# The Mitochondrial Nucleoid Protein, Mgm101p, of *Saccharomyces cerevisiae* Is Involved in the Maintenance of $\rho^+$ and *ori/rep*-Devoid Petite Genomes but Is Not Required for Hypersuppressive $\rho^-$ mtDNA

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ABSTRACT

The *Saccharomyces cerevisiae MGM101* gene encodes a DNA-binding protein targeted to mitochondrial nucleoids. *MGM101* is essential for maintenance of a functional $\rho^+$ genome because meiotic segregants, with a disrupted *mgm101* allele, cannot undergo more than 10 divisions on glycerol medium. Quantitative analysis of mtDNA copy number in a $\rho^+$ strain carrying a temperature-sensitive allele, *mgm101-1*, revealed that the amount of mtDNA is halved each cell division upon a shift to the restrictive temperature. These data suggest that mtDNA replication is rapidly blocked in cells lacking *MGM101*. However, a small proportion of meiotic segregants, disrupted in *MGM101*, have $\rho^-$ genomes that are stably maintained. Interestingly, all surviving $\rho^-$ mtDNAs contain an *ori/rep* sequence. Disruption of *MGM101* in hypersuppressive (HS) strains does not have a significant effect on the propagation of HS $\rho^-$ mtDNA. However, in petites lacking an *ori/rep*, disruption of *MGM101* leads to either a complete loss or a dramatically decreased stability of mtDNA. This discriminatory effect of *MGM101* suggests that replication of $\rho^+$ and *ori/rep*-devoid $\rho^-$ mtDNAs is carried out by the same process. By contrast, the persistence of *ori/rep*-containing mtDNA in HS petites lacking *MGM101* identifies a distinct replication pathway. The alternative mtDNA replication mechanism provided by *ori/rep* is independent of mitochondrial RNA polymerase encoded by *RPO41* as a HS $\rho^-$ genome is stably maintained in a *mgm101, rpo41* double mutant.

THE mitochondrial genome of eukaryotic cells encodes integral components of the energy-producing oxidative phosphorylation pathway. Central to the cell's energy metabolism is the need to maintain the organelle DNA (mtDNA). However, in the budding yeast *Saccharomyces cerevisiae*, a complete loss of mtDNA is not lethal provided that fermentable carbon sources such as glucose are supplied. Consequently, *S. cerevisiae* provides an excellent experimental vehicle for the identification of genes involved in mtDNA replication and transmission. Several proteins participating in mtDNA replication have been identified (for recent reviews, see Hermann and Shaw 1998; Shadel 1999; Contamine and Picard 2000; Lecrenier and Foury 2000). The pol-γ DNA polymerase, Mip1p (Foury 1989), the single-stranded DNA (ssDNA)-binding protein, Rim1p (Van Dyck *et al.* 1992), and a DNA ligase activity encoded by the *CDC9* gene (Willer *et al.* 1999) directly participate in mtDNA replication. Deleterious mutations in genes encoding these proteins lead to a complete loss of the mitochondrial genome ($\rho^o$).

*MGM101* is another *S. cerevisiae* gene whose disruption leads to the loss of a functional mitochondrial genome. The *MGM101* locus was initially identified in a genetic screen for mutations affecting the maintenance of mtDNA (Chen *et al.* 1993). It encodes a polypeptide of 269 residues rich in positively charged amino acids. A homolog of *MGM101* has been identified in the petite-negative yeast *Kluyveromyces lactis* by functional complementation of a *S. cerevisiae mgm101* mutant (Clark-Walker and Chen 1996). *K. lactis* cells carrying a disrupted *mgm101* allele form nonviable microcolonies due to loss of mtDNA. Subsequently, it has been found that the Mgm101 protein has a high affinity for DNA and is a component of mitochondrial nucleoids (Meeusen *et al.* 1999). Mgm101p can be specifically crosslinked to mtDNA *in vivo* (Kaufman *et al.* 2000). Functionally, it has been proposed that Mgm101p might be involved in the repair of oxidatively damaged mtDNA (Meeusen *et al.* 1999).

Yeast mitochondria contain a number of proteins required for the maintenance of the mitochondrial genome by their involvement in recombination and repair. The Pif1 helicase (Foury and Kolodynski 1983), the cruciform-cutting endonuclease Cce1p/Mgt1p (Kleff *et al.* 1992; Lockshon *et al.* 1995; White and Lilley 1996), the mitochondrial high-mobility group protein Abf2p (Diffley and Stillman 1992; Kao *et al.* 1993; MacAlpine *et al.* 1998; Zelenaya-Troitskaya *et al.* 1998), and a putative recombinase defined by the *MHR1* locus

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(Ling *et al.* 1995) are involved in mtDNA recombination. The Msh1 protein, homologous to bacterial MutS, is needed for mismatch repair (Reenan and Kolodner 1992; Chi and Kolodner 1994). In addition to their role in DNA recombination, the Pif1 helicase and the putative Mhr1 recombinase are also suggested to be involved in the repair of UV-damaged and spontaneously occurring DNA lesions (Foury and Kolodynski 1983; Ling *et al.* 2000).

*S. cerevisiae* has a mitochondrial genome of 85.8 kb (Foury *et al.* 1998). Respiratory-competent yeast cells contain 50–100 copies of mtDNA that are organized into punctate protein-DNA complexes called mitochondrial nucleoids (mt-nucleoids) with a diameter of 20–50 nm (Williamson and Fennell 1979; Miyakawa *et al.* 1987, 1995). Each individual nucleoid, estimated to contain 3–4 copies of mtDNA, has been proposed to be the unit of mtDNA inheritance (Lockshon *et al.* 1995). If this is the case, it could resolve the discrepancy between the number of segregating units for mtDNA being smaller than the number of mtDNA molecules (Dujon 1981; Gingold 1988). A subset of proteins that are enriched in mt-nucleoids purified by sedimentation has been identified (Miyakawa *et al.* 1987; Newman *et al.* 1996). *In organello* formaldehyde crosslinking studies have shown that up to 20 proteins are crosslinked to mtDNA (Kaufman *et al.* 2000). Among them are Abf2p, Rim1p, and Mgm101p, which are involved in mtDNA maintenance.

Despite the increasing number of genes identified as participating in mtDNA metabolism and the emergence of a better picture for the *in vivo* organization of the mitochondrial genome, initiation of mtDNA replication is not fully understood. It has been proposed that mtDNA replication in *S. cerevisiae* is a transcription-primed process similar to the mechanism observed in vertebrates (Schinkel and Tabak 1989; Schmitt and Clayton 1993). The mitochondrial genome is supposed to contain four active *ori/rep* sequences that are composed of three GC-rich clusters and a mitochondrial promoter. It is believed that transcription from the promoter by the Rpo41 RNA polymerase primes synthesis of the leading strand of DNA replication. This notion has gained support from biochemical experiments showing that DNA replication initiates from the *ori/rep* promoters and that the formation of transcription-dependent RNA/DNA hybrids is detected in these sequences (Baldacci *et al.* 1984; Xu and Clayton 1995; Graves *et al.* 1998; Van Dyck and Clayton 1998). Consistent with this notion is genetic evidence showing that *S. cerevisiae* mutants lacking either the mitochondrial RNA polymerase Rpo41p or the mitochondrial transcription initiation factor Mtf1p are unable to maintain a functional mitochondrial genome (Greenleaf *et al.* 1986; Lisowsky and Michaelis 1988). Furthermore, it is known that a subset of mtDNA deletion mutants (called petite or $\rho^-$ mutants), containing an *ori/rep* sequence amplified into tandem repeats, has a "replicative advantage" over the wild-type ($\rho^+$) genome. These particular $\rho^-$ mutants, called hypersuppressive (HS) mutants, can give rise to diploid progeny composed almost exclusively of petite mutants in a cross against a strain harboring a $\rho^+$ DNA, whereas $\rho^-$ genomes lacking *ori-rep* (or neutral petites) do not share the replicative advantage in similar genetic crosses.

However, several studies seem to contradict the transcription-dependent mtDNA replication model. First, an *ori/rep*-devoid $\rho^-$ genome of 35 bp, composed entirely of A·T base pairs, can be stably maintained (Fangman *et al.* 1989). Second, $\rho^-$ genomes can also be stably propagated in the absence of mtRNA polymerase (Fangman *et al.* 1990). Finally, it has been shown that transcription from the *ori/rep* sequences is not essential for the biased inheritance of HS genomes in HS $\rho^-$ × neutral $\rho^-$ crosses (Lorimer *et al.* 1995). These studies suggest that mtDNA replication is independent of *ori/rep* sequences and mtRNA polymerase-driven initiation. An alternative mode may exist for the replication of the $\rho^-$ genome devoid of *ori/rep* sequences. A recombination-mediated DNA replication process that converts a recombinational intermediate into a replication fork, as in the bacteriophage $T_4$, has been proposed to occur in the replication of *S. cerevisiae* mtDNA (Fangman *et al.* 1990; Preiser *et al.* 1996; MacAlpine *et al.* 1998). Support for such a replication mechanism comes from studies showing the presence of single- or double-stranded lariats and large concatemers (Maleszka *et al.* 1991; Bendich 1996) and the correlation between the level of recombinational intermediates and the mtDNA copy number and stability (MacAlpine *et al.* 1998; Zelenaya-Troitskaya *et al.* 1998).

In this study, we demonstrate that the mitochondrial genome maintenance gene, *MGM101*, is required for the propagation of $\rho^+$ and petite genomes lacking an *ori/rep* sequence but is dispensable for HS mtDNA. The results suggest that the replication of $\rho^-$ genomes devoid of *ori/rep* needs the participation of a distinct set of proteins, which can be spared by the presence of a highly amplified *ori/rep* sequence in HS strains. As the maintenance of a wild-type mitochondrial genome is also *MGM101* dependent (Chen *et al.* 1993; Meeusen *et al.* 1999), its replication is likely to be mediated by the proposed *ori/rep*-independent mtDNA replication mechanism.

## MATERIALS AND METHODS

**Media and strains:** Complete medium for growth of yeast (GYP) contains 0.5% Bacto yeast extract, 1% Bacto peptone, and 2% glucose. Glycerol medium (GlyYP) contains 2% glycerol instead of glucose. Minimal medium (GMM) contains 0.17% Difco yeast nitrogen base without amino acids, 0.5% ammonium sulfate, and 2% glucose. Nutrients essential for auxotrophic strains were added to GMM at 25 μg/ml for bases and 50 μg/ml for amino acids. GYP containing 5 and 10%

**TABLE 1**

**Genotype and source of *S. cerevisiae* strains used in this study**

| Strains | Relevant genotype | Source or reference |
|---|---|---|
| CS6 | *MAT*α/*MAT***a** *leu2/leu2 ura3/ura3 his4/+ ade2/+ ade3/+ mgm101::URA3/+* | This study |
| DS400/A3 | *MAT*α *met* ρ$^-$ | Macino and Tzagoloff (1979) |
| DS400/A3/Δabf2 | Same as DS400/A3, but *abf2*Δ*::kan* | This study |
| DS400/A3/Δcce1 | Same as DS400/A3, but *cce1*Δ*::kan* | This study |
| DS400/A3/Δmgm101 | Same as DS400/A3, but *mgm101*Δ*::kan* | This study |
| DS400/A3/Δmhr1 | Same as DS400/A3, but *mhr1*Δ*::kan* | This study |
| DS400/A3/Δrpo41 | Same as DS400/A3, but *rpo41*Δ*::kan* | This study |
| DS400/N1 | *MAT*α *met* ρ$^-$ | Nobrega and Tzagoloff (1980) |
| E3 | *MAT*α, *his1 trp1* ρ$^-$ | Sor and Fukuhara (1982) |
| E3-U | Same as E3, but *ura3* | This study |
| E3/Δabf2 | Same as E3, but *abf2*Δ*::kan* | This study |
| E3/Δcce1 | Same as E3, but *cce1*Δ*::kan* | This study |
| E3/Δmgm101 | Same as E3, but *mgm101*Δ*::kan* | This study |
| E3/Δmhr1 | Same as E3, but *mhr1*Δ*::kan* | This study |
| E3/Δrpo41 | Same as E3, but *rpo41*Δ*::kan* | This study |
| E3/m25 | Same as E3-U, but *mgm101-1*$^{ts}$ | This study |
| E3/m30 | Same as E3-U, but *mgm101-1*$^{ts}$ | This study |
| E3/m36 | Same as E3-U, but *mgm101-1*$^{ts}$ | This study |
| HSρ$^-$15 | *MAT*α *his3 trp1* [*ori2*, HS ρ$^-$] | G. D. Clark-Walker |
| HSρ$^-$15/Δmgm101 | Same as HSρ$^-$15, but *mgm101*Δ*::kan* | This study |
| HSρ$^-$15/Δabf2 | Same as HSρ$^-$15, but *abf2*Δ*::kan* | This study |
| HSρ$^-$15/Δmhr1 | Same as HSρ$^-$15, but *mhr1*Δ*::kan* | This study |
| HSρ$^-$15/Δcce1 | Same as HSρ$^-$15, but *cce1*Δ*::kan* | This study |
| HSρ$^-$15/Δrpo41 | Same as HSρ$^-$15, but *rpo41*Δ*::kan* | This study |
| HSρ$^-$15/Δrpo41Δmgm101 | Same as HSρ$^-$15/Δrpo41, but *mgm101*Δ*::HIS3* | This study |
| HSρ$^-$20 | *MAT*α *his3 trp1* [*ori5*, HS ρ$^-$] | G. D. Clark-Walker |
| HSρ$^-$20/Δmgm101 | Same as HSρ$^-$20, but *mgm101*Δ*::kan* | This study |
| HS416 | *MAT*α *his1 trp2* Leu$^-$ [*ori3*, HS ρ$^-$] | Blanc and Dujon (1980) |
| HS416/Δmgm101 | Same as HS416, but *mgm101*Δ*::kan* | This study |
| M2915-6A | *MAT***a**, *leu2 ade2 ura3* | Chen *et al.* (1993) |
| M2915-6A/ρ$^0$ | Same as M2915-6A, but ρ$^o$ | This laboratory |
| M2915-7C | *MAT***a**, *leu2 his4 ade2 ura3 mgm101.1* | Chen *et al.* (1993) |
| N1-U2 | Same as DS400/N1, but *ura3* | This study |
| N1-U2/Δmgm101 | Same as N1-U2, but *mgm101*Δ*::kan* | This study |
| N1-U2/Δabf2 | Same as N1-U2, but *abf2*Δ*::kan* | This study |
| N1-U2/Δmhr1 | Same as N1-U2, but *mhr1*Δ*::kan* | This study |
| N1-U2/Δcce1 | Same as N1-U2, but *cce1*Δ*::kan* | This study |
| N1-U2/Δrpo41 | Same as N1-U2, but *rpo41*Δ*::kan* | This study |

glucose, respectively, was used for mating and presporulation medium. Sporulation medium consists of 1% potassium acetate, 0.1% Bacto yeast extract, and 0.05% glucose. For solid medium, 2% Bacto agar was added. G418 medium is GYP supplemented with the drug at 200 μg/ml.

Yeast strains used in this study are listed in Table 1. CS6 is derived from CS4 (Chen *et al.* 1993) by disrupting *MGM101* using the *mgm101::URA3* cassette. N1-U2 and E3-U are derived from DS400/N1 and E3 with the introduction of the *ura3* mutation by selecting for 5-fluoroorotic acid (5-FOA)-resistant colonies on GMM supplemented with 5-FOA (Toronto Research Biochemicals) at 500 μg/ml and uracil at 10 μg/ml.

**Manipulation of *S. cerevisiae*:** Standard methods were used for cell culture, mating, and sporulation (Kaiser *et al.* 1994). Tetrad dissection was undertaken with a Singer MSM System (series 200, Singer Instruments, Somerset, UK) following brief treatment with Zymolyase (Seikaguku, Tokyo). Transformation of yeast cells was performed by the lithium acetate-dimethyl sulfoxide method (Gietz *et al.* 1995). Yeast colony hybridization and total DNA extraction were carried out according to standard procedures (Kaiser *et al.* 1994). Yeast colony PCR was conducted as previously described (Brown and Tuite 1998).

**Southern blot analysis and preparation of mtDNA probes:** Yeast total DNA was electrophoresed on a 1% agarose gel and Southern blot analysis was performed as described (Sambrook *et al.* 1989). For the preparation of mtDNA probes, the mitochondrial genomes were purified on a cesium chloride/bisbenzimide gradient. The ρ$^+$ mtDNA was purified from the strain W303-1B and the 0.43-kb DS400/N1 mtDNA was from DS400/N1. The probe specific for *ori3* was prepared from the *Pst*I-digested pSCM107 plasmid DNA containing the *ori3* sequence. mtDNAs were labeled with [$^{32}$P]dATP using the random-priming GigaPrime DNA labeling kit (Bresatec, Thebarton, Australia). Quantitation of hybridization signals was

**TABLE 2**

**Oligonucleotides used for disruption of *S. cerevisiae* genes**

| Name | Sequence[a] |
|---|---|
| ScABF2P4 | 5′-GAACAGTTACAGCCTATTAACTAGATCTTTCCACGA**CAGCTGAAGCTTCGTACGCT**-3′ |
| ScABF2P5 | 5′-GAGAGTACCGCGGTCTAGTTGAGAGGGTAGCGAGC**CTAGTGGATCTGATATCATC**-3′ |
| ScCCE1P1 | 5′-AGTAATATGTCGACAGCACAGAAAGCTAAGATATTG**CAGCTGAAGCTTCGTACGCT**-3′ |
| ScCCE1P2 | 5′-CAAGTTTGCGATCACCGCACAATTTCTTAGTCATTG**CTAGTGGATCTGATATCATC**-3′ |
| ScMHR1P1 | 5′-CGATATTTCAGGGCAAAAGCATGAAGGTAAACCAT**CAGCTGAAGCTTCGTACGCT**-3′ |
| ScMHR1P2 | 5′-CTACCTGCATTTGTATATCTAATGACGAAGCGTTC**CTAGTGGATCTGATATCATC**-3′ |
| ScRPO41P1 | 5′-TGATTCAAGTCGCTAAGGTATCTGTGCAAGGTGTT**CAGCTGAAGCTTCGTACGCT**-3′ |
| ScRPO41P2 | 5′-AGCAATTTGAAAAGTAGCCCCGACGTAGGTAACAC**CTAGTGGATCTGATATCATC**-3′ |
| ScMGM101HIS3P1 | 5′-AGGAAAGAAAGGACAAGTAGGAAGATCAGCGTACG<u>CGCTGCACGGTCCTGTTCC</u>-3′ |
| ScMGM101HIS3P2 | 5′-ACAGGGTCCCATAATTCAGAACCGACGCCGAGATC<u>TTTCCCGTTCCTCCATCTCTTTTA</u>-3′ |

[a] The sequences complementary to the *kan* module are in boldface type and those complementary to *HIS3* are underlined.

undertaken by using a phosphorimage analyzer (Molecular Dynamics, Sunnyvale, CA) with ImageQuant software (version 5.1).

**Plasmid construction and gene disruption:** The *mgm101::kan* cassette was isolated from the plasmid pScmgm101::kan/1 by digestion with *Sph*I and *Sac*I. The 2.4-kb cassette contains *MGM101* with the *kan* module (Güldener *et al.* 1996) inserted at codon 218. Yeast strains were transformed with the disruption cassette and selected for $G418^R$. Correct disruption of the gene was confirmed by Southern blot analysis of genomic DNA from stable $G418^R$ transformants, after digestion with *Sph*I and *Sac*I, by using a $^{32}$P-labeled 714-bp *Hin*dIII-*Sac*I fragment derived from *MGM101* as a probe. Disruptions of *ABF2*, *CCE1*, *MHR1*, and *RPO41* were conducted by using a PCR-based gene disruption method (Güldener *et al.* 1996; Brown and Tuite 1998). The oligonucleotide primers, listed in Table 2, contain 35–36 nucleotides on the 5′-end matching sequences in the target genes and 20–21 nucleotides complementary to the flanking region of the *kan* module in pFA6a-kanMX4 (Wach *et al.* 1994). The primers used for gene disruptions are as follows: ScABF2P4 and ScABF2P5 for *ABF2*; ScCCE1P1 and ScCCE1P2 for *CCE1*; ScMHR1P1 and ScMHR1P2 for *MHR1*; and ScRPO41P1 and ScRPO41P2 for *RPO41*. For disruption of *MGM101* in HSρ$^-$15/Δrpo41, the primers ScMGM-101HIS3P1 and ScMGM101HIS3P2 were used to amplify the *mgm101Δ::HIS3* cassette with *S. cerevisiae* genomic DNA as template. The PCR DNA was used to transform HSρ$^-$15/Δrpo41 by selecting for stable $His^+$ colonies, resulting in the *rpo41Δ::kan mgm101Δ::HIS3* double mutants. The correct disruption of *MGM101*, *ABF2*, *RPO41*, *MHR1*, and *CCE1* in the yeast strains was confirmed either by Southern blot analysis of genomic DNA or by PCR using appropriate primers.

To introduce the *mgm101-1$^{ts}$* allele into the E3 background, the plasmid pURA-mgm101.1, carrying the *mgm101-1$^{ts}$* allele, was integrated into the *Stu*I site of the *URA3* locus in E3-U by selecting for $Ura^+$ transformants. The wild-type *MGM101* allele was then disrupted by using the *mgm101::kan* cassette. As confirmed by Southern blot analysis, three independent transformants, named E3m25, E3m30, and E3m36, were found to have the *MGM101* locus correctly disrupted while retaining the *mgm101-1$^{ts}$* allele.

## RESULTS

***MGM101* is essential for the maintenance of a functional ρ$^+$ genome:** Several mitochondrial nucleoid proteins, including Abf2p, Ilv5p, Hsp60p, Kgd2p, and Rim1p, have been reported to be involved in the maintenance of mtDNA stability in *S. cerevisiae* (Kaufman *et al.* 2000). In the case of Abf2p and Ilv5p, although null mutants of the genes lose their ρ$^+$ mtDNA when grown in complete glucose medium, a functional mitochondrial genome can be maintained when cells are grown on a nonfermentable carbon source such as glycerol (Diffley and Stillman 1992; Megraw and Chae 1993; Zelenaya-Troitskaya *et al.* 1995). Consequently, these proteins are not directly involved in the essential steps of mtDNA replication or are functionally redundant with unidentified proteins. On the other hand, disruption of the *RIM1* gene, which encodes a mitochondrial single-stranded DNA-binding protein directly involved in mtDNA synthesis, results in cells that are unable to maintain mtDNA even on glycerol medium (Van Dyck *et al.* 1992). Previous studies have shown that inactivation of *MGM101* converts cells into respiratory-deficient mutants on glucose medium (Chen *et al.* 1993; Meeusen *et al.* 1999). To know whether *MGM101* participates in an essential step of mtDNA replication/maintenance, we dissected the strain CS6 (*mgm101::URA3/+, ura3/ura3*) on a glycerol plate. It was found that in addition to two large $Ura^-$ segregants, each tetrad gave rise to two spores that form microcolonies of 300–1000 cells. These spores are all $Ura^+$ when tested on GMM minus uracil medium but cannot be further propagated when transferred to a fresh GlyYP plate. This observation suggests that cells are unable to maintain a functional ρ$^+$ genome upon inactivation of *MGM101* even in the presence of a strong selective pressure against loss of mtDNA.

***mgm101* mutants are likely to be blocked in mtDNA replication:** The rapid cell growth arrest of *mgm101* mutants on glycerol medium favors the proposal that the primary role of Mgm101p might be a direct participation in mtDNA replication. To examine this possibility, we analyzed changes to the ρ$^+$ mitochondrial genome in a conditional *mgm101* mutant upon a shift to restrictive temperature. Figure 1A shows that the *mgm101* mutant

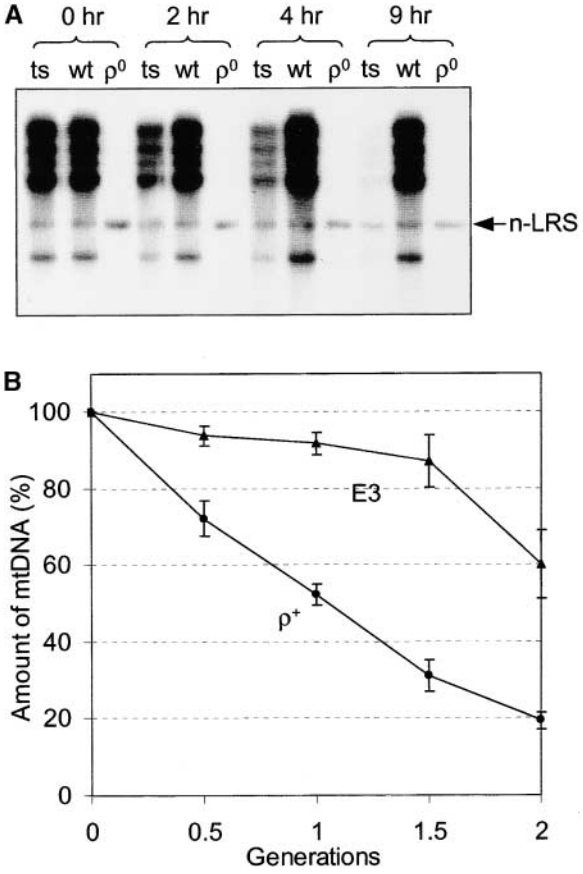


FIGURE 1.—Time course analysis showing the loss of the $\rho^+$ and the E3 $\rho^-$ genomes from *mgm101-1*$^{ts}$ cells upon a shift to the restrictive temperature. (A) A representative example of three independent Southern blot analyses of yeast total DNA showing the loss of the $\rho^+$ genome in M2915-7C (*mgm101-1*$^{ts}$). Yeast strains were grown at 25° overnight to a late exponential phase and diluted into fresh GYP to an $OD_{600}$ of ~0.5. The cultures were shifted to 37° and aliquots were removed at different time points before $OD_{600}$ reached 2.0. The harvested cell fractions were therefore all at the exponential phase within two cell divisions (not shown). Total DNA was extracted, digested with *Cfo*I, and analyzed by Southern blots using $^{32}$P-labeled $\rho^+$ mtDNA as a probe. The level of mtDNA in the strains relative to the n-LRS, which cross-hybridizes to mtDNA LRS (our unpublished observation), was quantified by phosphorimaging. ts, M2915-7C (*mgm101-1*$^{ts}$); wt, M2915-6A; $\rho^o$, M2915-6A/$\rho^o$. (B) The levels of the $\rho^+$ and the E3 $\rho^-$ genomes in M2915-7C (*mgm101-1*$^{ts}$) and E3/m25 (*mgm101-1*$^{ts}$) are plotted against the number of doubling times (generations) to show the kinetics of mtDNA decrease from growing *mgm101-1* cells under restrictive conditions. Three independent experiments were carried out for each strain and the standard errors for each time point are indicated.

has a decreasing mtDNA level under the restrictive condition of 37°. The mtDNA level relative to the nuclear large ribosomal subunit gene (n-LRS), which cross-hybridizes to mtDNA LRS (our unpublished observation), was estimated and plotted against cell doubling (Figure 1B). It was revealed that at the first doubling time, mtDNA decreased by 48%. At the second doubling time, only 19% of mtDNA was retained. The rapid decrease of total mtDNA in the cell population, which is reciprocally related to cell doubling, suggests that an efficient block in mtDNA synthesis occurs in cells inactivated in Mgm101p.

From Figure 1A, it appears that inactivation of *MGM101* does not change the mtDNA profile other than causing a decrease in the amount of mtDNA. When undigested DNA was subjected to Southern blot analysis, the hybridization signals from the *mgm101-1* mutants exposed to restrictive temperature were found mainly in a high-molecular-weight position as in a wild-type strain (not illustrated). No prominent bands were detected that could be interpreted as products of specific mtDNA deletion or degradation. In addition, it was found that the effect of Mgm101p inactivation is not manifested under a nongrowth condition. After the *mgm101-1* mutant, M2915-7C, was incubated in water at 37° for up to 24 hr, no significant increase in petite frequency was observed when cells were returned to the permissive temperature (data not shown). This finding suggests that the loss of Mgm101p function is reversible and that the mitochondrial genome remains intact under the nongrowth condition.

**Escape of $\rho^-$ genomes from *MGM101* disruption:** In Southern blot analysis of *mgm101*-disrupted cells, we detected the presence of mtDNA in some mutant strains (not illustrated). This raised the possibility that upon disruption of *MGM101*, a subpopulation of cells could have rearrangements in mtDNA giving rise to a $\rho^-$ genome that can be subsequently maintained. To examine this notion, we dissected CS6 (*mgm101::URA3*/+) on GYP plates (Figure 2A) followed by testing for the presence of mtDNA by colony hybridization (Figure 2B). It was found that after a long exposure for 7 days, ~25% of *mgm101::URA3* segregants had detectable signals when hybridized to a total mtDNA probe. These segregants are all respiratory deficient as they do not grow on GlyYP medium. The presence of mtDNA in the *mgm101::URA3* strains was further confirmed by Southern blot analysis. A total of 10 *mgm101::URA3* segregants were chosen at random and 4 of them were found to contain $\rho^-$ mtDNA hybridizing to a total mtDNA probe (data not shown).

To know whether the detection of the hybridization signals in *mgm101::URA3* segregants is due to a delayed loss of mtDNA, the strains were subcloned on GYP plates for three rounds, equivalent to ~60 generations. Individual colonies were subsequently examined for the presence of mtDNA by hybridization. It was found that all colonies gave rise to positive signals indicative of the presence of mtDNA (data not shown), suggesting that the detected $\rho^-$ genomes in the *mgm101::URA3* strains can be stably maintained.

The mtDNA-containing *mgm101::URA3* segregants were examined further by Southern blot analysis. It was found that all the retained $\rho^-$ genomes contained an *ori/rep* sequence (Figure 2C). The six clones, chosen at random, all contained sequences that hybridized to an *ori3* probe. These results suggest that an amplification

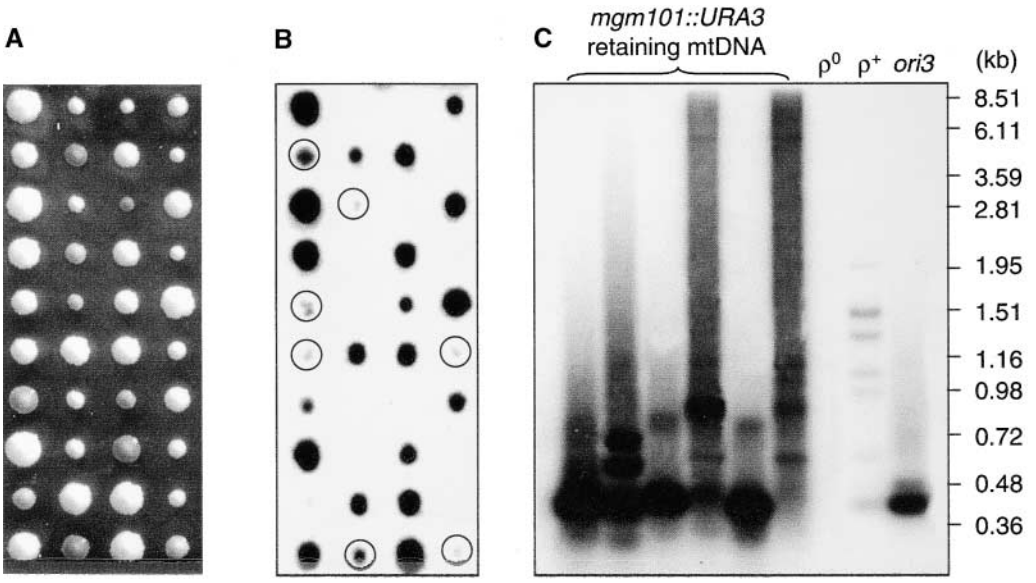


FIGURE 2.—Genome deletion/rearrangement leads to the escape of ρ⁻ genomes from the requirement for *MGM101* in meiotic segregants. (A) Dissection of asci from CS6 (+/*mgm101::URA3*) on GYP medium. (B) CS6 tetrads were analyzed for the presence of mtDNA by colony hybridization using a total mtDNA probe. Circled are the *mgm101::URA3* segregants giving rise to a positive signal on hybridization. (C) Southern blot analysis showing that the ρ⁻ genomes that escaped from *MGM101* disruption contain an *ori/rep* sequence. Total DNA was extracted from six mtDNA-containing *mgm101::URA3* segregants identified by the colony hybridization and digested with *Dra*I. After electrophoresis on a 1% agarose gel, DNA was transferred to nylon membrane and hybridized to a $^{32}$P-labeled *ori3* probe. As controls, M2915-6A/ρ° (ρ°), M2915-6A (ρ⁺), and HS416 (*ori3*) were included in the analysis.

of an *ori/rep* sequence could be required to escape the need for Mgm101 function.

**Mgm101p is dispensable for the maintenance of HS ρ⁻ genomes:** The above observations prompted us to investigate the role of *MGM101* in the maintenance of genetically well-defined HS petite genomes that contain an amplified *ori/rep* sequence. *MGM101* was disrupted in the strains HSρ⁻15, HS416, and HSρ⁻20, which contain an amplified *ori2*, *-3*, and *-5* of 1.9, 0.7, and 2.1 kb, respectively. The resulting strains, HSρ⁻15/Δmgm101, HS416/Δmgm101, and HSρ⁻20/Δmgm101, were examined by Southern blot analysis (Figure 3). It was found that disruption of *MGM101* does not significantly affect the mtDNA copy number in the cell populations. The hybridization pattern of mtDNA in the *mgm101*-disrupted strains is almost identical to their parental strains with a wild-type *MGM101*. These data indicate that loss of Mgm101 function does not have a significant effect on maintenance of the three HS genomes.

**Involvement of other genes in maintenance of HS ρ⁻ mtDNA:** The lack of a requirement for *MGM101* in maintenance of HS ρ⁻ mtDNA prompted us to examine whether other genes, especially ones involved in recombination, are similarly dispensable. We chose for this analysis *MHR1*, encoding a putative recombinase, and *CCE1*, encoding a cruciform-cutting endonuclease (KLEFF *et al.* 1992; LING *et al.* 1995, 2000; WHITE and LILLEY 1996). For comparison, as possible controls, we included the *ABF2* and *RPO41* genes that are required for maintenance of ρ⁺ mtDNA, but are not needed for ρ⁻ genomes (FANGMAN *et al.* 1990; ZELENAYA-TROITSKAYA *et al.* 1998; SEDMAN *et al.* 2000). Consequently, each of the above genes was disrupted in HSρ⁻15 and retention of the *ori/rep*-containing mtDNA was examined by Southern blot analysis before (Figure 4A) and after (Figure 4B) restriction enzyme digestion. Although all disrupted strains retain HSρ⁻15 mtDNA, the one lacking *CCE1* shows the greatest decline, retaining 14% of the mtDNA

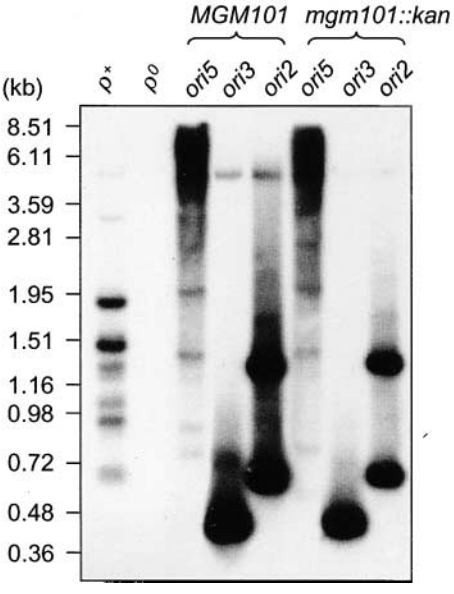


FIGURE 3.—Southern blot analysis showing that disruption of *MGM101* does not affect the maintenance of hypersuppressive ρ⁻ genomes. Total DNA was extracted from yeast strains, digested with *Dra*I, and probed with $^{32}$P-labeled total mtDNA. The strains examined include M2915-6A (ρ⁺), M2915-6A/ρ° (ρ°), HSρ⁻20 (*MGM101*, *ori5*), HS416 (*MGM101*, *ori3*), HSρ⁻15 (*MGM101*, *ori2*), HSρ⁻20/Δmgm101 (*mgm101::kan*, *ori5*), HS416/Δmgm101 (*mgm101::kan*, *ori3*), and HSρ⁻15/Δmgm101 (*mgm101::kan*, *ori2*).

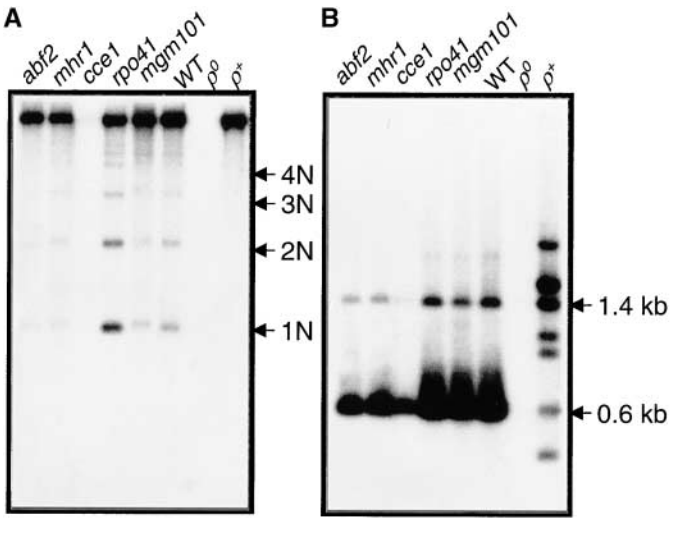


FIGURE 4.—Effect of gene disruptions on the abundance of the hypersuppressive HSρ⁻15 mtDNA. Total DNA of equal absorbance at 260 nm was analyzed by Southern blots before (A) and after (B) digestion with *Dra*I. The DNA samples are from HSρ⁻15 (WT) and its *abf2*, *mhr1*, *cce1*, *rpo41*, and *mgm101* disruption derivatives. ρ⁰ and ρ⁺ controls are M2915-6A/ρ⁰ and M2915-6A, respectively. The *ori3* sequence was used as a probe that identifies the 0.6- and 1.4-kb fragments released from the digestion of the HSρ⁻15 ρ⁻ mtDNA by *Dra*I. (C) Histogram for a comparison of the total amount of mtDNA in the strains disrupted in various genes, relative to their parent HSρ⁻15 (WT).

present in the parent (Figure 4C). In Figure 4A, showing unrestricted mtDNA, the presence of the *ori/rep* genome in the *CCE1* disrupted strain is barely detectable. The apparent lack of mtDNA in the unrestricted mutant sample could be due to the presence of networks, accumulating in the absence of the cruciform-cutting endonuclease, that prevent DNA from entering the gel.

***MGM101* is required for the propagation of ρ⁻ genomes devoid of *ori/rep*:** To know whether the formation of a ρ⁻ genome by the amplification of any mtDNA segment is sufficient to escape the requirement for Mgm101p, we examined the effect of *mgm101* mutation on ρ⁻ genomes that do not contain an *ori/rep* sequence. The petite mutants E3, DS400/A3, and DS400/N1 harbor ρ⁻ genomes of 1.6, 1.8, and 0.43 kb, respectively. *MGM101* was disrupted in the three mutants and mtDNA in the resulting strains was examined by Southern blot analysis. Determination of 15 independent *mgm101* isolates, each derived from E3 or DS400/A3, revealed that all clones have completely lost their mtDNA. An example is given in Figure 5A, which shows that the E3 and DS400/A3 ρ⁻ genomes are no longer detectable in E3/Δmgm101 and A3/Δmgm101. These results indicate that *MGM101* is essential for the maintenance of these two ρ⁻ genomes.

The requirement for Mgm101p in the maintenance of an *ori/rep*-devoid ρ⁻ genome was further confirmed by examining the loss of the E3 mtDNA in the *mgm101-1*$^{ts}$ background. The strains E3m25, E3m30, and E3m36 were grown at the restrictive temperature of 37° and loss of the E3 mtDNA in the cell population was monitored by Southern blot analysis (data not shown). It was found that at the first doubling time, the mtDNA level was decreased by only 8% (Figure 1B) while 60% of mtDNA was retained at the second doubling time. However, a complete loss of the E3 ρ⁻ genome was observed after growth for approximately five doubling times under the restrictive conditions (data not shown). From these data, it can be concluded that Mgm101p is essential for the stable maintenance of the E3 ρ⁻ genome. In contrast to the ρ⁺ mtDNA, the highly repeated E3 ρ⁻ genome has a delayed mtDNA loss in *mgm101* cells.

A different situation was observed with the DS400/N1 ρ⁻ genome. As shown in Figure 5B (left), hybridization of unrestricted total DNA from DS400/N1 (*MGM101*) with a probe specific for the ρ⁻ DS400/N1 repeat shows the presence of two species of mtDNA molecules: those in the form of circular oligomers and those in a distinct band of higher molecular weight that is most likely concatemers formed by DS400/N1 repeats. We found that upon the disruption of *MGM101*, cells still retain a measurable level of the DS400/N1 ρ⁻ mtDNA. When 12 independent *mgm101* isolates were examined by Southern blotting, the presence of a markedly reduced level of mtDNA, hybridizable to the DS400/N1 ρ⁻ genome, was detected in all clones (not illustrated). Quantitation by phosphorimaging showed that the total amount of mtDNA in N1/Δmgm101, as exemplified in Figure 5B (left), is reduced to 8–21% of a strain with a wild-type *MGM101*, depending on different isolates. The amount of mtDNA molecules represented in the circular oligomer and concatemer forms appears to be proportionally reduced. When total DNA was analyzed after digestion with *Sau*3AI, which has a single site in the DS400/N1 sequence, repeats were released as a band of 0.43 kb (Figure 5B, right). Phosphorimager analysis estimates a reduction of these repeats in N1/Δmgm101

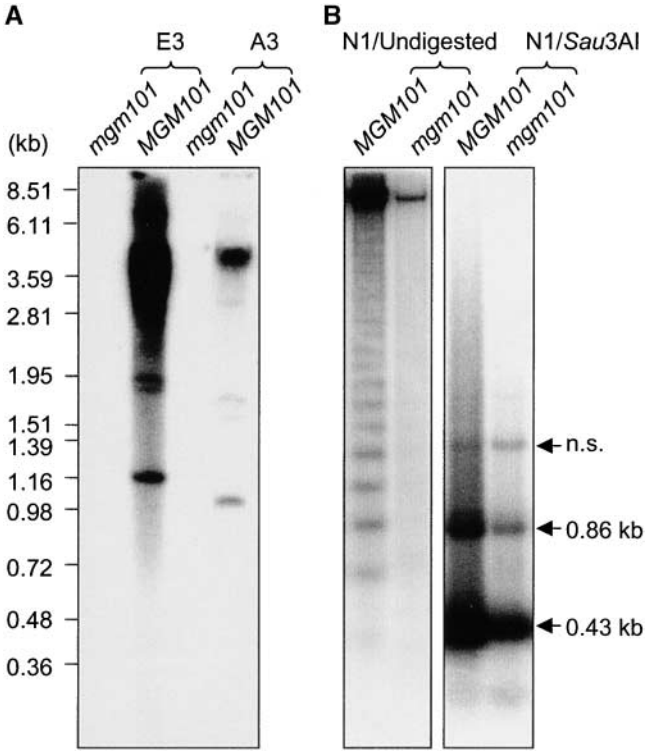


FIGURE 5.—Southern blot analysis showing that *MGM101* is required for the propagation of ρ⁻ mtDNA lacking an *ori/rep* sequence. (A) Equal amounts of total DNA from E3/Δmgm101 (E3, *mgm101*), E3 (E3, *MGM101*), DS400/A3/Δmgm101 (A3, *mgm101*), and DS400/A3 (A3, *MGM101*) were analyzed using a *S. cerevisiae* total mtDNA probe. (B) Total DNA was extracted from DS400/N1 (*MGM101*) and N1-U2/Δmgm101 (*mgm101*) and equal amounts of DNA, as estimated by $OD_{260}$, were analyzed before (left) and after (right) digestion with *Sau*3AI. The probe used was $^{32}$P-labeled DS400/N1 ρ⁻ mtDNA purified from a cesium chloride gradient. The 0.43-kb DS400/N1 repeat and the 0.86-kb recombination intermediate are indicated. n.s., a nonspecific band resulting from cross-hybridization between nuclear DNA and the DS400/N1 repeat.

(*mgm101Δ::kan*) to ~15% of the level in DS400/N1 (*MGM101*), which is comparable to the amount found from unrestricted DNA.

In the analysis of retained mtDNA in N1-U2/Δmgm-101 digested with *Sau*3AI (Figure 5B, right), a distinct band of 0.86 kb, which is double the size of the DS/400/N1 repeat, can be reproducibly detected. This band is inferred to be molecules comprised of two DS/400/N1 repeats linked by a recombination junction, as suggested by others (LOCKSHON *et al.* 1995; MACALPINE *et al.* 1998). It was found that although total mtDNA is decreased by over sixfold in N1-U2/Δmgm101 (*mgm101Δ::kan*) compared with DS/400/N1 (*MGM101*), both strains have the 0.86-kb recombination intermediate at 11% of the total mtDNA. From the observation that disruption of *MGM101* does not change the proportion between the circular oligomers and the linear concatemers, it can be concluded that *MGM101* does not noticeably affect the level of homologous recombination of the DS/400/N1 genome.

However, although the analysis of *Sau*3AI-restricted DNAs revealed the presence of the 0.43-kb DS/400/N1 repeats in N1/Δmgm101 (*mgm101Δ::kan*), which has an apparent size similar to ones in a wild-type *MGM101* culture, the mtDNAs in the two strains may not be identical. When the autoradiograph involving the analysis of unrestricted DNA was overexposed (not illustrated), the oligomers from N1/Δmgm101 did not appear to have the same gel mobility compared with those from DS400/N1. The DS400/N1 mtDNA in the *mgm101* mutant may have been subjected to some minor changes that alter the conformation of the oligomers.

The above observation raised the possibility that inactivation of *MGM101* may destabilize the DS400/N1 genome, leading to a rearranged or mutated version that is no longer dependent on *MGM101* for its replication. Indeed, when we subcultured N1-U2/Δmgm101 on GYP medium and examined 24 clones by Southern blotting, it was found that mtDNA could not be detected in 16 clones (66.7%) and that the eight remaining strains (33.3%) displayed variable levels of mtDNA (data not illustrated). The mtDNA also showed variable banding patterns as revealed by hybridization analysis of undigested DNA. It therefore appears that *MGM101* is required for maintenance of DS400/N1 mtDNA but possible mutations in this ρ⁻ genome can generate molecules that are stably maintained in the absence of Mgm101p.

**Involvement of other genes in maintenance of *ori/rep*-devoid ρ⁻ genomes:** The data described above indicate that *MGM101* is dispensable for the maintenance of *ori/rep*-containing ρ⁻ genomes but is essential for the propagation of *ori/rep*-devoid ρ⁻ mtDNA. To know whether this feature is unique to *MGM101* among the mtDNA maintenance genes, the effects of disrupting *ABF2*, *MHR1*, *CCE1*, and *RPO41* on the three nonsuppressive mtDNAs in DS400/N1, E3, and DS400/A3 were examined. As an example, the effects of the disruptions on the DS400/N1 ρ⁻ genome are illustrated in Figure 6, A and B. As summarized in Figure 6C, a substantial fraction of the three ρ⁻ mtDNAs is present in cells disrupted in *ABF2*, *MHR1*, and *RPO41*. As in the case of the HSρ⁻15 genome (Figure 4), strains disrupted in *CCE1* show a strong decline in mtDNA. These observations are in sharp contrast to the *mgm101* mutants in which the DS400/A3 and E3 ρ⁻ genomes are hardly measurable, while a low level of mutated mtDNA derived from the DS400/N1 mtDNA can still be detected (see above). It can therefore be concluded that *MGM101* is distinct in that it is dispensable for the replication of a HS ρ⁻ mtDNA but is essential for the propagation of the three *ori/rep*-devoid ρ⁻ genomes examined in this study.

***mgm101* and *rpo41* double mutants can stably maintain a HS ρ⁻ genome:** In light of the results described above, it is evident that Mgm101p participates in a mtDNA replication mechanism essential for the propagation of *ori/*

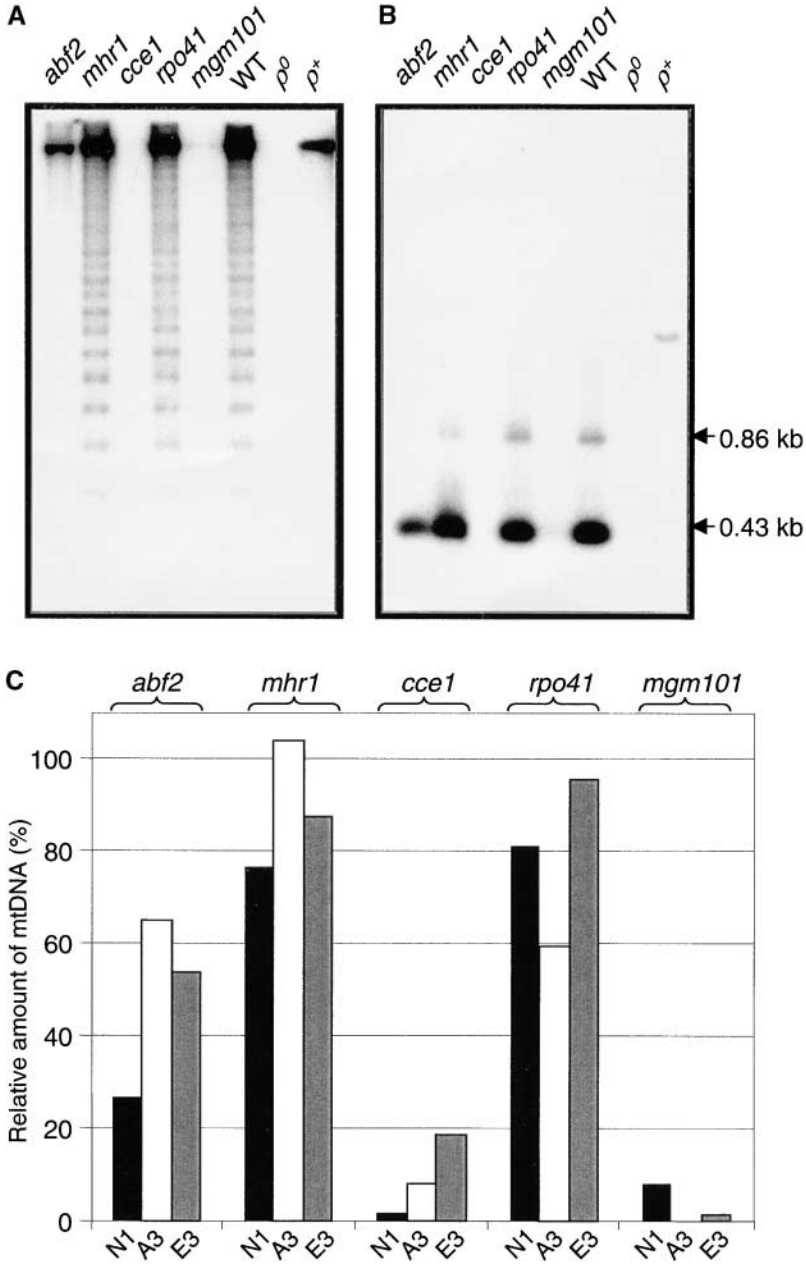


FIGURE 6.—Effect of gene disruptions on the abundance of the DS400/N1, DS400/A3, and E3 $\rho^-$ mtDNAs. (A and B) A representative Southern blot analysis showing the effect of gene disruptions on the DS400/N1 $\rho^-$ genome. Total DNA of equal absorbance at 260 nm was analyzed by Southern blot analysis before (A) and after (B) digestion with *Sau*3AI. The DNA samples are from DS400/N1 (WT) and its *abf2*, *mhr1*, *cce1*, *rpo41*, and *mgm101* disruption derivatives. $\rho^o$ and $\rho^+$ controls are M2915-6A/$\rho^o$ and M2915-6A, respectively. The probe, specific for the DS400/N1 genome, identifies the 0.43-kb repeat unit and the 0.86-kb recombination intermediate after digestion with *Sau*3AI. (C) Histogram for a comparison of the amount of mtDNA in the strains disrupted in various genes relative to that in the parental strains DS400/N1, DS/400/A3, and E3.

*rep*-devoid $\rho^-$ mtDNA. However, the HS $\rho^-$ genomes, containing an amplified *ori/rep*, could be replicated by a distinct mechanism involving transcription by the Rpo41 RNA polymerase. As individual disruption of *MGM101* and *RPO41* has little effect on the propagation of the HS $\rho^-$ genomes, the possibility was considered that replication of HS $\rho^-$ mtDNA may proceed either by an *MGM101*-based mechanism in the absence of *RPO41* or by a *RPO41*-mediated process in strains lacking *MGM101*. To test this possibility, we disrupted both *MGM101* and *RPO41* in HS$\rho^-$15-carrying *ori2*. Southern blot analysis of four independent double mutants showed that such strains have a level of HS$\rho^-$15 $\rho^-$ mtDNA comparable to that of *mgm101* or *rpo41* single mutants (not illustrated). Thus, a combination of the two mutations does not have a synergistic effect in destabilizing the HS $\rho^-$ genome.

## DISCUSSION

Initiation of mtDNA replication in yeast is still a matter of controversy (SHADEL 1999; LECRENIER and FOURY 2000). Central to the issue are the roles of *ori/rep* sequences and the mitochondrial RNA synthesis machinery. Studies have shown that synthesis of a RNA primer from the *ori/rep* sequences does not play a significant role in the replication of $\rho^-$ mtDNA (FANGMAN *et al.* 1990). An alternative model, implicating recombination, has been proposed (FANGMAN *et al.* 1990; PREISER *et al.* 1996; MACALPINE *et al.* 1998). However, because

the HS $\rho^-$ petites still retain their hypersuppressiveness in the absence of the mitochondrial RNA polymerase gene *RPO41* (Lorimer *et al.* 1995), the possibility was raised that the highly amplified *ori/rep* sequences might initiate DNA replication by a mechanism distinct from the one used by $\rho^+$ mtDNA and $\rho^-$ mtDNA devoid of *ori/rep*. This mechanism would not need transcriptional priming by the Rpo41 RNA polymerase.

In this study we have found that the mitochondrial genome maintenance gene, *MGM101*, is required for the replication of $\rho^+$ and *ori/rep*-devoid $\rho^-$ genomes but is not needed for HS $\rho^-$ mtDNA. The effect on the maintenance of $\rho^+$ and *ori/rep*-devoid $\rho^-$ genomes is likely to be through a direct role of the Mgm101 protein in mtDNA replication. Genes required for the maintenance of $\rho^+$ but not for $\rho^-$ mtDNA have previously been reported and include *ABF2*, *RPO41*, and *HMI1*, as well as ones involved in mitochondrial protein synthesis (Weislogel and Butow 1970; Myers *et al.* 1985). These genes, as described by other investigators and supported by this study, are not essential for the propagation of either HS $\rho^-$ or $\rho^-$ mtDNA devoid of *ori/rep* (Fangman *et al.* 1990; Van Dyck and Clayton 1998; Zelenaya-Troitskaya *et al.* 1998; Sedman *et al.* 2000). As mtDNA maintenance genes that discriminate between these two types of $\rho^-$ genomes have not been reported, *MGM101* is therefore the first gene of this category.

An indication that Mgm101p has a role in replication of mtDNA arises from analysis of the $\rho^+$ genome in the temperature-sensitive *mgm101-1* mutant. Upon inactivation of the mutant Mgm101p at the restrictive temperature, it was found that the total amount of mtDNA is almost halved at each cell division. This rapid diminution suggests that mtDNA replication is immediately blocked on the loss of Mgm101 function. In a recent report, it was shown clearly that Mgm101p does not play any appreciable role in mtDNA packaging and segregation/partitioning (Meeusen *et al.* 1999). However, by using an *in vivo* detection technique involving indirect immunofluorescence, these investigators found the incorporation of 5-bromodeoxyuridine into mitochondria when the *mgm101-2* mutant was transferred to the restrictive temperature, suggesting that mtDNA synthesis continues in the absence of a functional Mgm101p. It has been suggested that the observed phenomenon may reflect rolling circle replication that is independent of initiation (Meeusen *et al.* 1999). However, in view of the present results, the detection of nucleotide incorporation might have arisen from replication of *ori/rep*-containing $\rho^-$ mtDNA derived from the $\rho^+$ genome, since replication of such genomes is no longer dependent on *MGM101*. In support of this notion is the observation that *ori/rep*-containing $\rho^-$ mtDNAs occur in 25% of meiotic *mgm101* segregants.

The mtDNA replication mechanism, which involves Mgm101p, could be a rolling circle process initiated by recombination (Fangman *et al.* 1990; Maleszka *et al.* 1991; Preiser *et al.* 1996; MacAlpine *et al.* 1998). Recombination-dependent DNA replication has been well documented in *Escherichia coli* and the bacteriophage $T_4$ (Asai *et al.* 1994; for a recent review, see Kowalczykowski 2000). Following the initiation of recombination, DNA strand invasion by the 3′ end of ssDNA allows it to serve as a primer for DNA replication. A recombination-dependent mtDNA replication mechanism in yeast has drawn strong support from the correlation between the level of recombinational intermediates and mtDNA copy number and stability (MacAlpine *et al.* 1998; Zelenaya-Troitskaya *et al.* 1998). However, the adoption of such a mechanism leaves many intriguing questions to be answered. For example, how is a recombination intermediate converted into a replication fork? Is there any specific type of recombination intermediate required for replication initiation or is the one created by homologous recombination sufficient for the loading of the DNA replication machinery? If the Mgm101 protein is involved in mtDNA replication, does it intervene in the formation of a replication-competent recombination intermediate?

Because HS $\rho^-$ genomes can be replicated in a *MGM101*-independent manner, it is likely that *ori/rep* sequences participate in an alternative replication initiation mechanism. The *ori/rep* sequences are known to contain three GC-rich clusters designated A, B, and C and it has been well documented that GC clusters are highly recombinogenic (Dieckmann and Gandy 1987; Zinn *et al.* 1988; Clark-Walker 1989). Double-stranded DNA breaks can be readily detected in GC-rich sequences (Zinn *et al.* 1988; Van Dyck and Clayton 1998). Thus, one explanation for the initiation of mtDNA replication could be that the *ori/rep* sequences form structures that prime DNA synthesis. Establishment of these structures would not require Mgm101p or Rpo41p because HS genomes can be stably maintained in *rpo41 mgm101* double mutants. Alternatively, the *ori/rep* sequences may utilize a primase to initiate replication. A mitochondrial primase activity has been previously detected (Desai *et al.* 1989) but the identity of the encoding gene(s) remains to be established (reviewed by Lecrenier and Foury 2000).

Three *ori/rep*-devoid $\rho^-$ genomes have been examined for the effect of *MGM101* disruption. It is interesting to find that loss of the E3 $\rho^-$ genome occurs in *MGM101*-inactivated cells at a slower rate compared with the $\rho^+$ mtDNA. This observation could reflect the presence of preinitiated replicative circles derived from the highly repetitive $\rho^-$ genome that causes a lag in mtDNA loss upon *MGM101* inactivation. It is also interesting to find, in contrast to the E3 and DS400/A3 $\rho^-$ mtDNAs where the mitochondrial genomes are rapidly lost upon the inactivation of *MGM101*, that the DS400/N1 $\rho^-$ genome can be maintained in some *mgm101*Δ cells. Replication of a portion of mtDNA is apparently no longer dependent on Mgm101p, suggesting a possible change has occurred. Although it is known that the DS400/N1 se-

quence does not contain any recognizable GC clusters (Nobrega and Tzagoloff 1980), it is possible that a putative mutation in the $\rho^-$ genome has created a structure functionally resembling *ori/rep* that can initiate mtDNA replication in a Mgm101p-independent manner.

In summary, this study suggests that two distinct mtDNA replication initiation mechanisms operate in *S. cerevisiae*. The synthesis of $\rho^+$ and *ori/rep*-devoid $\rho^-$ genomes is dependent on Mgm101p whereas replication of *ori/rep*-containing $\rho^-$ mtDNA proceeds in its absence. In this context, the presence of three or four *ori/rep* sequences at low density in the $\rho^+$ genome may not play any significant role in mtDNA replication. Support for this notion comes from studies of another budding yeast, *K. lactis*, where mtDNA maintenance is dependent on *MGM101* but *ori/rep* sequences are lacking (Clark-Walker and Chen 1996).

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## LITERATURE CITED


Asai, T., D. B. Bates and T. Kogoma, 1994 DNA replication triggered by double-stranded breaks in *E. coli*: dependence on homologous recombination functions. Cell **78:** 1051–1061.

Baldacci, G., B. Cherif-Zahar and G. Bernardi, 1984 The initiation of DNA replication in the mitochondrial genome of yeast. EMBO J. **3:** 2115–2120.

Bendich, A. J., 1996 Structural analysis of mitochondrial DNA molecules from fungi and plants using moving pictures and pulsed-field gel electrophoresis. J. Mol. Biol. **255:** 564–588.

Blanc, H., and B. Dujon, 1980 Replicator regions of the yeast mitochondrial DNA responsible for suppressiveness. Proc. Natl. Acad. Sci. USA **77:** 3942–3946.

Brown, A. J. P., and M. F. Tuite, 1998 *Yeast Gene Analysis*. Academic Press, San Diego.

Chen, X. J., M. X. Guan and G. D. Clark-Walker, 1993 *MGM101*, a nuclear gene involved in maintenance of the mitochondrial genome in *Saccharomyces cerevisiae*. Nucleic Acids Res. **21:** 3473–3477.

Chi, N. W., and R. D. Kolodner, 1994 Purification and characterization of *MSH1*, a yeast mitochondrial protein that binds to DNA mismatches. J. Biol. Chem. **269:** 29984–29992.

Clark-Walker, G. D., 1989 *In vivo* rearrangement of mitochondrial DNA in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **86:** 8847–8851.

Clark-Walker, G. D., and X. J. Chen, 1996 A vital function for mitochondrial DNA in the petite-negative yeast *Kluyveromyces lactis*. Mol. Gen. Genet. **252:** 746–750.

Contamine, V., and M. Picard, 2000 Maintenance and integrity of the mitochondrial genome: a plethora of nuclear genes in the budding yeast. Microbiol. Mol. Biol. Rev. **64:** 281–315.

Desai, S. D., K. Pasupathy, K. G. Chetty and D. S. Pradhan, 1989 Evidence for the presence of DNA primase in mitochondria of *Saccharomyces cerevisiae*. Biochem. Biophys. Res. Commun. **160:** 525–534.

Dieckmann, C. L., and B. Gandy, 1987 Preferential recombination between GC clusters in yeast mitochondrial DNA. EMBO J. **6:** 4197–4203.

Diffley, J. F., and B. Stillman, 1992 DNA binding properties of an HMG1-related protein from yeast mitochondria. J. Biol. Chem. **267:** 3368–3374.

Dujon, B., 1981 Mitochondrial genetics and functions, pp. 505–635 in *The Molecular Biology of the Yeast Saccharomyces cerevisiae*, edited by J. N. Strathern, E. W. Jones and J. R. Broach. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Fangman, W. L., J. W. Henly and B. J. Brewer, 1990 *RPO41*-independent maintenance of [rho$^-$] mitochondrial DNA in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:** 10–15.

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.

Foury, F., 1989 Cloning and sequencing of the nuclear gene *MIP1* encoding the catalytic subunit of the yeast mitochondrial DNA polymerase. J. Biol. Chem. **264:** 20552–20560.

Foury, F., and J. Kolodynski, 1983 *pif* mutation blocks recombination between mitochondrial rho$^+$ and rho$^-$ genomes having tandemly arrayed repeat units in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **80:** 5345–5349.

Foury, F., T. Roganti, N. Lecrenier and B. Purnelle, 1998 The complete sequence of the mitochondrial genome of *Saccharomyces cerevisiae*. FEBS Lett. **440:** 325–331.

Gietz, R. D., R. H. Schiestl, A. R. Willems and R. A. Woods, 1995 Studies on the transformation of intact yeast cells by the LiAc/SS-DNA/PEG procedure. Yeast **11:** 355–360.

Gingold, E. B., 1988 The replication and segregation of yeast mitochondrial DNA, pp. 149–170 in *Division and Segregation of Organelles*, edited by S. A. Boffey and D. Lloyd. Cambridge University Press, Cambridge, UK.

Graves, T., M. Dante, L. Eisenhour and T. W. Christianson, 1998 Precise mapping and characterization of the RNA primers of DNA replication for a yeast hypersuppressive petite by in vitro capping with guanylyltransferase. Nucleic Acids Res. **26:** 1309–1316.

Greenleaf, A. L., J. L. Kelly and I. R. Lehman, 1986 Yeast *RPO41* gene product is required for transcription and maintenance of the mitochondrial genome. Proc. Natl. Acad. Sci. USA **83:** 3391–3394.

Güldener, U., S. Heck, T. Fielder, J. Beinhauer and J. H. Hegemann, 1996 A new efficient gene disruption cassette for repeated use in budding yeast. Nucleic Acids Res. **24:** 2519–2524.

Hermann, G. J., and J. M. Shaw, 1998 Mitochondrial dynamics in yeast. Annu. Rev. Cell Dev. Biol. **14:** 265–303.

Kaiser, C., S. Michaelis and A. Mitchell, 1994 *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kao, L. R., T. L. Megraw and C. B. Chae, 1993 Essential role of the HMG domain in the function of yeast mitochondrial histone HM: functional complementation of HM by the nuclear nonhistone protein NHP6A. Proc. Natl. Acad. Sci. USA **90:** 5598–5602.

Kaufman, B. A., S. M. Newman, R. L. Hallberg, C. A. Slaughter, P. S. Perlman *et al.*, 2000 *In organello* formaldehyde crosslinking of proteins to mtDNA: identification of bifunctional proteins. Proc. Natl. Acad. Sci. USA **97:** 7772–7777.

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.

Kowalczykowski, S. C., 2000 Initiation of genetic recombination and recombination-dependent replication. Trends Biochem. Sci. **25:** 156–165.

Lecrenier, N., and F. Foury, 2000 New features of mitochondrial DNA replication system in yeast and man. Gene **246:** 37–48.

Ling, F., F. Makishima, N. Morishima and T. Shibata, 1995 A nuclear mutation defective in mitochondrial recombination in yeast. EMBO J. **14:** 4090–4101.

Ling, F., H. Morioka, E. Ohtsuka and T. Shibata, 2000 A role for *MHR1*, a gene required for mitochondrial genetic recombination, in the repair of damage spontaneously introduced in yeast mtDNA. Nucleic Acids Res. **28:** 4956–4963.

Lisowsky, T., and G. Michaelis, 1988 A nuclear gene essential for mitochondrial replication suppresses a defect of mitochondrial transcription in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **214:** 218–223.

Lockshon, D., S. G. Zweifel, L. L. Freeman-Cook, H. E. Lorimer, B. J. Brewer *et al.*, 1995 A role for recombination junctions in the segregation of mitochondrial DNA in yeast. Cell **81:** 947–955.

Lorimer, H. E., B. J. Brewer and W. L. Fangman, 1995 A test of the transcription model for biased inheritance of yeast mitochondrial DNA. Mol. Cell. Biol. **15:** 4803–4809.

MacAlpine, D. M., P. S. Perlman and R. A. Butow, 1998 The high mobility group protein Abf2p influences the level of yeast mitochondrial DNA recombination intermediates *in vivo*. Proc. Natl. Acad. Sci. USA **95:** 6739–6743.

Macino, G., and A. Tzagoloff, 1979 Assembly of the mitochondrial

membrane system: partial sequence of a mitochondrial ATPase gene in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **76:** 131–135.

Maleszka, R., P. J. Skelly and G. D. Clark-Walker, 1991 Rolling circle replication of DNA in yeast mitochondria. EMBO J. **10:** 3923–3929.

Meeusen, S., Q. Tieu, E. Wong, E. Weiss, D. Schieltz *et al.*, 1999 Mgm101p is a novel component of the mitochondrial nucleoid that binds DNA and is required for the repair of oxidatively damaged mitochondrial DNA. J. Cell Biol. **145:** 291–304.

Megraw, T. L., and C. B. Chae, 1993 Functional complementarity between the HMG1-like yeast mitochondrial histone HM and the bacterial histone-like protein HU. J. Biol. Chem. **268:** 12758–12763.

Miyakawa, I., N. Sando, S. Kawano, S. Nakamura and T. Kuroiwa, 1987 Isolation of morphologically intact mitochondrial nucleoids from the yeast, *Saccharomyces cerevisiae*. J. Cell Sci. **88:** 431–439.

Miyakawa, I., S. Fumoto, T. Kuroiwa and N. Sando, 1995 Characterization of DNA-binding proteins involved in the assembly of mitochondrial nucleoids in the yeast *Saccharomyces cerevisiae*. Plant Cell Physiol. **36:** 1179–1188.

Myers, A. M., L. K. Pape and A. Tzagoloff, 1985 Mitochondrial protein synthesis is required for maintenance of intact mitochondrial genomes in *Saccharomyces cerevisiae*. EMBO J. **4:** 2087–2092.

Newman, S. M., O. Zelenaya-Troitskaya, P. S. Perlman and R. A. Butow, 1996 Analysis of mitochondrial DNA nucleoids in wild-type and a mutant strain of *Saccharomyces cerevisiae* that lacks the mitochondrial HMG box protein Abf2p. Nucleic Acids Res. **24:** 386–393.

Nobrega, F. G., and A. Tzagoloff, 1980 Assembly of the mitochondrial membrane system. DNA sequence and organization of the cytochrome b gene in *Saccharomyces cerevisiae* D273-10B. J. Biol. Chem. **255:** 9828–9837.

Preiser, P. R., R. J. Wilson, P. W. Moore, S. McCready, M. A. Hajibagheri *et al.*, 1996 Recombination associated with replication of malarial mitochondrial DNA. EMBO J. **15:** 684–693.

Reenan, R. A., and R. D. Kolodner, 1992 Isolation and characterization of two *Saccharomyces cerevisiae* genes encoding homologs of the bacterial HexA and MutS mismatch repair proteins. Genetics **132:** 963–973.

Sambrook, J., T. Maniatis and E. F. Fritsch, 1989 *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schinkel, A. H., and H. F. Tabak, 1989 Mitochondrial RNA polymerase: dual role in transcription and replication. Trends Genet. **5:** 149–154.

Schmitt, M. E., and D. A. Clayton, 1993 Conserved features of yeast and mammalian mitochondrial DNA replication. Curr. Opin. Genet. Dev. **3:** 769–774.

Sedman, T., S. Kuusk, S. Kivi and J. Sedman, 2000 A DNA helicase required for maintenance of the functional mitochondrial genome in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **20:** 1816–1824.

Shadel, G. S., 1999 Yeast as a model for human mtDNA replication. Am. J. Hum. Genet. **65:** 1230–1237.

Sor, F., and H. Fukuhara, 1982 Identification of two erythromycin resistance mutations in the mitochondrial gene coding for the large ribosomal RNA in yeast. Nucleic Acids Res. **10:** 6571–6577.

Van Dyck, E., and D. A. Clayton, 1998 Transcription-dependent DNA transactions in the mitochondrial genome of a yeast hypersuppressive petite mutant. Mol. Cell. Biol. **18:** 2976–2985.

Van Dyck, E., F. Foury, B. Stillman and S. J. Brill, 1992 A single-stranded DNA binding protein required for mitochondrial DNA replication in *S. cerevisiae* is homologous to *E. coli* SSB. EMBO J. **11:** 3421–3430.

Wach, A., A. Brachat, R. Pohlmann and P. Philippsen, 1994 New heterologous modules for classical or PCR-based gene disruptions in *Saccharomyces cerevisiae*. Yeast **10:** 1793–1808.

Weislogel, P. O., and R. A. Butow, 1970 Low temperature and chloramphenicol induction of respiratory deficiency in a cold-sensitive mutant of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **67:** 52–58.

White, M. F., and D. M. Lilley, 1996 The structure-selectivity and sequence-preference of the junction-resolving enzyme *CCE1* of *Saccharomyces cerevisiae*. J. Mol. Biol. **257:** 330–341.

Willer, M., M. Rainey, T. Pullen and C. J. Stirling, 1999 The yeast *CDC9* gene encodes both a nuclear and a mitochondrial form of DNA ligase I. Curr. Biol. **9:** 1085–1094.

Williamson, D. H., and D. J. Fennell, 1979 Visualization of yeast mitochondrial DNA with the fluorescent stain "DAPI." Methods Enzymol. **56:** 728–733.

Xu, B., and D. A. Clayton, 1995 A persistent RNA-DNA hybrid is formed during transcription at a phylogenetically conserved mitochondrial DNA sequence. Mol. Cell. Biol. **15:** 580–589.

Zelenaya-Troitskaya, O., P. S. Perlman and R. A. Butow, 1995 An enzyme in yeast mitochondria that catalyzes a step in branched-chain amino acid biosynthesis also functions in mitochondrial DNA stability. EMBO J. **14:** 3268–3276.

Zelenaya-Troitskaya, O., S. M. Newman, K. Okamoto, P. S. Perlman and R. A. Butow, 1998 Functions of the high mobility group protein, Abf2p, in mitochondrial DNA segregation, recombination and copy number in *Saccharomyces cerevisiae*. Genetics **148:** 1763–1776.

Zinn, A. R., J. K. Pohlman, P. S. Perlman and R. A. Butow, 1988 In vivo double-strand breaks occur at recombinogenic G + C-rich sequences in the yeast mitochondrial genome. Proc. Natl. Acad. Sci. USA **85:** 2686–2690.

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