ABNORMAL MITOCHONDRIAL GENOMES IN YEAST RESTORED TO RESPIRATORY COMPETENCE

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Manuscript received February 21, 1978
Revised copy received May 12, 1978

ABSTRACT

When crosses are performed between newly arisen, spontaneous *petite* mutants of *Saccharomyces cerevisiae*, respiratory competent (restored) colonies can form. Some of the restored colonies are highly sectored and produce large numbers of *petite* mutants. The high-frequency *petite* formation trait is inherited in a non-Mendelian manner, and elimination of mitochondrial DNA from these strains results in the loss of the trait. These results indicate that abnormal mitochondrial genomes are sometimes formed during restoration of respiratory competence. It is hypothesized that these abnormalities result either from recombination between mitochondrial DNA fragments to produce molecules having partial duplications contained on inverted or transposed sequences, or else recombinational "hot spots" have been expanded.

THE *petite* mutation in *Saccharomyces cerevisiae* is manifested by a lack of respiration and an absence of cytochromes $aa_3 + b$. The mutation, which occurs spontaneously at a high frequency (approximately 1 percent of cells per generation), is cytoplasmically inherited and is associated with large deletions from mitochondrial DNA.

In this laboratory, genetic studies have been undertaken to seek insight into the mechanism of deletion from the mitochondrial DNA. These studies have shown that when newly arisen ρ^- mutants* are mated, respiratory competent ρ^+ (restored) colonies can arise in some crosses (CLARK-WALKER and MIKLOS 1975, CLARK-WALKER *et al.* 1976). This result demonstrates that deletions do not always involve a common sequence.

However, since this study was undertaken, two new classes of cytoplasmically inherited respiratory deficient mutations have been described. These are the *syn*$^-$

* The symbols are defined as follows:
ρ^- mutants having defective mitochondrial function attributed to abnormality in mitochondrial DNA.
ρ° ρ^- mutants lacking mitochondrial DNA.
mit$^-$ ρ^- mutants that can carry out mitochondrial protein synthesis.
syn$^-$ ρ^- mutants unable to carry out mitochondrial protein synthesis and whose lesions appear to be restricted to a single site.
ρ^+ strains having wild-type mitochondrial DNA or mutants having altered mitochondrial DNA that still result in functional mitochondria.

and *mit*$^-$ mutants, the former being phenotypically indistinguishable from ρ^- (FLURY, MAHLER and FELDMAN 1974, TZAGOLOFF, AKAI and NEEDLEMAN 1975). As restoration to ρ^+ is observed only in newly arisen ρ^- mutants, a criticism is that it could be due to complementation between *syn*$^-$ or *mit*$^-$ mutants, which may transiently exist before larger deletions are formed. However, results described below render unlikely the possibility that restoration is due to *mit*$^-$ × *mit*$^-$ or even to *mit* × ρ^-.

It was observed during our previous studies that a proportion of the restored diploid colonies were highly sectored, suggesting that cells in these colonies were producing large numbers of *petite* mutants. This communication reports that colony sectoring is indeed associated with a high frequency of *petite* formation, which in turn can be attributed to the mitochondrial genome. These results imply that abnormal mitochondrial genomes are sometimes formed upon restoration of respiratory competence.

MATERIALS AND METHODS

Yeast strains

The strains used in this study are listed in Table 1. The strains F and T which have identical mitochondrial genomes, are those used in the original study on restoration of respiratory competence in *petite* crosses (CLARK-WALKER and MIKLOS 1975). The various strains isolated as a result of the complementation tests are numbered according to their coordinates.

Media

GYP—yeast extract 0.5% w/v, bactopeptone 1% w/v, KH_2PO_4 3 gm/l, glucose 2% w/v. GGYP—yeast extract 1.0% w/v, bactopeptone 1% w/v, KH_2PO_4 3 gm/l, glucose 0.2% w/v and glycerol 4% w/v. GlyYP—same as GGYP but with glycerol 4% w/v as carbon source. EthanolYP—same as GGYP, but with 4% v/v of 96% ethanol as carbon source. GlySV—contains per liter: 40 gm glycerol, 4.0 gm KH_2PO_4, 0.5 gm K_2HPO_4, 1.5 gm $(NH_4)_2SO_4$, 0.1 gm NaCl, 0.1 gm $CaCl_2 \cdot 2H_2O$, 0.5 gm $MgSO_4 \cdot 7H_2O$, 5.4 mg ferric citrate · $3H_2O$, 5 ml of a trace element solution containing per 5 ml; 0.2 mg $CuSO_4 \cdot 5H_2O$, 0.5 mg KI, 0.5 mg $ZnSO_4 \cdot 7H_2O$, 0.5 mg $Na_2MoO_4 \cdot 2H_2O$, 0.5 mg $Na_2B_4O_7 \cdot 10H_2O$, 1 mg $MnSO_4 \cdot 4H_2O$ and 5.7 mg of a vitamin mixture containing per 5.7 mg: 1 mg calcium pantothenate, 1 mg thiamine, 1 mg pyridoxine · HCl, 2 mg inositol, 0.5 mg nicotinic acid and 0.2 mg biotin. GluSV—same as GlySV but with 10 gm/l glucose as carbon source. GGSV—same as GlySV but with 2 gm glucose and 40 gm glycerol per litre as carbon source.

Presporulation medium: 1% w/v potassium acetate, 0.6 % w/v yeast nitrogen base, 0.5% w/v yeast extract, 0.5 w/v peptone, 1% w/v potassium biphthalate pH 5.0.

TABLE 1

Genotypes and origins of yeast strains

Strain	Genotype			Origin
F	n	**a**	*ade8–18 lys2*	See CLARK-WALKER and MIKLOS (1975)
T	n	α	*ade1 arg4*	See CLARK-WALKER and MIKLOS (1975)
410	n	α	*his1*	Derived from strain 41 of D. WILKIE
ure3R$_2$	n	**a**	*ade2*	Derived from strain MA1-spl (*ure3*) of M. AIGLE

Sporulation medium (*KAc*): anhydrous potassium acetate 5 gm/l. For plating, 1.5% Difco agar was added to all the media. In mating experiments, the glucose concentration was increased to 3% in order to promote mating.

Complementation tests

A full description of this procedure has appeared previously (CLARK-WALKER and MIKLOS 1975). In the experiments described herein, 22 spontaneous *petites* were usually isolated from each strain and grown for ten hr in 3% GYP liquid medium before being individually crossed to form a 22×22 matrix. After overnight incubation, each individual cross was dropped onto GlySV medium and incubated at 30° for seven days. On GlySV medium, only respiratory competent diploids can grow.

Reversion of sectoring

Sectored strains were grown for 48 hours in Ethanol YP liquid medium and plated on GlyYP at approximately 200 colonies per plate. After seven days at 30°, plates were scored for sectored and unsectored colonies.

Sporulation and ascus dissection

Strains to be sporulated were grown in 2% GYP liquid medium to late exponential phase, followed by growth for 16 hr in presporulation medium. Aliquots of 0.1 ml were sporulated by spreading on KAc agar. After five to seven days at 25°, asci were briefly digested with snail enzyme and dissected using a de Fonbrune micromanipulator. Dissected ascospores were incubated at 30° for three days and samples of the resulting colonies dropped onto selective medium to determine growth requirements.

Ethidium bromide mutagenesis

Yeast strains lacking detectable mitochondrial DNA were generated by the "margin-of-growth" technique, which ensures exposure to the highest concentration of the drug compatible with growth (CLARK-WALKER 1972). A drop of ethidium bromide solution (10 mg/ml) was placed 1 to 2 cm from the perimeter of a GGYP plate and allowed to dry. A loopful of an actively growing culture was drawn over the ethidium bromide drop. After two to three days incubation at 30°, the colonies growing nearest to the margin of the drop were substreaked. After a further two to three days at 30°, respiratory deficient *petite* colonies could be distinguished by their small size from the larger wild-type colonies on this medium.

Petite *frequency determination*

For $\rho+$ strains, a small inoculum from an overnight GYP culture has transferred to 4% Ethanol YP liquid medium and grown for 48 hr at 30° with shaking. For plating, cultures were diluted to give a final concentration of approximately 100 colonies per plate. Strains were plated on GGYP medium and incubated at 30° for three days, by which time *petite* colonies could be distinguished from the wild type by size and color. According to OGUR *et al.* (1959), the mutant frequency, as determined from plates, is equal to the mutation rate once the population has reached equilibrium in a medium totally selective against *petites*. Exponential growth for 48 hr in ethanol medium is sufficient to establish this equilibrium, hence the *petite* frequencies obtained are reliable estimates of the mutation rate.

For determination of *petite* frequencies of crosses between $\rho+$ and ρ° strains, the $\rho+$ strains were grown overnight in 50 ml 2% GYP liquid medium at 30° with shaking and then inoculated into 50 ml 4% Ethanol YP medium and shaken for 48 hr at 30°. The ρ° strains were grown overnight in 50 ml 2% GYP liquid medium at 30° with shaking. At the time of mating, an inoculum of each strain was added to 3 ml 3% GYP liquid medium to give an $OD_{640\,nm} = 1.0$. The mating mixture was shaken at 30° for four hr and allowed to stand for one hr. Cultures were serially diluted and plated on GGSV plates and incubated at 30° for three days before counting.

RESULTS

Occurrence of sectored colonies in complementation tests

Respiratory competent diploid colonies can arise when spontaneous *petite* mutants of recent origin are crossed. (CLARK-WALKER and MIKLOS 1975). A proportion of the respiratory competent colonies can be both highly sectored and variable in size on GlySV medium (Figure 1), see also Figure 1 of CLARK-WALKER and MIKLOS 1975). By contrast, sectored colonies are not observed

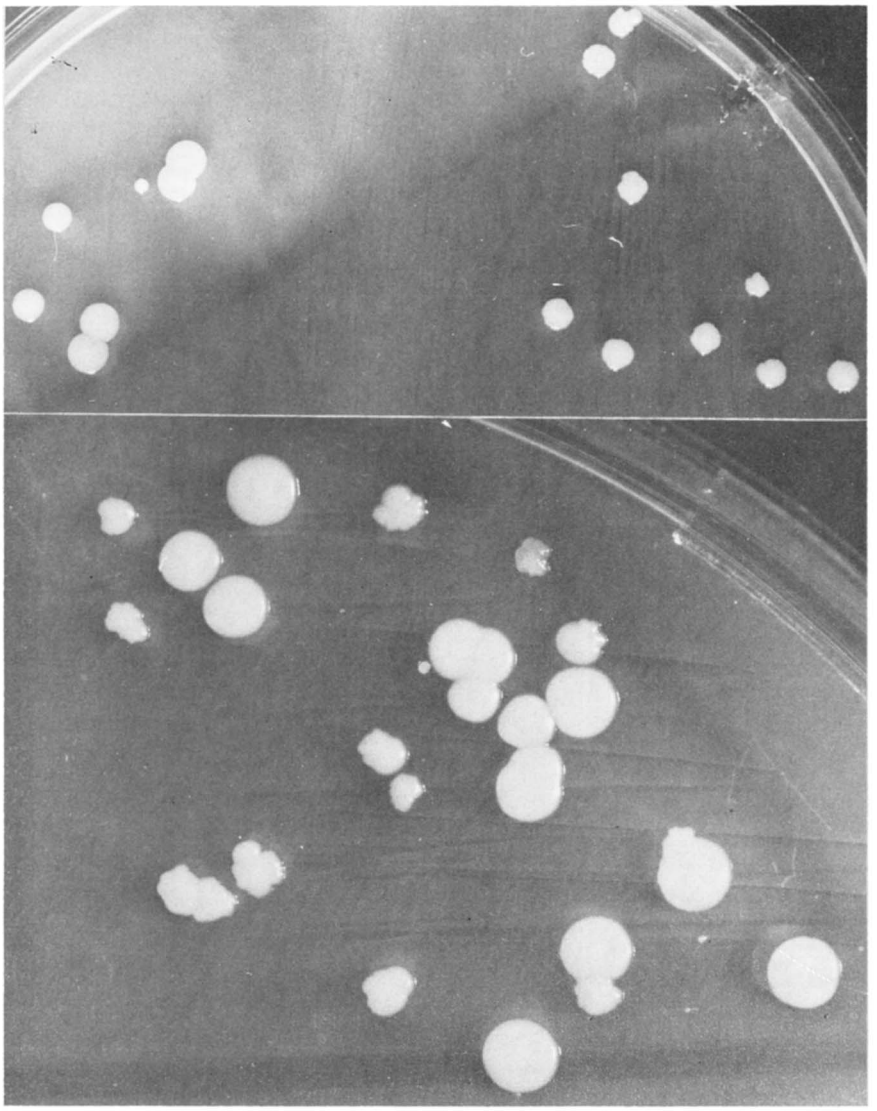

FIGURE 1.—$\rho+$ colonies on GlySV medium arising from separate $\rho^- \times \rho^-$ crosses. In the upper panel of the figure are crosses showing all-unsectored or all-sectored colonies, while the lower panel shows a cross producing both sectored and unsectored colonies.

among diploid colonies arising from crosses between the two respiratory competent parent strains F and T, nor are *petite* colonies of these strains sectored on GYP or GluSV media. Therefore, colony sectoring is not associated with peculiarities of the F and T strains themselves, but has arisen during the restoration of respiratory competence.

The frequency of sectored colonies in a complementation test also varies (Figure 2). In those positions in the matrix where respiratory competent colonies occur (termed "hits"), the total number of colonies is shown above the double bar and the percentage of sectored colonies below the bars. Out of a total of 41 hits, 12 have all sectored colonies, six show all unsectored colonies and 23 have mixtures of both sectored and unsectored colonies. Apart from *petite* T1, which gives rise to hits having all sectored-colonies, there is no correlation between *petite* progenitors and the presence or absence of sectored colonies. Thus, looking at rows or columns, it is seen that individual F or T *petite* colonies can give rise to hits which show all sectored, all unsectored, or mixtures of both colony types.

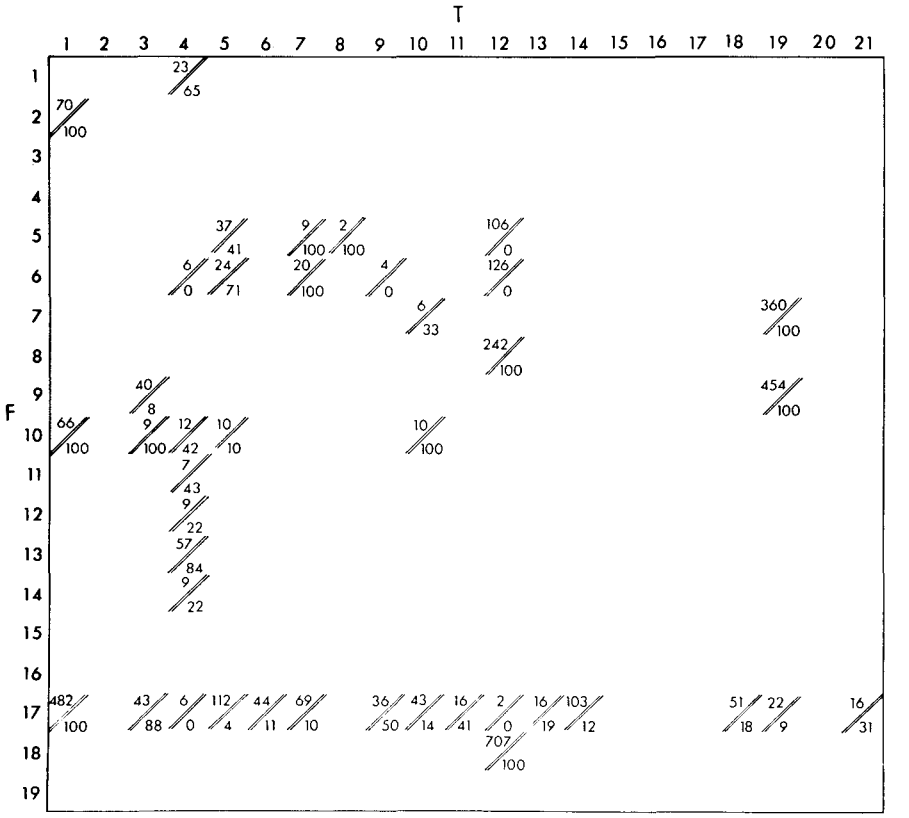

FIGURE 2.—Complementation pattern observed between individual ρ^- isolates from strains F (ordinate) and T (abscissa). The number above the double bar is the total of all colonies, while the number below the double bar is the percentage of sectored colonies. Blank areas indicate no $\rho+$ colonies.

TABLE 2

Percentage of sectored colonies in complementation tests

Matrix size $F\rho^- \times T\rho^-$	Total number of ρ^+ colonies	Percentage of "hits"	Percentage of colonies showing sectoring
$18\rho^- \times 21\rho^-$	3486	11	76*
$18\rho^- \times 21\rho^-$	256	13	42
$10\rho^- \times 20\rho^-$	2847	15	28
$16\rho^- \times 21\rho^-$	2128	22	13

* Experiment illustrated in Figure 2.

Furthermore, in this experiment 76% (2650/3245) of all colonies are sectored, while in three other experiments the percentage of sectored-colonies ranged from 13% to 42% (Table 2).

Additional features of this experiment are that the 11% level of hits is similar to that reported previously (CLARK-WALKER and MIKLOS 1975), and again *petites* are present that show patterns of hits which are either overlapping, non-overlapping or simple reductions.

Association of colony sectoring with high frequency petite *formation*

To test our main assumption that colony sectoring on glycerol medium is due to high frequency *petite* formation, sectored colonies were picked from various positions in a complementation test matrix and plated on GGYP medium, which permits a limited growth of *petite* colonies. On initial sampling, *petite* frequencies of five highly sectored colonies ranged from 46 to 89% (Table 3a). Repeated subculture in ethanol medium, which prevents the proliferation of *petites* during serial culture and therefore permits a reliable estimation of the mutation rate (OGUR *et al.* 1959), shows that the high frequency of *petite* formation is maintained for approximately 120 divisions (Table 3a). Similar testing of unsectored colonies from this experiment gave *petite* frequencies of 0.5 to 5% comparable to diploids arising from crossing the respiratory competent parental strains (Table 3b). Less highly sectored colonies had *petite* frequencies ranging upwards from approximately 5%.

TABLE 3A

Petite *frequencies* (%) *upon subculture of respiratory competent diploid sectored colonies*

Strain	Transfer number 0	3rd	6th
47.31	88	86	83
12.32	80	80	79
11.9	80	83	81
38.24	89	86	86
18.24	46	45	41

TABLE 3B

Petite *frequencies* (%) *upon crossing* $\rho+$ *and* ρ° *strains of F and T*

$F\rho+ \times T\rho+$	1.46
$F\rho^\circ \times T\rho+$	2.62
$F\rho+ \times T\rho^\circ$	2.91

Reversion of sectoring

Three of the sectored strains were examined for reversion to unsectored morphology (Table 4). In all three strains, revertants appeared at a frequency close to 1 in 10^4 colonies.

Segregation of the high frequency petite formation trait

Three strains were selected for segregation analysis of the high frequency *petite* forming trait. Included in this study was a diploid (30.3) showing unsectored colony morphology and having a low frequency of *petite* formation (0.9%). Segregation ratios of ρ^+ to ρ^- upon tetrad dissection are recorded in Table 5, which shows that there is no Mendelian segregation of ρ^+, whereas nuclear genes give 2:2 ratios. In strains 47.31 and 12. 32, which show the highest frequency of *petite* formation, the majority of asci contain respiratory deficient spores. Indeed with strain 47.31, only one ascus out of 35 had four respiratory competent ascospores. By contrast, in strain 30.3, which has a low *petite* frequency, all tetrads were respiratory competent.

In Table 6 are listed the frequencies of *petite* formation of complete ρ^+ tetrads from the three strains. All haploids still have higher *petite* frequencies than the parental strains F and T (0.5 and 1.5%). Although there is no Mendelian segregation of the high *petite* frequency trait, there is some variation of *petite* frequency within single tetrads. This variation within asci may reflect segregation of nuclear factors associated with the production of *petite* mutants as detailed below.

Association of high frequency petite formation with mitochondrial DNA

As mitochondrial DNA can be entirely eliminated from *S. cerevisiae* by treatment with ethidium bromide (forming ρ°), it is possible to examine the influence of this genome in a constant nuclear background by crossing ρ^+ and ρ° strains in a reciprocal manner.

TABLE 4

Reversion of sectored colonies

Strain	Number of sectored colonies	Number of unsectored colonies	Frequency (%) of unsectored colonies
47.31	17,100	2	0.012
12.32	18,500	3	0.016
18.24	19,700	2	0.010

TABLE 5

Tetrad analysis of sectored strains

Strain	*Petite* frequency (%)	Number of asci dissected	Segregation ratios $\rho^+ : \rho^-$ 4:0	3:1	2:2	1:3	0:4
47.31	86	35	1	4	4	12	14
12.32	71*	29	3	4	4	8	10
18.24	46	25	10	8	2	4	1
30.3	0.9	20	20	0	0	0	0

* At the time of assay, the *petite* frequency of this strain had changed slightly from that recorded in Table 3.

TABLE 6

Frequency of petite *formation in* 4:0 ($\rho+ : \rho-$) *asci*

Strain	*Petite* frequencies (%) of ascospores numbered 1–4 1	2	3	4†
47.31 (86%)*	86	85	81	64
12.32 (71%)	47	43	39	24
	46	38	21	17
	41	40	33	21
18.24 (46%)	91	30	22	13
	44	43	20	13
	38	38	20	14
	25	14	14	13

* Spontaneous *petite* frequencies of diploid strains given in brackets.
† Ascospores within a single ascus have been listed in order of *petite* frequency.

TABLE 7

Petite *frequencies* (%) *of diploids after crossing* $\rho+$ *and* $\rho°$ *strains of a complete tetrad from 47.31 with* $\rho+$ *and* $\rho°$ *isolates of normal strains*

Normal tester strains		Sectored strains from 47.31			
		1—α lys2 (81%)*		*2—α ade1 arg4 lys2* (86%)	
		$\rho+$	$\rho°$	$\rho+$	$\rho°$
ure3R_2	$\rho+$	12.2	1.7	11.1	2.8
a *ade2* (0.6%)	$\rho°$	63.1		60.4	
	*3—***a** *ade1*† *ade8–18 arg4* (64%)			*4—***a** *ade8–18* (85%)	
410-*α his1* (9%)		$\rho+$	$\rho°$	$\rho+$	$\rho°$
	$\rho+$	10.8	5.7	12.7	4.4
	$\rho°$	78.6		71.2	
		Control crosses			
		ure3R_2 $\rho+$ × 410 $\rho+$		10.4	
		ure3R $\rho°$ × 410 $\rho+$		5.8	
		ure3R_2 $\rho+$ × 410 $\rho°$		10.6	

* Spontaneous *petite* frequencies given in brackets.
† The presence of *ade1*$^-$ detected by red colony coloration, is masked by the *ade8* mutation, which blocks a prior step in adenine biosynthesis.

This is illustrated in Table 7, which records *petite* frequencies obtained on crossing ρ^+ and ρ° isolates from a single tetrad of 47.31 with ρ^+ and ρ° isolates of two wild-type strains 410 and ure3R2. It can be seen that elimination of mitochondrial DNA from haploids of 47.31 results in the frequency of *petite* formation returning to normal levels in zygotes. For instance, in the cross of ure3R2 ρ^+ with 1α ρ°, the *petite* frequency is 1.7%, whereas the reciprocal cross of ure3R2 ρ° with 1α ρ^+ gives 63% *petites*. Moreover, it is apparent from the $\rho^+ \times \rho^+$ cross, where the *petite* frequency is 12.2%, that the normal mitochondrial genome "dominates" the abnormal one in the majority of zygotes.

Notwithstanding these results, nuclear factors can influence, to some extent, the frequency of *petite* formation. In the control crosses between 410 and ure3R2 (Table 7), it can be seen that there is a dominant nuclear factor(s) that influences the frequency of *petites* in zygotes regardless of what mitochondrial DNA is present. Thus, the variation in frequency of *petite* formation within asci noted in Table 5 could be associated with the segregation of such a nuclear factor(s).

It is interesting to note that nuclear factors influencing the frequency of *petite* formation were first reported by EPHRUSSI (EPHRUSSI and HOTTINGUER 1951) and have since been described in *rad* mutants (MOUSTACCHI 1971) and as a recessive factor in a wild-type laboratory strain (LUSENA and JAMES 1976).

DISCUSSION

Sectoring of colonies arising from zygotes that have been restored to respiratory competence is associated with high frequency *petite* formation. This trait, which persists upon repeated subculture of diploids, is transmitted to haploid progeny in a non-Mendelian manner and is lost when mitochondrial DNA is eliminated from the cell. We therefore conclude that abnormal mitochondrial genomes are present in cells having a high frequency of *petite* formation and that the abnormal forms have appeared during the mating of newly arisen *petite* mutants. Furthermore, abnormal mitochondrial genomes would not be expected if restoration to ρ^+ were due to crosses involving *mit*$^-$ mutants, where ample opportunity would exist for internal rearrangement to produce the wild-type mitochondrial genome (see below).

Three proposals can be advanced to account for the presence of abnormal mitochondrial genomes in restored cells. First, separate defective but complementary mitochondrial DNA molecules may co-exist (associate) without recombining. It can be imagined that in such heteroplasmic cells the associating molecules may readily dissociate into buds, thereby giving rise to high-frequency *petite* formation. If this were so, it could be supposed that sectored colonies may contain unrecombined molecules, whereas unsectored colonies may contain recombined single-molecule genomes. Consistent with the suggestion that separate complementary molecules could co-exist in restored cells is the observation termed "zygotic gene rescue," whereby mitochondrial DNA has been found to undergo transcription and translation without recombination, upon mating of a *petite* with a *grande* (STRAUSBERG and BUTOW 1977).

A second proposal for the occurrence of abnormal mitochondrial genomes, and more consistent with the observation of segregation and persistence of the abnormal trait, is that restoration of respiratory competence in both sectored and unsectored colonies follows recombination, but only in the latter case is the original wild-type mitochondrial DNA molecule reformed. In both cases, however, we envisage that a hybrid molecule containing duplicated sequences would be formed following a single recombination event because the deletions in the DNA of the mating *petites* would rarely be reciprocal if the initial deletions are random. However, depending on the nature of the deletions and the site and orientation of the recombination event, it is hypothetically possible to reform the wild-type genome. A possible pathway for the reformation of wild-type mitochondrial DNA is illustrated in Figure 3(a), which involves an initial homologous-site recombination between molecules having single nonoverlapping deletions. The resulting partially duplicated hybrid molecule could now undergo internal homologous-site recombination in the duplicated regions indicated by the arrows to produce the original mitochondrial DNA. In this instance, the partially duplicated molecule would be only transiently formed en route to establishment of the normal mitochondrial DNA.

Other circumstances can be imagined, however, where internal rearrangement of a partially duplicated molecule to produce the wild type is not possible without first producing defective molecules. These situations are illustrated in Figure 3(b,c,d) and can come about by: (b) formation of an inversion, (c) formation of a translocation, and (d) occurrence of two or more deletions in one or both of the recombining defective molecules (effectively a translocation). More complex situations may occur where the incoming defective molecules in the *petites* could have already undergone inversions, translocations and differential amplification prior to mating and restoration of respiratory competence (see below).

It can be seen in Figure 3b-d that mitochondrial DNA having any of these structural peculiarities would form defective molecules following internal homologous-site recombination in the duplicated regions. Furthermore cells having such abnormal mitochondrial DNA may readily produce more *petite* mutants than normal because of the intramolecular sequence duplications that would be expected to promote the instability of the mitochondrial genome by providing regions of sequence homology where recombination would be facilitated.

There is evidence from studies on mitochondrial DNA from both wild type and *petite* mutants to support the occurrence of each of the three structural rearrangements listed above (b-d). First, inverted sequences have been demonstrated in mitochondrial DNA of *petite* mutants (Locker, Rabinowitz and Getz 1972; Faye *et al.* 1973). Second, translocations could occur in the same manner or at the same sites involved in the formation of *petite* mutant deletions. Here, the formation of deletions either must involve internal recombination at sites of partial sequence homology (Clark-Walker and Miklos 1974; Prunell and Bernardi 1974) or else recombination is independent of sequence homology. The latter suggestion, which at present lacks experimental support, would imply

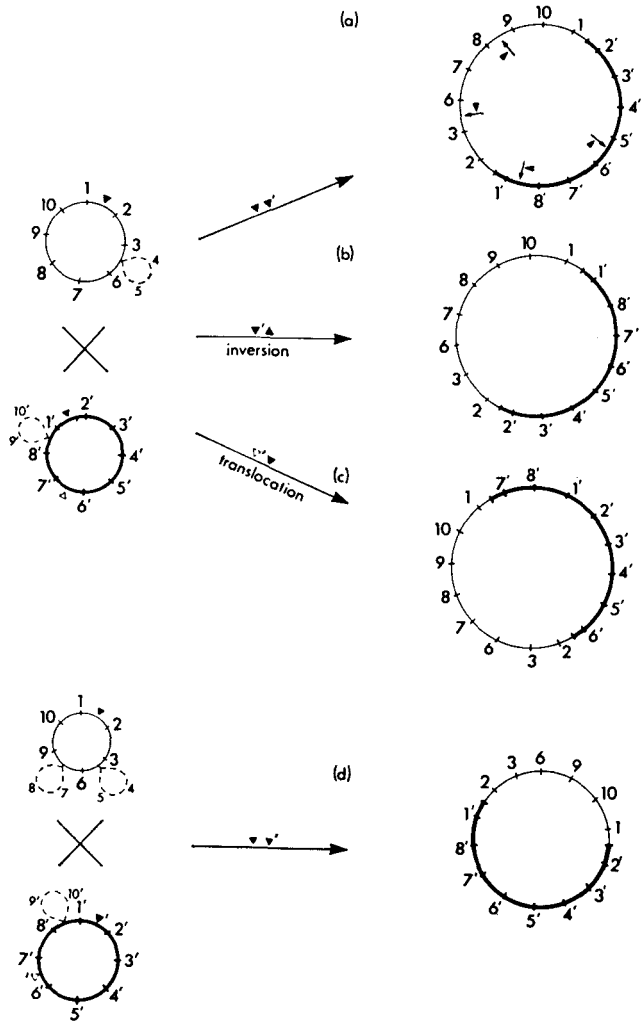

FIGURE 3.—Formation of partially duplicated hybrid DNA molecules by recombination of defective molecules at sites indicated by the triangles (▼ or ▽) The complete genome is represented by numbers 1–10, while deletions are represented by dotted lines. (a) Formation of a hybrid molecule by homologous-site recombination, with retention of gene polarity. This molecule is capable of undergoing homologous-site recombination in regions indicated by the arrows to excise the duplication. (b) Formation of a hybrid molecule containing an inversion by a reversed recombination event at an homologous site. (c) Formation of a hybrid molecule containing a translocation, but preserving gene polarity by recombination at a region other than an homologous site. (d) Formation of a hybrid molecule containing a translocation by homologous-site recombination between defective molecules, one of which contains two deletions. In the hybrid molecules b-d, internal homologous-site recombination in duplicated regions leads to defective molecules.

that translocations could occur anywhere in the DNA. Alternatively, both the observation of the circularization of mitochondrial DNA fragments *in vitro* (SHAPIRO *et al.* 1968) and the demonstration of separated inverted repeat sequences in *grande* mitochondrial DNA (LOCKER and RABINOWITZ 1976) imply that partially homologous sequences do exist that could be sites facilitating recombination. Finally, multiple deletions have been detected in *petite* mutants by restriction enzyme digestion of mitochondrial DNA (MORIMOTO *et al.* 1975), by DNA-DNA hybridization (SRIPRAKASH *et al.* 1976) and by genetic tests (SCHWEYEN *et al.* 1976).

A third model to account for the high-frequency *petite* forming trait is based on favored sites of recombination or "hot spots." It can be imagined that deletion from the wild-type mitochondrial DNA is not random, but occurs from a limited number of hot spots. Excision from such hot spots may not be precise because of unequal crossing over, so that families of deleted molecules may be produced that contain greater or lesser amounts of the sequence from the hot spots. If restoration to ρ^+ involves crossing over between reciprocally deleted molecules having greater or lesser proportions of the same hot spots, then such sites could be further expanded or contracted relative to the wild-type molecule. Indeed recent studies by BERNARDI and co-workers have shown that unequal crossing over appears to be a common occurrence in yeast mitochondrial DNA (FONTY *et al.* 1978). Therefore, in the high *petite* frequency strains it is envisaged that the mitochondrial DNA would have one or two expanded hot spots, but would not necessarily contain large duplications or rearranged sequences.

The hot spot model can be extended to include damage to the *tsm-8* (HANDWERKER *et al.* 1973) or *uvsρ* 72 (CHANET, WILLIAMSON and MOUSTACCHI 1973) mitochondrial genes, which influence the spontaneous mutation rate to ρ^-. If hot spots occur within these genes, they could well be damaged by unequal crossing over. Thus, even though ρ^+ could be restored, the resulting strains may have higher mutation rates to ρ^-.

Fortunately, by using restriction endonuclease digestion of mitochondrial DNA or genetically marked mitochondrial genomes, it should be possible to distinguish between the three models outlined above. These studies are in progress.

We thank W. HAYES and G. L. G. MIKLOS for critical discussion of the manuscript and F. SHERMAN for suggesting definitions for the various mitochondrial mutants. K. M. OAKLEY acknowledges support from a Commonwealth Post-graduate Scholarship.

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Corresponding editor: F. SHERMAN