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The Transmission Disadvantage of Yeast Mitochondrial Intergenic Mutants Is Eliminated in the *mgt1* (*cce1*) Background

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A *trans*-acting element, *MGT1* (also called *CCE1*), has previously been shown to be required in *Saccharomyces cerevisiae* for the preferential transmission of petite mitochondrial DNA (mtDNA) molecules over wild-type mtDNA molecules. In the present study a possible role of this nuclear gene in the transmission of mtDNA from various respiration-competent mutants was studied. Several of these mutants, lacking one or the other of two biologically active mitochondrial intergenic sequences, were employed in genetic crosses. When these deletion mutants were crossed to the parental wild-type strain in the *MGT1*/*CCE1* background, the progeny contained predominantly wild-type mtDNA molecules. When crosses were performed in the *mgt1*/*cce1* background, the parental molecules interacted in zygotes and underwent homologous recombination but wild-type and intergenic-deletion alleles were transmitted with equal frequencies.

The average cell of *Saccharomyces cerevisiae* has about 50 copies of the mitochondrial DNA (mtDNA) molecule (5). The coding regions represent only one-fifth of the mitochondrial genome, and the rest is made up largely of alternating and nonalternating adenine-thymine (A-T) sequences and various guanine-cytosine (G-C) clusters, such as *ori*/*rep*/*tra* sequences (4). The process of transmission of mtDNA to the progeny can be roughly subdivided into two steps: (i) propagation and (ii) physical segregation of mtDNA molecules into new cells. Two kinds of elements, *cis*-acting mitochondrial sequences and *trans*-acting gene products, are involved in the transmission process (for a review see reference 14). The use of various mitochondrial mutants in genetic crosses has revealed some of the basic rules governing inheritance of the yeast mitochondrial genome. Transmission to the progeny is not just a random flow but includes a kind of competition among mtDNA molecules (12, 15). The *cis*-acting elements promoting transmission are represented by intergenic regions, especially *ori*/*rep*/*tra* sequences (1, 3, 9, 12). On the other hand, much less is known about the character of the *trans*-acting elements which determine the competition rules. A mutant that prevents displacement of wild-type mtDNA molecules by petite mtDNA molecules has been isolated by Zweifel and Fangman (17). The corresponding wild-type gene, *MGT1* (also called *CCE1*) was shown to be necessary for the biased transmission of hypersuppressive mtDNA during zygotic growth (10, 11, 17). Apparently, the *mgt1*/*cce1* defect causes clustering of petite mtDNA molecules, which decreases the number of active hereditary units (11). In this note, studies of the *MGT1*/*CCE1* gene have been conducted by crossing strains bearing wild-type mtDNA with strains containing intergenic deletions in their mtDNA. The results indicate that the action of the *MGT1*/*CCE1* gene is not limited to petite mtDNA molecules alone but has a general role in the inheritance of the mitochondrial genome.

The generation of petite mutants and the transmission capacity of yeast mtDNA are strongly influenced by environmental as well as genetic factors. Therefore, it is important to perform transmission experiments with yeast strains that have well-defined nuclear backgrounds. Details of the origins and descriptions of the parental *S. cerevisiae* strain and the respiration-competent mitochondrial mutants employed in this study (Table 1) can be found elsewhere (7, 12, 13). T3/3 is the parental strain with wild-type mtDNA (Fig. 1). The other strains, which are *rho*$^+$ (*ori*/*rep*/*tra* deficient), have deletions of some mitochondrial intergenic sequences. Strains with an E-type mtDNA, E14.9 and E15.7, lack parts of mitochondrial intergenic sequences which map between the tRNA^{Glu} and *oli2* genes. The deletions include *ori2* or both *ori2* and *-7*. R-type mtDNAs, R0.54, R2.9, and R3.1, lack mitochondrial sequences located between the tRNA^{Pro} and *SrRNA* genes and encompassing *ori1* (Fig. 1). These strains were transformed so that their *MGT1*/*CCE1* genes were knocked out by gene disruption through homologous recombinations. Disruption of the *MGT1*/*CCE1* gene was achieved by insertion of the *ATP1* gene, allowing selection of yeast transformants with the disrupted gene (Fig. 2). The plasmid YEp352Sub8-2, which contains a 2.1-kb *Sac*I/*Sph*I insert carrying the entire yeast *MGT1*/*CCE1* gene, was obtained from S. Kleff, State University of New York at Stony Brook (10). pCH216 is a plasmid (from Chris Harfield, University of Leicester, Leicester, United Kingdom) which contains a 2-kb insert of the *ATP1* gene conferring resistance to geneticin at a concentration of 150 mg/liter. A plasmid bearing a disrupted *MGT1*/*CCE1* gene was constructed by using unique restriction sites. The 1.3-kb *Sph*I/

TABLE 1. Yeast strains used in this study

Strain	Nuclear marker[a]	mtDNA type[b]
T3/3	*MAT***a** *ade1 arg4-16*	Wild type
E14.9/7	*MAT***a** *ade1*	E (3.80)
E15.7/13	*MAT*α *his3-532 trp1*	E (3.05)
R0.54/1	*MAT***a** *ade1 his3-532*	R (4.95)
R2.9/10	*MAT*α *arg4-16*	R (2.55)
R3.1/6	*MAT*α *arg4-16*	R (2.40)

[a] For each strain listed, an isogenic strain which carries the *mgt1*/*cce1*::*APT1* marker was constructed (Fig. 2).

[b] The sizes of mitochondrial intergenic deletions, in kilobases, are given in parentheses. The mtDNA molecule and both the E and R types of deletions are shown in Fig. 1.

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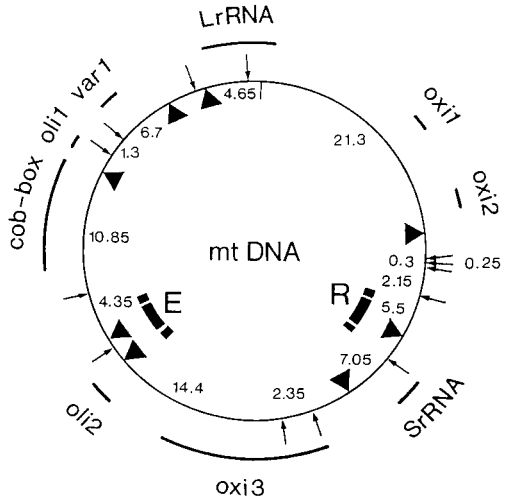

FIG. 1. *Cfo*I restriction map of the wild-type mtDNA molecule, showing some of the coding regions and the positions of the deletions E and R. The *Cfo*I sites are indicated by arrows, and the sizes of the fragments are given in kilobases. Arrowheads indicate the positions of the *ori*/*rep*/*tra* sequences.

*Eco*RI fragment from the YEp352Sub8-2 plasmid containing the *MGT1*/*CCE1* gene was subcloned into the pUC18 plasmid, producing the plasmid pJP020. This plasmid was digested at the unique *Cla*I site at the 5′ end of the *MGT1*/*CCE1* coding region, was blunt ended with the Klenow fragment of DNA polymerase I, and subsequently was also cut at the unique *Sal*I site, which maps approximately 45 bp 5′ to the *Cla*I site. The *Sal*I site is located between the 2nd and 3rd amino acid positions of the *MGT1*/*CCE1* open reading frame. Thus, the vector contained a blunt end and a *Sal*I sticky end. The insert, conferring resistance to geneticin, was isolated as the 2-kb *Eco*RV/*Sal*I fragment from the pCH216 plasmid and cloned into the vector described above, producing a new plasmid, pJP027. This plasmid contains a disrupted *MGT1*/*CCE1* gene which could be cut out of pJP027 as a 3.3-kb fragment by *Eco*RI and *Sph*I (Fig. 2). This fragment was subsequently used for transformation of the yeast mitochondrial mutant strains by the standard yeast transformation techniques. When total DNA originating from transformed clones was digested with *Eco*RI and *Sph*I and was hybridized with pJP020, a specific change in the hybridization pattern was observed. In the case of yeast strains carrying the wild-type copy of *MGT1*/*CCE1*, only a 1.3-kb segment hybridized with the probe, while in the

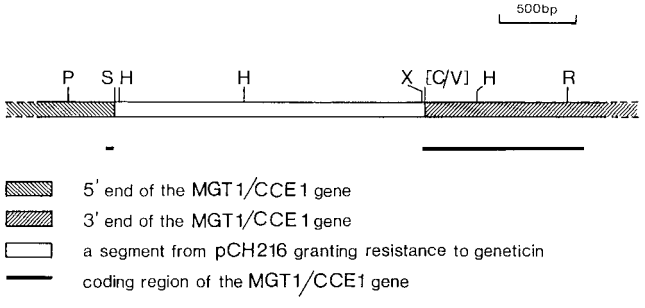

FIG. 2. Map of a portion of the plasmid pJP027, showing the disrupted *MGT1*/*CCE1* gene and the inserted *APT1* gene conferring resistance to geneticin. Restriction sites are designated as follows: C, *Cla*I; H, *Hin*dIII; P, *Sph*I; R, *Eco*RI; S, *Sal*I; V, *Eco*RV; X, *Xho*I. The insert is located between the 2nd and 3rd amino acids of the *MGT1*/*CCE1* open reading frame.

TABLE 2. Spontaneous generation of petite mutants in two different nuclear backgrounds

Strain	Petite clones[a] in nuclear background:	
	MGT1/*CCE1*	*mgt1*/*cce1*
T3/3	2.4	18.6
E15.7/13	6.2	16.0
R2.9/10	0.8	8.1
R0.54/1	0.5	11.0
R3.1/6	2.4	16.8

[a] Expressed as percentages of petite clones in the culture.

case of the transformed clones, only a 3.3-kb fragment appeared (Fig. 2 and data not shown). Thus, these yeast clones contain only a disrupted copy of the *MGT1*/*CCE1* gene.

Previously, it has been shown that the *MGT1*/*CCE1* gene plays a role in the heritability of petite mutations (17). Cells of *S. cerevisiae* constantly produce petite mutants which have gross alterations in the primary structure of their mtDNA (1). Petite-mutant frequency was recorded as a percentage of respiration-deficient colonies on the selective plates (with GGlyPD medium, containing 2% glycerol, 0.1% glucose, 1% Bacto Peptone, 0.1% yeast extract, and 1.5% agar). For the mitochondrial, respiration-competent, intergenic mutants employed in this study, the frequency of spontaneous petite-mutant formation ranged from 0.5 to 6.2% in the *MGT1*/*CCE1* background (Table 2). The variation is attributed to the facts that these strains carry different mitochondrial intergenic deletions and that their nuclear genomes also differ slightly. However, in each corresponding *mgt1*/*cce1* strain, the frequency of petite mutants was elevated dramatically (Table 2). For example, in the case of the strain carrying the wild-type mitochondrial genome, T3/3, the frequency of petite mutants was elevated from 2.4 to 18.6% by disruption of the *MGT1*/*CCE1* gene. These results agree with the previous observation that the original *mgt1*/*cce1* strain exhibited elevated petite-mutant frequency (10), but they also imply that a lack of some *ori*/*rep*/*tra* does not influence this phenotype.

The role of *MGT1*/*CCE1* in the transmission of mtDNA was investigated further by comparing the transmission efficiencies of various mitochondrial genomes following crosses in the *MGT1*/*CCE1* and *mgt1*/*cce1* nuclear backgrounds. A mitochondrial cross involved mass mating of yeast cells and selection for the zygotes (5, 12). For mtDNA analyses of daughter clones arising from crosses, isolated total-yeast DNA (12) was digested with the restriction enzyme *Cfo*I and hybridized to labelled mtDNA isolated from T3/3 or, after the R0.54 × R3.1 cross, to the mitochondrial *SrRNA* gene (16). The digestion patterns of the progeny lines were then compared with the patterns of the parental strains, and the output frequencies of certain alleles and genomes were calculated. In a cross of the wild-type strain to an E mutant (E15.7) performed in the *MGT1*/*CCE1* background, the wild-type alleles were preferentially transmitted to the progeny (12) (Table 3). However, when the same mitochondrial cross was performed in the *mgt1*/*cce1* background, the results were substantially different (Table 3). This indicates that the output of the cross reflected the input of the mitochondrial alleles when in the *mgt1*/*cce1* background. After another set of crosses involving two overlapping R deletion mutants (R0.54/1 × R3.1/6), the progenies were analyzed. In the *MGT1*/*CCE1* background the R0.54 deletion, which is much larger than the R3.1 deletion, was transmitted poorly (12) (Table 3). When the cross was performed with the strains carrying the mutant *mgt1*/*cce1* allele, only a slight, prob-

TABLE 3. Progenies of mitochondrial crosses performed in the *MGT1*/*CCE1* and *mgt1*/*cce1* backgrounds

Cross	mtDNA type of progeny[a]	% (no.) of clones in nuclear background:	
		MGT1/*CCE1*	*mgt1*/*cce1*
T3/3[b] × E15.7/13	Wild type	83 (25)	52 (28)
	E15.7	17 (5)	48 (26)
R0.54/1 × R3.1/6	R0.54	15 (3)	44 (22)
	R3.1	85 (17)	56 (28)
R2.9/10 × E14.9/7[c]	R2.9	18 (4)	47 (27)
	E14.9	18 (4)	26 (15)
	Recombinant		
	Wild type	64 (14)	17 (10)
	Double mutant	0	10 (6)

[a] mtDNAs of progeny clones were analyzed and classified as wild type, mutant, or recombinant.

[b] Wild type.

[c] The results expected if selection did not operate among different mtDNA molecules and transmission followed the rules predicted by the random model (2, 6) would be that R2.9 and E14.9 clones would each account for 37.5% of the progeny, while recombinant wild-type and double-mutant clones would each account for 12.5%.

ably insignificant bias was observed (Table 3). Therefore, that allele did not exhibit a drastic transmission disadvantage in the *mgt1*/*cce1* nuclear background. The third cross, between R2.9/10 and E14.9/7, was performed with mitochondrial genomes carrying nonoverlapping and nonlinked deletion alleles. In theory, four different mtDNA molecules could appear in the progeny, the two parental and two recombinant types (12). One recombinant type would carry both deletions, R and E, and would thus have the smallest genome. The other recombinant would be the wild-type molecule. According to the Dujon model (5, 6), such a cross should produce the following distribution of daughter mtDNA molecules: 37.5% of the molecules should represent each of the parental types and 12.5% should represent each recombinant type. However, these results would be expected only if there were no competition among various mtDNA types. After R × E crosses performed in the *MGT1*/*CCE1* nuclear background, the output substantially violated the above predictions. Recombinant wild-type mtDNA was found in approximately 50% of the daughter cells, while a double-deletion recombinant was only rarely represented (12, 13) (Table 3). Previously it was shown that transmission of genetically unlinked markers in coding regions was also substantially reduced when an intergenic deletion was present on the same genome (13). Thus, the intergenic regions seem to confer a selective advantage on mtDNA molecules carrying these sequences, and the "unfit" recombinant mtDNA molecules lacking these sequences are simply "washed out" of the genetic pool (14). In other words, the output does not quantitatively reflect expected reciprocal recombination events. The *mgt1*/*cce1* mutation switched preferential transmission to less-biased transmission in the R × E cross also (Table 3). The parental molecules, R and E, were found in 73% of the progeny examined, but there was a bias in favor of R2.9 mtDNA. On the other hand, both recombinants were present at comparable frequencies, 17% for the wild-type recombinant and 10% for the double-deletion mutant (Table 3). The appearance of both types of reciprocal recombinant mtDNA molecule in the progeny demonstrates that mixing and fusion of parental mitochondria are not blocked. In addition, homologous recombination can still take place in the *mgt1*/*cce1* background also. Furthermore, in this cross the intergenic sequences at the R and E loci did not promote biased transmission; only 17% wild-type progeny resulted. The results obtained fit well with the Dujon (6) or random flow model of transmission (Table 3). Therefore, it can be concluded that the *mgt1*/*cce1* mutation leads to a nonbiased transmission mode, so that the output of mtDNA molecules quantitatively reflects the inferred input and recombination events predicted by the Dujon model.

A nuclear mutation, *mgt1*, was shown to abolish biased transmission of petite mtDNAs in matings of wild-type to hypersuppressive petite strains (17). *MGT1* was shown to be allelic to *CCE1*, which encodes a protein with DNA cruciform-cutting endonuclease activity (10) and is located in the mitochondria (8). It has been proposed that the *mgt1*/*cce1* defect causes clustering of mtDNA molecules so that they stay linked together by recombination junctions. This change in the structure, which supposedly affects mainly petite mtDNA molecules, would account for the changed genetic properties of petite mtDNA molecules in genetic crosses (11). In this study an *mgt1*/*cce1* mutation was shown to elevate the frequency of petite mDNA molecules in various respiration-competent strains (Table 2), regardless the absence of some *ori*/*rep*/*tra*. In addition, it was shown that the action of the *MGT1*/*CCE1* gene is not limited to petite mutants. When mitochondrial crosses between certain respiration-competent mitochondrial genomes were performed in the *mgt1*/*cce1* background, fusion of mitochondria from both parents must have occurred in zygotes because homologous recombination took place (Table 3). when the outcomes of crosses performed in the *mgt1*/*cce1* and *MGT1*/*CCE1* backgrounds were compared, it was evident that different transmission rules operate in the two cases (Table 3). While the output does not reflect the input in the case of *MGT1*/*CCE1*, random distribution of mtDNAs seems to operate in the mutant background (Table 3). These results are in accord with the previous results (11, 17) suggesting that *MGT1*/*CCE1* is involved in the selection mechanism. Our results also confirm the previously proposed Lockshon model, which suggests that the product of *MGT1*/*CCE1* only indirectly influences transmission (11). Upon zygote formation, both *rho*$^+$ and *rho*$^+$ (*ori*/*rep*/*tra* deficient) mtDNA molecules can recombine independently of *MGT1*/*CCE1*, but subsequently both types of molecule become insufficiently processed in the *mgt1*/*cce1* background and clustering occurs. As a consequence of clustering (11), the number of free competing units from both parents is drastically decreased, and this results in diminished competition among different free mtDNA molecules. In this way transmission in *mgt1*/*cce1* resembles a random flux rather than a selective process, and even molecules carrying extensive deletions of otherwise biologically active intergenic sequences get transmitted to the progeny (Table 3). Apparently, the action of *MGT1*/*CCE1* is not specifically dependent on the *ori*/*rep*/*tra* sequences.

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REFERENCES

1. **Bernardi, G.** 1979. The petite mutation in yeast. Trends Biochem. Sci. **4:** 197–201.
2. **Birky, C. W., R. L. Strausberg, P. S. Perlman, and J. L. Foster.** 1978. Vegetative segregation of mitochondria in yeast: estimating parameters using a random model. Mol. Gen. Genet. **158:**251–261.
3. **Blanc, H., and B. Dujon.** 1980. Replicator regions of the yeast mitochondrial

DNA responsible for supressiveness. Proc. Natl. Acad. Sci. USA **77:**3942–3946.
4. **deZamaroczy, M., and G. Bernardi.** 1985. Sequence organization of the mitochondrial genome of yeast—a review. Gene **37:**1–17.
5. **Dujon, B.** 1981. Mitochondrial genetics and functions, p. 505–635. *In* J. N. Strathern, E. W. Jones, and J. R. Broach (ed.), Molecular biology of the yeast *Saccharomyces cerevisiae*: life cycle and inheritance. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
6. **Dujon, B., P. P. Slonimski, and L. Weill.** 1974. Mitochondrial genetics. IX. A model for recombination and segregation of mitochondrial genomes in *Saccharomyces cerevisiae*. Genetics **78:**415–437.
7. **Evans, R. I., K. M. Oakley, and G. D. Clark-Walker.** 1985. Elevated levels of petite formation in strains of *Saccharomyces cerevisiae* restored to respiratory competence. I. Association of both high and moderate frequencies of petite mutant formation with the presence of aberrant mitochondrial DNA. Genetics **111:**389–402.
8. **Ezekiel, U. R., and H. P. Zasenhaus.** 1993. Localization of a cruciform cutting endonuclease to yeast mitochondria. Mol. Gen. Genet. **240:**414–418.
9. **Fangman, W. L., J. W. Henly, G. Churchill, and B. J. Brewer.** 1989. Stable maintenance of a 35-base-pair yeast mitochondrial genome. Mol. Cell. Biol. **9:**1917–1921.
10. **Kleff, S., B. Kemper, and R. Sternglanz.** 1992. Identification and characterization of yeast mutants and the gene for a cruciform cutting endonuclease. EMBO J. **11:**699–704.
11. **Lockshon, D., S. G. Zweifel, L. L. Freeman-Cook, H. E. Lorimer, B. J. Brewer, and W. L. Fangman.** 1995. A role for recombination junctions in the segregation of mitochondrial DNA in yeast. Cell **81:**947–955.
12. **Piskur, J.** 1988. Transmission of yeast mitochondrial loci to progeny is reduced when nearby intergenic regions containing *ori*/*rep* sequences are deleted. Mol. Gen. Genet. **214:**425–432.
13. **Piskur, J.** 1989. Transmission of the yeast mitochondrial genome to the progeny: the impact of intergenic sequences. Mol. Gen. Genet. **218:**161–168.
14. **Piskur, J.** 1994. Inheritance of the yeast mitochondrial genome. Plasmid **31:**229–241.
15. **Rayko, E., R. Goursot, B. Cherif-Zahar, R. Melis, and G. Bernardi.** 1988. Regions flanking *ori* sequences affect replication efficiency of the mitochondrial genome of *ori*-plus petite mutants from yeast. Gene **63:**213–226.
16. **Sor, F., and H. Fukuhara.** 1980. Sequence nucleotidique du gene de l'ARN ribosomique 15S mitochondrial de la levure. C. R. Acad. Sci. (Paris) **291:** 933–936.
17. **Zweifel, S. G., and W. L. Fangman.** 1991. A nuclear mutation reversing a biased transmission of yeast mitochondrial DNA. Genetics **128:**241–249.