The Mitochondrial Genome Integrity Gene, *MGI1*, of *Kluyveromyces lactis* Encodes the β-Subunit of F_1-ATPase

Xin Jie Chen and G. Desmond Clark-Walker

Molecular and Population Genetics Group, Research School of Biological Sciences, The Australian National University, GPO Box 475, Canberra, ACT2601, Australia

Manuscript received April 29, 1996
Accepted for publication September 18, 1996

ABSTRACT

In a previous report, we found that mutations at the mitochondrial genome integrity locus, *MGI1*, can convert *Kluyveromyces lactis* into a petite-positive yeast. In this report, we describe the isolation of the *MGI1* gene and show that it encodes the β-subunit of the mitochondrial F_1-ATPase. The site of mutation in four independently isolated *mgi1* alleles is at Arg435, which has changed to Gly in three cases and Ile in the fourth isolate. Disruption of *MGI1* does not lead to the production of mitochondrial genome deletion mutants, indicating that an assembled F_1 complex is needed for the "gain-of-function" phenotype found in *mgi1* point mutants. The location of Arg435 in the β-subunit, as deduced from the three-dimensional structure of the bovine F_1-ATPase, together with mutational sites in the previously identified *mgi2* and *mgi5* alleles, suggests that interaction of the β- and α- (*MGI2*) subunits with the γ-subunit (*MGI5*) is likely to be affected by the mutations.

Saccharomyces cerevisiae and some related yeasts readily produce respiratory deficient mutants (petites) spontaneously and on treatment with DNA targeting drugs (EPHRUSSI 1953; BULDER 1964a,b; DE DEKEN 1966; CLARK-WALKER *et al.* 1981). Such mutants lack mtDNA or have large deletions in this genome (FAYE *et al.* 1973). By contrast, most yeasts and all other eukaryotes do not lose their mtDNA on exposure to mutagens (BULDER 1964a,b; DE DEKEN 1966). However, it has been found in the petite-negative yeasts *Kluyveromyces lactis* (CHEN and CLARK-WALKER 1993) and *Schizosacchromyces pombe* (HAFFTER and FOX 1992) that mutations in nuclear genes permit mitochondrial genome deletion mutants to be obtained. For *K. lactis*, we named these nuclear genes *MGI* for mitochondrial genome integrity because we imagined that their function could be to prevent ectopic recombination in mtDNA leading to deletions (CHEN and CLARK-WALKER 1993). However, contrary to our expectations, we have found that two *MGI* genes in *K. lactis* encode the α- and γ-subunits of the mitochondrial inner membrane F_1F_o-ATP synthase (CHEN and CLARK-WALKER 1995).

The mitochondrial F_1F_o-ATP synthase is involved in energy conversion in eukaryotic cells by using a protonmotive force, generated across the inner membrane during respiration, to drive the synthesis of ATP (for review, see CROSS 1981; SENIOR 1988; FUTAI *et al.* 1989; FILLINGAME 1990; COX *et al.* 1992; HATEFI 1993). Yeast mitochondrial F_1F_o-ATP synthase consists of two essential domains, namely, the extrinsic and intrinsic membrane complexes, F_1 and F_o. When protons move from the intermembrane space to the matrix side of the inner membrane through F_o, the released energy is transmitted to F_1 by conformational changes in the complex to catalyze ATP synthesis. The F_1 portion of the complex contains the catalytic site for ATP synthesis and is composed of five proteins with the stoichiometry $3\alpha:3\beta:1\gamma:1\delta:1\epsilon$. In contrast to the F_o sector, where three genes for subunits 6, 8 and 9 are present in yeast mtDNA, all five subunits of F_1 are encoded by the nuclear genome. In *Escherichia coli*, the α-subunit of F_1 has been proposed to be involved in nucleotide binding and enzyme cooperativity as well as in the binding of F_1 to F_o (MAGGIO *et al.* 1988; SOGA *et al.* 1989) while the β-subunit contains the catalytic site (FUTAI and KANAZAWA 1983). The γ-subunit has been implicated in the assembly of the ATP synthase in yeast (PAUL *et al.* 1994), the synthesis or hydrolysis of ATP and the control of proton flow through the membrane via F_o in chloroplasts and *E. coli* (WEISS and MCCARTY 1977; MORONEY and MCCARTY 1979; MORONEY *et al.* 1980; IWAMOTO *et al.* 1990; SHIN *et al.* 1992).

At present, it remains unclear how mutations in F_1 subunits of the mitochondrial ATP synthase affect recovery of petites in *K. lactis*. However, it has been found that specific mutations in *MGI2*, encoding the α-subunit, and *MGI5*, encoding the γ-subunit are needed as disruption of either gene does not lead to a Mgi^- phenotype (CHEN and CLARK-WALKER 1995). These observations qualify the *mgi2* and *mgi5* alleles as "gain-of-function" mutations. In other words, the F_1 complex with a mutant α- or γ-subunit, has gained a novel function that permits the recovery of petites.

Corresponding author: G. Desmond Clark-Walker, Molecular and Population Genetics Group, Research School of Biological Sciences, The Australian National University, GPO Box 475, Canberra City, ACT2601 Australia. E-mail: dcw@rsbs-central.anu.edu.au

TABLE 1

Genotype and source of yeast strains

Strain	Relevant genotype	Source or reference
K. lactis		
2359/152	**a**, *metA1*	WESOLOWSKI-LOUVEL *et al.* (1982)
WM52	α, *ade1*, *adeX*, *his7*	TINGLE *et al.* (1968)
WM52/Gly$^-$2.10	as above, but *mgi1-1*, [ρ^o]	CHEN and CLARK-WALKER (1993)
WM52/Gly$^-$23.4	as above, but *mgi1-2*, [ρ^o]	CHEN and CLARK-WALKER (1993)
PM6-7A	**a**, *adeT-600*, *uraA1*	CHEN *et al.* (1992)
PM6-7A/UV1	as above, but *mgi1-3*, [ρ^o]	This study
PM6-7A/ER27	as above, but *mgi1-4*, [ρ^o]	This study
CW1	as above, but *mgi1::URA3*	This study
CW10	α/**a**, *ade1/+*, *lysA1/+*, *uraA1/uraA1*, *+/adeT-600*, *MGI1/mgi1-1::pMGI1/2.10*	This study
CK14-1A	**a**, *his7*	This laboratory
CK24-1D	**a**, Ade$^-$, *his7*, *uraA1*, *mgi1-2*	CHEN and CLARK-WALKER (1993)
CK56-7A	α, *ade1*, *lysA1*, *uraA1*, *mgi1-1*	This study
CK56-7A/tr3	α, *ade1*, *lysA1*, *uraA1*, *mgi1-1::pMGI1/2.10*	This study
CK56-16C	α, *ade1*, *lysA1*, *uraA1*	This laboratory
CK98-8A	α, *ade1*, *metA1*, *uraA1*, *mgi1-3*	This study
CK98-8A/tr1	α, *ade1*, *metA1*, *uraA1*, *mgi1-3::pMGI1/2.10*	This study
CK145-22A	α, *ade1*, *uraA1*, *mgi5-1*	CHEN and CLARK-WALKER (1995)
CK151-1	α, *ade1*, *lysA1*, *uraA1*, *mgi1-4*	This study
CK188b	α,, *ade1*, *metA1*, *uraA1*, *mgi1-3*, *MGI2::pURA-MGI2/HP1*	This study
CK197	α/**a**, *ade1/+*, *lysA1/+*, *+/adeT-600*, *uraA1/uraA1*, *+/mgi1-4*	This study
CK197-4D	**a**, *lysA1*, *uraA1*, *mgi1-4*	This study
CK197-13A	α, *ade1*, *uraA1*, *mgi1-4*	This study
CK208	α, *ade1*, *metA1*, *uraA1*, *mgi1-3*, *MGI5::pURA-MGI5*	This study
CK218-11C	**a**, *his7*, *mgi1-4*	This study
CK217	α, *ade1*, *uraA1*, *mgi5-1*, *mgi1::URA3*	This study
CK218	α/**a**, *ade1/+*, *uraA1/+*, *+/his7*, *mgi1-4/+*	This study
CK225	α, *ade1*, *lysA1*, *uraA1*, *mgi1-1*, *mgi5::URA3*	This study
CK226	α, *ade1*, *metA1*, *uraA1*, *mgi1-3*, *mgi5::URA3*	This study
CK227	α, *ade1*, *uraA1*, *mgi1-4*, *mgi5::URA3*	This study
CK261	α/**a**, *ade1/+*, *metA1/+*, *uraA1/uraA1*, *+/adeT-600*, *mgi1-3/mgi1-4*	This study
CK277	α/**a**, *ade1/+*, *metA1/+*, *uraA1/uraA1*, *+/adeT-600*, *MGI1/mgi1-3::pMGI1/2.10*	This study
S. cerevisiae		
MDY2102	**a**, *leu2-3, 112*, *ura3-52*, *suc2-9*, *his4-519*, *gal2*, *atp2::LEU2*	M. DOUGLAS
AH22	**a**, *leu2-3, 112*, *his4*	G. FINK

As the F_1 complex is composed of five different subunits our observations raise a question as to whether *mgi* mutations are confined to the α- and γ-subunit proteins? Here we report that mutations occurring in the β-subunit also produce a Mgi$^-$ phenotype. This result supports the idea that a Mgi$^-$ phenotype is conferred by an assembled but abnormal F_1 particle. The location of the mutations in the central core regions of the α- and β-subunit proteins suggests that their interaction with the γ-subunit is affected.

MATERIALS AND METHODS

Strains and media: Yeast strains used in this study are listed in Table 1. The *S. cerevisiae* strain, MDY2102, contains an insertional mutation of *ATP2* and was kindly provided by D. M. CYR from the collection of M. G. DOUGLAS.

The complete medium (GYP) contains 0.5% Bacto yeast extract, 1% Bacto Peptone, and 2% glucose. Glycerol medium (GlyYP) contains 2% glycerol in place of glucose. Minimal medium (GMM) contains 0.67% Difco yeast nitrogen base without amino acids and 2% glucose. Nutrients essential for auxotrophic strains were added at 25 μg/ml for bases and 50 μg/ml for amino acids. Ethidium bromide (EB) medium is GYP plus EB at 16 μg/ml. For sporulation of *K. lactis*, ME medium contains 5% malt extract and 2% Bacto agar.

Isolation of additional *mgi1* mutants and nomenclature: In a previous report, we described the isolation of two mutant alleles, *mgi1-1* and *mgi1-2*, derived from WM52/gly$^-$2.10 and WM52/gly$^-$23.4 (CHEN and CLARK-WALKER 1993). In this study, we identified two novel *mgi1* isolates. The first one, PM6-7A/UV1, was identified as a Gly$^-$, [ρ^o] colony after ultraviolet light mutagenesis of PM6-7A followed by EB-margin-of-growth treatment as described in a previous paper (CHEN and CLARK-WALKER 1995). Lack of mtDNA in the isolates was confirmed by gel electrophoresis of DNA digested with *Hae*III followed by Southern transfer and hybridization with [^{32}P]-labeled *K. lactis* mtDNA. The presence of a *mgi* mutation in PM6-7A/UV1 was demonstrated by crossing to the wild-type strain WM52, followed by successive crossing of the resulting Mgi$^-$ segregants to 2359/152 and PM6-7A. In the three crosses, the Mgi$^-$ phenotype segregated 2$^+$:2$^-$ in all 26 tetrads

analyzed, indicating that the *mgi* mutation is a nuclear monogenic trait. As this *mgi* isolate is allelic to *mgi1* (see below), it was designated *mgi1-3*. The second *mgi1* isolate, PM6-7A/ER27, was obtained as a EB-resistant colony after incubation of PM6-7A for 19 days at 28° on GYP medium containing 16 μg/ml EB. For the purification of the *mgi* allele, PM6-7A/ER27 was crossed to the wild-type strain CK56-16C. After sporulation, one of the random spores, CK151-1, was crossed to PM6-7A to give the diploid CK197. After sporulation of the diploid CK197 a Mgi^- segregant, CK197-13A, was crossed to CK14-1A to produce the diploid CK218. The *mgi* mutation segregated $2^+:2^-$ in all tetrads analyzed (21 from CK197 and 20 from CK218). This mutation also fell into the *MGI1* locus (see below) and was designated *mgi1-4*.

Manipulations of *K. lactis*: Genetic techniques used to construct and analyze *K. lactis* strains have been described in a previous paper (CHEN and CLARK-WALKER 1994). Random spore analysis was performed as previously reported (CHEN and CLARK-WALKER 1995). Spontaneous petite frequency was determined according to CHEN and CLARK-WALKER (1993). Transformation of *K. lactis* was performed by the lithium acetate-dimethyl sulfoxide method (HILL *et al.* 1991; GIETZ *et al.* 1992) as described in detail (CHEN and CLARK-WALKER 1994). Total DNA from *K. lactis* was extracted as described by SHERMAN *et al.* (1983).

DNA manipulation and plasmid construction: Standard techniques were used for generating recombinant DNAs and performing DNA and RNA blot hybridizations (SAMBROOK *et al.* 1989). Construction of the integrative plasmids pURA-KlMGI2/HP1 and pURA-MGI5, containing the *MGI2* and *MGI5* genes, have been described in a previous paper (CHEN and CLARK-WALKER 1995). To construct the plasmid pCXJ22-MGI1, the *S. cerevisiae*/*K. lactis*/*E. coli* shuttle vector pCXJ22 (CHEN 1996) was digested with *Sal*I followed by cloning of the 4-kb *Xho*I fragment containing *MGI1* (isolated from pMGI1/B19, see below).

Isolation of the *MGI1* gene: Strain CK56-7A containing *mgi1-1* was transformed with a *K. lactis* partial *Sau*3AI genomic library (WESOLOWSKI-LOUVEL *et al.* 1986) cloned in the *K. lactis*/*E. coli* shuttle vector KEp6 (CHEN *et al.* 1988). About 5400 Ura^+ transformants were scored. After incubation at 4° for 7 mon, colonies were replica-plated onto GlyYP and incubated for 3 days at 35°. Three survivors were scored from which plasmids were rescued by transformation of *E. coli* with total DNA extracted from the yeast transformants. Digestion by restriction enzymes revealed that the three plasmids contain an identical insert of 6.1 kb. One of the plasmids, pMGI1/601, was subjected to further analysis. Restriction fragments from the inserted DNA were subcloned into the *K. lactis* integrative vector pCXJ4 (X. J. CHEN, unpublished data). pCXJ4 contains *URA3* and *K. lactis LEU2* genes so that the resulting plasmids can be opened at the unique *Cla*I site within *KlLEU2* and targeted onto the chromosome of CK56-7A by selecting for Ura^+ transformants. Complementation of the *mgi1-1* mutation was examined by measuring the spontaneous petite frequency of stable integrants.

By a similar strategy we obtained the plasmid pMGI1/B19 after complementation of the *mgi1-4* mutant CK197-4D. pMGI1/B19 was confirmed to contain *MGI1* and to overlap with the insert in pMGI1/601.

Nucleotide sequence determination: For nucleotide sequence analysis of *MGI1*, appropriate DNA fragments were subcloned into the sequencing vectors pTZ18U and pTZ19U (Pharmacia). Single- and double-stranded DNA templates were prepared for sequencing by the dideoxy-chain termination method (SANGER *et al.* 1977) using "universal primer" (Amersham) and a series of synthetic oligonucleotides covering the total coding sequence of the gene (data not shown). The deduced protein sequence was compared with sequences in the Swissprot data bases by access through the Australian National Genomic Information Service. The entire coding region of the four mutant *mgi1* alleles were directly sequenced using PCR-amplified DNAs as templates and synthetic oligonucleotides as primers. Primers used for PCR amplification are shown in Figure 3. To confirm mutated bases, two or three independent PCR products were sequenced in the region containing the mutations. The sequence of *MGI1* in the parental strains PM6-7A and WM52, used for generating the *mgi1* mutants, were also determined and found to be identical to that from the genomic library (2359/152).

Gene disruption: Gene disruption was carried out by the one-step replacement method of ROTHSTEIN (1983). A plasmid containing a disrupted *MGI1* gene was constructed by taking advantage of a unique *Eco*RV site, which is located within the coding region of the gene. A 1.55-kb *Acc*I fragment, including the 3′ half of the *MGI1* sequence, was inserted into *Acc*I cut pTZ18U (Pharmacia). The resulting plasmid, pKlMGI1.2, has a unique *Eco*RV site that was used for insertion of a 1.1-kb fragment containing *URA3* that had been blunt ended by Klenow polymerase. Disruption of *MGI1* in the wild-type strain PM6-7A and the *mgi5-1* mutant CK145-22A was achieved by a *mgi1::URA3* cassette produced by *Msc*I and *Sph*I digestion of the resulting plasmid. Transformants were selected for Ura^+ and correct insertion was verified by digestion of genomic DNA with *Xho*I and hybridization with [^{32}P]-labeled pKlMGI1.2.

Disruption of *MGI5* in both CK56-7A (*mgi1-1*) and CK98-8A (*mgi1-3*) was performed as follows. The *mgi5::URA3* cassette, containing the *MGI5* coding region disrupted by the insertion of *URA3*, was isolated from pMGI5::URA3 (CHEN and CLARK-WALKER 1995) and introduced into CK56-7A and CK98-8A by transformation. Stable Ura^+ Gly^- transformants were scored and correct chromosomal integration of the construct was confirmed by PCR amplification using primers MGI5P1 and MGI5P4 matching to the *MGI5* sequence (CHEN and CLARK-WALKER 1995).

Integration of *MGI1* into chromosomal DNA: A 3-kb fragment, obtained from plasmid pTZ-MGI1/X2 (a subclone of pMGI1/B19 in pTZ19U) by digestion with *Eco*RI and partial digestion with *Hin*dIII, was cloned into the integrative plasmid pUC-URA3/4 (CHEN and FUKUHARA 1988) cut with *Eco*RI and *Hin*dIII. The resulting plasmid, pMGI1/2.10, was cut at the unique *Msc*I site in the *MGI1* gene followed by transformation of strains CK56-7A (*mgi1-1*) and CK98-8A (*mgi1-3*). Correct integration was determined by digesting genomic DNA with *Xho*I and hybridization with a *S. cerevisiae ATP2* gene obtained by PCR amplification.

Amplification by polymerase chain reaction: Amplification of *MGI1* and its mutant alleles was carried out as described by MULLIS and FALOONA (1987) using the thermostable *Taq* DNA polymerase kit provided by Promega. Target DNAs used for amplification of the mutant *mgi1* alleles were from strains CK56-7A (*mgi1-1*), CK24-1D (*mgi1-2*), CK98-8A (*mgi1-3*) and CK197-13A (*mgi1-4*). The forward primer sequence located just upstream of the gene and the reverse primer located downstream of the gene are shown in Figure 3. The amplified DNAs were separated from the reaction buffer using the PCR Spinclean DNA purification kit (Progen).

Amplification of the *S. cerevisiae ATP2* gene was carried out as above using 50 ng of DNA from AH22 and oligonucleotide primers ScATP2P1 [5′-(+238)GTTTTGGAAGT(A/T)GC(T/C)CAAC(+256)-3′] in the forward direction and ScATP2P2 [5′-(+1482)ACCAACCATATAGAAGC(+1465)-3′] in the reverse orientation.

Nucleotide sequence accession number: The nucleotide

sequence of *MGI1* has been assigned GenBank accession number U37764.

RESULTS

Isolation of *mgi1* alleles: In addition to the *mgi1-1* and *mgi1-2* alleles described in our previous report (CHEN and CLARK-WALKER 1993), we identified two other mutants, PM6-7A/ER27 and PM6-7A/UV1, that are allelic to *mgi1*. The first step in identification of the mutants was achieved by eliminating *mgi2* and *mgi5* mutants. By introduction of plasmids containing *MGI2* or *MGI5* into the unclassified *mgi* mutants, we have been able to establish that the *mgi* mutation in CK98-8A (derived from PM6-7A/UV1) does not occur at either locus. For example, the integrative plasmids pURA-MGI2/HP1 and pURA-MGI5, containing the complete *MGI2* and *MGI5* genes, were linearized by *Bgl*II (which cuts both the *MGI2* and *MGI5* coding regions) and introduced into CK98-8A by selecting Ura^+ transformants. The correct chromosomal targeting of the plasmids was confirmed by Southern-blot analysis (data not shown). The resulting strains, CK188b and CK208, were examined for production of spontaneous petite mutants. In 4000–5000 colonies screened for each strain, CK188b and CK208 were found to form spontaneous petite mutants at frequencies of 0.3 and 0.4%, respectively, which do not differ significantly from the parental strain (0.2%). In other words, the Mgi^- phenotype of CK98-8A is not complemented by either of the wild-type *MGI* genes. This is based on the observation that the *mgi* mutation in CK98-8A is recessive so that spontaneous petites are not observed in a heterozygous diploid *MGI/mgi* strain. Similar experiments showed that the *mgi* mutation derived from PM6-7A/ER27 also cannot be complemented by the *MGI2* and *MGI5* genes (data not shown).

Allelism testing between the two isolates was performed by crossing CK218-11C, a *mgi* derivative of PM6-7A/ER27, to CK98-8A derived from PM6-7A/UV1. As the resulting diploid, CK261, showed poor sporulation, only four tetrads were dissected. However examination of the segregants showed that the Mgi^- phenotype segregated $4^-:0^+$. Analysis of random spores revealed that 80 isolates, marked by the red *ade1* mutation, were all Mgi^-. As previous genetic analysis indicated that *ade1* is not linked to these *mgi* mutations, we concluded that the two *mgi* mutations are allelic.

Allelism of the two novel *mgi* mutations to the previously described *mgi1-1* and *mgi1-2* (CHEN and CLARK-WALKER 1993) was demonstrated by complementation of the *mgi* mutation derived from the four independent isolates by the cloned *MGI1* gene and the genetic linkage between the *MGI1* gene and the chromosomal *mgi* loci (see below). The mutation in PM6-7A/UV1 was thus designated as *mgi1-3* and that in PM6-7A/ER27 as *mgi1-4*. Both *mgi1-3* and *mgi1-4* mutants are respiratory competent and form spontaneous petites on GYP plates at frequencies of 0.2 and 2.1%, respectively. The production of petites from CK98-8A (*mgi1-3*) by the margin-of-growth technique using EB is illustrated in Figure 1.

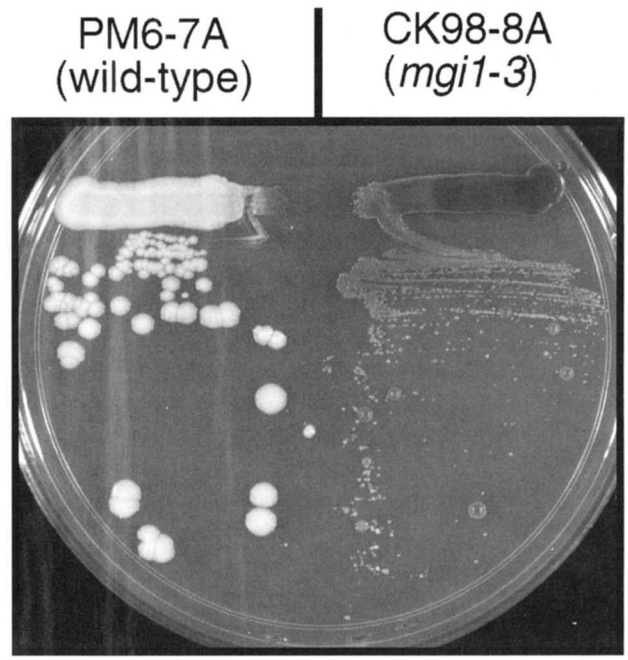
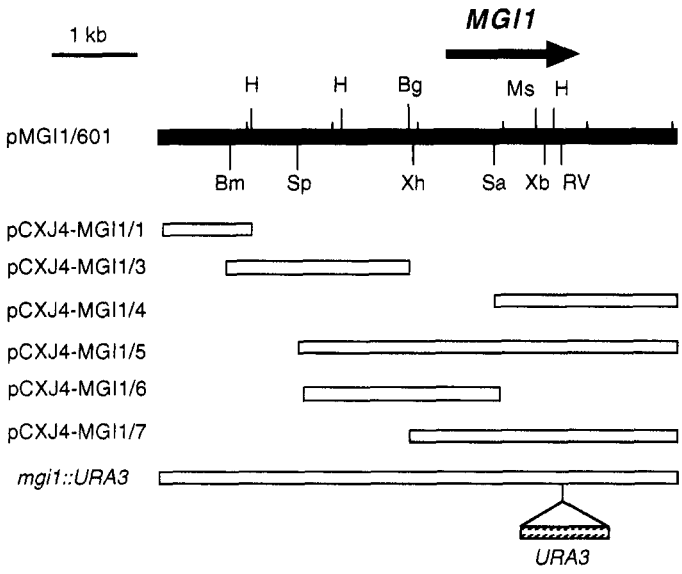


FIGURE 1.—Production of petites from CK98-8A (*mgi1-3*) after treatment with EB. The wild-type parental strain PM6-7A was included as a control. Yeast strains were grown in GYP to stationary phase and streaked across a 25 μl drop of EB (2.5 mg/ml). After incubation for 24 hr at 28°, the surviving cells on the margin between growth and nongrowth were substreaked down the plate, followed by incubation for 4 days before photography. The big colonies produced from the wild-type strain PM6-7A are respiratory competent and have an intact mitochondrial genome. The small colonies (petites) produced from CK98-8A are respiratory deficient (Gly^-) and have either lost mtDNA or have a deleted mitochondrial DNA as revealed by Southern blot analysis (not illustrated).

Cloning of *MGI1*: Cloning of the *MGI1* gene was based on the observation that colonies of the *mgi1* mutants have a very low viability on plates after a long period of incubation at 4°. By using the two different host strains, CK56-7A (*mgi1-1*) and CK197-4D (*mgi1-4*), two overlapping plasmids, pMGI1/601 and pMGI1/B19, were identified that allow cells to survive at low temperature.

To map the location of the complementing activity on the insert DNA of pMGI1/601, DNA fragments were subcloned into the *K. lactis* integrative vector pCXJ4. The resulting plasmids were linearized by *Cla*I within the *KlLEU2* gene of the vector and targeted into the chromosomal *LEU2* locus of the *mgi1-1* mutant CK56-7A. Transformants with a stable Ura^+ phenotype were selected and examined for spontaneous petite frequency. If a fragment of DNA complements the *mgi1* mutation, we would expect that no spontaneous petites should be produced because the *mgi1-1* allele is recessive (CHEN and CLARK-WALKER 1993). As shown in Fig-



	Total colonies scored	Number of petites	Spontaneous petite frequency (%)
pCXJ4-MGI1/1	2,580	25	0.97
pCXJ4-MGI1/3	2,275	18	0.79
pCXJ4-MGI1/4	2,010	12	0.60
pCXJ4-MGI1/5	18,040	0	0
pCXJ4-MGI1/6	760	8	1.05
pCXJ4-MGI1/7	4,000	0	0
mgi1::URA3	N/A	N/A	N/A

FIGURE 2.—Physical map of *K. lactis* genomic DNA containing the *MGI1* gene and complementation of the *mgi1-1* mutation by subclones derived from the DNA of pMGI1/601. The arrow represents the *MGI1* ORF and indicates the direction of transcription. Bm, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; Ms, *Msc*I; RV, *Eco*RV; Sa, *Sal*I; Sp, *Sph*I; Xb, *Xba*I; Xh, *Xho*I. DNA fragments from pMGI1/601 were cloned into the *K. lactis* integrative vector pCXJ4. The resulting plasmids, from pCXJ4-MGI1/1 to pCXJ4-MGI1/7, were linearized by digestion with *Cla*I within the *KlLEU2* gene of the vector and targeted to the *LEU2* locus of the *mgi1-1* mutant CK56-7A. Stable Ura$^+$ transformants were selected and examined for spontaneous petite frequency by plating onto GYP plates. Petites were scored as small and white colonies as CK56–7A carries the *ade1* mutation and forms red colonies on GYP plates. The genomic structure of the *mgi1* allele, disrupted by the insertion of *URA3*, is also illustrated. N/A, not applicable.

ure 2, the plasmid pCXJ4-MGI1/7, containing a 3.0-kb fragment, is able to complement the *mgi1-1* mutation as the transformed cells carrying this fragment do not produce spontaneous petites.

The ability of the cloned gene to prevent production of spontaneous petites in the *mgi* mutants CK24-1D (*mgi1-2*), CK98-8A (*mgi1-3*) and CK197-13A (*mgi1-4*), which are derived from three other independent isolates, was examined by integrating the plasmid pCXJ4-MGI1/7, containing *MGI1*, into the *LEU2* locus as described above. In each case, the integrants did not show spontaneous petites in 5000 colonies scored. The data indicate that the mutations in the four isolates described above can be complemented by *MGI1*.

Confirmation that the complementing fragment contains *MGI1* was obtained by genetic linkage analysis. Plasmid pMGI1/2.10, containing *URA3* and *MGI1*, was targeted onto the *mgi1* locus after cleavage in *MGI1*. The resulting stable transformants, CK56-7A/tr3 and CK98-8A/tr1 containing *URA3* integrated at the *mgi1-1* and *mgi1-3* loci, were crossed to wild type PM6-7A. Sporulation and tetrad analysis of the resulting diploids CK277 and CW10 showed that Ura$^+$ cosegregated with a Mgi$^-$ phenotype in 21 and 23 tetrads respectively. As previously observed (CHEN and CLARK-WALKER 1993), a Mgi$^-$ phenotype in heterozygous *MGI1/mgi* strains is dominant in the presence of EB induction and is manifested by growth of petite mutants on 16 μg/ml EB. We conclude that pMGI1/2.10 containing the *URA3* gene has integrated at *mgi1-1* and *mgi1-3*, and that *MGI1* is allelic to the mutant gene at this locus.

***MGI1* encodes the β-subunit of F_1-ATPase:** Sequence determination of the *MGI1* gene showed the presence of a 1515-bp open reading frame encoding a protein of 505 amino acids (Figure 3). The deduced Mgi1 protein is six amino acids shorter than the F_1-ATPase β-subunit from *S. cerevisiae*. However the two proteins are identical at 449 positions, with the greatest variation located at the amino terminal. This region has characteristics of a mitochondrial targeting signal, being rich in basic and hydrophobic amino acids. Starting at position 184 is a sequence, GlyGlyAlaGlyValGlyLysThr, termed the P-loop that is conserved between β-subunits from *E. coli* to mammals. Also present is a motif starting at 215, GlyGluArgThrArgGlu, with the underlined amino acids being highly conserved and implicated in the catalytic activity of the β-subunit.

To confirm that *MGI1* is the functional homologue of the β-subunit of F_1-ATPase, *S. cerevisiae* strain MDY2102, disrupted in the β-subunit encoding gene *ATP2*, was transformed with the plasmid pCXJ22-MGI1 that carries the *MGI1* gene. The transformants were found to be respiratory competent as they grew on GlyYP, indicating that *MGI1* complements the *ATP2* deficiency in *S. cerevisiae* (data not shown).

Southern-blot hybridization indicates that *MGI1* is a unique gene in *K. lactis*. Northern (RNA) blot analysis, using the *MGI1* coding sequence as a probe, detected a single mRNA of ~2.0 kb (data not shown). There was no significant difference in mRNA levels in cells grown in GYP or GlyYP, indicating that there is no glucose repression of *MGI1* transcription.

```
                                                                    TCGAGAACGTC  -435
TGTTAACACCGGGTAATACCGTGCATGTCCAATACGTGTGTCCCCTTTGTATCTTTGAATAATCGAAAAAAAATTTTCG  -356
CTCAAAATCCACAGAAAGAACTGTAGAAAATACCGCGAATTTTATCCAAAATTACGAAATTTGGCAAAAAATGTGTTTT  -277
CGCCCTTTCCCAGGTCAAGCGCTCTATTTGTCTGTTCAATTATATTTTTAAATTTTTTTTTTTTTTCCCTCGATTCGA  -198
TTTTTCTGGGTTTTTGGGTTTGATCTCATAGGTTCAAACGGATTTCATTGAAAAAGTTGGTGAAAAGTTGGGAAAATTA  -119
TATATTGAGATTATAAAAGATATGTTTTCTCCTTTAACCAACTACTTGTTCAAATTCTTGCTGGTCAATTGCATAGTGA   -40
CGATAAGGATTTAGGTAAGACAAGGACAATTTTTTAAGA ATG GTC TTG CCA AGA TTT TAT GCT GCT TCA   +30
                                        M   V   L   P   R   F   Y   A   A   S     10
TCT CGT GCA GCT TTG CAA GCT GCG AGA CGT GCT GTT CCC TTC ACC GGT GTG AGA GGT TAT   +90
 S   R   A   A   L   Q   A   A   R   R   A   V   P   F   T   G   V   R   G   Y     30
GCT GCT GCT GCC TCT TCT CAA GGT AAG GTT AGA GCC GTT ATT GGT GCT ATT GTT GAT GTT  +150
 A   A   A   A   S   S   Q   G   K   V   R   A   V   I   G   A   I   V   D   V     50
CAA TTT GAA CAA GGT CAA TTG CCA GCT ATT TTG AAC GCT TTG GAA ATC GAC ACT CCA GAA  +210
 Q   F   E   Q   G   Q   L   P   A   I   L   N   A   L   E   I   D   T   P   E     70
GGA AAG TTG GTT TTG GAA GTC GCT CAA CAT TTG GGT GAA AAC ACT GTC AGA ACC ATT GCT  +270
 G   K   L   V   L   E   V   A   Q   H   L   G   E   N   T   V   R   T   I   A     90
ATG GAT GGT ACT GAA GGT TTA GTC CGT GGT GAA AAC GTT TCT GAC ACT GGT GCT CCA ATT  +330
 M   D   G   T   E   G   L   V   R   G   E   N   V   S   D   T   G   A   P   I    110
TCC GTC CCA GTT GGT AGA GAA ACC TTG GGT AGA ATT ATC AAC GTT ATT GGT GAG CCA ATT  +390
 S   V   P   V   G   R   E   T   L   G   R   I   I   N   V   I   G   E   P   I    130
GAC GAA AGA GGT CCA ATC AAC TCC AAG ATG AGA AAG CCA ATT CAT GCT GAT CCT CCA TTA  +450
 D   E   R   G   P   I   N   S   K   M   R   K   P   I   H   A   D   P   P   L    150
TTC GTT GAA CAA TCC ACT GCT GCT GAA GTT TTG GAA ACT GGT ATC AAG GTT GTC GAC TTG  +510
 F   V   E   Q   S   T   A   A   E   V   L   E   T   G   I   K   V   V   D   L    170
TTG GCC CCA TAC GCC AGA GGT GGT AAG ATT GGT TTG TTC GGT GGT GCC GGT GTC GGT AAG  +570
 L   A   P   Y   A   R   G   G   K   I   G   L   F   G   G   A   G   V   G   K    190
ACC GTT TTC ATC CAA GAA TTG ATT AAC AAC ATT GCC AAG GCT CAT GGT GGT TTC TCT GTC  +630
 T   V   F   I   Q   E   L   I   N   N   I   A   K   A   H   G   G   F   S   V    210
TTC ACT GGT GTC GGT GAA AGA ACC AGA GAA GGT AAC GAT TTG TAC CGT GAA ATG AAG GAA  +690
 F   T   G   V   G   E   R   T   R   E   G   N   D   L   Y   R   E   M   K   E    230
ACT GGT GTT ATC AAC TTG GAA GGT GAT TCT AAG GTC GCG TTG GTT TTC GGT CAA ATG AAC  +750
 T   G   V   I   N   L   E   G   D   S   K   V   A   L   V   F   G   Q   M   N    250
GAA CCT CCT GGA GCT AGA GCT AGA GTC GCC TTG ACT GGT TTG ACC ATC GCT GAA TAC TTC  +810
 E   P   P   G   A   R   A   R   V   A   L   T   G   L   T   I   A   E   Y   F    270
AGA GAT GAA GAA GGT CAA GAT GTC TTG TTG TTT ATC GAC AAC ATT TTC AGA TTC ACA CAA  +870
 R   D   E   E   G   Q   D   V   L   L   F   I   D   N   I   F   R   F   T   Q    290
GCC GGT TCC GAA GTG TCC GCT TTG TTG GGT CGT ATT CCA TCC GCT GTC GGT TAT CAA CCA  +930
 A   G   S   E   V   S   A   L   L   G   R   I   P   S   A   V   G   Y   Q   P    310
ACT TTG GCC ACC GAT ATG GGT TTG TTG CAA GAA AGA ATT ACT ACC ACC AAG AAG GGT TCC  +990
 T   L   A   T   D   M   G   L   L   Q   E   R   I   T   T   T   K   K   G   S    330
GTC ACT TCT GTC CAA GCC GTT TAC GTG CCA GCT GAT GAT TTG ACT GAT CCT GCT CCA GCT +1050
 V   T   S   V   Q   A   V   Y   V   P   A   D   D   L   T   D   P   A   P   A    350
ACC ACT TTC GCG CAT TTG GAT GCC ACC ACT GTG TTG TCC AGA GGG ATC TCT GAA TTG GGT +1110
 T   T   F   A   H   L   D   A   T   T   V   L   S   R   G   I   S   E   L   G    370
ATC TAC CCA GCT GTC GAT CCT TTG GAT TCC AAA TCT AGA TTG TTG GAT GCT GCC GTC GTT +1170
 I   Y   P   A   V   D   P   L   D   S   K   S   R   L   L   D   A   A   V   V    390
GGT CAA GAA CAT TAC GAT GTC GCT ACT CAA GTT CAA CAA ACT TTG CAA GCT TAC AAG TCT +1230
 G   Q   E   H   Y   D   V   A   T   Q   V   Q   Q   T   L   Q   A   Y   K   S    410
TTG CAA GAT ATC ATT GCC ATT TTG GGT ATG GAT GAA TTG TCC GAA CAA GAT AAG TTG ACC +1290
 L   Q   D   I   I   A   I   L   G   M   D   E   L   S   E   Q   D   K   L   T    430
GTC GAA AGA GCT AGA AAG ATT CAA AGA TTC TTG TCT CAA CCT TTC GCT GTC GCT GAA GTC +1350
 V   E   R   A   R   K   I   Q   R   F   L   S   Q   P   F   A   V   A   E   V    450
TTC ACT GGT ATC CCA GGT AGA TTG GTC AGA TTA AAG GAC ACC ATC TCT TCT TTC AAG GCT +1410
 F   T   G   I   P   G   R   L   V   R   L   K   D   T   I   S   S   F   K   A    470
GTC TTG GAC GGT AAG TAC GAT CAC TTG CCA GAA AAC GCC TTC TAC ATG GTT GGT GGT ATT +1470
 V   L   D   G   K   Y   D   H   L   P   E   N   A   F   Y   M   V   G   G   I    490
GAA GAT GTT GTT GCT AAG GCT GAA AAG TTG GCT GCT GAA GCT AAT TAAGAACCTCTCACTCCCT +1526
 E   D   V   V   A   K   A   E   K   L   A   A   E   A   N   *                    505
CTGCTCTTTTAAATTGAAAAGAAAAGATGACAGAAACTAGAAGGGAATAATAACATCTC                     +1585
```

FIGURE 3.—Nucleotide sequence of the *K. lactis MGI1* gene and deduced amino acid sequence. Underlined are sequences corresponding to the oligonucleotides used for PCR amplification of *MGI1* and *mgi1* alleles. Genebank accession U37764.

Disruption of *MGI1*: Stable Ura$^+$ transformants of PM6-7A were shown to have *URA3* inserted in the *MGI1* gene by analysis of chromosomal DNA (Figure 4A). Such disrupted strains are respiratory deficient although they still retain intact mtDNA as demonstrated by hybridization (not illustrated). However, disrupted strains do not grow on 16 μg/ml EB (Figure 4B) and no loss of mtDNA occurs when cultures are treated with the drug by the margin-of-growth technique (see Figure 1; CLARK-WALKER 1972; HARDY *et al.* 1989). Consequently it appears from these observations that disruption of *MGI1* produces respiratory deficiency but does not result in a Mgi$^-$ phenotype.

Previous work has shown that an assembled F_1 is required for the expression of the Mgi$^-$ phenotype of *mgi* mutants. To see whether the β-subunit of F_1-ATPase is required for the recovery of petites from a *mgi* strain, *MGI1* was disrupted in the *mgi5-1* mutant CK145-22A. The *mgi5-1* mutation is a conversion of Thr275→Ala in the γ-subunit of F_1-ATPase (CHEN and CLARK-WALKER 1995). Correct disruption of *MGI1* was confirmed by PCR amplification of the disrupted locus (not illustrated). The *mgi5-1*, *mgi1::URA3* double mutant, CK217, was respiratory deficient but did not grow on EB at 16 μg/ml on GYP plates. Treatment with EB by the "margin-of-growth" technique did not produce pe-

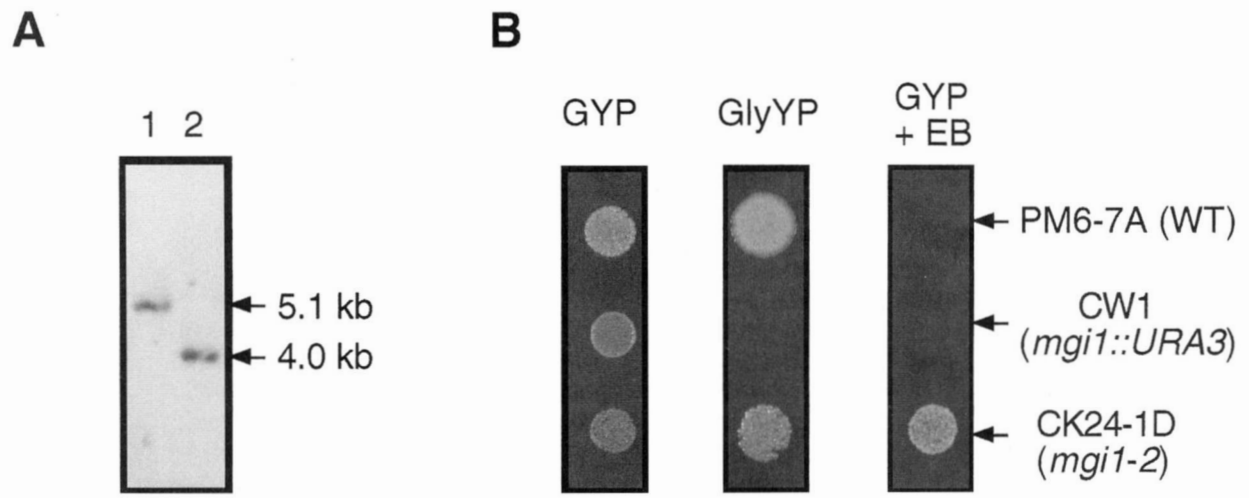


FIGURE 4.—Southern-blot analysis of *K. lactis MGI1*-disrupted strains and phenotype of the cells on EB medium. (A) Autoradiography showing the disruption of *MGI1*. Genomic DNA from the wild-type PM6-7A (lane 2) and the *MGI1*-disrupted strain CW1 (lane 1) was digested with *Xho*I, transferred to nylon membrane and hybridized with [^{32}P]-labeled pKlMGI1.2. Sizes of the hybridizing bands correspond to 4 and 5.1 kb in the wild-type and *MGI1*-disrupted strains respectively. (B) Sensitivity of *K. lactis* strains to EB. Cells were grown to stationary phase, diluted to 2×10^6 cells/ml, and 10 μl aliquots were applied to GYP, GlyYP and GYP plus EB at 16 μg/ml. Plates were incubated at 28° for 4–6 days before being photographed.

tites with deletions in mtDNA, indicating that CK217 is Mgi$^+$ and unable to form mitochondrial deletion mutants. Likewise, the γ-subunit encoding *MGI5* was disrupted by insertion of the *URA3* gene in the strains CK56-7A (*mgi1-1*), CK98-8A (*mgi1-3*) and CK197-13A (*mgi1-4*) as previously described (CHEN and CLARK-WALKER 1995). The resulting strains CK225, CK226 and CK227 were also respiratory deficient, EB sensitive and exhibit a Mgi$^+$ phenotype.

Mutations in the *mgi1* alleles: The coding region of the *mgi1* mutant alleles were amplified by PCR from the strains CK56-7A (*mgi1-1*), CK24-1D (*mgi1-2*), CK98-8A (*mgi1-3*) and CK151-1 (*mgi1-4*). Sequence analysis revealed that the *mgi1* allele in CK56-7A, CK24-1D and CK151-1 have the same mutation that converts the codon 435 from AGA to GGA and results in the substitution of Arg435 by a Gly residue in the stretch 434-AlaArgLysIleGln-438 (Figures 5 and 6). The mutation in the *mgi1-3* allele in CK98-8A was found in the same codon, but the change from AGA to ATA converts Arg435 to Ile (Figures 5 and 6).

DISCUSSION

In this paper, we describe the cloning of the *MGI1* gene of the petite-negative yeast *K. lactis*, by complementation of reduced viability of *mgi1* mutants at low temperature after a long period of incubation. *MGI1* is a nuclear gene that encodes a polypeptide of 505 amino acids with high homology to the β-subunit of the mitochondrial F_1-ATPase from others species. The cloned *MGI1* gene can functionally complement a null mutant of the *ATP2* gene in *S. cerevisiae* that encodes the β-subunit of F_1. Creation of a null mutation in *MGI1* by insertion of an auxotrophic marker gene leads to a respiratory deficient phenotype. It is thus concluded that the *MGI1* gene of *K. lactis* encodes the β-subunit of the mitochondrial F_1-ATPase.

In a previous paper, we proposed a model to explain how *mgi* mutations allow recovery of mtDNA deletion mutants in *K. lactis* (CHEN and CLARK-WALKER 1993). In this model, we suggest that the presence of one (or more) of the mitochondrially encoded ATP6, 8 and 9 subunits is critical for cell viability. Loss of these subunits, caused by mtDNA elimination by DNA targeting drugs or large scale deletions in the mitochondrial genome, is lethal due to the creation of a proton permeable pore on the mitochondrial inner membrane through the interaction of a wild-type F_1 with the membrane lacking the three F_o subunits. However, lethality can be suppressed by *mgi* mutations through the interaction between an altered F_1 particle and the inner membrane. Consistent with this model are data described in this paper. We found that only specific point mutations in *MGI1*, but not a null allele, predisposes cells to the formation of mtDNA deletion mutants. The *mgi1* alleles are thus "gain-of-function" mutations. The F_1 complex with an altered β-subunit must have gained a novel function that is likely to block the proton permeable pore on the membrane created by the loss of mtDNA-encoded F_o subunits. In addition, when the *MGI5* gene, encoding the γ-subunit of F_1, is disrupted in the *mgi1-1* and *mgi1-3* background, the resulting strains showed a Mgi$^+$ phenotype and do not form mtDNA deletion mutants upon treatment with EB. Likewise, when the *MGI1* gene is disrupted in a *mgi5-1* strain, the disruptants are also Mgi$^+$. These data clearly indicate that the novel function of the *mgi* mutations is through an assembled F_1. Disrupting one of the three large subunits of F_1 might affect the assembly of the

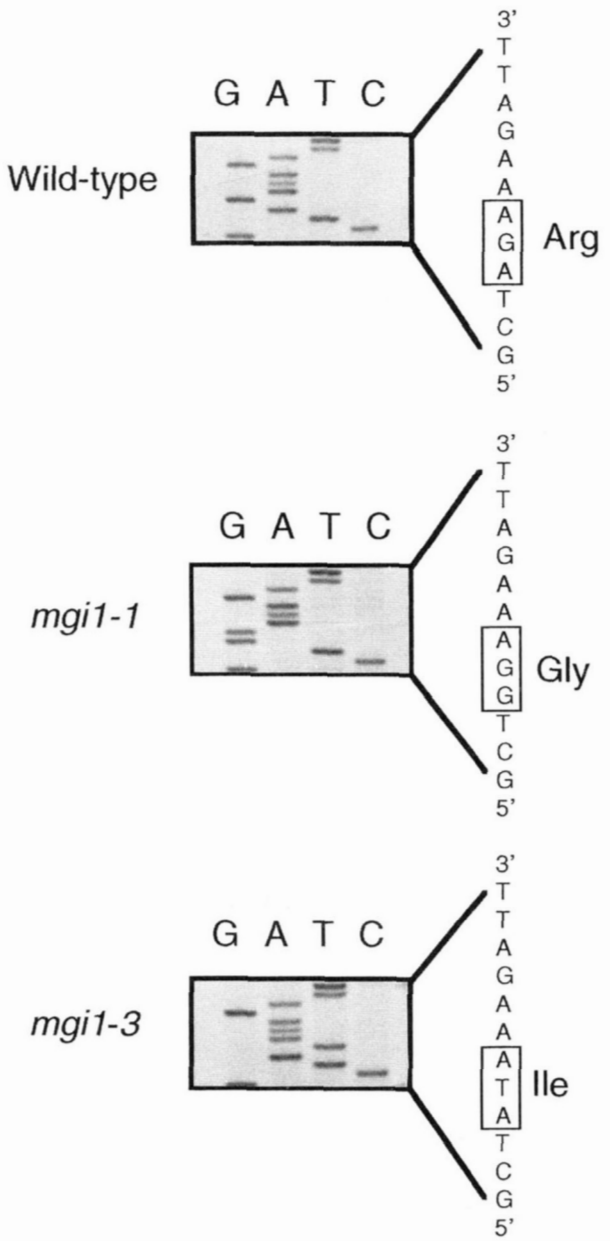


FIGURE 5.—Identification of mutations in *mgi1-1* and *mgi1-3* alleles. PCR products from CK56-7A (*mgi1-1*), CK98-8A (*mgi1-3*) and the wild-type strain PM6-7A were directly sequenced using the synthetic oligonucleotide KlATP2P11 [5′-(+1188)TGTCGCTACTCAAGTTC(+1204)-3′] as primer. The codon Arg435 in the wild-type strain and the mutant codons in *mgi1-1* and *mgi1-3* are boxed.

complex or a subcomplex and the interaction with the membrane. Hence null mutants of a F_1 subunit gene do not have the same phenotype as point mutations. The "gain-of-function" model is also consistent with the dominant feature of the *mgi1* alleles in a heterozygous *MGI/mgi* strain in the presence of EB. However, it remains unclear why in such strains *mgi* alleles are recessive in the absence of the drug and spontaneous petite mutants are not formed.

A striking aspect of the *mgi1* alleles described in this

E.coli	387	-	KLMVARARKIQRFLS	- 401
Cow	401	-	KLTVSRARKIQRFLS	- 415
S. cerevisiae	434	-	KLTVERARKIQRFLS	- 448
K.lactis	428	-	KLTVERARKIQRFLS	- 442
mgi1-1	428	-	KLTVERAGKIQRFLS	- 442
mgi1-3	428	-	KLTVERAIKIQRFLS	- 442

FIGURE 6.—Comparison of amino acid sequences from various organisms corresponding to the flanking regions of the mutational sites in *mgi1-1* and *mgi1-3*. Bold letters are the mutated Arg435s in the mutant alleles.

paper is that the four independent isolates have a mutation in the same codon. Three of them, *mgi1-1*, *-2* and *-4*, have a substitution of Arg435 by Gly while the *mgi1-3* allele has a conversion of the same Arg residue to Ile. Comparison of the amino acid sequences flanking this residue from *E. coli*, Cow, *S. cerevisiae*, and the wild-type *K. lactis* is illustrated in Figure 6. In fact, examination of all F_1 β-subunit sequences from 72 different species, including those from chloroplasts, bacteria and mammalian mitochondria, reveals that this Arg residue is totally conserved. According to the secondary structure prediction of the F_1 β-subunit (DUCAN *et al.* 1986) and the crystal structural analysis of the bovine mitochondrial F_1 complex (ABRAHAMS *et al.* 1994), Arg435 of the *K. lactis* gene, corresponding to Arg408 in the bovine protein, is located in helix 2 at the carboxyl terminus (ABRAHAMS *et al.* 1994). Close to this helix is a loop that has been proposed by the same authors to contain a "catch" that forms hydrogen bonds with the γ-subunit. Thus it is possible that mutations in *mgi1-1* and *mgi1-3*

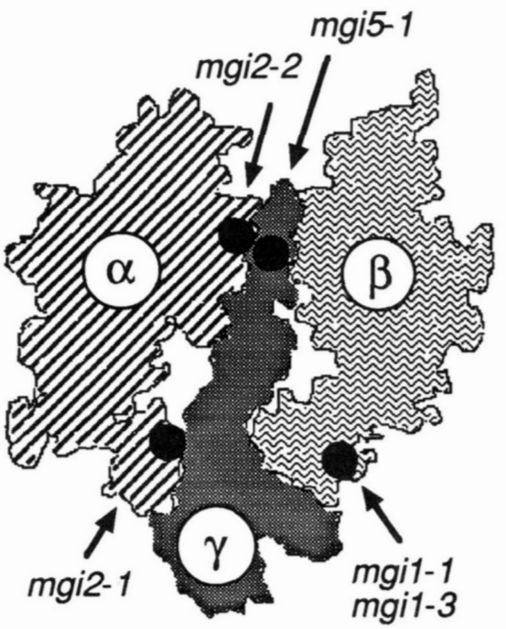


FIGURE 7.—Approximate locations of *mgi* mutations in the F_1 complex. The three patches, representing the α-, β- and γ-subunits in a diagrammatic cross-section of the bovine mitochondrial F_1-ATPase, are drawn according to the crystal structure reported by ABRAHAMS *et al.* (1994). The black circles represent the positions of the *mgi* mutations.

in Arg435 affect the interaction between the β- and γ-subunits.

The approximate positions of the *mgi1* mutations in the F_1 complex , together with those of the previously identified *mgi2* and *mgi5* alleles, are shown in Figure 7. One can see from this diagram that the *mgi2-1, mgi2-2* and *mgi5-1* mutations are all located on the interface between α- and γ-subunits. The *mgi2-2* and *mgi5-1* mutations probably represent the same contact point between the two subunits. Although the Arg residue where the *mgi1-1* and *mgi1-3* mutations occur is not in direct contact with the γ-subunit, these mutations may alter the structural conformation in the carboxyl terminus of the β-subunit and affect its interaction with the γ-subunit. Taken together, it seems that altering interaction between α- and γ-subunits or between β- and γ-subunits could be the common feature of the five identified *mgi* alleles.

Although the three *MGI* genes so far characterized encode the α-, β- and γ-subunits of the F_1 complex, it is known that the yeast mitochondrial F_1F_o-ATP synthase is composed of at least eleven different subunits. Eight of the subunits are encoded by the nuclear genome, including the five subunits of F_1 (α, β, γ, δ and ϵ) and three F_o proteins namely P25 (*ATP4,* VELOURS *et al.* 1988), OSCP (*ATP5,* UH *et al.* 1990) and P18 (*ATP7,* NORAIS *et al.* 1991). It will be important to determine, in the context of our model for proton permeability of the ATP synthase, whether *mgi* mutations are confined to the α-, β- and γ-subunits of F_1 or if other components of the complex can be involved.

We thank M. G. DOUGLAS and D. M. CYR for providing the *S. cerevisiae* strain MDY2101 and Lijun Ouyang for technical assistance.

LITERATURE CITED

ABRAHAMS, J. P., A. G. W. LESLIE, R. LUTTER and J. E. WALKER, 1994 Structure at 2.8 Å resolution of F_1-ATPase from bovine heart mitochondria. Nature **370:** 621–628.

BULDER, C. G. E. A., 1964a Induction of petite mutation and inhibition of synthesis of respiratory enzymes in various yeasts. Antonie Leeuwenhoek **30:** 1–9.

BULDER, C. G. E. A., 1964b Lethality of the petite mutation in petite negative yeasts. Antonie Leeuwenhoek **30:** 442–445.

CHEN, X. J., 1996 Low and high copy number shuttle vectors for replication in the budding yeast *Kluyveromyces lactis.* Gene **172:** 131–136.

CHEN, X. J., and G. D. CLARK-WALKER, 1993 Mutations in *MGI* genes convert *Kluyveromyces lactis* into a petite-positive yeast. Genetics **133:** 517–525.

CHEN, X. J., and G. D. CLARK-WALKER, 1994 *sir2* mutants of *Kluyveromyces lactis* are hypersensitive to DNA-targeting drugs. Mol. Cell. Biol. **14:** 4501–4508.

CHEN, X. J., and G. D. CLARK-WALKER, 1995 Specific mutations in α- and γ-subunits of F_1-ATPase affect mitochondrial genome integrity in the petite-negative yeast *Kluyveromyces lactis.* EMBO J. **14:** 3277–3286.

CHEN, X. J., and H. FUKUHARA, 1988 A gene fusion system using the aminoglycoside 3′-phosphotransferase gene of the kanamycin-resistance transposon Tn*903*: use in the yeasts *Kluyveromyces lactis* and *Saccharomyces cerevisiae.* Gene **69:** 181–192.

CHEN, X. J., M. WESOLOWSKI-LOUVEL, C. TANGUY-ROUGEAU, M. M BIANCHI, L. FABIANI *et al.*, 1988 A gene-cloning system for *Kluyveromyces lactis* and isolation of a chromosomal gene required for killer toxin production. J. Basic Microbiol. **28:** 211–220.

CHEN, X. J., M. WESOLOWSKI-LOUVEL and H. FUKUHARA, 1992 Glucose transport in the yeast *Kluyveromyces lactis.* II. Transcriptional regulation of the glucose transporter gene *RAG1.* Mol. Gen. Genet. **233:** 97–105.

CLARK-WALKER, G. D., 1972 Isolation of circular DNA from a mitochondrial fraction from yeast. Proc. Natl. Acad. Sci. USA **69:** 388–392.

COX, G. B., R. J. DEVENISH, F. GIBSON, S. M. HOWITT and P. NAGLEY, 1992 The structure and assembly of ATP synthase, pp. 283–315 in *Molecular Mechanisms in Bioenergetics,* edited by L. ERNSTER. Elsevier Science Publication B.V., Amsterdam.

CROSS, R. L. A., 1981 The mechanism and regulation of ATP synthesis by F_1- ATPase. Annu. Rev. Biochem. **50:** 681–714.

DE DEKEN, R. H., 1966 The Crabtree effect and its relation to the petite mutation. J. Gen. Microbiol. **44:** 157–165.

DUCAN, T. M., D. PARSONAGE and A. E. SENIOR, 1986 Structure of the nucleotide-binding domain in the β-subunit of *Escherichia coli* F_1-ATPase. FEBS Lett. **208:** 1–5.

EPHRUSSI, B., 1953 *Nucleocytoplasmic Relations in Microorganisms.* Clarendon Press, Oxford.

FAYE, G., H. FUKUHARA, C. GRANDCHAMP, J. LAZOWSKA, F. MICHEL *et al.*, 1973 Mitochondrial nucleic acids in the *petite colonie* mutants: deletions and repetitions of genes. Biochimie **55:** 779–792.

FILLINGAME, R. H., 1990 Molecular mechanics of ATP synthesis by F_1F_o-type H^+- transporting ATP synthase, Vol. XII pp. 345–391 in *The Bacteria,* edited by T. A. KRULWICH. Academic Press Inc., New York.

FUTAI, M., and H. KANAZAWA, 1983 Structure and function of proton-translocating adenosine triphosphatase (F_oF_1): biochemical and molecular biological approaches. Microbiol. Rev. **47:** 285–312.

FUTAI, M., T, NOUMI and M. MAEDA, 1989 ATP synthase (H^+-ATPase): results by combined biochemical and molecular biological approaches. Annu. Rev. Biochem. **58:** 111–136.

GIETZ, D., A. S. JEAN, R. A. WOODS and R. H. SCHIESTL, 1992 Improved method for high efficiency transformation of intact yeast cells. Nucleic Acids Res. **20:** 1425.

HAFFTER, P., and T. D. FOX, 1992 Nuclear mutations in the petite-negative yeast *Schizosaccharomyces pombe* allow growth of cells lacking mitochondrial DNA. Genetics **131:** 255–260.

HARDY, C. M., C. L. GALEOTTI and G. D. CLARK-WALKER, 1989 Deletions and rearrangement in *Kluyveromyces lactis* mitochondrial DNA. Curr. Genet. **16:** 419–428.

HATEFI, Y., 1993 ATP synthesis in mitochondria. Eur. J. Biochem. **218:** 759–769.

HILL, J., K. A. I. G. DONALD, and D. E. GRIFFITHS, 1991 DMSO-enhanced whole cell yeast transformation. Nucleic Acids Res. **19:** 5791.

IWAMOTO, A., J. MIKI, M. MAEDA and M. FUTAI, 1990 H^+-ATPase g subunit of *Escherichia coli*: role of the conserved carboxyl-terminal region. J. Biol. Chem. **265:** 5043–5048.

MAGGIO, M. B., D. PARSONAGE and A. E. SENIOR, 1988 A mutation in the a- subunit of F_1-ATPase from *Escherichia coli* affects the binding of F_1 to the membrane. J. Biol. Chem. **263:** 4619–4623.

MORONEY, J. V., and R. E. MCCARTY, 1979 Reversible uncoupling of photophosphorylation by a new bifunctional maleimide. J. Biol. Chem. **254:** 8951–8955.

MORONEY, J. V., C. S. ANDREO, R. H. VALLEJOS and R. E. MCCARTY, 1980 Uncoupling and energy transfer inhibition of photophosphorylation by sulfhydryl reagents. J. Biol. Chem. **255:** 6670–6674.

MULLIS, K. B., and F. A. FALOONA, 1987 Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods Enzymol. **155:** 335–350.

NORAIS, N., D. PROME and J. VELOURS, 1991 ATP synthase of yeast mitochondria: characterization of subunit d and sequence analysis of the structural gene *ATP7.* J. Biol. Chem. **266:** 16541–16549.

PAUL, M.-F., S. ACKERMANN, J. YUE, G. ARSELIN, J. VELOURS and A. TZAGOLOFF, 1994 Cloning of the yeast *ATP3* gene coding for the γ-subunit of F_1 and characterization of *atp3* mutants. J. Biol. Chem. **269:** 26158–26164.

ROTHSTEIN, R. J., 1983 A one step gene disruption in yeast. Methods Enzymol. **101:** 202–210.

SAMBROOK, J., E. F. FRITSCH and T. MANAITIS, 1989 *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SANGER, F. S., S. NICKLEN and A. R. COULSON, 1977 DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:** 5463–5467.

SENIOR, A. E., 1988 ATP synthesis by oxidative phosphorylation. Physiol. Rev. **68:** 177–231.

SHERMAN, F., G. F. FINK and J. B. HICKS, 1983 *Methods in Yeast Genetics.* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

SHIN, K., R. K. NAKAMOTO, M. MAEDA and M. FUTAI, 1992 F_oF_1-ATPase γ subunit mutations perturb the coupling between catalysis and transport. J. Biol. Chem. **267:** 20835–20839.

SOGA, S., T. NOUMI, M. TAKEYAMA, M. MAEDA and M. FUTAI, 1989 Mutational replacements of conserved amino acid residues in the a subunit change the catalytic properties of *Escherichia coli* F_1-ATPase. Arch. Biochem. Biophys. **268:** 643–648.

TINGLE, M., A. HERMAN and H. O. HALVORSON, 1968 Characterization and mapping of histidine genes in *Saccharomyces lactis.* Genetics **58:** 361–371.

UH, M., D. JONES and D. M. MUELLER, 1990 The gene coding for the yeast oligomycin sensitivity-conferring protein. J. Biol. Chem. **265:** 19047–19052

VELOURS, J., P. DURRENS, M. AIGLE and B. GUERIN, 1988 *ATP4,* the structural gene for yeast F_oF_1-ATPase subunit 4. Eur. J. Biochem. **170:** 637–642.

WEISS, M. A. and R. E. MCCARTY, 1977 Cross-linking within a subunit of coupling factor 1 increases the proton permeability of spinach chloroplast thylakoids. J. Biol. Chem. **252:** 8007–8012.

WESOLOWSKI, M., A. A. ALGERI, P. GOFFRINI and H. FUKUHARA, 1982 Killer plasmids of the yeast *Kluyveromyces lactis.* I. Mutations affecting the killer phenotype. Curr. Genet. **5:** 191–197.

WESOLOWSKI-LOUVEL, M., C. TANGUY-ROUGEAU and H. FUKUHARA, 1986 A nuclear gene required for the expression of the linear DNA-associated killer system in the yeast *Kluyveromyces lactis.* Yeast **4:** 71–81.

Communicating editor: K. J. NEWTON