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A Nuclear Mutation Reversing a Biased Transmission of Yeast Mitochondrial DNA

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ABSTRACT

The highly biased transmission of ρ^- mitochondrial DNA that occurs in hypersuppressive matings between ρ^- and ρ^+ cells of the yeast *Saccharomyces cerevisiae* is thought to be a consequence of the replication advantage of the ρ^- mtDNA. A nuclear gene, *MGT1*, that is required for this displacement of ρ^+ mtDNA from zygotic clones has been identified through mutation. When one haploid parent carries the *mgt1* allele, transmission of ρ^- mtDNA is substantially reduced. When both haploid parents carry the *mgt1* allele, ρ^- mtDNA is essentially eliminated from the zygotic progeny. Thus in the absence of the *MGT1* gene there is a switch in the transmission bias; ρ^+ mtDNA rather than the hypersuppressive ρ^- mtDNA is inherited by most zygotic clones. In contrast to its semi-dominant behavior in haploid matings, *mgt1* behaves as a recessive allele in diploid matings since the ρ^+ genome in *MGT1/mgt1* diploids is efficiently displaced when mated with a *MGT1/mgt1* hypersuppressive ρ^- diploid strain. We find that ρ^+ genomes can be comaintained along with hypersuppressive ρ^- mtDNA for extended periods in clonal lines derived from *MGT1* × *mgt1* matings. However, as expected from the recessive nature of the *mgt1* mutation, these ρ^+ genomes are eventually eliminated. Our work indicates that *MGT1* plays a crucial role in the competition for inheritance between hypersuppressive ρ^- mtDNAs and the ρ^+ mitochondrial genome. The *MGT1* gene product may be a component of a mtDNA replication system that acts preferentially at the *rep* sequences found in hypersuppressive mtDNAs.

ZYGOTE formation and the establishment of a diploid clone in the yeast *Saccharomyces cerevisiae* is a developmental process that involves, among other processes, the transmission of mitochondrial genomes. Although the transmission of mitochondrial DNA (mtDNA) during mating and postzygotic divisions in yeast has been studied extensively [reviewed by DUJON (1981) and GINGOLD (1989)], little is known about the mechanisms involved. When two genetically marked ρ^+ strains are mated, the resulting diploids segregate pure parental and recombinant ρ^+ genomes within a few cell divisions [reviewed by BIRKY *et al.* (1982)]. Thus the zygotic colony consists of a mixture of pure cell types; some cells represent the mitochondrial genotype of one parent and some cells represent the mitochondrial genotype of the other parent. Such a rapid transition from the heteroplasmic to homoplasmic state is remarkable, since with about 100 mtDNA molecules initially present in the zygote a random partitioning during cell division would seldom produce a pure mitochondrial cell type. This rapid purification of mitochondrial genomes is also observed in matings between ρ^+ strains and mitochondrial DNA mutants, called ρ^-. These ρ^- mutants are deletion variants that usually have lost most of the 80 kilobases (kb) of ρ^+ mtDNA sequence and are therefore respiration deficient. In some cases the ρ^+ and ρ^- genomes segregate as do two ρ^+ genomes; that is, the zygotic clones contain a mixture of pure ρ^+ cell types and pure ρ^- cell types. However, in other $\rho^+ \times \rho^-$ matings the ρ^- genome exhibits preferential transmission, such that a large fraction of the zygotic clones contain only the original ρ^- mtDNA as the ρ^+ genomes are somehow eliminated during the growth of the clone. This phenomenon, called suppressiveness, was first described 35 years ago (EPHRUSSI, HOTTINGUER and ROMAN 1955). The fraction of zygotic diploid clones that are composed entirely of ρ^- cells is characteristic of a particular ρ^- mutant and is referred to as the degree of suppressiveness. The ρ^- mutants that exhibit >95% zygotic suppressiveness are termed hypersuppressive (BLANC and DUJON 1980; GOURSOT *et al.* 1980).

The mtDNA sequences retained by ρ^- mutants can arise from dozens of different locations on the ρ^+ genome [reviewed by DUJON (1981)]. Hypersuppressive ρ^- strains are a subset of these ρ^- mutants, retaining a specific intergenic sequence that is found in three or four (depending on the strain studied) regions of the ρ^+ genome. These sequences contain a 300-bp stretch of relatively G + C-rich DNA that is 80%

We dedicate this paper to the memory of HERSCHEL L. ROMAN who established the Department of Genetics at the University of Washington and its tradition of commitment to graduate training, and who helped discover the phenomenon of mitochondrial suppressiveness.

homologous among the three or four locations (BLANC and DUJON 1980; DE ZAMAROCZY *et al.* 1981). These conserved regions, called *rep* or *ori*, are thought to be primary replication origins in the ρ^+ genome, and it is proposed that the biased transmission of hypersuppressive ρ^- mtDNA reflects a replication advantage. This replication advantage model is based on the observation that the mtDNA sequences retained in the ρ^- mutants are amplified, largely as tandem arrays, to a level that is equivalent to the normal amount of mtDNA per cell (10–20% of the nuclear DNA content). Thus, while a specific mtDNA sequence is present only once per 80 kb in the ρ^+ genome, the same sequence will be present multiple times in an equivalent mass of ρ^- mtDNA. In cells with hypersuppressive ρ^- mtDNAs the tandemly repeated *rep* sequences presumably compete more efficiently for the replication initiation machinery than the smaller number of *rep* sequences in the ρ^+ genome. While a number of observations support the hypothesis that *rep* sequences are origins of replication (FANGMAN, HENLY and BREWER 1990), only one direct test of the replicative advantage model has been made (CHAMBERS and GINGOLD 1986). Replicating ρ^+ and ρ^- mtDNAs were measured in newly formed zygotes using radiolabeled precursor. A moderately suppressive (70%) ρ^- mtDNA incorporated more label than did the ρ^+ mtDNA. It remains to be seen whether this preferential synthesis is observed for other suppressive ρ^- mtDNAs and whether it is required for the biased transmission.

The genes required for mitotic and zygotic transmission of mtDNA are encoded in the nucleus [reviewed by DUJON (1981) and GINGOLD (1989)]. As a genetic approach to understanding the biased inheritance of hypersuppressive ρ^- genomes we have identified a nuclear mutation that disrupts the transmission bias. The mutation, called *mgt1*, prevents the displacement of the ρ^+ mtDNA by the hypersuppressive ρ^- genome during mating and zygotic colony formation. When present in one of the haploid parents (either ρ^+ or ρ^-), the *mgt1* mutation greatly reduces the fraction of pure ρ^- diploid clones. When both the ρ^+ and ρ^- haploid parents carry the *mgt1* allele the biased transmission is completely reversed such that only the ρ^+ mtDNA is inherited. We propose that the wild-type *MGT1* gene may specify a component of a mtDNA replication initiation system that acts at the *rep* sequences.

MATERIALS AND METHODS

Yeast strains, genetic methods and media: The *Saccharomyces cerevisiae* strains used in this study are listed in Table 1. Procedures for mating, sporulation, tetrad dissection and scoring of nutritional markers have been described previously (MORTIMER and HAWTHORNE 1969). All tetrad analyses were performed on a collection of independent zygotes rather than from a single cloned zygotic diploid. Homozygous *MAT*α/*MAT*α or MAT**a**/*MAT***a** diploids were generated from the initial heterozygous MAT**a**/MATα diploid strains by X-ray-induced mitotic recombination.

Rich media for yeast contained, per liter, 10 g yeast extract and 20 g peptone supplemented with 20 g dextrose (YEPD). YEPG plates were made by replacing the glucose in YEPD with 3% glycerol. For YEPG + diuron plates, 35 mg of diuron was added to one liter of YEPG medium. For YEPG + chloramphenicol plates, 4 g of chloramphenicol (dissolved in water and filter sterilized) were added to one liter of YEPG medium. Synthetic drop-out media and standard methods for the growth and manipulation of yeast strains have been described previously (SHERMAN, FINK and HICKS 1986).

Mutant screen: The haploid yeast strain N248–1C was mutagenized with ethyl methanesulfonate to 50% viability, according to published methods (SHERMAN, FINK and HICKS 1986). Colonies grown on YEPD plates were replica plated to a lawn of the hypersuppressive ρ^- strain HS3324. After incubating at 30° for 24 hr, these mating plates were replica plated to minimal medium containing glucose in order to select for diploid cells. Patches of diploid growth were present on the selective plates after two days of incubation at 30°. The identification of diploid clones that contained a reduced fraction of respiratory deficient cells was made by replica plating to YEP plates containing glycerol as the carbon source (YEPG plates). These plates were incubated at 30° for three days, and then examined for patches that grew more vigorously than the unmutagenized control.

Quantitative determination of the hypersuppressive response: The yeast strains used in the liquid culture mating were grown to mid-log phase in either YEP + 2% glucose (HS3324 ρ^- tester) or YEP + 3% glycerol (ρ^+ strains). From each of the two haploid cultures used in the mating, 3×10^6 cells were placed into 4 ml of minimal medium and vortexed briefly to ensure mixing. The cells were collected onto a 25 mm diameter Millipore filter disk (type HA 0.45 μM). The filters were incubated at 23° for 3 hr on plates containing complete medium plus noble agar in order to maximize the frequency of zygote formation. The filters were removed from the plates and placed into a test tube containing 5 ml of minimal medium. The cultures were vortexed briefly in order to resuspend the mating mixture of cells. The filter was removed, and the remaining cell suspension sonicated for 5 sec. The culture was plated on medium that was selective for zygotic cells. After approximately 3 days of growth at 30° the resulting diploid colonies were either replica plated or patched onto YEPD and YEPG plates. A colony was scored as containing only ρ^- cells if, after three days of incubation, no significant growth (other than small isolated papillae) was observed on the YEPG plates. The degree of suppressiveness was calculated as the fraction of ρ^- diploid colonies issuing from the particular cross.

Southern hybridization analysis of mtDNA: The presence of either the ρ^+ or the ρ^- (HS3324) mitochondrial DNA in yeast cells was determined by DNA hybridization analysis. Total DNA was isolated from 5 ml cultures by methods described previously (DAVIS *et al.* 1980). The DNA preparations were cleaved with the restriction endonuclease *Bgl*II and separated by electrophoresis on a 1.4% agarose gel. The *Bgl*II enzyme cuts once within the 963-bp HS3324 mtDNA sequence producing a single band of approximately 1 kb in length. Cutting the ρ^+ mtDNA with the *Bgl*II enzyme produces a much larger (approximately 15 kb) fragment that hybridizes with the HS3324 sequence (this work). It was thus possible to determine the presence of either the ρ^+, the ρ^-, or a mixture of both types of mtDNA in cultures. Standard procedures were used for Southern blotting and DNA manipulations (MANIATIS, FRITSCH and

TABLE 1

Yeast strains used in this study

Strain	Genotype	Source
N248-1C	*MATα his2 trp1 ura3 met14 ade1 leu1 MGT1* ρ^+	D. Hawthorne
YB1	*MATα his2 trp1 ura3 met14 ade1 leu1 mgt1* ρ^+	Mutagenesis of N248-1C
KL14-4a	*MAT***a** *his1 trp2 leu2 MGT1* ρ^+ (*diu*^{S} *cap*^{R})	B. Dujon
KL14-4a: HS3324	*MAT***a** *his1 trp2 leu2 MGT1* ρ^- (*rep2*)	B. Dujon
SG501	*MAT***a** *his1 trp2 leu2 ura3 met14 MGT1* ρ^- (HS3324)	Segregant from SG101 × HS3324
SG502	*MAT***a** *leu2 ura3 met14 mgt1* ρ^- (HS3324)	Segregant from SG101 × HS3324
KL14-4a: HS416	*MAT***a** *his1 trp2 leu2 MGT1* ρ^- (*rep1*)	B. Dujon
KL14-4a: HS137	*MAT***a** *his1 trp2 leu2 MGT1* ρ^- (*rep3*)	B. Dujon
BS127	*MAT***a** *ade1 ade2 leu2 trp1 ura3 MGT1* ρ^+	R. Sclafani
D243-4A	*MAT***a** *ade1 lys2 cyh*^{R} *MGT1* ρ^+ (*diu*^{R} *cap*^{S})	D. Hawthorne
SG101	*MATα ura3 trp1 ade1 his2 met14 mgt1* ρ^+	Segregant from YB1 × BS127
8617-17	*MAT***a** *ura3 trp1 leu2 cyh2 cdc16 MGT1* ρ^+	L. Hartwell
SG102	*MATα ura3 trp1 ade1 his2 met14 mgt1* ρ^+	Segregant from YB1 × BS127
SG103	*MATα ura3 trp1 ade1 his2 MGT1* ρ^+	Segregant from N248-1C × BS127
SG104	*MAT***a** *ura3 trp1 ade1 leu2 met14 mgt1* ρ^+	Segregant from YB1 × BS127
SG105	*MAT***a** *ura3 trp1 ade1 leu2 MGT1* ρ^+	Segregant from N248-1C × BS127
SG106	*MATα ura3 trp1 ade1 his1 met14 mgt1* ρ^+	Segregant from YB1 × KL14-4a
SG201	*MATα/α ura3/ura3 trp1/trp1 ade1/ade1 his2/+ leu2/+ MGT1/MGT1* ρ^+	Mating SG103 × SG105
SG202	*MATα/α ura3/ura3 trp1/trp1 ade1/ade1 his2/+ leu2/+ met14/+ MGT1/mgt1* ρ^+	Mating SG103 × SG104
SG203	*MATα/α ura3/ura3 trp1/trp1 ade1/ade1 met14/met14 his2/+ leu2/+ mgt1/mgt1* ρ^+	Mating SG102 × SG104
SG204	*MAT***a/a** *ura3/+ trp1/+ trp2/+ ade1/+ his1/his1 leu2/+ met14/+ MGT1/mgt1* ρ^-	Mating SG106 × HS3324
SG301	*MAT***a** *ura3 leu2 ade1 his2 met14 mgt1* ρ^- (HS3324)	Segregant from SG101 × HS3324
SG401	*MAT***a** *trp1,2 ura3 leu2 his1,2 MGT1* ρ^+ (*diu*^{S} *cap*^{R})	Segregant from KL14-4A × SG101
SG402	*MAT***a** *leu2 met14 mgt1* ρ^+ (*diu*^{S} *cap*^{R})	Segregant from KL14-4A × SG101
SG403	*MATα ade1 leu1 MGT1* ρ^+ (*diu*^{R} *cap*^{S})	Segregant from YB1 × D243-4A
SG404	*MATα trp1 his2 met14 mgt1* ρ^+ (*diu*^{R} *cap*^{S})	Segregant from YB1 × D243-4A

Sambrook 1982). Mitochondrial DNA was probed with the nick-translated (^{32}P-labeled) plasmid pCT10. Plasmid pCT10 contains the 963 bp HS3324 sequence linearized at its unique *Eco*RV site and cloned into the *Eco*RV site of pBR322 (from B. Brewer). Under the hybridization conditions used there appeared to be very little cross-hybridization to the other *rep* sequences found in the ρ^+ genome.

RESULTS

Identification and quantitation of the *mgt1* mutation: The screen used in this study was designed to detect a reduction in the frequency of respiration deficient diploid cells issuing from a mating between a wild type ρ^+ strain and a hypersuppressive ρ^- strain. A population of ρ^+ haploid yeast cells (strain N248-1C) was mutagenized with EMS, plated, and the resulting colonies mated by replica plating to a lawn of the hypersuppressive ρ^- strain KL14-4a:HS3324 (abbreviated HS3324). HS3324 contains the *rep2* mtDNA region along with flanking sequences (Blanc 1984), and in quantitative tests with the unmutagenized ρ^+ strain, HS3324 is about 98% suppressive. After selecting for zygotes, the patches of zygotic clones were replica plated to medium that contained the non-fermentable carbon source glycerol (YEPG plates). Presumptive mutants were identified as those giving rise to patches of vigorous (respiration competent) growth. With the unmutagenized control, the diploid patches gave rise to weak growth on the final YEPG plates. This limited growth reflects the small fraction of respiration competent diploid cells that arise from a mating with the hypersuppressive ρ^- strain. However, with the mutagenized population of cells, one of the test patches gave rise to strong growth on the YEPG plate indicating that a large proportion of the diploid cells were respiration competent. This phenotype suggested that the original ρ^+ cell contained a mutation that rendered it resistant to the highly biased transmission of the hypersuppressive ρ^- mtDNA during zygote formation and subsequent mitotic growth. Our screening method assumes that the mutant will act in a dominant or semidominant fashion during matings between ρ^+ and ρ^- cells. The mutant strain is designated YB1 and the mutation is called *mgt1* (for mitochondrial genome transmission). That the *mgt1* mutation results in reduced transmission of the hypersuppressive HS3324 ρ^- mtDNA was confirmed by measuring the fraction of diploid clones containing only ρ^- cells that arise from the $\rho^+ \times \rho^-$ matings (MATERIALS AND METHODS). In scoring over 850 zygotes from the YB1 × HS3324 mating we found that only 14% of the diploid clones were res-

piration deficient (14% suppressive). This value was a substantial reduction from the 98% suppressiveness determined using the original N248–1C strain.

***mgt1* is a nuclear gene located on chromosome *XI*:** The phenotype conferred by the *mgt1* mutation and the method by which it was isolated did not provide any evidence as to whether the mutation was mitochondrial or nuclear. If hypersuppressiveness involves the physical interaction of ρ^+ and ρ^- mtDNA, then an alteration of the ρ^+ mitochondrial genome might prevent the ρ^- mtDNA from monopolizing the zygotic progeny. Alternatively, *mgt1* might define a nuclear gene whose product is required for the biased transmission of hypersuppressive mtDNA. To distinguish between a cytoplasmic or nuclear location for the *mgt1* mutation, the pattern of meiotic segregation of the *mgt1* phenotype was examined in diploid cells. Strain YB1 (*mgt1* ρ^+) was mated to a wild type strain of the opposite mating type, BS127 (*MGT1* ρ^+), and the resulting diploids were induced to sporulate. Twelve *MAT*α ρ^+ spore colonies were chosen at random and tested for the presence of the *mgt1* mutation by suppressiveness tests with the ρ^- strain HS3324. In seven of the spores the ρ^+ mtDNA was susceptible to displacement by the hypersuppressive ρ^- mtDNA (MGT$^+$) and in five of the spores the ρ^+ mtDNA was resistant (*mgt*$^-$). Furthermore, all of the susceptible spores were Met$^+$ (*MET14* was carried by the BS127 parent) whereas all of the resistant spores were Met$^-$ (*met14* was carried by the YB1 parent). This result suggests that the *mgt1* mutation is closely linked to the known chromosomal gene, *MET14*. The *MET14* gene has been mapped to the right arm of chromosome *XI*, tightly linked to the centromere (MORTIMER and SCHILD 1986). Also linked to *MET14*, but on the opposite side of the centromere, is the *CDC16* gene.

To confirm the chromosomal location of *mgt1* and to accurately position it on the yeast genetic map, a three point cross was carried out with *met14*, *cdc16*, and *mgt1*. The complete genotypes of the two strains used in this mapping study, SG101 and 8617–17, are listed in Table 1. The meiotic products of 54 diploid cells derived from the SG101 × 8617–17 mating were examined by tetrad analysis. The *MAT* locus, the *CDC16* gene, and all nutritional markers segregated as expected (data not shown). The presence of the *mgt1* mutation was determined for each spore colony by a suppressiveness test with a ρ^- tester of the opposite mating type, either HS3324 (*MAT*a) or SG501 (*MAT*α). In all 54 of the tetrads examined there was 2:2 segregation for *MGT1:mgt1*. These data are consistent with *mgt1* defining a nuclear gene, not a mitochondrial gene. Furthermore, the recombination data provided the following genetic map order: *mgt1* is on the left arm of chromosome *XI*, 8.3 map units to the right of *cdc16*, and 2.8 map units to the left of the *met14*/centromere region (no recombination events were observed between the centromere and *met14* as determined by the segregation of a second centromere linked marker, *leu2*).

TABLE 2

Expression of *mgt1* in ρ^+ and ρ^- cells

ρ^+ strain	ρ^- tester[a]	Percent ρ^- diploid clones[b]
N248-1C (*MGT1*)	HS3324 (*MGT1*)	99
N248-1C (*MGT1*)	SG301 (*mgt1*)	21
SG101 (*mgt1*)	HS3324 (*MGT1*)	28
SG101 (*mgt1*)	SG301 (*mgt1*)	<1

[a] Contains the hypersuppressive ρ^- HS3324 mtDNA.
[b] Determined by replica plating a minimum of 350 zygotic colonies onto YEPD and YEPG plates.

Expression of *mgt1* in a ρ^- cell: Since *mgt1* was isolated in a ρ^+ mitochondrial background, it is possible that the mutation acts by preconditioning the resident ρ^+ mitochondrial genome so that it will be resistant to displacement by the hypersuppressive ρ^- mtDNA in future matings. It follows that if the *mgt1* gene were carried by the hypersuppressive ρ^- parent in a haploid mating there should be no decrease in the hypersuppressive response. This possibility is eliminated since we were able to isolate a hypersuppressive ρ^- strain, SG301 (Table 1), that was shown through mating tests to contain the nuclear *mgt1* mutation. DNA hybridization experiments showed that SG301 contained HS3324 ρ^- mtDNA in amounts equivalent to its HS3324 *MGT1* parent. Table 2 summarizes the suppressiveness tests from four $\rho^+ \times \rho^-$ matings in which various combinations of *mgt1* and *MGT1* are examined. The first mating (*MGT1* ρ^+ × *MGT1* ρ^-) illustrates the normal high degree of suppressiveness displayed by the HS3324 ρ^- mitochondrial genome. The second and third matings demonstrate that the *mgt1* mutation reduces the biased transmission of the hypersuppressive ρ^- mtDNA whether it was present in the ρ^+ or the ρ^- haploid parent. This result rules out the possibility that the *mgt1* mutation preconditions only the ρ^+ genome. The last mating, in which both parents contain the *mgt1* gene, demonstrates that *mgt1* behaves as a semidominant mutation in haploid matings since the suppressiveness is much lower (<1%) than in either of the *MGT1* × *mgt1* matings (21–28%). Thus, it appears that in the absence of the *MGT1* gene in the zygote there is essentially no transmission of the hypersuppressive ρ^- mtDNA to the zygotic progeny.

***mgt1* affects transmission of *rep1*, *rep2* and *rep3* ρ^- genomes:** The *mgt1* mutant was identified in a screen using the hypersuppressive ρ^- strain KL14-4a:HS3324. This strain contains a 963 bp reiterated mitochondrial genome that includes the *rep2* sequence (BLANC 1984). Two other *rep* sequences (*rep1* and *rep3*) that are found in the ρ^+ mtDNA of strain KL14-4a are also hypersuppressive when present in short repeated ρ^- DNAs. Given the similarity among the

TABLE 3

$mgt1$ is a recessive mutation: diploid × haploid matings

ρ^+ strain	ρ^- tester[a]	Percent ρ^- triploid clones[b]
SG201 (*MGT1/MGT1*)	HS3324 (*MGT1*)	100
SG202 (*MGT1/mgt1*)	HS3324 (*MGT1*)	98
SG203 (*mgt1/mgt1*)	HS3324 (*MGT1*)	2

[a] Contains the hypersuppressive ρ^- HS3324 mtDNA.
[b] Determined by patching 100 of the zygotic colonies from each matings onto YEPD and YEPG plates.

TABLE 4

$mgt1$ is a recessive mutation: diploid × diploid matings

ρ^+ strain	ρ^- tester[a]	Percent ρ^- tetraploid clones[b]
SG201 (*MGT1/MGT1*)	SG204 (*MGT1/mgt1*)	98
SG202 (*MGT1/mgt1*)	SG204 (*MGT1/mgt1*)	99
SG203 (*mgt1/mgt1*)	SG204 (*MGT1/mgt1*)	14

[a] Contains the hypersuppressive ρ^- HS3324 mtDNA.
[b] Determined by replica plating a minimum of 200 tetraploid colonies for each mating onto YEPD and YEPG plates.

rep sequences, we were interested in knowing whether the *mgt1* mutation also affected the biased transmission of ρ^- mtDNAs containing *rep1* and *rep3*. Therefore, two additional hypersuppressive strains were examined; KL14-4a:HS416, which contains a 700-bp tandemly repeated mtDNA sequence that includes *rep1*, and KL14-4a:HS137, which contains an approximately 640 bp tandemly repeated mtDNA sequence that includes *rep3* (BLANC and DUJON 1980, 1982). For each of the $\rho^+ \times \rho^-$ matings examined, a minimum of 200 zygotes were scored for respiration competence. Compared to the *MGT1* ρ^+ × *MGT1* ρ^- matings (95–99% suppressiveness), *mgt1* ρ^+ × MGT1 ρ^- matings gave varying reduced suppressiveness values for HS416 (59–88%) and for HS137 (71–91%). Some of this variation is probably due to differences in nuclear background since the stocks used in these tests were not congenic. However, *mgt1* ρ^+ × *mgt1* ρ^- matings were unambiguous in showing that the mutation eliminates the biased transmission of all three hypersuppressive genomes. As with the HS3324 strain, both HS416 and HS137 matings produced fewer than 1% pure ρ^- zygotic clones.

***mgt1* is a recessive mutation:** The original selection and characterization of the *mgt1* mutation demanded that it act at least partially dominant during haploid matings and subsequent zygotic growth. However, this observation does not necessarily mean that the *mgt1* allele inhibits wild type *MGT1* function as would a true dominant allele. The critical test for dominance or recessiveness is whether the mutant allele is expressed when present in the heterozygous condition. Therefore, a series of suppressiveness tests were carried out with the following *MAT*α/α ρ^+ diploid strains: SG201 (*MGT1/MGT1*), SG202 (*MGT1/mgt1*), and SG203 (*mgt1/mgt1*). These diploid strains were mated to the HS3324 ρ^- haploid tester strain (*MGT1*) and the resulting triploid zygotic clones tested for respiration competence. The results in Table 3 indicate that *mgt1* is a recessive mutation, since a reduction in the hypersuppressive response was only observed in matings with the homozygous ρ^+ parent (*mgt1/mgt1*). When present in the heterozygous condition (*MGT1/mgt1* ρ^+ parent) the *mgt1* allele is not expressed.

One weakness in the above experiment is that gene dosage may play a role in the expression of the *mgt1* phenotype during mating and zygotic colony formation. In the mating *MGT1/mgt1* ρ^+ × *MGT1* ρ^-, the resulting triploid zygotes contain two copies of the *MGT1* gene and only one copy of the *mgt1* gene. To rule out a possible gene dosage effect, we examined the degree of suppressiveness of *MAT*α/α diploid strains SG201, SG202, and SG203 when mated to the *MAT***a**/**a** diploid hypersuppressive strain SG204 (*MGT1/mgt1* ρ^-). Table 4 shows that neither a homozygous wild type (*MGT1/MGT1*) nor a heterozygous (*MGT1/mgt1*) ρ^+ strain is resistant to the biased transmission of the ρ^- mtDNA. However, a substantial reduction in the fraction of ρ^- tetraploid progeny is seen with the homozygous mutant (*mgt1/mgt1*) ρ^+ diploid strain. Since the *MGT1/mgt1* heterozygous ρ^+ strain displays no resistance to the hypersuppressive ρ^- genome even though there are equivalent copies of the *MGT1* and *mgt1* genes in the tetraploid zygotes we conclude that *mgt1* is indeed a recessive mutation.

Transient heteroplasmy in *MGT1* × *mgt1* diploids: Since *mgt1* is a recessive allele, as the *MGT1/mgt1* zygotes grow and divide the diploid cells should approach the phenotype of the wild-type (*MGT1/MGT1*) diploids. More specifically, if cells derived from zygotes from a *mgt1* ρ^+ × *MGT1* ρ^- mating contain a mixture of both ρ^+ and ρ^- mtDNA, one would expect that over time the wild type phenotype will be expressed and any residual ρ^- mitochondrial genomes will eventually displace the ρ^+ genomes within the cell. In support of this idea we have observed that respiration competent zygotic colonies arising from *mgt1* ρ^+ × *MGT1* ρ^- matings show noticeably weaker growth on YEPG plates than do colonies arising from *MGT1* ρ^+ × *MGT1* ρ^+ matings (data not shown). This finding suggested that significant numbers of respiration deficient (ρ^-) cells are segregated during the mitotic growth of the initial *MGT1/mgt1* ρ^+ diploids. It should be pointed out that in contrast to these *MGT1* × *mgt1* matings, zygotic colonies arising from *mgt1* ρ^+ × *mgt1* ρ^- matings exhibit vigorous growth on YEPG plates indicating little or no segregation of ρ^- cells. In order to verify this model of transient comaintenance of ρ^+ and ρ^- mtDNAs in the *MGT1/mgt1* heterozygote, we designed an experiment to measure the fraction of ρ^- diploid clones at various times after zygote formation.

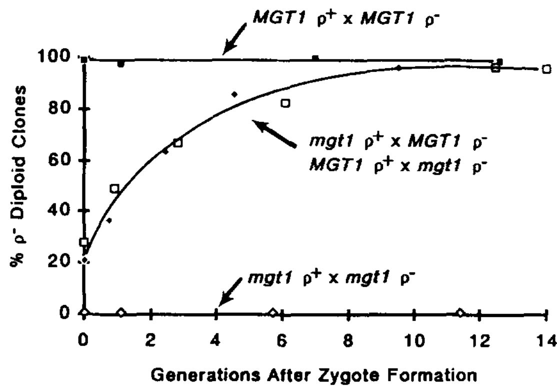

FIGURE 1.—Postzygotic segregation of hypersuppressive ρ^- genomes. Diploid clones from each of the $\rho^+ \times \rho^-$ matings (see Table 2) were tested at various times after zygote formation for the presence of stable respiration competent cells. A minimum of 200 diploid colonies were patched onto YEPD and YEPG plates for each time point in order to determine the per cent ρ^- colonies. *MGT1* $\rho^+ \times$ *MGT1* ρ^- (■); *mgt1* $\rho^+ \times$ *MGT1* ρ^- (♦); *MGT1* $\rho^+ \times$ *mgt1* ρ^- (□); *mgt1* $\rho^+ \times$ *mgt1* ρ^- (◇).

Cells were allowed to mate on filters for three hours then diluted into liquid medium selective for the zygotes and incubated at 30°. Samples of each culture were removed at various times and plated onto medium selective for diploid cells. After the diploid clones had grown up, the fraction of pure ρ^- colonies was determined by replica plating to YEPG medium. Figure 1 shows the percent of ρ^- diploid clones as a function of generations after zygote formation. The *MGT1* $\rho^+ \times$ *MGT1* ρ^- mating, as expected, produced an initially high percentage of pure ρ^- diploid clones and no significant change was found throughout the experiment. In the *mgt1* $\rho^+ \times$ *MGT1* ρ^- and *MGT1* $\rho^+ \times$ *mgt1* ρ^- matings the production of pure ρ^- diploid clones followed a pattern that would be predicted from the recessive nature of the *mgt1* allele. When the initial zygotes from these two crosses were tested there was a relatively low percentage of respiration deficient diploid colonies (22–28% ρ^-). However, when the zygotes were allowed to grow and divide, the fraction of respiration deficient zygotic clones increased over time. After approximately eight generations of postzygotic growth the percentage of pure ρ^- colonies was equivalent to that observed for the *MGT1* × *MGT1* mating. This result implies that a large fraction of the original *MGT1/mgt1* diploid cells contained a mixture of ρ^+ and ρ^- mtDNAs and that during growth and cell division preferential transmission of the ρ^- mtDNA was reestablished.

Finally, in the *mgt1* $\rho^+ \times$ *mgt1* ρ^- mating essentially all of the zygotic clones remained respiration competent throughout the eleven generations of postzygotic growth. This result can be interpreted in one of two ways. The first explanation is that the absence of the *MGT1* gene in a diploid cell eliminates mtDNA segregation so that the ρ^+ and ρ^- mtDNA are comaintained within the same cell. Only by the random partioning of mtDNAs would a cell segregate a respiration deficient (pure ρ^-) daughter cell. An alternative explanation is that in the absence of the *MGT1* gene the ρ^+ mtDNA is preferentially transmitted to the zygotic progeny and the ρ^- mtDNA is eliminated early in postzygotic growth. These possibilities can be distinguished by determining whether the respiration competent *mgt1/mgt1* diploid cells contain a mixture of ρ^+ and ρ^- mtDNA (similar to the *MGT1/mgt1* diploids) or only the ρ^+ mtDNA.

TABLE 5

Tetrad analysis of $\rho^+ \times \rho^-$ matings

Tetrad types	*MGT1* × *MGT1*	*mgt1* × *MGT1*	*mgt1* × *mgt1*
4 ρ^+: 0 ρ^-	20	3	26
3 ρ^+: 1 ρ^-	0	2	0
2 ρ^+: 2 ρ^-	0	4	0
1 ρ^+: 3 ρ^-	0	3	0
0 ρ^+: 4 ρ^-	0	10	0

Mass matings for each of the three crosses were generated by cross stamping the two haploid strains. After allowing growth on nonselective media for 2 days, the mating plates were replica plated to nutrient drop-out media. Patches of diploid growth were then streaked onto sporulation inducing media. The data represents the results for tetrads in which all four spore colonies were viable.

No evidence for heteroplasmy in *mgt1* × *mgt1* matings: Evidence for heteroplasmy in *mgt1* $\rho^+ \times$ *mgt1* ρ^- matings was first sought by examining the sporulation products of respiration competent diploid cells. For a diploid yeast cell to undergo sporulation it must be active in respiration at the time of transfer to sporulation medium (KUENZI, TINGLE and HALVORSON 1974). A cell containing a mixture of ρ^+ and ρ^- mtDNAs should contain enough wild type genetic information to carry out cellular respiration and thus be capable of sporulation. However, since a meiotic spore packages only a fraction of the total mtDNA (BREWER and FANGMAN 1980), some spores might contain only ρ^- genomes. These spore colonies would be signaled by their inability to grow on YEPG plates. Asci from sporulating diploid cells were dissected and tested for the presence of respiration deficient spore colonies. In the control experiment, N248–1C (*MGT1* ρ^+) and HS3324 (*MGT1* ρ^-) were mated and the resulting population of diploid cells sporulated. Although the majority of diploid clones from this cross are respiration deficient and thus unable to sporulate, it was possible to identify the small fraction of sporulating cells by microscopic analysis. We estimate that approximately 15–20 generations of postzygotic growth had occurred during the selection of the diploid cells and growth on sporulation medium. The first column in Table 5 shows that the spore colonies from all 20 asci examined were respiration competent (designated as 4 ρ^+:0 ρ^-). As expected, there was no evidence for the comaintenance of ρ^+ and ρ^- mtDNA in the small fraction of respiration competent diploid cells arising from this mating.

For the mating between strains SG101 (*mgt1* ρ^+) and HS3324 (*MGT1* ρ^-) the second column in Table 5 shows that among 22 asci examined, 19 contained one or more spores that were respiration deficient. We confirmed that the respiration deficient spore colonies contained the ρ^- mtDNA by isolating total DNA from ten of them (each from a different ascus) and probing with HS3324 mtDNA (data not shown). Since a sporulating diploid cell must be capable of respiration, we propose that in a large fraction of sporulating *mgt1/MGT1* diploid cells both ρ^+ and ρ^- mtDNAs had been present in their recent postzygotic history. Thus, in haploid matings between an *MGT1* hypersuppressive ρ^- and a *mgt1* ρ^+ strain, many of the respiration competent zygotic clones are able to maintain both the ρ^+ and the ρ^- mtDNA for an extended period of time. The third column in Table 5 presents the data from a mating between two *mgt1* mutant strains, SG101 (*mgt1* ρ^+) and strain SG502 (*mgt1* ρ^-). Interestingly, when both of the haploid parents carry the *mgt1* allele, there is no evidence for the comaintenance of ρ^+ and ρ^- mtDNA. All 26 of the asci dissected gave rise to four spore colonies that were capable of growth on YEPG plates (4 ρ^+:0ρ^-). This result is consistent with the idea that the respiration competent *mgt1/mgt1* diploid cells are of pure ρ^+ mtDNA type.

Direct physical evidence for the type of mtDNA present in the diploid cells was obtained by DNA hybridization analysis. Zygotic diploid cells were selected from the matings presented above and recloned onto YEPG plates. Ten respiration competent colonies from each mating were picked and grown for total DNA isolation. Cutting the DNA preparations with the restriction enzyme *Bgl*II and hybridizing with radioatively labeled HS3324 mtDNA allowed us to distinguish between the ρ^+ mitochondrial genome and the tandemly repeated 963-bp HS3324 genome. When cut with *Bgl*II the HS3324 mitochondrial genome yields an approximately 1-kb DNA fragment while the ρ^+ mtDNA produces an approximately 15-kb fragment that contains the HS3324 sequence. Figure 2 is an autoradiogram from an experiment in which total DNA from ten *mgt1/MGT1* respiration competent diploid cultures was probed with HS3324 labeled DNA. Seven out of the ten isolates clearly contain both the 1-kb and the 15-kb restriction fragment. Similar DNA hybridization experiments with ten respiration competent diploid colonies derived from the *mgt1* × *mgt1* mating provided no evidence for maintenance of a heteroplasmic state; only the 15-kb fragment was observed (Figure 3). Thus the inability to find ρ^- spores from sporulating *mgt1/mgt1* diploid cells (Table 5) appears to be due to the absence of ρ^- mtDNA rather than to a failure of diploids to transmit ρ^- mtDNA to sporulation products.

Mitochondrial fusion is unaffected by *mgt1*: If the ρ^- and ρ^+ mtDNAs need to be within the same mitochondrion in order for the hypersuppressive genome to exert a transmission advantage, then the absence of organelle fusion might produce the phenotype associated with *mgt1*. To examine the effect of *mgt1* on organelle fusion we determined the rate of recombination between two mitochondrial markers. Genetic exchange presumably requires the mixing of mtDNA molecules in the same intramitochondrial matrix and should thus provide a means of assessing the interaction between mitochondrial genomes. To measure generalized mitochondrial recombination, *MGT1* and *mgt1* strains were constructed that are either resistant or sensitive to the drugs diuron and chloramphenicol (Table 1). Mass matings were carried out, zygotes plated on selective plates, and diploid cells were recloned on selective plates. The final colonies were then tested for diuron and chloramphenicol resistance to identify the segregated parental (*diu*^{R}*cap*^{S} and *diu*^{S}*cap*^{R}) and recombinant classes (*diu*^{S}*cap*^{S} and *diu*^{R}*cap*^{R}). Table 6 shows that in the *MGT1* × *MGT1* mating the recombinant classes account for 18% of the homoplasmic cell types. No significant difference was seen in the *MGT1* × *mgt1* or *mgt1* × *mgt1* matings (20% and 16% recombinants, respectively). This result argues against the possibility that the *mgt1* mutation blocks mitochondrial fusion and therefore the mixing of mtDNAs.

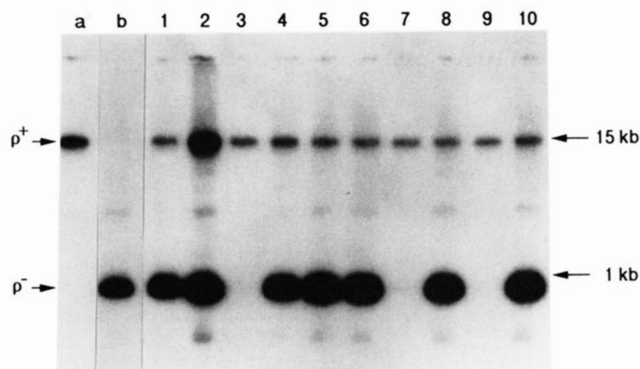

FIGURE 2.—mtDNA analysis of *mgt1/MGT1* diploid cells. Total DNA was isolated from ten respiratory competent *mgt1/MGT1* diploid colonies (SG101 × HS3324). The DNA samples were digested with the restriction enzyme *Bgl*II, separated on an agarose gel, blotted onto Nytran filter membrane, and probed with ^{32}P-labeled HS3324 mtDNA. Lane **a** is total DNA isolated from the ρ^+ haploid parent, SG101. Lane **b** is the total DNA isolated from the ρ^- haploid parent, HS3324. Lanes **1–10** represent the DNA isolated from the respiration competent diploid colonies that issued from the *mgt1* ρ^+ × *MGT1* ρ^- mating.

DISCUSSION

To identify the nuclear gene products responsible for the biased transmission of hypersuppressive ρ^- mtDNA, we screened a collection of EMS treated ρ^+ yeast cells for a mutant that was resistant to displacement of ρ^+ mtDNA during zygote formation and subsequent diploid growth. The mutation is called *mgt1* to denote its effect on mitochondrial genome

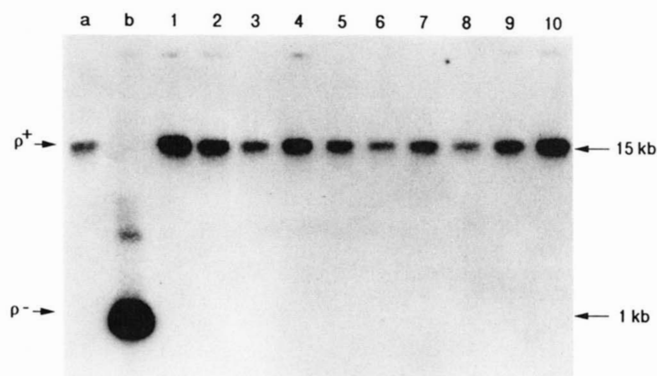

FIGURE 3.—mtDNA analysis of *mgt1/mgt1* diploid cells. Lane **a** is the total DNA isolated from the ρ^+ haploid parent, SG101. Lane **b** is the total DNA isolated from the ρ^- haploid parent, SG301. Lanes **1–10** represent the DNA isolated from ten independent respiration competent diploid colonies.

transmission. We have shown that the *mgt1* mutation defines a nuclear gene that maps near the centromere of chromosome *XI,* and is expressed as a semidominant mutation during haploid matings. If one of the haploid parents carried the *mgt1* allele (*MGT1* × *mgt1*), there was a substantial reduction in the fraction of respiratory deficient diploid colonies (approximately 15–30% of diploid colonies are ρ^-). When both of the haploid parents contained the *mgt1* mutation (*mgt1* × *mgt1*) virtually no respiration deficient diploid zygotic clones were produced (<1%). The expression of the *mgt1* phenotype was not limited to the ρ^+ haploid cell. The frequency of ρ^- diploid clones arising from haploid mating was the same whether the *mgt1* mutation was brought in by the ρ^+ parent (*mgt1* ρ^+ × *MGT1* ρ^-) or the ρ^- parent (*MGT1* ρ^+ × *mgt1* ρ^-). This result argues against the specific preconditioning of the ρ^+ mtDNA so that it is somehow resistant to the hypersuppressive mtDNA. In contrast to the semidominant behavior in haploid matings, when the *mgt1* mutation was analyzed in the heterozygous condition during diploid matings (*MGT1/mgt1* ρ^+ × *MGT1/mgt1* ρ^-) it behaved as a recessive allele. Only when the *mgt1* gene was present in the homozygous condition in the ρ^+ parent (*mgt1/mgt1* ρ^+ × *MGT1/mgt1* ρ^-) was there a reduction in the transmission of the hypersuppressive mtDNA.

The analysis of sporulating diploids derived from matings of *mgt1* ρ^+ × *MGT1* ρ^- strains suggested that a large fraction of the respiration competent diploid cells maintained both the ρ^+ and the ρ^- mtDNA even after 15–20 generations of clonal growth. DNA hybridization experiments from *MGT1/mgt1* diploids confirmed that they did indeed contain both mtDNAs. To assess the segregation of the ρ^+ and ρ^- mtDNAs during zygote formation and clonal growth, we monitored the production of respiratory deficient diploid cells at various times after zygote formation for three different matings. In a *MGT1* ρ^+ × *MGT1* ρ^- mating, as expected, the production of pure ρ^- cell types

TABLE 6

Genomes segregated in ρ^+ × ρ^+ matings

Mating	Percent segregation			
	Parental		Recombinant	
	$diu^R\ cap^S$	$diu^S\ cap^R$	$diu^S\ chl^S$	$diu^R\ cap^R$
MGT1 × *MGT1* (SG401/SG403)	32	50	10	8
MGT1 × *mgt1* (SG401/SG404)	35	45	9	11
mgt1 × *mgt1* (SG402/SG404)	41	43	6	10

Each mitochondrial cross consisted of a mass mating between two haploid strains. After allowing for mtDNA segregation and the production of homoplasmic cells, the zygotic progeny were pooled and plated onto media selective for diploids. Mitochondrial genotypes were determined by replica plating onto YEPG + diuron and YEPG + chloramphenicol. Total zygotic clones examined: cross #1, 436; cross #2, 382; cross #3, 280.

occured in most zygotic clones soon after zygote formation. With either *mgt1* ρ^+ × *MGT1* ρ^- or *MGT1* ρ^+ × *mgt1* ρ^- matings a substantial fraction of diploid clones contained respiration competent cells when the initial zygotes were plated. However, when the diploid cells were grown a number of generations after zygote formation we found that a smaller and smaller fraction of the diploid clones contained respiration competent cells. After about eight to ten generations of postzygotic growth, the fraction of ρ^- diploids in the *MGT1/mgt1* cultures reached a value similar to that obtained with the *MGT1/MGT1* control (>95% ρ^-). Since ρ^- cells do not have a growth advantage, these results must mean that ρ^+ genomes were being displaced by ρ^- genomes and lost from the zygotic clones. The initial high fraction of colonies with respiration competent cells found when only one of the haploid parents contained the *mgt1* mutation (*mgt1* ρ^+ × *MGT1* ρ^- or *MGT1* ρ^+ × *mgt1* ρ^-) can be explained by assuming that the two-fold reduction in the level of the *MGT1* gene product that occurs at zygote formation compromises the preferential transmission of the ρ^- mtDNA. Since *MGT1* is a dominant allele, the levels of the wild-type gene product could be returned to normal levels after a few generations of postzygotic growth (possibly by some form of feedback regulation). Once this optimum concentration of *MGT1* gene product is reached, the biased transmission of the hypersuppressive ρ^- mtDNA would once again be established.

Our results indicate that the *MGT1* gene product is necessary for the transmission of the hypersuppressive ρ^- mtDNA during zygotic growth. In matings where both parents carried the *mgt1* allele (*mgt1* ρ^+ × *mgt1* ρ^-) no diploid colonies were found that contained only ρ^- cells. In addition, essentially no ρ^- mtDNA was detected in ten independent *mgt1/mgt1* diploid clones. The *mgt1* mutation is remarkable, therefore, in that it switches the highly biased transmission of the hy-

persuppressive ρ^- mtDNA found with wild-type (*MGT1* × *MGT1*) matings to a highly biased transmission of ρ^+ mtDNA. Furthermore, the results on recombination of mtDNA suggest that fusion of mitochondria and mixing of mtDNAs are not blocked by the *mgt1* mutation. Three different hypersuppressive ρ^- strains with *rep* sequences derived from different regions of the ρ^+ genome were similarly affected by the *mgt1* mutation. This result suggests that there is a conserved sequence found in the *rep* region that interacts with the *MGT1* gene product and is therefore essential for the preferential transmission of hypersuppressive ρ^- mtDNA. Although it is possible that the hypersuppressive ρ^- genome outcompetes the ρ^+ genome by a strict segregation mechanism, our results are also consistent with a replication advantage mechanism where the *MGT1* gene product is responsible for ensuring the preferential replication of the ρ^- mtDNA when in competition with the ρ^+ genome. The *mgt1* mutation may, therefore, be revealing a component of a mtDNA replication initiation system that acts at *rep* sequences. Since the maintenance of neither mtDNA type in vegetative cells is affected in *mgt1* haploid strains, it is necessary to propose that there is a second mechanism by which both ρ^+ and ρ^- mtDNAs are replicated. Indeed, many ρ^- genomes are maintained that do not contain *rep* sequences [reviewed by DUJON (1981)] indicating that the ρ^+ genome contains many other sites where replication can be initiated. This alternative mechanism must be more efficient at replicating the ρ^+ genome than the hypersuppressive ρ^- genome because in *mgt1* ρ^+ × *mgt1* ρ^- matings only the ρ^+ genome is transmitted to the zygotic clones. A possible target for this alternate initiation system could be A + T-rich intergenic sequences, which are abundant in the ρ^+ genome and are capable of autonomous replication in the mitochondrion (FANGMAN *et al.* 1989).

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