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Segregation and Transmission of Mitochondrial Markers in Fusion Products of the Asporogenous Yeast *Torulopsis glabrata*

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Summary. Using a protoplast fusion technique we have been able to locate to the mitochondrial genome of the asporogenous yeast *Torulopsis glabrata* mutations conferring resistance to oligomycin, antimycin and diuron. When two strains differing in the size of their mtDNAs were fused the mitochondrial markers from the parent with the larger mtDNA (71-91) were transmitted predominantly among the fusion products. Both genetical and physical evidence support the occurrence of recombination in *T. glabrata* mitochondrial genome. Segregation of the mitochondrial genome appears to take place before the separation of the first bud from the fusion product.

Key words: Protoplast – Fusion – Mitochondrial – Genetics

Introduction

With a view to understanding the evolution of mitochondrial genomes among different yeasts, we have undertaken to identify by genetic techniques as many as possible of the mitochondrial genes of *Torulopsis glabrata*. This yeast has mtDNA of only 6 μm (O'Connor et al. 1976) in contrast with 25 μm mtDNA in *Saccharomyces cerevisiae* (Hollenberger et al. 1969). We were interested to see if the difference in the size can, in part, be accounted for by an absence of some of the genes in the smaller mtDNA. At the same time as this study was undertaken we sought to determine by physical methods the locations of the mitochondrial genes. The results of such studies to be described elsewhere show that *T. glabrata* mtDNA contains sequences hybridizing to probes for the three subunits of cytochrome oxidase, 2 subunits of ATPase and cytochrome b in addition to large and small rRNAs and tRNAs (Clark-Walker et al. 1980). To supplement the physical methods and to get insight into functionality of the genes, we isolated and characterised mitochondrial mutations in *T. glabrata*. However unlike *S. cerevisiae, T. glabrata* which is an asporogenous yeast has no mating system and therefore is not easily amenable to genetic studies. Protoplast fusion was employed to overcome this problem. Yet another disadvantage of this system is that haploidisation of the fusion products was not readily possible. As a result, fusion experiments involving mutant alleles in *cis* and *trans* configurations could not be performed. Despite these difficulties these studies revealed that certain characteristics of mitochondrial genetics were shared between this organism and *S. cerevisiae*. They include: high recombination and gene conversion, mitotic segregation and preferential transmission in the fusion products of the mitochondrial mutations from one parent. It was also found that segregation occurs before the first bud separates from the fusion product.

Materials and Methods

Strains. The strains of *T. glabrata* CBS 138 and 71-91 were described earlier (Galeotti et al. 1981). Strains 72-28, 72-33, 75-1 were obtained from Dr. Phaff, and YB4025, Y2242, Y7337 were from the US Department of Agriculture. Auxotrophic mutants were isolated after mutagenesis with U.V. and were selected on magdala red plates (Horn and Wilkie 1966). Spontaneous mutants resistant to oligomycin and diuron were isolated in strains carrying double auxotrophic markers on Gly YP plates containing 10 μg/ml oligomycin and 100 μg/ml diuron respectively. As most of the spontaneous mutations conferring resistance

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Table 1. *T. glabrata* mutants used in this study

Strain	Nuclear Markers	Mitochondrial markers	Parents
138-11-1	*ade pro*	ρ^+	CBS 138
138-0-1	*ade pro*	ρ^+ *oli*^{r}1	138-11-1
7191-C-5	*arg his*	ρ^+	71-91
7191-C-6	*arg ade*	ρ^+	71-91
138-OA-11	*ade pro*	ρ^+ *oli*^{r}*1*, *ana*^{r}1	138-0-1
7191-D-1	*arg his*	ρ^+ *diu*^{r}1	7191-C-5
7191-DA-1	*arg his*	ρ^+ *diu*^{r}1, *ana*^{r}2	7191-D-1
7191-DAO-1	*arg his*	ρ^+ *diu*^{r}1, *ana*^{r}2, *oli*^{r}2	7191-DA-1
138-11-1 eρ°	*ade pro*	ρ° [a]	138-11-1
138-0-1 eρ°	*ade pro*	ρ°	138-0-1
7191-C-5 eρ^{-6}	*arg his*	ρ^-	7191-C-5
138-OA-11 eρ^{-1}	*ade pro*	ρ^-	138-OA-11
7191-D-1 eρ^{-1}	*arg his*	ρ^-	7191-D-1
7191-DA-1 eρ^{-1}	*arg his*	ρ^-	7191-DA-1
7191-DAO-1 eρ^{-1}	*arg his*	ρ^-	7191-DAO-1
138-OA-10	*ade pro*	ρ^+ *oli*^{r}1 (resistant to antimycin)	138-0-1
138-OA-10 eρ^{-1}	*ade pro*	ρ^-	138-OA-10

ρ^+ denotes respiratory competence and intact mitochondrial DNA. ρ° and ρ^- denote respiratory deficient cytoplasmic mutations caused by partial (ρ^-) or complete (ρ°) loss of mtDNA.
(a) DNA preparation showed no detectable mitochondrial DNA band in CsCl-ethidium bromide gradients

to antimycin were not mitochondrial (see below) we employed a different strategy to obtain mitochondrial mutants using mutagenesis with manganese (Putrament et al. 1973). In this procedure cells were grown in liquid GYP medium containing 0.5 mM $MnCl_2$. After overnight growth the cells were washed and plated on Gly YP containing antimycin (1 μg/ml) and sodium dodecyl sulphate (SDS) (0.05 mM); the latter was included to select against membrane mutants. Resistant colonies which appeared in 2 to 3 days were then subcloned on to Gly YP + antimycin (1 μg/ml) medium.

The cytoplasmic respiratory deficient mutants which lack detectable mtDNA were isolated according to the procedure described by Clark-Walker (1972). Table 1 summarises the strains used in this study.

GYP, Gly YP, MMK, minimal medium were described earlier (Galeotti et al. 1981; Oakley and Clark-Walker 1978).

Protoplast Fusion

Cells from exponentially growing cultures were harvested and washed once with 0.05 M Tris/HCl pH 9.1. After resuspending in the same buffer, the cells were treated with 0.015 M β mercaptoethanol for 20 min at room temperature and washed with 'osmotic support buffer' (OSB) containing 0.05 M maleate – NaOH buffer (pH 5.8) and 0.6 M KCl as osmotic support. After washing, cells were resuspended in OSB and treated with 2–5% (V/V) snail enzyme (Industrie Biologique Francaise) until conversion into protoplasts was greater than 95%. The period of incubation required to give 95–100% protoplasts, as measured by a 60–80% decrease in optical density at 640 nm of 30-fold diluted samples, varied from 20 min to 60 min for different strains. Protoplasts from the two strains to be fused were washed once with OSB, resuspended in OSB at a cell density of 10^9/ml and equal volumes of protoplasts were mixed (approx. 10^8 protoplasts for each auxotroph). To this mixture was added 0.24 ml of 0.5 M $CaCl_2$ and 2 ml of 30% polyethylene glycol (PEG MW 4000, (Sigma)). The fusion mix was incubated at 30 °C for 20 min, spun and the pellets gently resuspended in 2 ml 1:1 mixture of OSB and 0.5 M $CaCl_2$. Suspensions were plated on MMK medium.

Biochemical Methods. Isolation of mtDNA, agarose gel electrophoresis, extraction of DNA from gels and autoradiography were as described previously (Clark-Walker et al. 1980; 1981). DNA was labelled using random primers and the Klenow fragment of DNA polymerase I. The details of this will be published elsewhere. Briefly, the primer was made by digesting calf thymus DNA with DNAse I, heat denaturing digested DNA and chromatography on a DEAE-column. The primer (50 μg) was added to 1–2 μg of the DNA to be labelled and the mixture heated to 90 °C for 5 min and quickly cooled. The reaction mixture in a final volume of 50 μl contained 20 mM Tris pH 7.5, 5 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1.25 μg each of dCTP, dGTP and dTTP, 30 μci $\alpha^{32}P$ dATP, 2 units of DNA polymerase I and the denatured DNA and primer mixture. Incubation was at 37 °C for 2 h and the reaction was terminated by extraction with phenol and chloroform. The newly synthesised DNA was separated from unreacted $\alpha^{32}P$ dATP on a column of Sephadex G50 (0.6 x 15 cm) previously equilibrated with 20 mM Tris pH 7.5 and 10 mM EDTA.

Yeast colony hybridization was similar to the procedure described by Blanc et al. (1978). In this method, yeast colonies were suspended in 20 μl of buffered snail enzyme (1 ml snail enzyme + 2 ml of 50 mM EDTA, 100 mM potassium phosphate buffer pH 6.8 and 0.5 M β-mercaptoethanol) and placed in the wells of a Microtest-tissue culture plate (Becton & Dickinson Company). After 2 h at room temperature 20 μl 0.5 N NaOH was added to each well and incubation continued at 37 °C for 1 h. The contents of each well were then neutralised with 20 μl of 0.5 N HCl. A multipronged inoculator was used to transfer the colony lysates to a nitrocellulose sheet presoaked in 6 x SSC (0.9 N NaCl, 0.09 M Trisodium citrate). The nitrocellulose sheet was dried and baked at 80 °C in vacuuo for 2 h. the hybridization conditions were the same as described by Clark-Walker et al. (1980).

Results

Screening of Different Strains of T. glabrata for Antibiotic Sensitivity

In a preliminary experiment *T. glabrata* CBS 138 and 71-91 were found to be resistant to chloramphenicol, erythromycin and paromomycin. These strains were sensitive to antimycin, diuron and oligomycin. Subsequently we screened a further six independent isolates of *T. glabrata.* The results (Table 2) show that all the strains exhibited the same spectrum of antibiotic sensitivity. The levels of sensitivity to oligomycin, antimycin and diuron were comparable to those observed in *S. cerevisiae* (Avner and Griffiths 1973; Michaelis 1976; Colson et al. 1977). CBS 138 and 71-91 were chosen for further studies because preliminary experiments on isolation of auxotrophic mutants, fusion of protoplasts and characterization of fusion products were done with these strains (Galeotti et al. 1981).

Isolation of Antibiotic Resistance Mutants

Mutants resistant to oligomycin, diuron and antimycin could be obtained spontaneously on Gly YP + antibiotic plates. The concentration of the drugs used in the isolation of mutants were: 1 μg/ml for oligomycin, 1 μg/ml for antimycin, 30 μg/ml for diuron. About 10^7 cells were plated and in each case several colonies grew on antibiotic plates after 3–5 days. Some of these colonies were subcloned and tested once again for the resistant phenotype. We found, that in general the level of resistance for oligomycin was 10 μg/ml and for diuron 100 μg/ml. Growth of antimycin resistant cells at higher drug concentrations than 1 μg/ml was not tested. The characterization of these mutants described below suggested that all 10 spontaneous antimycin resistant mutants were nonmitochondrial. Therefore we employed mutagenesis using manganese which is known to specifically induce mitochondrial mutations (Putrament et al. 1973). A preliminary study revealed that *T. glabrata* is more sensitive to Mn^{++} than *S. cerevisiae* as 1 mM of $MnCl_2$ completely inhibited growth. For mutagenesis we used 0.5 mM of $MnCl_2$. and in the selection plates we incorporated 0.05 mM SDS in order to select against permeability mutants which are likely to be nuclear in origin. Using this modified procedure, mitochondrial antimycin resistant mutants can be isolated routinely.

Table 2. Sensitivity of *T. glabrata* towards different antibiotics

Antibiotic	Inhibitory concentration (μg/ml)
Oligomycin	0.5
Antimycin	<0.05
Diuron	25
Chloramphenicol	>3,000
Erythromycin	>3,000
Paromomycin	>2,000

T. glabrata strains CBS 138, 71-91, 72-28, 72-33, 71-1, YB4025, Y2242 and Y7337 were tested for the sensitivity to oligomycin, antimycin, diuron, chloramphenicol, erythromycin and paromomycin on GlyYP + antibiotic medium. The inhibitory concentrations of these antibiotics are the same for all strains

Characterisation of Antibiotic Resistant Mutants

In a fusion that involves a respiratory competent (grande) parent and a respiratory deficient (petite) parent in which the mtDNA was deleted by treatment with high concentrations of ethidium bromide, the respiratory competent fusion products must transmit the mitochondrial genome from the first parent and thereby its markers. If an antibiotic resistance mutation is mitochondrially inherited one expects all the fusion products to be resistant to the antibiotic in the above experiment in which the grande parental cells are resistant. Conversely, if mtDNA was deleted from this strain and then fused with sensitive grande cells then all the fusion products should be sensitive to the antibiotic. A deviation from this pattern is suggestive that the given antibiotic marker is not mitochondrially inherited. Such experiments are described in Table 3. It is evident that the results of experiments 1–4 in Table 3, are consistent with the conclusion that the antibiotic markers tested are mitochondrially inherited, whereas experiment 5 suggests that the spontaneous antimycin resistance mutation in 138-OA-10 is not a mitochondrial mutation.

Analysis of Individual Fusion Products

Some of the important properties of mitochondrial inheritance are vegetative segregation and heterogeneity of zygotic clones. It was therefore of interest to study the behaviour of mitochondrial genomes in individual fusion products. Colonies arising from separate fusion were grown individually in liquid minimal medium and plated to give single colonies. Then 80–100 colonies were tested for the mitochondrial markers. The results presented in Table 4 show that the phenotype of all the subclones from a fusion product is the same; both parental and recombinant phenotypes were recoverd. Hence it appears that mtDNA in *T. glabrata* undergoes segregation before the first bud separates from its mother. This conclusion was confirmed subsequently with 32 fusion products by analysing five subclones from each

Table 3. Characterization of antibiotic resistance markers

Experiment No.	Fusion	Parental phenotypes	No. of fusion products scored	Phenotype of fusion products
1	138-0-1 x 7191-C-5 $e\rho^{-}6$	$O^R \times \rho^\circ$	115	All O^R
	138-0-1 $e\rho^\circ 1$ x 7191-C-5	$\rho^\circ \times O^S$	105	All O^S
2	138-OA-11 x 7191-C-5 $e\rho^{-}6$	$O^RA^R \times \rho^\circ$	10	All A^RO^R
	138-OA-11 $e\rho^{-}1$ x 7191-C-5	$\rho^\circ \times O^SA^S$	32	All A^SO^S
3	7191-D-1 x 138-11-1 $e\rho^\circ$	$D^R \times \rho^\circ$	81	All D^R
	7191-D-1 $e\rho^{-}1$ x 138-11-1	$\rho^\circ \times D^S$	30	All D^S
4	7191-DAO-1 x 138-0-1 $e\rho^\circ 1$	$D^RA^RO^R \times \rho^\circ$	19	All $D^RA^RO^R$
	7191-DAO-1 $e\rho^{-}1$ x 138-11-1	$\rho^\circ \times D^SA^SO^S$	20	All $D^SA^SO^S$
5	138-OA-10 x 7191-C-5 $e\rho^{-}6$	$O^RA^R \times \rho^\circ$	31	All O^R 29 were A^R
	138-OA-10 $e\rho^{-}$ x 7191-C-5	$\rho^\circ \times O^SA^S$	27	All are $O^RA^RD^R$

Each fusion product growing on MMK plate was subcloned on minimal medium and five subclones from each were scored for the mitochondrial markers on GlyYP medium containing oligomycin (10 μg/ml) antimycin (1 μg/ml) or diuron (30 μg/ml). The plates were scored after 48 h growth. O^RA^R and D^R refer to resistance phenotype for oligomycin, antimycin and diuron respectively. O^S, A^S, and D^S correspond to sensitive phenotype for the same antibiotics

Table 4. Segregation of mitochondrial markers in fusion products

Fusion	Number of fusion products checked	Number of subclones checked	Phenotypes recovered
138-OA-11 x 7191-D-1	2	80	All are $O^SA^SD^R$
		80	All are $O^SA^SD^R$
7191-DA-1 x 138-0-1	4	100	All are $O^SA^RD^R$
		100	All are $O^SA^RD^R$
		80	All are $O^SA^RD^R$
		80	All are O^RA^DR

The fusion products were grown individually in 50 ml of liquid minimal medium overnight and plated on minimal medium. About 80–100 colonies were scored for the mitochondrial markers as described in Table 3

original clone (data not shown). Once again, the subclones from a given fusion product had the same mitochondrial markers. Thus vegetative segregation does occur for mitochondrial genes among the fusion products. The individual clone from the fusion product is, however, pure. A similar observation was reported for the fusion of *Schizosaccharomyces pombe* (Luckemann et al. 1979). These authors concluded that delay in regeneration of the protoplast fusion product is the cause. This explanation could well apply in *T. glabrata,* as the same technique of protoplast fusion has been employed.

Transmission of Drug Resistance Mutations Among Fusion Products

The fusion experiments involving multiply marked *T. glabrata* mitochondrial genomes are summarised in Table 5. In these experiments about 40 fusion products were pooled and the progeny were analysed. Since the data presented above suggest that the mitochondrial genome segregates before the first bud separates from its mother cell, the analysis of the pool of fusion products described in this section represents an average of the data for individual fusion products, assuming that there is no selective replicative advantage of any particular genotype over the others. It is apparent that when 71-91 derived strains fused with CBS 138 derived strains, the mitochondrial markers of the former are recovered predominantly among the fusion products (experiments 1–3, Table 5). In experiment 4, where both the parents are derived from the same predecessor, no preferential recovery of drug resistance alleles was observed and hence this phenomenon can not be attributed to an intrinsic property of the drug resistance alleles.

Table 5. Fusions involving multiple mitochondrial markers

Experiment No.	Strains fused	Mitochondrial markers	Phenotypes recovered		Frequency %
1	7191-D1 x 138-OA-11	*oli*^{s}, *ana*^{s}, *diu*^{r}1 x *oli*^{r}1, *ana*^{r}1, *diu*^{s}	$O^SA^SD^R$	Parental	79.5
			$O^RA^RD^S$		13.8
			$O^RA^SD^R$	Recombinants	4.8
			$O^SA^SD^S$		1.9
			Other recombinants		0
2	7191-DA-1 x 138-0-1	*oli*^{s}, *ana*^{r}2, *diu*^{r}1 x *oli*^{r}1, *ana*^{s}, *diu*^{s}	$O^SA^RD^R$	Parental	67.8
			$O^RA^SD^S$		10.5
			$O^RA^RD^R$	Recombinants	17.5
			$O^SA^SD^S$		4.2
			other recombinants		0
3	7191-DAO-1 x 138-11-1	*oli*^{r}2, *ana*^{r}2, *diu*^{r}1 x *oli*^{s}, *ana*^{s}, *diu*^{s}	$O^RA^RD^R$	Parental	76.0
			$O^SA^SD^S$		13.5
			$O^SA^RD^R$	Recombinants	3.75
			$O^RA^SD^S$		6.75
			other recombinants		0
4	7191-DAO-1 x 7191-C-6	*oli*^{r}2, *ana*^{r}2, *diu*^{r}1 x *oli*^{s}, *ana*^{s}, *diu*^{s}	$O^RA^RD^R$	Parental	31.05
			$O^SA^SD^S$		65.45
			$O^RA^SD^S$	Recombinant	3.5
			other recombinants		0

About 40 fusion products from each experiment growing on MMK medium were pooled into 250 ml of liquid minimal medium and grown for 14–16 h. The cells were plated on minimal medium and 300–400 colonies were scored for the mitochondrial markers as described in the legend to Table 3

Heterogeneity in mtDNAs of CBS 138 and 71-91

Prompted by the genetic behaviour of mitochondrial markers in the fusion between strains 71-91 and CBS 138, we undertook to investigate heterogeneity of mtDNAs of these strains. The pattern of migration in gels of mtDNAs of CBS 138 and 71-91 after digestion with restriction endonucleases HindIII and BclI, is shown in Fig. 1 (Channels 1 and 3). It is clear that there are several differences in the mtDNAs of these two strains; the striking difference being in the band c′ of 71-91. This band is larger than its counterpart in CBS 138 (band c) by 1,520 bp. Overall the mtDNA of 71-91 is larger than that of CBS 138 by 1,500 bp.

Transmission of Insertion Sequence in Fusion Products

The 1,520 bp additional sequence in one of the parents described above can be used as a physical marker in the study of the transmission of mitochondrial genome in the fusion products. 28 fusion products obtained by fusing 138-0-1 and 7191-D-1 were pooled into 250 ml liquid minimal medium, grown for 6–8 generations and plated. 74 colonies, comprising all 4 possible combinations for the 2 mitochondrial markers were probed for the presence of the insertion sequence. This was done by hybridizing labelled probe to the lysates of colonies transferred to nitrocellulose. To specifically isolate labelled probe, mtDNA of 71-91 was digested with HindIII + BclI and the 3.4 Kbp band (c′ in Fig. 1) was isolated. After labelling with α^{32}P dATP the labelled DNA was sonicated, denatured and hybridized to an excess of filter bound mtDNA from CBS 138 to remove common sequences. The unhybridized DNA was used as a probe to determine the transmission of the insert among the fusion products. The results are shown in Fig. 2. Lysates of 71-91 and CBS 138 were also included as positive and negative controls. It is apparent that 80% of the fusion products have the insertion. This figure is an underestimation, since some of the colonies which did not hybridize to the probe did indeed have the insertion

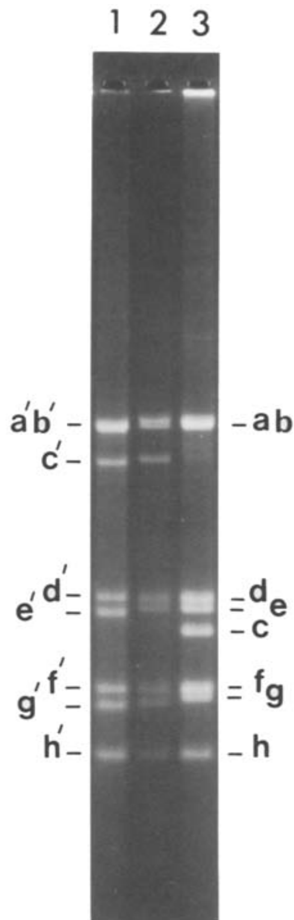

Fig. 1. BclI and HindIII restriction endonuclease digestion of mtDNAs from 71-91 (channel 1), recombinant fusion product (channel 2) and CBS 138 (channel 3). The first band (ab and a'b') is a composite band

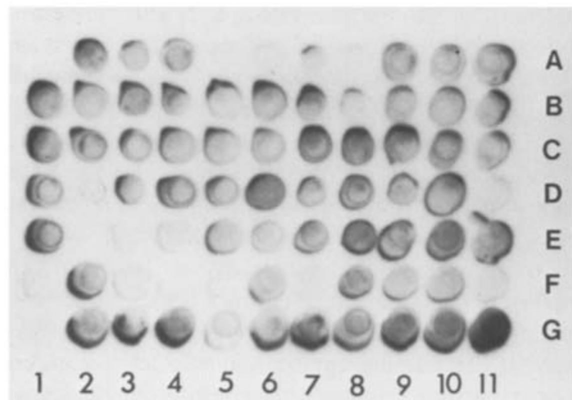

Fig. 2. Colony lysates were transferred to a nitrocellulose sheet and hybridised to the labelled probe prepared from the insertion sequence. The filter was then washed and autoradiographed. Spot A-1 had no lysate; spots G-1 and G-11 had colony lysates from CBS 138 and 71-91 respectively

Table 6. Transmission of insertion sequence
Fusion: 138-0-1 x 7191-D-1

Phenotype of clones arising	Number of fusion products	Number of fusion products transmitting insertion sequence
O^RD^R	16	14
O^SD^S	9	6
O^RD^S	9	6
O^SD^R	40	33

The fusion products were scored for mitochondrial markers as described under legend to Table 3. The data on the transmission of insertion sequence were derived from Fig. 2

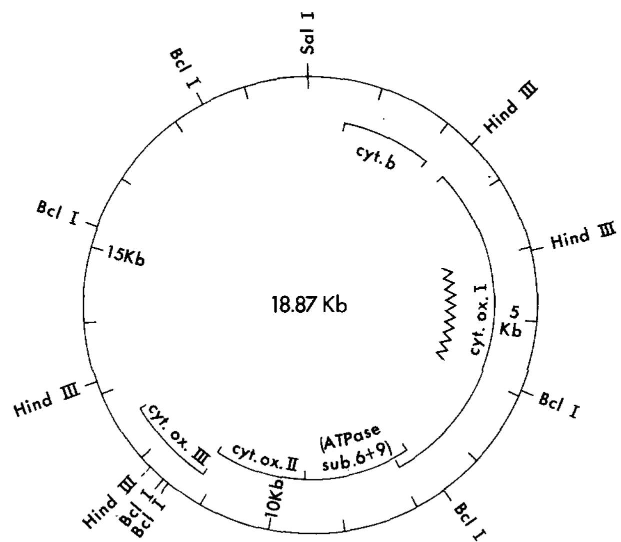

Fig. 3. Physical map of the genes in mtDNA of *T. glabrata*, representing approximate position of genes. Zig-zag line indicates position of 1,520 bp insertion in the strain 71-91. (From Clark-Walker and Sriprakash, in preparation)

sequence, as judged by the restriction pattern of isolated mtDNA (data not shown). This discrepancy is probably due to inefficient protoplasting of some colonies in the colony hybridization procedure. Further analysis of the distribution of the insertion sequence among the fusion products showed that the same proportion of recombinant and parental types for the mitochondrial markers transmitted the insertion sequence (Table 6). The data of physical mapping (see Fig. 3 and discussion) suggest that the two drug resistant markers used in this study flank the 1,520 bp insertion sequence. Therefore, it appears that conversion at the region containing the insertion sequence is independent of the recombination for the outside markers. Moreover, at least two thirds of the O^RD^S parental type transmitted the insertion. This could arise by either unequal double cross-over, asymmetric gene conversion or transposon mechanism.

Physical Evidence for Recombination in T. glabrata mtDNA

In the light of our previous study suggesting the lack of a recombination-repair system for the nuclear DNA of *T. glabrata* (Galeotti et al. 1981) we were surprised by the high frequency of mitochondrial recombination observed between the oligomycin and diuron resistance loci. Therefore, we sought for physical evidence of recombination. The restriction pattern of mtDNA isolated from an $O^R D^R$ recombinant issued in the cross described in Table 6, is shown in Fig. 1 (channel 2). The pattern is different from either of the parents (channels 1 and 3). Like 71-91, the recombinant had the large insertion in band c′ and the bands corresponding to d and e of CBS 138 were present in the recombinant. These results provide physical evidence for recombination.

Discussion

In this study we have unequivocally established the occurrence of mitochondrial mutations conferring resistances to diuron, antimycin and oligomycin in *T. glabrata.* In *S. cerevisiae,* resistance to the former two drugs affect the gene for cytochrome b, whereas oligomycin resistances have been mapped in the genes for the two subunits of ATPase (Sebald et al. 1979; Macino and Tzagoloff 1980; Colson and Slonimski 1979). By analogy, we postulate that *T. glabrata* mtDNA encodes a functional cytochrome b gene and at least one functional gene of ATPase. Molecular hybridization studies revealed (Clark-Walker and Sriprakash, manuscript in preparation), in fact, that all the identifiable gene sequences in *S. cerevisiae* mtDNA are present in *T. glabrata* mtDNA. The locations of these genes are shown in Fig. 3.

In contrast to *S. cerevisiae* we have not been able to isolate in *T. glabrata* chloramphenicol, erythromycin and paromomycin resistant mutants because of the high intrinsic resistance of this yeast to these antibiotics. Hence the study of transmission of resistance markers had to be confined essentially to two functional regions in *T. glabrata.*

The results of the analysis of single clones from fusion products show that the mitochondrial genome undergoes segregation before the separation of the first bud. Thus clones of individual fusion products almost certainly are pure with respect to the mitochondrial genome. In experiments involving mitochondrial chlorampenicol and erythromycin resistances in *S. cerevisiae* Christensen (1979) recovered the four possible combination of the two markers among the fusion products. However, the individual primary clone was pure with respect to the mitochondrial markers. These results indicate that mitochondrial segregation occurs earlier than the separation of the first bud from fusion products in *S. cerevisiae* as well. Similarly, the transmission patterns of mitochondrial markers in the individual fusion clones and individual zygotes which were starved in order to delay the first cell division, were the same in *Schizosaccharomyces pombe* (Luckemann et al. 1979); a majority of the clones transmitted uniparental markers. Since the segregation in *S. pombe* appears to be a slow process in normal zygotes (Seitz-Myer et al. 1978) Luckemann et al. (1979) concluded that the delay in the first cell division in fusion product as a result of the time needed for regeneration of the cell wall, is mainly responsible for the uniparental inheritance. During this period, there is a panmictic mixing of the mitochondrial genome followed by recombination and segregation. Our results with *T. glabrata* fusion products are consistent with these conclusions. Moreover, the segregation patterns of the mitochondrial genomes were similar in isonuclear and isomitochondrial fusion in *T. glabrata* and hence rule out the possibility of strain differences as a cause for pure fusion clones. As hypothesised by Lancashire and Mattoon (1979), soon after the segregating unit makes its way into the first bud, the residual heteroplasmic fusion product might become inactive or die.

Earlier work from our laboratory (Galeotti et al. 1981) showed that in *T. glabrata* the fusion products and their parents are sensitive to ionizing radiation to the same extent, and the dose response curves are similar, whereas in the same study it was found that the fusion products of *S. cerevisiae* and *S. unisporus* are more resistant to the radiation than their respective parents. These observations led us to postulate that *T. glabrata* can not repair radiation damage by recombination pathways. In this communication we report the isolation of a fusion product with mtDNA different from either of the parents, as judged by the restriction pattern. These results provide conclusive evidence for the proficiency of recombination in *T. glabrata* mitochondrial genome.

Assuming that oligomycin resistance affects the structural genes for subunits 6 and 9 of ATPase, and antimycin and diuron resistances map within the cytochrome b gene as are the cases in *S. cerevisiae* mtDNA then from the physical mapping data (Fig. 3) we might argue that oligomycin resistance locus maps around the 5–6 o'clock position and the latter two antibiotic resistance loci map around the 1 o'clock position in *T. glabrata* mtDNA relative to the unique SalI site. The distance between these two positions is 7–8 Kbp.

The frequency of recombination between *oli*^{r}1 and *diu*^{r}1 is 21.7 (experiment 2, Table 5) and between *oli*^{r}2 and *diu*^{r}1 is 10.5 (experiment 3, Table 5). These

estimates, although somewhat variable, (see experiments 1 and 4, Table 5), are similar to the recombination frequency observed for markers separated by similar distances in *S. cerevisiae* (Trembath et al. 1976; Strausberg et al. 1978), we conclude that recombination in mitochondria may be a separate process to recombination in the nucleus.

The frequency of spontaneous petite formation is variable among different petite positive yeasts. The data of Clark-Walker et al. (1981) clearly show that petite frequency is not a linear function of the size of mtDNAs. Among other explanations for the differences in the petite frequencies in different yeasts is the levels of recombination activity. Our results presented in this paper, however, indicate that spontaneous petite frequency may not be simply correlated with recombination proficiency.

Yet another interesting feature in *T. glabrata* mitochondrial genetics is preferential transmission of the insertion element. However, the process by which the preferential transmission of insertion sequence occurs is not known. Asymmetric gene conversion has been reported to occur in the regions of heterogeneity in *S. cerevisiae* mtDNA (Strausberg et al. 1978). Heterogeneity of mtDNA in *T. glabrata* presumably within the cytochrome oxidase subunit 1 gene (see Fig. 3) lends itself to the study of processing and possible occurrence of polymorphism of this gene product in this yeast.

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