results that a great deal of recombination takes place, but from the observation (6) it must be concluded that a mechanism exists for preserving an intact parental genome and for its rapid predominance in the zygote.

TABLE 1

Mitochondrial phenotypes in clones from zygote daughter cells (buds in the cross 41 $O^S C^R_9 E^R_{76}$ × 4a $O^R_1 C^S E^S$) expressed as R/S to O, C and E in that order. Respective frequencies of each mitochondrial type in cases of mixed clones are given in parenthesis

Zygote lineage	BUDS 1	1a	1b	1c	1d	2	2a	3	4	Zygote*
A	SRR	SRR (.94)	...	...	...	...	...	...	...	RSS
	...	SRS (.06)	...	...	...	RRS	...	...	...	...
B	RSS	...	...	...	...	RRS (.53)	...	...	...	RSS
	...	...	...	...	...	RRR (.47)	...	SSS	...	...
C	SSR (.61)	...	...	...	...	RSS	...	...	...	RSS
	RSR (.33)	...	...	...	...	...	...	...	...	...
	RSS (.06)	...	...	...	...	...	...	...	...	...
	SSS (.04)	...	...	...	...	...	...	...	...	...
D	RSS	RSS	...	...	...	RRR (.35)	...	RRR (.46)	RSS (.99)	RSS
	...	...	...	...	...	RSS (.29)	...	RRS (.27)	RRR (.01)	...
	...	...	...	...	...	RRS (.22)	...	RSS (.26)	...	...
	...	...	...	...	...	RSR (.13)	...	RSR (.01)	...	...
E	×†	...	...	...	...	SRR (.99)	SRR (.99)	...	...	RSS (.80)
	...	...	...	...	...	RRR (.01)	RRR (.01)	...	...	RSR (.18)
	...	...	...	...	...	...	...	...	...	RRS (.02)
F	RSS (.39)	RSS (.83)	...	...	...	×	...	RRR (.91)	RSR (.60)	RSS (.65)
	RSR (.59)	RSR (.16)	...	...	...	...	...	RRS (.09)	RSS (.40)	RSR (.35)
	RRS (.02)	RRR (.01)	...	...	...	...	...	...	...	...
G	SRR	SRR	SRR (.99)	...	...	SRR (.95)	...	SRR	...	SRR
	...	...	RRR (.01)	...	...	RRR (.05)	...	...	...	...
H	SRR	SRR (.99)	...	...	...	SRR	...	SRR (.98)	...	SRR (.99)
	...	RRR (.01)	...	...	...	...	...	RRR (.02)	...	SSR (.01)
I	RSS (.69)	SRR	SRR	RSR (.66)	RSS (.83)	...	...	...	...	RSR (.82)
	SRR (.21)	...	...	RSS (.34)	SRR (.15)	...	...	...	...	RRR (.18)
	RRS (.10)	...	...	...	RSR (.02)	...	...	...	...	...
J	RRS (.68)	RSS (.19)	...	...	...	RRS (.98)	...	RRS	...	×
	RSS (.02)	ρ— (.81)‡	...	...	...	ρ— (.02)	...	...	...	...
	ρ— (.30)	...	...	...	...	...	...	...	...	...
K	RSS	RSS	RSS	...	...	RSS	...	RSS	RSS	RSS

* The zygote after removal of initial buds was incubated to give a clone derived from subsequent buds.
† × = Did not survive.
‡ ρ— = Respiratory deficient; dissection carried out on glucose medium in this lineage.

TABLE 2

Total phenotypes obtained from individual zygotes from cross I (See Table 1)

Phenotypes	Zygote lineage A	B	C	D	E	F	G	H	I	J	K	Totals	Random diploids*
O C E													
S R R	+	..	..	..	+	..	+	+	+	..	..	5	24
R S S	+	+	+	+	+	+	..	..	+	+	+	9	128
S S S	..	+	+	..	..	..	..	..	..	..	..	2	13
R R R	..	+	..	+	+	+	+	+	+	..	..	7	18
S R S	+	..	..	..	..	..	..	..	..	..	..	1	8
R S R	..	..	+	+	+	+	..	..	+	..	..	5	33
S S R	..	..	+	..	..	..	..	+	..	..	..	2	4
R R S	+	+	..	+	+	+	..	..	+	+	..	7	11

* Cells sampled from a culture on minimal (diploid-selecting) medium obtained from a mass mating.

The occurrence of the different classes in zygote lineage is summarized in Table 2.

When random diploid cells from a culture obtained by mass-mating the two strains were plated, resulting colonies each showed a single mitochondrial phenotype when tested. The frequencies of the various classes obtained are also listed in Table 2. It can be readily appreciated why there is an excess of parental types using this procedure, since it was apparent from the micromanipulation results that proliferation of the zygote beyond the first few buds leads to the production of parental type buds only. The excess of the RSS parental type is also consistent with the dissection experiments. Among recombinant types from the random diploids, reciprocal products in the class SRS/RSR are not in equal amounts, indicating that a selection mechanism is operating in this case as well as for parental type. This selection is not apparent in the other recombinant classes. In the analysis of a clone derived from a zygote, the relative frequencies of parentals and recombinants seems to depend on how quickly the parental genome becomes established in the zygote, an extreme case being zygote K, Table 1. Before speculating further on the mechanism of mitochondrial inheritance, the results of zygote cell lineage analysis in a second cross will be presented.

A multiple resistant strain was produced from strain D22 with resistance levels of oligomycin–2.5 μg/ml, erythromycin–5 mg/ml and paromomycin–2 mg/ml and checked for cytoplasmic inheritance of these resistance markers. From strain *41* a mutant with chloramphenicol resistance of 4 mg/ml was isolated. Cross II (*D22* $O^R_6C^SE^R_9P^R_{701} \times$ *41* $O^SC^R_3E^SP^S$) was set up and zygotes and buds were analyzed mainly as described for cross I. The results of a particular series of experiments which typifies the general findings in this cross are given in Table 3. Zygotes in this case were obtained by pairing up cells with the micromanipulator. The most strikingly difference between these results and those of cross I is that, although the zygote usually transmits one particular mitochondrial type after removal of the first few buds, this is not generally a parental type but can be any one of the sixteen possible types. In other respects the results are similar in the

Table 3. Mitochondrial phenotypes in clones from zygote buds in the cross 41 $O^S C^R_3 E^S P^S$ x D22 $O^R_6 C^S E^R_9 P^R_{701}$. Respective frequencies in cases of mixed clones are given in parenthesis.

Zygote Lineage	BUDS (expressed as R/S to OCEP) 1	1a	1b	2	2a	3	3a	4	5	6	7	8	ZYGOTE
1	RRSR(.005)	*RSRR	*RSRR	RRRR		RRRR	RSRR	RRRR(.70)	RRRR	RRRR			RRRR
	RRRR(.995)							RRRS(.30)					
2	SSRR(.53)	SSRRS		*SRSS(.95)		RRRS		SRRR	RSRS				RSRS
	SSRS(.47)			SRRS(.05)									SRRS
3	SSRS			SRRS				RRRR	RSRS	RRRR(.03)			SSRS
4	x			RRRS(.01)	RRRS	RSRS				RRRS(.97)			
				SRRR(.05)				RRRR	RRRR	RRRS	RRRR		SRRR
				SRRS(.94)				RSRR	RRRR	RRRR	SRRR		RRRR
5	SRRR			SRRR		SRRR		SRSR	RRSR				*SRSS
6	*RSRR	SSRR		x		SRRS							SRSR(0.58)
7	SRRR(.02)			SRRR(0.48)	SSRS(0.34)	SRRS							SRRR(0.41)
	SRRS(.98)			SRSR(0.52)	SSSS(0.61)								RRRR(0.01)
8	RSRR(0.28)	RSSR	SRRR(0.93)	SRRR	SRRS	SRRS(0.64)		RRSR(0.94)	SRSS(0.91)				RSSR
	RSSR(0.72)		SRSR(0.07)			*SRSS(0.36)		RRRR(0.06)	SRRS(0.09)				
9	x			x		RRRR	RSSR						x
						RRSS		SSRS(0.42)	RRRR				RRRS
10	RRRR	RRSS(0.58)		RRSS		*SRSS		SSSS(0.58)					
		RRRS(0.42)											
11	RRRS(0.79)	RRSS		x		x							
	RRSS(0.21)												
12	RSRS(0.68)			RRRS									RRRS
	SSSS(0.21)												· RRRR
	RSSS(0.11)							RRRS					RRRR(0.73)
13	†RRSR			†RRSR		†RRSR							SRRR(0.27)
14	SRRS	RRRS		†RSSS	†RRRS	†RRSS		SRRS	RRRS	RSRS	x	RRRS(0.68)	SRRS
E1	RRRS			RRRS		RSRS						RRSS(0.32)	
E2	RRRR(0.97)	RRRR		RRRR		RRRR		*RSRR					RRRR
	RRRS(0.03)												RRRR
E3	RRRS(0.35)							RSRR	RSRR				SRRR
	SRRS(0.65)			*RSRR									x
E4	RRRR			SSRR		*RSRR							RRRR
E5	RRRS	RRRS		RRRS	RRRS	RRRS							RRRS(0.58)
E6	*RSRR	*RSRR		RSRR		RSRR							SRRS(0.42)
E7	x			x									

* Parental type.

† Haploid.

two crosses: recombinant types issue from every zygote and are restricted in number from any one zygote; all recombinant types are seen (in this particular series the recombinant type SSSR is missing but appears in other series—see Table 4).

The daughter cells isolated from zygotes 13 and 14 are of particular interest, since some of these were found to be haploid. They were first detected as red (ade_2) colonies and mated with other haploid strains of mating type α when further analyzed. It was concluded that these zygotes resulted from matings between two parental cells, one of which (*D22*) contained two haploid nuclei that had undergone nuclear division just prior to nuclear fusion. After mating, the zygote would contain both a haploid and a diploid nucleus. The mitochondrial types present in the haploid clones vary and include RSRR, RRSS, SSRS and

TABLE 4

Total phenotypes obtained from individual zygotes in the cross
$41\ O^S C^R_3 E^S P^S \times D22\ O^R_6 C^S E^R_9 P^R_{701}$

Phenotypes O C E P	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	Totals
S R S S	+	.	.	+	.	.	+	.	+	.	.	+	+	.	.	+	+	+	.	.	+	.	.	+	.	.	.	+	+	+	.	+	.	.	15
R S R R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	+	.	.	.	.	+	.	+	.	.	.	.	.	.	+	6
S S R R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.	.	.	.	+	.	.	.	+	.	.	4
R R S S	.	.	.	+	.	.	+	.	.	.	+	+	.	.	.	.	.	+	.	.	.	+	+	+	.	+	.	.	.	.	.	.	.	.	9
S R R R	.	.	.	.	.	.	.	.	.	.	.	.	.	+	+	+	+	+	.	+	.	+	.	.	.	+	.	.	.	.	.	+	.	+	10
R S S S	+	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	4
S R S R	.	+	.	.	.	.	.	.	.	.	.	.	+	.	.	+	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	5
R S R S	+	.	+	.	.	.	.	+	+	.	.	.	.	+	.	.	.	.	.	.	.	+	.	+	.	+	.	.	.	.	.	+	.	.	9
S S S S	+	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	.	.	+	.	.	+	.	.	+	.	.	.	+	+	.	.	+	.	9
R R R R	.	+	.	.	.	.	+	.	.	+	.	.	+	+	+	.	+	+	.	+	.	+	+	+	.	+	.	.	+	.	.	.	.	+	15
S S R S	+	.	+	.	.	+	.	+	.	.	.	.	.	+	.	+	.	.	+	.	.	+	+	.	.	.	.	.	.	+	+	+	.	.	12
R R S R	.	+	.	.	.	.	+	.	.	+	.	.	+	.	.	+	.	+	.	.	.	.	+	.	.	+	.	.	+	.	.	.	.	+	10
S R R S	.	.	.	+	+	+	+	.	.	.	.	.	.	+	.	+	+	+	.	+	+	.	.	.	.	.	.	.	+	.	+	.	.	.	12
R S S R	.	+	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+	+	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	5
S S S R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	1
R R R S	+	.	.	+	+	.	+	+	+	.	+	.	.	+	+	.	.	.	.	.	.	+	+	+	+	+	.	.	.	.	.	+	+	+	17

RSRS. These findings provide further evidence of the extra-nuclear location of these factors (WRIGHT and LEDERBERG 1957).

Other zygote analyses were carried out in some cases with zygote formation, bud dissection and clone development on yeast extract-glucose medium. Results were similar to those already described for glycerol medium and the collective data for the non-selective medium (without erythromycin) are presented in Table 4, in which all phenotypes obtained from individual zygotes are listed. The maximum number of phenotypes from one zygote was eight (seen only in one case), while a minimum of two phenotypes was scored for several zygotes. In this four-point cross, there are eight classes of reciprocal pairs including parental class. It can be seen that: (1) all possible arrangements of markers occur; (2) reciprocal products appear together in six out of 34 zygote lineages analyzed; (3) the frequency of any one reciprocal pair is much the same as the frequency of any other pair; (4) the frequency of the RRRS segregant is significantly higher than the frequency of its reciprocal SSSR, but other reciprocal segregants are not significantly different than 1:1. These results are indicative of multiple recombinational events taking place in each zygote and from which a mainly random sample of resultant genomes survive or are selected to form a pool. Reciprocal products are available, but on this interpretation only a proportion of zygotes would retain both products in the pool.

Various segregant diploids from the above crosses were sporulated and tetrads were dissected. Low spore viability restricted the analysis, but in 20 ascospore clones obtained, all showed the phenotype of the diploid from which they were derived. Thus the mitochondrial phenotype was stable through meiotic as well as mitotic division in these cases.

DISCUSSION

Cytoplasmic inheritance of resistance factors and their association with the mitochondrial rho factor provide good evidence that these determinants are located in the mitochondrial DNA. Quantitative studies by other investigators lead to the conclusion that individual mitochondria probably possess more than one genome—that is, have several copies of the basic DNA molecule (see BORST 1972). It may be argued, then, that the different mitochondrial phenotypes that segregate among the vegetative diploid progeny of zygotes result from reassortment and not recombination of genomes of the two parents and interact in reconstituted mitochondria. However, one would have to postulate an extremely complicated system of dominance/recessivity of resistance factors so that mitochondria could show resistance to all drugs in one cell for example, but in other cells could show multiple sensitivity. An even more complicated system would have to be envisaged if the results were to be explained along the lines that a mixture of intact parental mitochondria are inherited from the zygote and that they interact within a common cytoplasm to produce the various phenotypes.

The findings are more readily ascribed to genetic recombination in which mitochondrial DNA of each parent is involved in exchanges. To prove this it is necessary to show that new DNA molecules arise in zygotes and are made up of

parts of each parental molecule. Experiments along these lines are in progress in which the cells of one strain are pre-grown in a heavy-labelled medium prior to crossing, in the expectation that resulting zygotes will have mitochondrial DNA molecules of intermediate buoyant densities.

It is evident from the results that the zygote begins to transmit a single mitochondrial type after the first few buds have been formed. In cross I this was a parental type in almost all zygotes analyzed, while in cross II it was quite random whether a parental or recombinant type became the predominant one. In other words, the mixture of mitochondrial types of the early zygote was either short-lived or the contribution of the two parents of mitochondrial genomes to the cross was grossly unequal. This can also be said of the early buds which gave mixed clones, the individual cells of which were pure with respect to mitochondrial type. There may be occasional exceptions to this general rule, as indicated in the erythromycin selection series in which E-sensitive mitochondria continue to segregate out on the drug medium. It is apparent that zygotes of cross I tend to stabilize their mitochondrial types sooner than those of cross II.

From electron microscope studies (D. G. SMITH, K. C. SRIVASTAVA and D. WILKIE 1972, in press), there are indications that mitochondria undergo dedifferentiation and loss of internal structure on zygote formation. Perhaps this releases their DNA molecules which then proceed to recombine extensively. The speed with which mitochondria reform in budding zygotes and incorporate the new genomes (resynthesis of mitochondria is indicated in the e.m. studies) could determine how quickly the mitochondrial type becomes stabilized, on the assumption that genomes not incorporated are eventually eliminated. The continuity and integrity of a parental genome in cross I zygotes may simply depend on an attachment site on a membrane for the DNA molecule. This site would be absent in cross II, which would mean that the site-conferring capacity is the property of strain *4a*. The preference for *4a* genomes may be attributable to a territorial advantage of these genomes' being located in that part of the early zygote containing the attachment site. Once attached, its role may be to replicate while other, recombinant genomes can only replicate following their incorporation into a developing mitochondrion; this would apply to all genomes in cross II zygotes.

Two observations which may be of significance in the mechanism of mitochondrial transmission are: (1) the striking similarities in the frequency of mitochondrial types seen in buds from the same zygote; and (2) the apparent passage through a bud of a mitochondrial type apparently without replication or expression but which then replicates to form the major mitochondrial type in the next bud. It is difficult to reconcile these observations without involving either segregation which is directed (the equivalent of nuclear chromosome division, but with all like genomes tending to segregate together) or a heterozygous structure inherited by a bud, with segregation occurring during cell division at equivalent times in different buds. The model could include the supposition that mitochondrial genomes undergo recombination at each cell division; in cells with a mixture of mitochondrial types we can detect this recombination. Such a recombinational event could have an evolutionary significance in ensuring a uniformity of mito-

chondrial genomes in cells and the elimination from a population of those cells containing mitochondria with deleterious mutations such as the ρ^- mutation.

Extensive analyses of crosses involving mainly two mitochondrial markers, E^R and C^R, have been published (COEN *et al.* 1970; BOLOTIN *et al.* 1971). Zygote clones were analyzed by these authors mainly by the method of random diploids from mass matings (see Table 2). They also found preferential transmission of one parental genome in some crosses but not in others. Where there is preferential transmission, they use the term "polarity" and attribute the phenomenon to "mitochondrial sex", drawing a close analogy between mitochondrial and bacterial recombination. The parent contributing the preferred genome is given the annotation ω^- and called female, with ω^+ denoting the male. When strains of the same "sex" are crossed, no polarity is seen ("homosexual" cross). In "heterosexual" crosses the frequency of recombinant cells from zygotes is generally higher than in homosexual crosses. The presence or absence of polarity extends to recombinant classes; that is, in heterosexual crosses there is inequality of reciprocal products but not in homosexual crosses. Of course, in a 2-point cross there is only one reciprocal pair of recombinants and, as was apparent from our results, one reciprocal pair may behave quite differently from another in the same cross.

From our results, cross I could be termed a heterosexual cross with strain *4a* as the ω^+ parent, and cross II a homosexual cross between two ω^- strains. The main difference between these crosses, as we see it, is the presence in cross I of a mechanism for maintaining the integrity of a parental genome in the face of recombinational events going on elsewhere in the zygote. These events are frequent, although the products may not always be seen (see Table 1, zygote K).

In this method of whole zygote clone analysis, it could be assumed that each mitochondrial type observed in a clone was transmitted initially to only one zygote bud, but to no other zygote bud. COEN *et al.* (1970) favor this hypothesis, claiming in support that the distribution of mitochondrial types in the cells of a clone statistically followed a $(1/2)^n$ pattern. Our results from the micromanipulation of zygote buds show this relationship to be fortuitous. Individual buds can inherit more than one mitochondrial type from the zygote, and these segregate later in the vegetative progeny. Also the same complement of types may be transmitted at different times, that is in different buds from the same zygote. However, it can be said that the segregational types which form the major part of any clone must have segregated early in the development of that clone. Those present in low frequencies, for example $< 1\%$, would not necessarily be derived from the final buds of the zygote as proposed by COEN *et al.* Rather, they can be carried on through more than one division of the primary bud and segregate later into vegetative cells during clone development. To assign any formal mechanism such as mitochondrial sex in an analogy with the bacterial system to the phenomenon of mitochondrial recombination is, as yet, premature. More information on the behavior of mitochondrial DNA in zygotes is clearly necessary before these problems on the mechanism of recombination and genome transmission can be solved.

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