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Note

A Single Nucleotide Polymorphism in the DNA Polymerase Gamma Gene of *Saccharomyces cerevisiae* Laboratory Strains Is Responsible for Increased Mitochondrial DNA Mutability

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ABSTRACT

In the *Saccharomyces cerevisiae* strains used for genome sequencing and functional analysis, the mitochondrial DNA replicase Mip1p contains a single nucleotide polymorphism changing the strictly conserved threonine 661 to alanine. This substitution is responsible for the increased rate of mitochondrial DNA point mutations and deletions in these strains.

THE yeast *Saccharomyces cerevisiae* is one of the few eukaryotic organisms that can survive in the absence of mitochondrial DNA (mtDNA). When grown on glucose-containing medium, *S. cerevisiae* produces respiratory-deficient mutants that form small colonies and thus are referred to as "cytoplasmic *petites*" (Slonimski and Ephrussi 1949). They either contain deletions in their mtDNA (*rho*$^-$) or are devoid of mtDNA (*rho*^{0}) (Dujon 1981). *petite* frequency varies within a large range among laboratory strains (Marmiroli *et al.* 1980), a not surprising feature with regards to the numerous genes that control *petite* accumulation (Contamine and Picard 2000). One of these is *MIP1*, which encodes the mitochondrial polymerase (DNA polymerase gamma) (Foury 1989). Previous studies using the *MIP1* gene isolated from a genomic library constructed with strain Σ1278b (Grenson *et al.* 1966), referred to as *MIP1*[Σ], have shown that Mip1p is an accurate replicase (Foury and Vanderstraeten 1992; Hu *et al.* 1995; Vanderstraeten *et al.* 1998). However, recent works using the *MIP1* allele from S288c-related strains, referred to as *MIP1*[S], have pointed to higher mutation rates of the mitochondrial genome (Baruffini *et al.* 2006; Stuart *et al.* 2006). Here we show that this increase in mtDNA instability results from a single nucleotide substitution in the *MIP1* gene, changing a strictly conserved threonine at position 661 to alanine.

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***MIP1*[S] allele increases mtDNA instability:** *petite* mutant frequency was determined in the DWM-5A-Δmip1 strain (Baruffini *et al.* 2006) carrying pFL39 plasmid-borne versions of either *MIP1*[S] or *MIP1*[Σ]. A fourfold increase in *petite* accumulation was observed at 28° in the presence of *MIP1*[S] compared to *MIP1*[Σ] (Figure 1A). Moreover, in the *MIP1*[S] context, and in contrast to the *MIP1*[Σ] context, *petite* accumulation was highly dependent on temperature (Figure 1A). On the basis of the capacity of independent *petite* clones to restore *mit*$^-$ mutations to wild type (Slonimski and Tzagoloff 1976), it was concluded that, in both *MIP1* contexts, most *petites* had retained mtDNA fragments and were *rho*$^-$, even though, in the *MIP1*[S] context, the fraction of *rho*^{0} clones at 36° was significantly higher (Figure 1B). The frequency of Ery^R mutants, which are caused by mtDNA mutations in the 21S rRNA gene (Sor and Fukuhara 1984), gives an estimate of the frequency of mtDNA point mutations. A sixfold increase in Ery^R mutants was observed in the *MIP1*[S] context compared to the *MIP1*[Σ] context (Figure 1C). These data show that in the same nuclear background the mitochondrial genome is less stable in the presence of the *MIP1*[S] allele, suggesting that this trait could be ascribed to differences in the amino acid sequence of the two Mip1p isoforms.

T661A substitution in the *MIP1*[S] allele is the cause of temperature-sensitive *petite* accumulation: DNA sequence analysis revealed 26 single nucleotide polymorphisms (SNPs) between *MIP1*[S] and *MIP1*[Σ] alleles (Table 1). The *MIP1* sequence was identical in W303-1B and S288c, confirming the close origin of these strains

(Schacherer *et al.* 2007). Sixteen SNPs were silent and 10 produced nonsynonymous substitutions. Multiple amino acid alignments with several fungi and animal pol g sequences showed that these modifications affected mostly poorly conserved residues (data not shown). However, Thr661, which is found in Mip1p[Σ] and is strictly conserved in all species from yeasts to humans, is changed to alanine in Mip1p[S] due to an A-to-G transition at position 1981 of the *MIP1* sequence. Moreover, Thr661 has been found in two recently sequenced *S. cerevisiae* strains, YJM789 (Gu *et al.* 2005) and RM11-1a (Lee *et al.* 2006), which have a *MIP1*[S]-like allele.

To determine whether the T661A substitution was the cause of *petite* accumulation in the *MIP1*[S] context, a *MIP1*[S]^{A661T} variant was constructed. In the presence of this new allele, *petite* frequency was similar to that observed with the *MIP1*[Σ] allele and, moreover, the temperature-sensitive trait had disappeared (Figure 1A). The frequency of Ery^R mutants was also reduced (Figure 1C). In a heteroallelic *MIP1*[S]^{A661T}/*MIP1*[S] strain, *petite* and Ery^R mutant frequencies were similar to those observed in a strain containing *MIP1*[S]^{A661T} only, indicating that the *MIP1*[S] allele is recessive (data not shown).

We measured *petite* frequency in several laboratory strains. Σ1278b, D273-10B/A1 (Sherman 1964), and FL100 (Lacroute 1968), which possess the *MIP1*[Σ] allele (E. Baruffini, unpublished data), had low levels of *petites* (Table 2). W303-1B (Thomas and Rothstein 1989) and BY4742 (Brachmann *et al.* 1998), which possess the *MIP1*[S] allele (and thus Ala661), accumulated *petites* at higher levels and in a temperature-dependent manner (Table 2). To further demonstrate that increased instability of the mitochondrial genome could be ascribed to Ala661 rather than to the genetic background of these strains, the pFL38 plasmid-borne *MIP1*[S]^{A661T} and *MIP1*[S] alleles were introduced in a *mip1*Δ derivative of D273-10B/A1. *petite* frequency was low in the presence of *MIP1*[S]^{A661T} and increased in the presence of *MIP1*[S] (Table 3). Altogether, these data demonstrate that the T661A substitution in Mip1p is the cause of the higher accumulation of *petites* in S288c, BY4742, and W303-1B strains.

Natural isolates contain a threonine residue at position 661: To determine whether T661A was a widespread mutation, we analyzed 20 strains isolated from grapes of different Italian regions or used as industrial starters for wine or bread production. Adenine at position

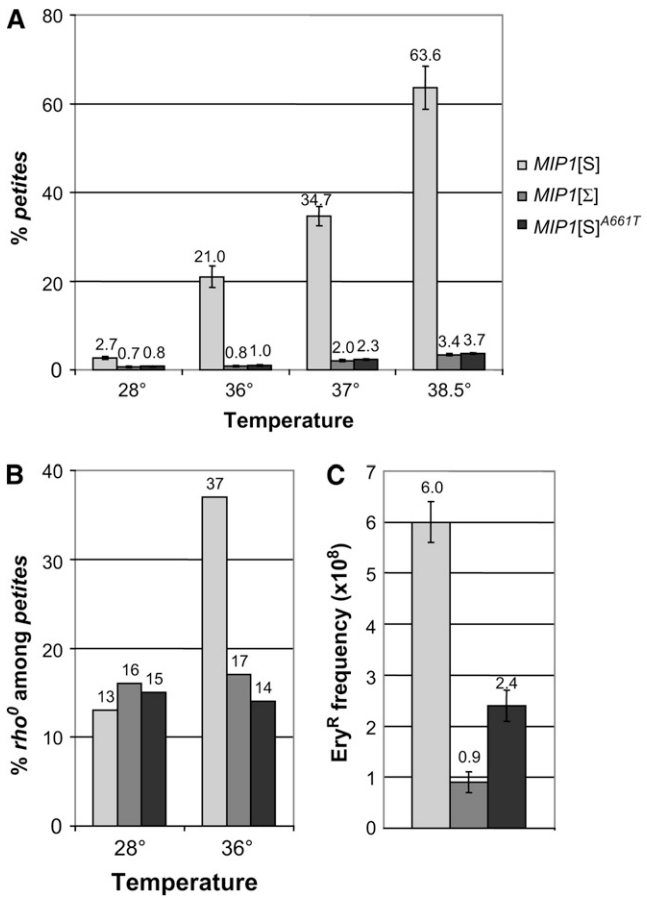

Figure 1.—Mitochondrial DNA mutation frequency with different *MIP1* alleles. DWM-5A-Δmip1, an haploid W303 derivative (Baruffini *et al.* 2006), was transformed with different *MIP1* alleles. The *MIP1* alleles were inserted into the *Sac*I and *Sal*I sites of the centromeric pFL39 plasmid (Bonneaud *et al.* 1991). The *MIP1*[S]^{A661T} allele was produced by site-directed mutagenesis using the PCR overlap extension technique (Ho *et al.* 1989). (A) *petite* mutant frequency. Cells were pregrown at 28° on solid SC medium (6.7 g/liter yeast nitrogen base supplemented with a mixture of amino acids) supplemented with 2% ethanol. After 60 hr, the strains were replica plated on SC medium supplemented with 2% glucose and grown at the specified temperature. After 24 hr, strains were replica plated again on this medium. After 24 hr, cells were plated for single colonies on SC medium supplemented with 2% ethanol and 0.3% glucose. *petite* frequency was defined as the percentage of colonies showing the *petite* phenotype after 5 days at 28°. For each strain, at least 4000 clones were analyzed. Values are means of three independent experiments. (B) Percentage of *rho*^{0} mutants. The *rho*$^-$ clones containing mtDNA-deleted molecules and the *rho*^{0} clones devoid of mtDNA were distinguished as follows. At least 200 independent *petite* clones from each haploid *mip1* strain were crossed with *cox2*, *cox3*, and two *cob mit*$^-$ mutants of opposite mating type on YPA plates (1% yeast extract, 2% bacto-peptone, and 40 mg/liter adenine) supplemented with 2% glucose, and after 2 days at 28° they were replica plated on YPA plates supplemented with 3% glycerol to identify *rho*$^+$ diploids. In this work, a clone unable to complement any of the *mit*$^-$ mutants was arbitrarily defined as *rho*^{0}. (C) Ery^R mutant frequency. Two independent series of 10 independent colonies grown on YPA plates supplemented with 3% glycerol were inoculated in 2.5 ml YPA medium. After 48 hr at 28°, 5–8 × 10^7 cells were plated on YPAEG-ery medium (YPA supplemented with 3% ethanol, 3% glycerol, 3 g/liter erythromycin, and 25 mM potassium phospate buffer at pH 6.5) and grown at 28° for 9 days. An aliquot of each culture was plated for single colonies on YPA plates supplemented with 3% glycerol to determine the exact number of *rho*$^+$ cells present in the culture.

TABLE 1

SNPs and amino acid substitutions in *MIP1*[Σ] and *MIP1*[S] alleles

SNP[a]	Amino acid substitution[b]	SNP	Amino acid substitution	SNP	Amino acid substitution
T23 → C[c]	*F8 → S*	T1299 → C	Silent (P433)	*A1847 → G*	*N616 → S*
G103 → A	*A35 → T*	T1590 → C	Silent (S530)	*A1981 → G*	*T661 → A*
G219 → T	Silent (L73)	G1617 → A	Silent (R539)	C2166 → T	Silent (C722)
G627 → A	Silent (A209)	*T1519 → C*	*M540 → T*	*C2932 → T*	*P978 → S*
G664 → A	*V222 → I*	*A1521 → C*	*N541 → H*	*G2957 → A*	*S986 → N*
G792 → A	Silent (Q264)	T1671 → C	Silent (P557)	T3345 → C	Silent (I1115)
A1069 → G	*K357 → E*	C1680 → G	Silent (P560)	G3384 → A	Silent (E1128)
T1161 → A	Silent (L387)	T1692 → C	Silent (C564)	A3516 → G	Silent (P1172)
A1221 → G	Silent (Q407)	T1794 → G	Silent (G598)		

[a] The first nucleotide refers to *MIP1*[Σ], the second to *MIP1*[S].
[b] The first amino acid refers to Mip1[Σ], the second to Mip1[S].
[c] Nonsynonymous SNPs and corresponding amino acid substitutions are in italics.

1981 of the *MIP1* gene is associated with a *Bsa*AI site while with guanine the *Bsa*AI site is lost. To distinguish these polymorphisms, a DNA fragment encompassing nucleotides 1046–2329 was amplified and digested by *Bsa*AI. The presence of adenine at position 1981 generates two DNA fragments of 937 and 347 bp, whereas guanine generates a single DNA fragment of 1284 bp (Figure 2A). In all strains, the *MIP1* gene possessed the *Bsa*AI site and thus Thr661.

On the other hand, the partial genome sequence of haploid segregants from 36 strains of different origins has recently been published (Saccharomyces Genome Resequencing Project at the Sanger Institute, http://www.sanger.ac.uk/Teams/Team71/durbin/sgrp/index.shtml). Most Western isolates have the *MIP1*[S]^{A661T} allele; the *MIP1* gene of Asian isolates is a complex mosaic of the *MIP1*[S]^{A661T} and *MIP1*[Σ] alleles, but none of the strains has the *MIP1*[Σ] allele, raising the question of the origin of this allele. However, all isolates have a threonine at position 661.

In contrast, analysis of the *MIP1* gene of the diploid EM93 strain, which is the progenitor of S288c and derivative strains and has been estimated to share 88% of its genome with S288c (Mortimer and Johnston 1986), revealed that, in addition to the two DNA fragments generated by the *Bsa*AI digest, an undigested fragment of 1284 bp was also present (Figure 2A), a sign of heterozygosity at the *Bsa*A1 site. *MIP1* gene sequencing and tetrad analysis (data not shown) confirmed that both A and G were present at position 1981 (Figure 2B). Therefore, EM93 is heterozygous for the *MIP1* allele. These data led to the conclusion that the T661A substitution is unique and specific to EM93, the founder of several commonly used laboratory strains.

Concluding remarks: This work provides an explanation for the high frequency of mtDNA deletions and point mutations occurring in commonly used laboratory strains: a missense mutation in the mitochondrial DNA replicase brought about by EM93, the founder strain (Mortimer and Johnston 1986). The fact that S288c and its derivatives BY4741, BY4742, and BY4743, the strains used in the genome sequencing and large-scale functional analysis projects, contain the *MIP1*[S] allele should be taken into consideration in genomic studies focused on alterations of the mitochondrial metabolism. Moreover, the *MIP1*[S] allele has previously been used to establish the impact of human pathological *POLG* mutations on the stability of mtDNA (Baruffini *et al.* 2006; Stuart *et al.* 2006). It must be stressed that

TABLE 2

***petite* accumulation in different laboratory strains**

Strains	% *petites* 28°	% *petites* 36°	Amino acid at position 661
D273-10B/A1	0.8 ± 0.1	0.9 ± 0.2	Threonine
FL100	0.5 ± 0.1	0.8 ± 0.1	Threonine
Σ1278b	0.7 ± 0.1	0.8 ± 0.2	Threonine
W303-1B	2.3 ± 0.2	15.1 ± 2.1	Alanine
BY4742	2.1 ± 0.2	11.3 ± 1.1	Alanine

Experimental conditions are as in Figure 1A.

TABLE 3

***petite* accumulation in strain D273-CD3-Δmip1 transformed with different *MIP1* alleles**

D273-CD3-Δmip1	% *petites* 28°	% *petites* 36°
MIP1[S]	5.1 ± 0.4	22.6 ± 3.4
MIP1[S]^{A661T}	2.2 ± 0.2	2.6 ± 0.3

D273-CD3-Δmip1 is a spontaneous *ura3* mutant of D273-10B/A1 in which the *MIP1* gene has been replaced by a *Kan*^{R} deletion cassette in the presence of the pFL38 (Bonneaud *et al.* 1991) plasmid-borne *MIP1*[S] or *MIP1*[S]^{A661T} to keep mtDNA. Experimental conditions are as in Figure 1A.

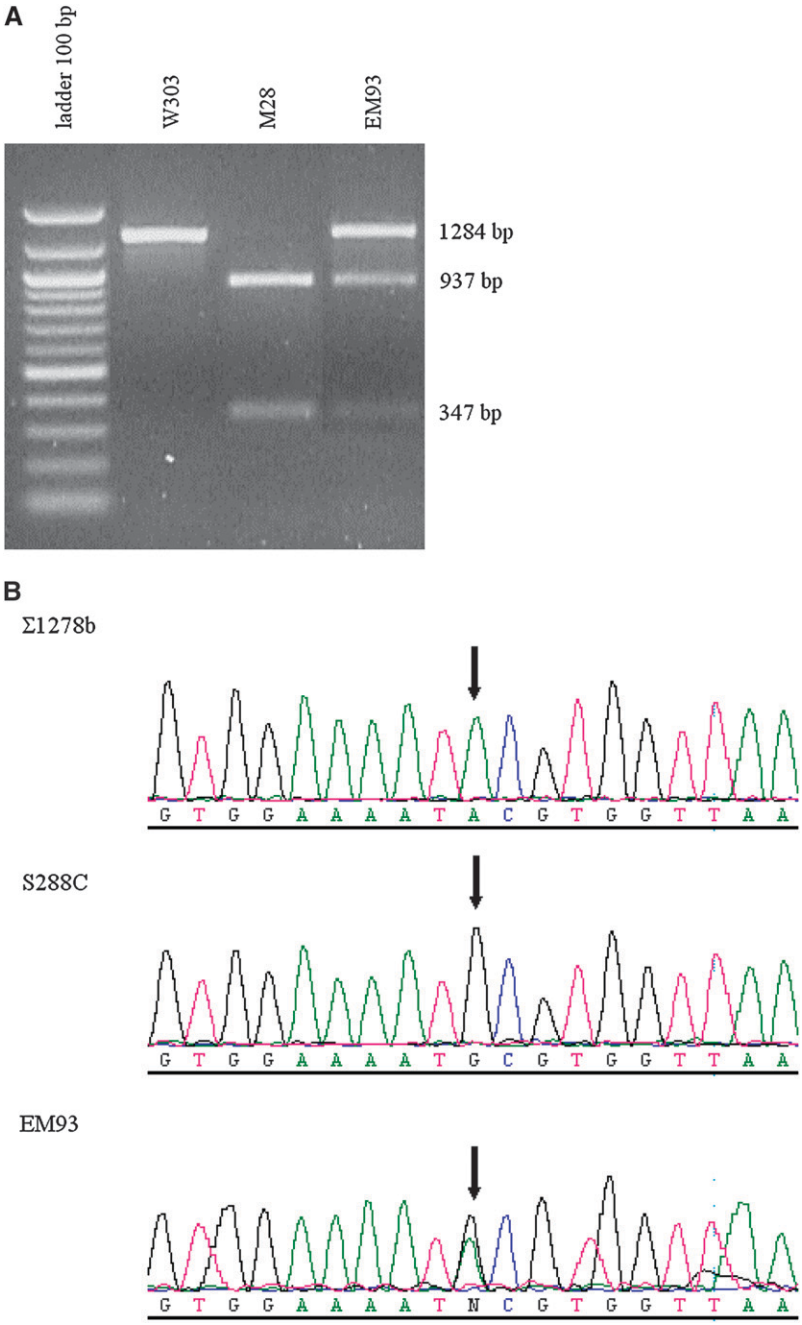

FIGURE 2.—Analysis of the polymorphism at position 1981 of the *MIP1* nucleotide sequence. (A) *Bsa*AI restriction analysis of the DNA fragment from nucleotides 1046 to 2329. All natural isolates analyzed produced the same pattern as the M28 strain, an isolate from grapes in a Tuscan vineyard (CAVALIERI *et al.* 2000). (B) DNA sequence of the segment encompassing position 1981.

even though this allele, which exacerbates certain defects, can be useful in detecting subtle effects of *mip1* mutations, it may also cause unreliable phenotypes.

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