Functions of the High Mobility Group Protein, Abf2p, in Mitochondrial DNA Segregation, Recombination and Copy Number in *Saccharomyces cerevisiae*

Olga Zelenaya-Troitskaya,[1] Scott M. Newman,[1] Koji Okamoto, Philip S. Perlman and Ronald A. Butow

Department of Molecular Biology and Oncology, University of Texas Southwestern Medical Center, Dallas, Texas 75235-9148

Manuscript received September 3, 1997
Accepted for publication December 24, 1997

ABSTRACT

Previous studies have established that the mitochondrial high mobility group (HMG) protein, Abf2p, of *Saccharomyces cerevisiae* influences the stability of wild-type (ρ^+) mitochondrial DNA (mtDNA) and plays an important role in mtDNA organization. Here we report new functions for Abf2p in mtDNA transactions. We find that in homozygous Δ*abf2* crosses, the pattern of sorting of mtDNA and mitochondrial matrix protein is altered, and mtDNA recombination is suppressed relative to homozygous *ABF2* crosses. Although Abf2p is known to be required for the maintenance of mtDNA in ρ^+ cells growing on rich dextrose medium, we find that it is not required for the maintenance of mtDNA in ρ^- cells grown on the same medium. The content of both ρ^+ and ρ^- mtDNAs is increased in cells by 50–150% by moderate (two- to threefold) increases in the *ABF2* copy number, suggesting that Abf2p plays a role in mtDNA copy control. Overproduction of Abf2p by ≥10-fold from an *ABF2* gene placed under control of the *GAL1* promoter, however, leads to a rapid loss of ρ^+ mtDNA and a quantitative conversion of ρ^+ cells to petites within two to four generations after a shift of the culture from glucose to galactose medium. Overexpression of Abf2p in ρ^- cells also leads to a loss of mtDNA, but at a slower rate than was observed for ρ^+ cells. The mtDNA instability phenotype is related to the DNA-binding properties of Abf2p because a mutant Abf2p that contains mutations in residues of both HMG box domains known to affect DNA binding *in vitro*, and that binds poorly to mtDNA *in vivo*, complements Δ*abf2* cells only weakly and greatly lessens the effect of overproduction on mtDNA instability. *In vivo* binding was assessed by colocalization to mtDNA of fusions between mutant or wild-type Abf2p and green fluorescent protein.These findings are discussed in the context of a model relating mtDNA copy number control and stability to mtDNA recombination.

THE segregating unit of mitochondrial DNA (mtDNA) is generally believed to be a protein–DNA complex that can be visualized in cells with DNA-specific dyes as punctate-staining, cytoplasmic structures termed nucleoids or chondriolites (Williamson and Fennell 1979). The mechanisms by which mtDNA is transmitted to progeny cells to achieve respiratory competence of the population are not well understood. Previous studies on the kinetics of sorting of mtDNA and mitochondrial matrix proteins in zygotes of the yeast *Saccharomyces cerevisiae* suggested that mtDNA is associated with a putative segregation apparatus that controls the movements and transmission of mtDNA independently of the flow of bulk mitochondrial matrix proteins (Azpiroz and Butow 1993).

Mitochondria of wild-type yeast cells contain an abundant 20-kD protein that plays an important role in mtDNA maintenance. This protein was first identified by Caron *et al.* and called HM. The gene was later cloned and named *ABF2* by Diffley and Stillman (1991), who showed that the phenotype of the null allele (Δ*abf2*) is a loss of wild-type (ρ^+) mtDNA from cells grown on rich dextrose medium. They observed, however, that when Δ*abf2* cells were grown on medium with glycerol, a nonfermentable carbon source, ρ^+ mtDNA could be maintained indefinitely. These findings indicate that Abf2p is not essential for mtDNA replication or gene expression.

Abf2p is a member of the family of high mobility group (HMG) proteins and it contains two HMG box domains. Because a truncated protein containing only one HMG box supplies the Abf2p function for mtDNA stability, probably only one is essential (Kao *et al.* 1993). HMG box proteins are a functionally diverse class of DNA-binding proteins that can bend and wrap DNA, and they have particular affinity for non-B DNA conformations, including Holliday junctions (reviewed in Landsman and Bustin 1993). The instability of ρ^+ mtDNA in Δ*abf2* cells can be suppressed by other HMG proteins, including the yeast nuclear protein NHP6A (Kao *et al.* 1993) and the human mitochondrial homologue of Abf2p, h-mtTFA (Parisi *et al.* 1993), when targeted to mitochondria. In addition, the *Escherichia coli* DNA-packing protein, HU, although not an HMG

Corresponding author: Ronald A. Butow, Department of Molecular Biology and Oncology, University of Texas SW Med. Center, 5323 Harry Hines Blvd., Dallas, TX 75235-9148.
E-mail: butow@swmed.edu

[1] These authors contributed equally to this work.

box protein, can also suppress the mtDNA instability phenotype when it is targeted to mitochondria of Δ*abf2* cells (Megraw and Chae 1993), suggesting that the function of Abf2p is related mostly, if not entirely, to its DNA-binding properties. Finally, the mtDNA instability phenotype of Δ*abf2* cells can be partially suppressed by overexpression of the mitochondrial enzyme acetohydroxy acid reductoisomerase, the product of the *ILV5* gene, which functions in branched chain amino acid biosynthesis (Troitskaya *et al.* 1995). The mechanism of suppression of the Δ*abf2* phenotype by acetohydroxy acid reductoisomerase, which has no obvious DNA-binding motifs, is unknown.

Recent studies have suggested that ρ^+ mtDNA in Δ*abf2* cells grown on glycerol medium is organized differently than in wild-type *ABF2* cells (Newman *et al.* 1996). First, ρ^+ mtDNA in glycerol-grown Δ*abf2* cells stains more diffusely with the DNA-binding dye 4′,6′-diamino-2-phenylindole (DAPI) than does ρ^+ mtDNA in *ABF2* cells, which shows a characteristic bright, punctate staining pattern. Second, some mtDNA sequences are four- to fivefold more sensitive to digestion by DNase I in permeabilized mitochondria from Δ*abf2* cells than in similarly prepared mitochondria from wild-type cells. Finally, a comparison of the protein profiles of purified mtDNA nucleoids isolated from Δ*abf2* and *ABF2* cells showed that, in addition to the expected absence of Abf2p, the nucleoids from Δ*abf2* cells lack polypeptides of 60 and 46 kD, both of which are present in amounts comparable to that of Abf2p in nucleoids from wild-type cells. Together, these findings suggest that Abf2p plays an important role in the organization of mtDNA.

To better understand the role of Abf2p in mtDNA maintenance, we have analyzed the sorting, copy number, and transmission properties of ρ^+ and ρ^- mtDNAs in wild-type cells and in cells that either lack Abf2p or contain elevated levels of the protein. Our experiments indicate that both ρ^+ and ρ^- mtDNA copy number can be modulated by the level of Abf2p; however, very high levels of Abf2p result in mtDNA instability, an effect that probably requires the DNA-binding properties of the protein. Surprisingly, unlike ρ^+ mtDNA, ρ^- mtDNAs are stable in Δ*abf2* cells when grown on rich dextrose medium. These results, together with data showing that mtDNA recombination is reduced in Δ*abf2* cells, suggest that Abf2p influences both the replication and segregation of mtDNA, perhaps through a role in forming or stabilizing mtDNA recombination intermediates.

MATERIALS AND METHODS

Strains, growth media, and growth conditions: Strains of *S. cerevisiae* used in this study are listed in Table 1. *MAT*α derivatives of 14WW and 14WWΔ*abf2* were made by mating-type switching induced by expression of the HO gene under galactose control (Herskowitz and Jensen 1991). For mtDNA recombination studies, strains 70 and 43 were constructed by cytoduction (Conde and Fink 1976) to contain mitochondrial genomes with the genotypes, respectively, ρ^+ ω^+ C^r O^r and ρ^+ ω^+ C^s O^s, with markers conferring resistance or sensitivity to chloramphenicol (C) or oligomycin (O) [see Azpiroz and Butow (1993) for details about those mtDNAs]. ρ^0 derivatives of strains were obtained by growth of cells in rich medium containing 2% dextrose and 25 μg/ml ethidium bromide. The mitochondrial genome of the HS40 ρ^- strain is a 760-bp repeat containing ori5, a putative origin of mtDNA replication (Parikh *et al.* 1989); that strain is a hypersuppressive petite that, when crossed to ρ^+ tester strains, >98% of the diploid progeny are ρ^- petites with the HS40 mitochondrial genome. The mitochondrial genome of the ρ^- VAR1 strain is a 2-kb repeat encompassing the VAR1 gene, which was first analyzed in strain 5D2-33 (Lopez *et al.* 1981); it is only moderately suppressive, yielding ~20% ρ^- diploid progeny when crossed to ρ^+ testers. These ρ^- genomes were introduced into α14WW and α14WWΔ*abf2* by cytoduction; the desired cytoductants were identified by colony hybridization or by Southern blotting with petite genome–specific probes. All standard yeast genetic analyses were performed as described in Rose *et al.* (1988).

YP medium contains 1% yeast extract and 2% Bacto peptone, and either 2% dextrose (YPD), 2% glycerol (YPG), or 2% galactose (YPGal). YNB medium contains 0.67% yeast nitrogen base without amino acids and either 4% dextrose (YNBD), 4% dextrose and 1% casamino acids (YNBD+cas), 2% glycerol (YNBG), 2% glycerol and 1% casamino acids (YNBG+cas), or 2% galactose and 1% casamino acids (YNBGal+cas). All YNB media were supplemented with the requisite nutritional requirements. RG medium contains 0.2% yeast extract, 0.2% Bacto peptone, 0.05% NaCl, 0.1% $(NH_4)_2SO_4$, 0.05% $MgCl_2$, 0.05% KH_2PO_4, pH 6.5, and 3.2% glycerol. Solid media contained 2% Bacto agar. Cells were grown at 30°. Colonies were scored as ρ^+ or ρ^- by the tetrazolium overlay method (Ogur *et al.* 1957); for adenine-requiring strains, it was also possible to score ρ^+ and ρ^- by the red colony color that ρ^+ *ade*1 and *ade*2 strains develop.

Plasmids: Plasmid DNAs were prepared by alkaline lysis using *E. coli* strains DH5α or XL1-blue.

pGCS1 is a *CEN-URA3* plasmid containing the *CIT1* coding region under control of the *GAL1* promotor (Azpiroz and Butrow 1993). pAM1A20 is a pUC119-based ARS1-*CEN4*-*URA3* plasmid containing the 1.6-kb *Eco*RI fragment of yeast DNA that includes the *ABF2* gene (Diffley and Stillman 1991). pAM1A::TRP1 contains a *TRP1* disruption of the *ABF2* gene described by Diffley and Stillman (1991). YCpABF2 is the CEN-*URA3* plasmid YCp50 containing the wild-type *ABF2* gene that was transferred from pAM1A20.

To construct plasmid pGAL68/ABF2, a 0.9-kb fragment of DNA containing the entire *ABF2* coding sequence flanked by restriction sites 5′-*Bam*HI and 3′-*Hin*dIII was generated by PCR using the primers 5′-GTAAACAGATTAACAAAGGATCCAATCAATTACAACAAC-3′ and 5′-TCGTAAAGAAGCTTTGTAAAGGTGAGGACG-3′. The PCR product was digested with *Bam*HI and *Hin*dIII, and the fragment containing the coding region of *ABF2* plus 24 bp of 5′ and 376 bp of 3′ sequences was gel purified and ligated into the *Bam*HI-*Hin*dIII site of pGAL68, which was previously called pSEYC68-Gal (Vida *et al.* 1990).

Plasmid YIp356/ABF2 was constructed by cloning the 1.6-kb *Eco*RI fragment of plasmid pAM1A20 containing the *ABF2* gene into the *Eco*RI site of plasmid YIp356 (Myers *et al.* 1986). Plasmid YIp356/ABF2/TRP1 was constructed by cloning the *Pst*I-*Sma*I fragment from plasmid YDp-W (Berben *et al.* 1991), which contains the *TRP1* gene, into the *Pst*I-*Sma*I sites of the multicloning site in plasmid YIp356/ABF2.

Plasmid pRS416/Abf2-GFP (*CEN URA3*) was constructed by ligating a 0.8-kb *Eco*RI-*Xho*I fragment containing the 5′

TABLE 1

***S. cerevisiae* strains used in this study[a]**

Strain	Genotype	Reference
14WW	*MAT***a** *ade2-1 ura3-52 trpl leu2-3,112 cit1::LEU2* ρ^+	Troitskaya *et al.* (1995)
α14WW	*MAT*α *ade2-1 ura3-52 trpl leu2-3,112 cit1::LEU2* ρ^+	This study
14WW/ABF2	*MAT***a** *ade2-1 ura3-52 trpl leu2-3,112 cit1::LEU2 ura3::YIp356/ABF2* ρ^+	This study
14WW/2ABF2	*MAT***a** *ade2-1 ura3-52 trpl leu2-3,112 cit1::LEU2 ura3::YIp356/ABF2 trpl::YI-356ABF2* ρ^+	This study
14WW/GAT	*MAT***a** *ade2-1 ura3-52 trpl leu2-3,112 cit1::LEU2 ura3::YIp356/GAT* ρ^+	This study
14WWΔ*abf2*	*MAT***a** *ade2-1 ura3-52 trp1 leu2-3,112 cit1::LEU2 abf2::TRP1* ρ^+	Troitskaya *et al.* (1995)
α14WWΔ*abf2*	*MAT*α *ade2-1 ura3-52 trp1 leu2-3,112 cit1::LEU2 abf2::TRP1* ρ^+	This study
15WW	*MAT*α *his3 ura3-52 trpl leu2-3,112 cit1::LEU2* ρ^+	This study
15WW Δ*abf2*	*MAT*α *his3 ura3-52 trp1 leu2-3,112 cit1::LEU2 abf2::TRP1* ρ^+	This study
70	*MAT***a** *ade2 ura3 trp1 leu2-3,112 abf2::TRP1 cit1::LEU2* ρ^+ ω^+ C^r O^r	This study
43	*MAT*α *his3 trp1 leu2-3,112 abft::TRP1 cit1::LEU2* ρ^+ ω^+ C^S O^S	This study
HS40 ρ^-	*MAT*α *ade2-1 ura3-52 trpl leu2-3,112 cit1::LEU2 HS40* ρ^-	This study and Parikh *et al.* (1989)
VARI ρ^-	*MAT*α *ade2-1 ura3-52 trp1 leu2-3,112 cit1::LEU2* VARI ρ^-	This study and Lopez *et al.* (1981)
14WW/GAT (ρ^-HS40)	*MAT***a** *ade2-1 ura3-52 trp1 leu2-3,112 cit1::LEU2 ura::YIp356/GAT*	This study
HS40 ρ^- Δ*abf2*	*MAT*α *ade2-1 ura3-52 trp1 leu2-3,112 cit1::LEU2 abf2::TRP1* HS40 ρ^-	This study and Parikh *et al.* (1989)
VAR1 ρ^- Δ*abf2*	*MAT*α *ade2-1 ura3-52 trp1 leu2-3,112 cit1::LEU2 abft2::TRP1* VAR1 ρ^-	This study and Lopez *et al.* (1981)

[a] Genealogies available upon request.

untranslated region (UTR) and coding region of *ABF2* in frame to a 0.73-kb *Xho*I-*Kpn*I fragment containing the coding region of green fluorescent protein (GFP), followed by a 1-kb *Kpn*I-*Eco*RI fragment of the *ABF2* 3′ UTR, and cloned into the *Eco*RI site of pRS416. The mutant allele, *abf2*-1$^-$2$^-$ (see below), was cloned into pRS416 in the same way to yield plasmid pRS416/Abf2-1$^-$2$^-$-GFP. pRS416/CS1-GFP was constructed by ligating a 0.9-kb *Eco*RI-*Xho*I fragment of the *CIT2* gene containing the 5′ UTR and the region of the open reading frame encoding the first 52 amino acids of citrate synthase 1 (CS1) fused to the *Xho*I-*Kpn*I fragment of the GFP-coding region, followed by a 0.5-kb *Kpn*I-*Hin*dII fragment of the *CIT2* 3′ UTR, and cloned into the *Eco*RI-*Hin*dIII site of pRS416.

Construction of mutant *abf2*-1$^-$2$^-$: The 1.6-kb *Eco*RI fragment from pAM1A20 containing the *ABF2* gene was cloned into pRS416 (to generate pRSABF2) and site-specific mutations were placed in the two HMG boxes. Primers corresponding to box 1 (5′-TAAGAAATAAGCAGATGTGGGGGCGCC AGGAACCCTGTTTTATCAATTC-3′) and box 2 (5′ CTTATT GAAGGGTCCTGCTGGGCCGGCTGGAGGAAGTTTTTCGT CAAAC-3′) were used to mutate $Lys^{44}Arg^{45}$ and $Lys^{117}Lys^{118}$ to GlyAla and AlaGly, respectively. Mutagenized plasmids (referred to as pRSabf2-1$^-$2$^-$) were propagated in *E. coli* strain CJ236, and the presence of the mutations was monitored by screening for the *Nar*I and *Nae*I restriction sites, respectively (underlined).

Yeast transformations were carried out by the lithium acetate method (Chen *et al.* 1992).

Strain constructions: YIp356/ABF2 was linearized at the *Nco*I site in the *URA3* gene and transformed into strain 14WW ρ^+; integration of the plasmid into the *URA3* locus yielded strain 14WW/ABF2 with two genomic copies of the *ABF2* gene. Plasmid YIp356/ABF2/TRP1 was linearized at an internal *Xba*I site in the *TRP1* gene and integrated into the genomic *TRP1* gene by transformation of strain 14WW/ABF2; the resulting strain, 14WW/2ABF2, has three genomic copies of the *ABF2* gene. To generate strain 14WW/GAT, in which one copy of the *ABF2* gene is under control of the *GAL1* promoter, a 1.6-kb *Eco*RI-*Hin*dIII fragment of plasmid pGAL68/ABF2 containing the *ABF2* gene under control of the *GAL1* promotor was cloned into the multicloning site of plasmid YIp356, yielding YIp356/GA. A Klenow-filled *Bam*HI fragment of plasmid YDP-W containing the *TRP1* gene was cloned into a Klenow-filled *Hin*dIII site of plasmid YIp356/GA. The resulting plasmid, YIp356/GAT, was linearized at the *Nco*I site, gel purified, and transformed into strain 14WW ρ^+. Ura^+ and Trp^+ transformants were selected, and integration was verified by Southern blot analysis.

Cells were cured of URA3 plasmids using standard methods (Rose *et al.* 1988).

mtDNA recombination: Δ*abf2* strains 70 (*MAT***a** C^r O^r) and 43 (*MAT*α C^s O^s) and YCpABF2 transformants of those strains were grown on YPG medium and mated in the four possible combinations for 12–16 hr on solid YPG medium. Diploids were selected by replica plating on appropriately supplemented YNBG medium. Diploid cells were then plated for single colonies on RG medium. Resistance or sensitivity to chloramphenicol (C) and oligomycin (O) was tested by replica plating to RG medium containing either 3 mg/ml chloramphenicol or 3 μg/ml oligomycin, and plates were scored after 4 days incubation at 30°.

Analysis of mtDNA and Abf2p content: Total cellular DNA from the various yeast strains was prepared by the rapid glass bead method of Hoffman and Winston (1987). DNA was digested with *Hae*III for ρ^+ mtDNA, *Dra*I for HS40 ρ^- mtDNA, or *Hin*cII for VAR1 ρ^- mtDNA, and was fractionated on 1% agarose gels. Blotted samples were hybridized with the following 5′ ^{32}P-end labeled probes: VAR1, 5′-ATGGTATCTTAAC TAATTATCAACGTA-3′; HS40, 5′-GATAAACAAGAAGATA TCCGGGTC-3′. For some experiments, HS40 DNA samples were hybridized with random-primed HS40 DNA (Random Primed DNA Labeling Kit; Boehringer Mannheim Biochemi-

cals, Indianapolis) purified from strain 14WW ρ^- HS40 and gel purified after cleavage with *Eco*RV. A 679-bp PCR-amplified fragment of the *COXII* gene (nt 1335–2014 of GenBank accession number J01485) was labeled by random priming and used as the probe for ρ^+ genomes. For each digest, the level of the single-copy *ACT1* gene was determined by hybridization with a random-primed fragment of the *ACT1* gene [the 0.6-kb *Xba*I-*Pst*I internal fragment from the *ACT1* gene from pGEM-actin (Ng and Abelson 1980)]. Signals were quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA), and the content of mtDNA was determined relative to the *ACT1* signal.

The amount of Abf2p in whole-cell extracts was determined by FluorImager (Molecular Dynamics, Sunnyvale, CA) scanning of Western blots using a polyclonal antibody to Abf2p that was expressed and purified from *E. coli* as described (Newman *et al.* 1996). Protein inputs were determined by Bradford analysis (Bio-Rad, Richmond, CA).

Staining and microscopy: To analyze the sorting of the mitochondrial matrix protein CS1 and mtDNA in zygotes, fixed cells (4% formaldehyde for 1 hr at 30°) were washed in P buffer (40 mm KH2PO4, pH 6.5, and 0.5 mm MgCl2). Spheroplasts were prepared and placed on poly-l-lysine–coated multiwell slides as described in Azpiroz and Butow (1993). DNA was stained by brief incubation with 1 μg/ml DAPI in PS buffer (1.2 m sorbitol in P buffer). After washing in PS buffer, samples were air dried and mounted. Microscopy was performed using an Axioplan microscope (Carl Zeiss, Thornwood, NY) equipped for epifluorescence using filter set G365/FT395/LP420. Synchronized matings, labeling of parental cells with mitochondrial CS1, immunofluorescence, and zygote scoring were performed as described in Azpiroz and Butow (1993). The percentage of ρ^o cells in cultures was determined by direct count of DAPI-stained cells. For microscopic analysis of GFP fusion proteins, ρ^+ cells of strain 14WW were transformed with pRS416/ABF2-GFP, pRS416/abf2-1$^-$2$^-$-GFP, or pRS416/CS1-GFP, and were grown in YNBR+cas medium to midlogarithmic phase. Cells were collected by centrifugation and resuspended in sterile distilled water to a density of 5×10^6 cells/ml. A small drop of the cell suspension (2–3 μl) was placed on a microscope slide, covered with a cover slip (22 × 22 mm), sealed with 0.5% agarose, and observed under the microscope. Cells were observed by fluorescence with a cooled CCD (model C5810; Hamamatsu Photonic Systems, Bridgewater, NJ) camera on a DMRXE microscope (Leica, Deerfield, IL) equipped with the following filter set: excitation, 450–490 nm; dichroic, 510 nm; barrier, 515 LP, an HBO 100W/2 mercury arc lamp, and a ×100 Plan-Apochromat objective. Fluorescence and differential interference contrast (DIC) digitized images were acquired using Adobe Photoshop (Adobe Systems, Mountain View, CA).

RESULTS

Altered sorting of mitochondrial constituents in zygotes formed from Δ*abf2* parents: We previously developed a system to follow the kinetics of sorting of mtDNA and mitochondrial matrix proteins in zygotes from synchronously mated cells (Azpiroz and Butow 1993). We showed that mtDNA movements can be distinguished from the movements of mitochondrial matrix proteins, suggesting the existence of an independent mtDNA segregation apparatus. In those experiments, a mitochondrial matrix protein, such as CS1, was transiently expressed in one of the two parents of a cross, *e.g.*, between ρ^+ cells or between a ρ^o petite and a ρ^+ strain, and the sorting of the protein was then followed at different time intervals after synchronous zygote formation by indirect immunoflourescence using anti-CS1 antiserum. Sorting of mtDNA was determined either by pedigree analysis of the mitochondrial genotype of zygote end buds or by direct vizualization of mtDNA by DAPI staining.

An important finding of those experiments was that in a $\rho^+ \times \rho^+$ cross, the matrix protein marker equilibrated throughout the zygotes after ~4 hr of zygote maturation while the ρ^+ parental mtDNAs remained largely unmixed (detected as progeny of end buds that were homogeneous for one parental mtDNA or the other). In the $\rho^+ \times \rho^o$ cross, an intermediate in the sorting process was observed as a population of zygote forms in which the matrix marker protein, initially present only in the mitochondria of the ρ^o parent, had quantitatively moved to the ρ^+ end of the zygote before equilibrating with mtDNA throughout the zygote and into the emerging diploid bud. This unusual zygote form was called "asymmetic" (A). We concluded in those studies that A-form zygotes were not the result of the transfer of bulk mitochondria from the ρ^o to the ρ^+ end because the CS1 fluorescence and DAPI staining of mtDNA were colocalized in one end of the zygote. Although the mechanism accounting for this quantitative transfer of the matrix protein from the ρ^o to the ρ^+ end of the zygote is not known, the formation of A-form zygotes requires the presence of ρ^+ mtDNA in one parent of the cross, although that parent need not be respiratory competent (Azpiroz and Butow 1993). Together, those experiments indicated that mtDNA sorting can occur independently of the sorting of matrix protein.

The appearance of A-form zygotes in $\rho^o \times \rho^+$ crosses, as well as the subsequent equilibration of matrix protein and mtDNA throughout the cell, which yields mixed, M-form zygotes, provides a convenient assay to determine whether Abf2p plays a role in the sorting process. To examine this possibility, we first verified that the wild-type (*ABF2*) strains used in the present study show the same zygote-sorting properties described previously by Azpiroz and Butow (1993) for a different set of parental strains. Figure 1A shows the kinetics of formation and disappearance of the various zygote forms derived from a synchronous mating between strains 14WWρ^+ (CS1$^-$) and 15WWρ^o (CS1$^+$), each of which has a wild-type *ABF2* allele. Unmixed, U-form zygotes containing CS1 in the ρ^o end and mtDNA in the ρ^+ end are present initially. By 4 hr after mating, the U-form zygotes have been largely replaced by mixed, M-form zygotes in which CS1 and mtDNA have equilibrated in the cell. Two intermediates in the mixing process are observed: first, partially mixed, P-form zygotes are present in which CS1 has begun to move to the ρ^+ end; later, asymmetric, A-form zygotes are seen in which essentially all of the CS1 from the ρ^o end has moved into the ρ^+ end of the zygote and colocalizes with mtDNA. These zygote forms and the

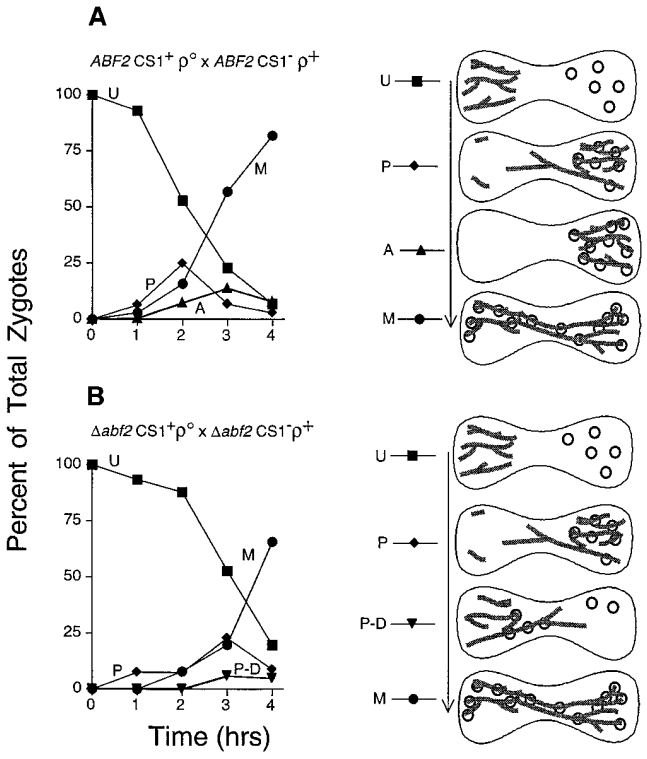

Figure 1.—Altered pattern of sorting of mitochondrial constituents in zygotes lacking Abf2p. Shown is the time course of appearance and disappearance of various zygote forms obtained from a synchronous mating between the *ABF2* strains, 15WW ρ^o CS1$^+$, and 14WW ρ^+ CS1$^-$ (A), and their Δ*abf2* derivatives (B). In both crosses, the ρ^o parent, transformed with plasmid pGCS1, which carries the CS1 gene under control of the *GAL1* promoter, was prelabeled with the mitochondrial matrix protein CS1 by growth in YPGal medium for 2 hr. Subsequent matings and microscopic analysis of zygotes were carried out as described in Azpiroz and Butow (1993) with the modifications described in materials and methods. Diagramatic representation of the various zygote forms and the designation assigned to each (type U, unmixed; type P, partially mixed for protein; type A, asymmetric; type P-D, partially mixed for protein and DNA; type M, mixed) are indicated to the right of the figure. All of these zygote forms, plotted as fractions of the total zygotes scored, were observed by direct microscopic analysis and are indistinguishable from the zygote forms that were fully documented for another pair of strains in Azpiroz and Butow (1993). In the diagrams of zygotes, the thick lines refer to CS1-labeled mitochondrial profiles visualized by indirect immunofluorescence, and the circles refer to mtDNA visualized by DAPI staining. At least 100 zygotes were scored for each time point.

kinetic pattern of their appearance and disappearance are identical to those described previously using other strains (Azpiroz and Butow 1993).

To determine whether the absence of Abf2p affects the sorting of mtDNA and matrix proteins, we repeated the $\rho^+ \times \rho^o$ cross with Δ*abf2* derivatives of the parental strains used above. As summarized in Figure 1B, no A-form zygotes were detected in the population of this homozygous Δ*abf2* cross. Instead, a small population of the P-D-form zygotes was observed in which CS1 from the ρ^o end of the zygote and mtDNA from the ρ^+ end moved toward each other and appeared colocalized in the neck region of the zygote. We have previously observed P-D-form zygotes only in crosses between ρ^o and ρ^- cells (Azpiroz and Butow 1993), but not in crosses between ρ^o and ρ^+ cells, even if the ρ^+ cells were respiratory deficient. We previously interpreted the A-form zygotes as indicating the association of ρ^+ mtDNA with a segregation apparatus, and the P-D-form zygotes as reflecting a poorer association of ρ^- mtDNA with the putative segregation apparatus. Thus, the absence of A-form zygotes and the presence of P-D-form zygotes in crosses between ρ^o and ρ^+ cells lacking Abf2p show that Abf2p influences the distribution and mixing of mitochondrial DNA and protein in crosses. Finally, other than the differences in the sorting patterns noted above, we have not detected any obvious changes in the mitochondrial structure of Δ*abf2* cells.

mtDNA recombination is reduced in crosses between Δ*abf2* strains: Recombination of mtDNA requires the mixing and subsequent pairing of parental mtDNA molecules in zygotes. The alterations in the "normal" pattern of both mtDNA and protein sorting in zygotes lacking Abf2p described in the preceding section raise the possibility that mtDNA recombination might also be affected. To examine this, crosses were carried out with the Δ*abf2* strains 70 and 43 that either lacked or contained the CEN plasmid YCpABF2 encoding the wild-type *ABF2* gene. The mtDNAs in these strains contain markers conferring either resistance (strain 70) or sensitivity (strain 43) to chloramphenicol and oligomycin (C^r O^r and C^s O^s, respectively). In standard crosses, these two markers yield the maximum level of ~25% recombination and, therefore, appear to be unlinked. The actual level of recombination observed, however, is lower than that maximum in so-called "biased" crosses where the parents have unequal inputs of mtDNA (Dujon 1981). The maximum of 25% recombination is not expected here because all four of these crosses shown have unequal inputs of markers from the two parents.

Table 2 shows that the homozygous *ABF2* cross (cross I) yields ~11% C-O recombinants. Similar values were obtained in the reciprocal heterozygous crosses (crosses II and III) where one parent is Δ*abf2* and the other is *ABF2*. In the homozygous Δ*abf2* cross (cross IV), however, recombination was reduced five- to sevenfold. Although there are differences in the input bias associated with the Δ*abf2* allele (see below), the comparison of crosses III and IV, where the parental allele outputs are essentially identical, shows clearly that the absence of Abf2p suppresses mtDNA recombination. We conclude that Abf2p is important for efficient recombination of mtDNA. These results and those of the zygote-sorting experiments above establish new phenotypes for the Δ*abf2*-null allele.

ρ^- mtDNA is stable in Δ*abf2* cells: The mitochondrial genomes of ρ^- petite mutants have sustained large dele-

TABLE 2

Recombination of mtDNA in homozygous and heterozygous *ABF2* and Δ*abf2* crosses

Cross	*N*	C^rO^r	C^sO^s	C^rO^s	C^sO^r	% Rec.	Output[a]
I. *ABF2* C^sO^s × *ABF2* C^rO^r	518	126	333	28	31	11.4	0.30
II. *ABF2* C^sO^s × Δ*abf2* C^rO^r	545	178	315	22	30	10.1	0.37
III. Δ*abf2* C^sO^s × *ABF2* C^rO^r	551	434	73	33	11	7.9	0.83
IV. Δ*abf2* C^sO^s × Δ*abf2* C^rO^r	546	463	74	8	1	1.6	0.86

The wild-type (*ABF2*) and Δ*abf2* derivatives of the ρ^+ strains 70 (C^rO^r) and 43 (C^sO^s) used in the experiment were mated on YPG medium in both homozygous and heterozygous configurations of *ABF2* and Δ*abf2*, and the resulting diploid progeny were scored for parental and recombinant combinations of alleles of CAP1 and OLI1 loci. The crosses and marker scoring were carried out as described in materials and methods. *N* equals the total number of diploid progeny scored in the cross.

[a] Output is given as $1/2(C^r + O^r)/N$.

tions of ρ^+ mtDNA. Despite differences in the extent of deletion of ρ^+ mtDNA sequences, the proportion of total cellular DNA represented by ρ^- mtDNAs is comparable to that of ρ^+ mtDNA (Nagley and Linnane 1972; Hall *et al.* 1976). It has also been generally observed that ρ^- mtDNAs are often stable in genetic backgrounds where ρ^+ mtDNA is unstable. For instance, Lorimer *et al.* (1995) showed that ρ^- genomes are stably maintained in cells containing a null allele of *RPO41*, the gene encoding mitochondrial RNA polymerase, whereas ρ^+ mtDNA is unstable in such cells. Those experiments underscore the well-documented but poorly understood observation that mitochondrial gene expression, including protein synthesis, is required for the maintenance of ρ^+ mtDNA (Weislogel and Butow 1970). With these considerations in mind, we tested whether Abf2p is required for the stability of ρ^- mtDNA, as it is for ρ^+ mtDNA. Petites containing two different ρ^- mitochondrial genomes (HS40 and VAR1) were analyzed. These ρ^- genomes were chosen because they retain similar-sized small fractions of the ρ^+ genome (0.8–2 kb) and have a similar A + T content; however, they have no sequences in common and they differ in the extent of suppressiveness (see materials and methods for more details). Each ρ^- mtDNA was transferred by cytoduction into cells of strain 14WWΔ*abf2*, which contained the *ABF2* gene on the plasmid YCpABF2. The plasmid was then cured from each strain, and the stability of the ρ^- genomes was analyzed during growth of the strains on YPD medium. As controls, these ρ^- genomes were also introduced into the parent (*ABF2*) strain and tested for stability in the same way.

The *ABF2* and Δ*abf2* ρ^- strains, grown in liquid YPD, and the control ρ^+ strains, grown in liquid YPG, were inoculated into liquid YPD and sampled at various times. Total cellular DNA was isolated from the samples, cleaved with the appropriate restriction enzymes, blotted, and hybridized with probes specific for the ρ^+ and ρ^- mtDNAs, as described in materials and methods and in the legend to Figure 2. A specific probe to the single-copy nuclear *ACT1* gene was included to allow normalization of the mtDNA content to that of nuclear DNA. Figure 2 shows a progressive decrease in ρ^+ mtDNA level in the Δ*abf2* cells during growth on YPD medium. The time course of loss of ρ^+ mtDNA has not been analyzed previously, and it is worth noting that the ρ^+ mtDNA is lost at a rate that is slower than would be expected if cells, upon shift to YPD medium, immediately failed to replicate their mtDNA. If that were the case, by six generations of growth on YPD medium, the population would have contained only 1–2% of the normalized mtDNA content of the starting population, which was clearly not observed in the data of Figure 2. In contrast to these results for ρ^+ Δ*abf2* cells, neither the HS40 nor the VAR1 Δ*abf2* ρ^- strains had any appreciable decrease in mtDNA content relative to the *ACT1* signal when grown on YPD medium for the same number of generations as the ρ^+ strain. Indeed, we find that these ρ^- mtDNAs can be maintained indefinitely in Δ*abf2* strains grown on YPD medium. Inspection of the data of Figure 2 also shows that there is no significant difference between the *ABF2* and Δ*abf2* strains in the amount of the HS40 and VAR1 ρ^- mtDNAs. On the other hand, as seen in the lanes marked "0 Generations," glycerol-grown ρ^+ Δ*abf2* cells have about half as much mtDNA as do ρ^+ *ABF2* cells (see below). Together, these data show that ρ^- mtDNAs respond differently to a lack of Abf2p than does ρ^+ mtDNA both in terms of mtDNA stability and copy number.

The *ABF2* gene dosage influences mtDNA copy number: To investigate further the influence of Abf2p on ρ^+ mtDNA copy number, we examined the effects of increased dosages of *ABF2* on the amount of ρ^+ mtDNAs in cells grown on glycerol or dextrose medium, and on the levels of VAR1 and HS40 ρ^- mtDNAs in cells grown on dextrose medium. For these experiments, we analyzed derivatives of ρ^+ strain 14WW (*ABF2*) carrying an average of two extra copies of the *ABF2* gene on a low copy number plasmid, YCpABF2 (based on plasmid copy number estimates, data not shown), or two extra copies of *ABF2* integrated into the nuclear genome, one near the *TRP1* gene and another near the *URA3* gene.

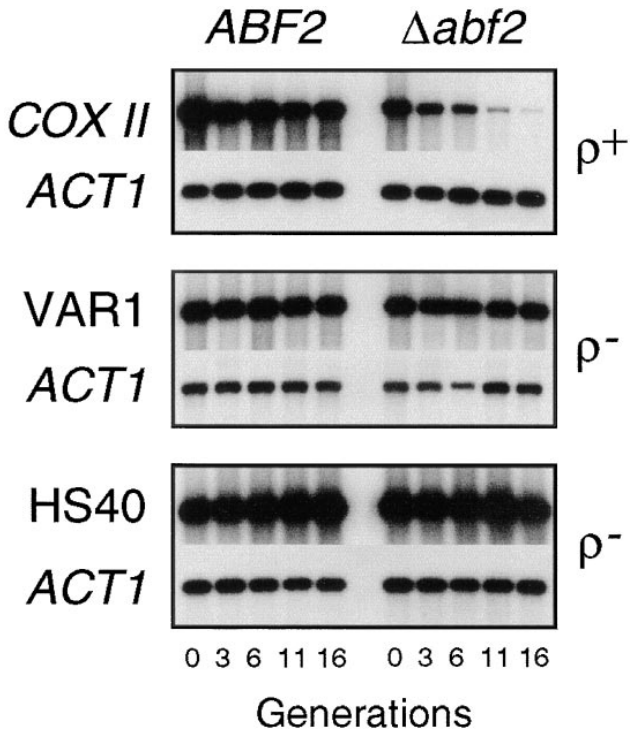

Figure 2.—Stability of mtDNAs in Δ*abf2* cells. Strains were constructed with ρ^+ or ρ^- mtDNAs containing (14WW) or lacking (14WW Δ*abf2*) a functional copy of the *ABF2* gene. The ρ^- mitochondrial genomes HS40 and VAR1 contain, respectively, 760 bp of mtDNA, including the ori5 sequence (Parikh *et al.* 1989), and 2 kb of mtDNA, including the VAR1 gene (Lopez *et al.* 1981). The ρ^+ *ABF2* and Δ*abf2* strains were maintained on YPG medium and shifted to liquid YPD medium at time zero. Cultures of the ρ^- strains were grown in liquid YPD medium throughout the experiment. Aliquots from the different YPD cultures were removed at various times, total DNA was extracted, and Southern blot analysis was carried out to determine the level relative to *ACT1* DNA sequences of the ρ^+ and ρ^- mtDNAs by using a COXII probe for the ρ^+ mtDNAs and HS40 and VAR1 sequences as probes for the ρ^- mtDNAs, as described in materials and methods.

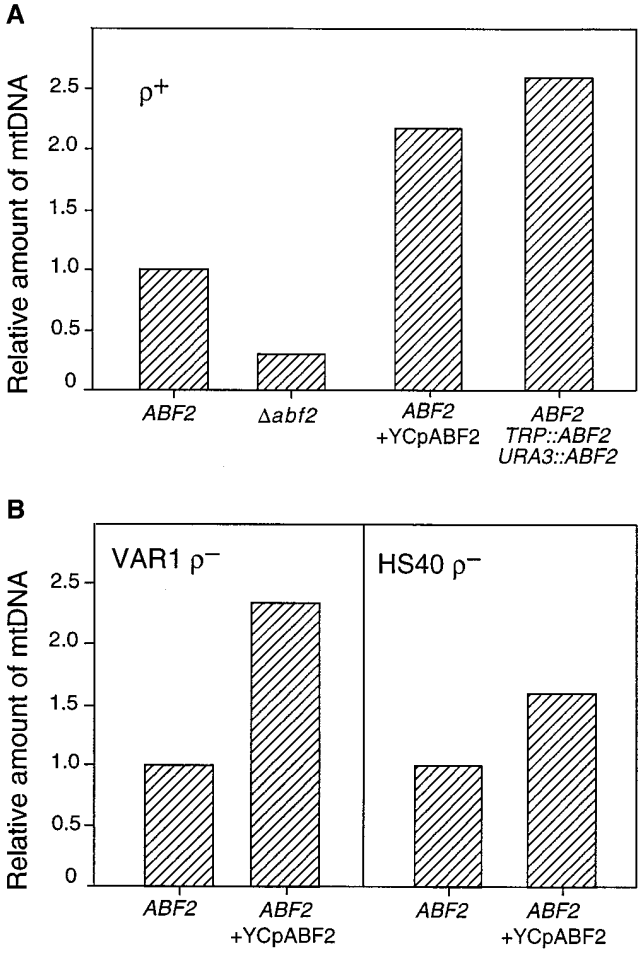

Figure 3.—*ABF2* dosage modulates mtDNA copy number. (A) ρ^+ mtDNA. Strains 14WW, 14WWΔ*abf2*, and 14WW transformed with YCpABF2 or one copy of the *ABF2* gene integrated into each of the *TRP1* and *URA3* loci to yield strain 14WW/2ABF2 (see materials and methods) were grown to an OD_{600} of ~0.8 in liquid YPG medium. Total DNA was extracted, and the relative abundance of mtDNA relative to the *ACT1* gene, normalized to a value of 1.0, was determined by quantitative Southern blot analysis using *COXII* DNA sequences as probes. (B) ρ^- mtDNAs. The relative mtDNA content in the VAR1 and HS40 ρ^- petites grown in dextrose medium was determined as for ρ^+ mtDNA using petite DNA–specific probes in strains with or without the plasmid YCpABF2. The probes and restriction digests used are described in materials and methods.

We analyzed derivatives of the ρ^- strains containing YCpABF2. Aliquots from logarithmic phase cultures of these strains were removed, and the amount of mtDNA was determined by quantitative Southern blot analysis. Figure 3, A and B, are representative experiments showing that increasing the gene dosage of *ABF2* by two- to threefold (and Abf2p in parallel, as determined by dot blot analysis) results in a 50–150% increase in the amount of mtDNA. Similar results were obtained when these cells were grown in dextrose medium. In six independent experiments that we have carried out with the ρ^+ and ρ^- strains with the integrated or plasmid-borne extra copies of *ABF2*, the overall average increase in mtDNA copy number was 100%, ranging from 30 to 330%, with no discernible difference between the ρ^+ and ρ^- mtDNAs. The data of Figure 3 also show that the ρ^+ mtDNA content is reduced ~70% in these Δ*abf2* cells grown in YPG medium, as was evident in an independent experiment shown in Figure 2. This decrease is not likely to be caused by a large accumulation of ρ^0 petites in the population because >95% of colonies of Δ*abf2* cells pregrown on glycerol medium contain some ρ^+ cells when grown on medium containing dextrose (data not shown). Thus, we conclude that *ABF2* not only plays a role in the maintenance of ρ^+ mtDNA, but also influences the amount of mtDNA in both ρ^+ and ρ^- cells.

High levels of *ABF2* expression result in loss of ρ^+ and ρ^- mtDNA: Megraw and Chae (1993) noted that overexpression of *ABF2* from a galactose-regulated promoter resulted in the formation of petite mutants. They did not report, however, the extent of Abf2p overpro-

duction or the kinetics of petite formation in those experiments. To reconcile their findings with the results presented here, showing that a moderate (two- to three-fold) increase in the level of Abf2p *increases* mtDNA content with no apparent compromise in the stability of mtDNA, we have overexpressed Abf2p in ρ^+ cells of strain 14WW containing an extra copy of *ABF2* under the control of the *GAL1* promoter integrated near the *URA3* gene. This strain, 14WW/GAT, and the parental strain, 14WW, were grown in YPG medium, shifted to YPGal, and monitored for the formation of petites by direct plating on YPD medium, for the level of mtDNA by quantitative Southern blotting, and for the amount of Abf2p by Western blot analysis.

Figure 4 shows that after the shift of the 14WW/GAT cells from YPG to YPGal medium, the Abf2p content increases by 8–10-fold by two generations and by about another twofold after a total of four generations. Coincident with the increase in Abf2p is a rapid decrease of mtDNA content and a concomitant production of petite mutants, which account for 100% of the cell population by four generations. Inspection of the kinetics of decrease in mtDNA content suggests that net synthesis of mtDNA ceases at high levels of Abf2p because the normalized loss of mtDNA roughly follows cell doublings. No mtDNA is detected by DAPI staining of the petite colonies (data not shown), indicating that this regime induces ρ^0 petites. Growth of the parental strain, 14WW (having just one copy of the *ABF2* gene), in YPGal medium caused no increase in Abf2p, no induction of petite mutants, and no loss of mtDNA other than what can be accounted for by the shift from glycerol to galactose medium. These experiments show that although moderate increases in Abf2p result in an increase in the copy number of ρ^+ mtDNA (Figure 3), larger increases have the opposite effect, causing a striking failure of mtDNA maintenance that leads to petite formation.

To determine whether hyperexpression of *ABF2* also destabilizes ρ^- mtDNA, we introduced the HS40 ρ^- mitochondrial genome by cytoduction to yield strain 14WW/GAT(ρ^- HS40) with an extra copy of *ABF2* under galactose control. These cells were grown in YPD medium, transferred to YPGal medium, and monitored for the level of HS40 mtDNA by Southern blot hybridization. Figure 5 shows that after a brief lag, there is a progressive decrease in the ratio of ρ^- HS40 mtDNA to nuclear DNA during the time course of Abf2p overproduction, with the mtDNA content falling to ~20% of the starting level after six generations of growth on galactose. A comparison of the data of Figures 4 and 5 shows that the rate and extent of HS40 mtDNA loss is lower than for ρ^+ mtDNA: the amount of mtDNA in ρ^+ cells has dropped by >50% between one and two generations of growth on galactose, whereas there is very little decrease in ρ^- mtDNA during the first two generations of

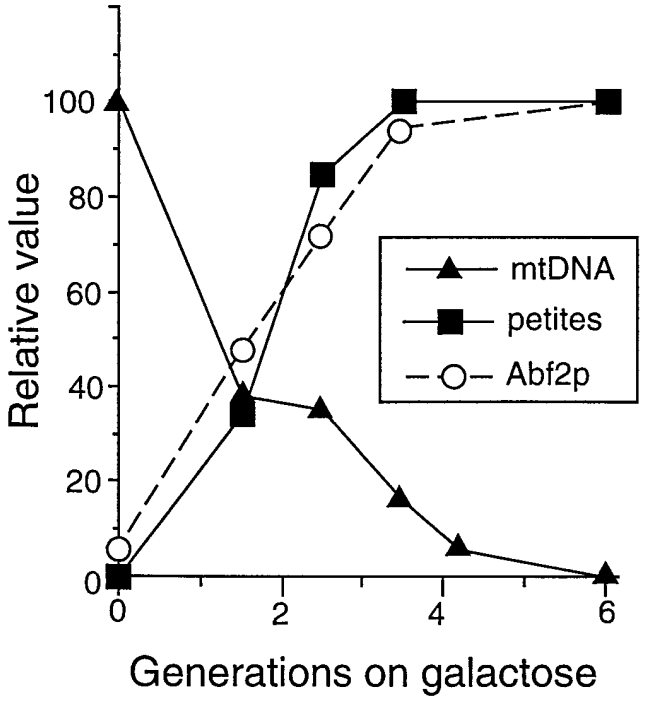

Figure 4.—Instability of ρ^+ mtDNA in cells overexpressing Abf2p. Strain 14WW/GAT, containing a single integrated copy of the *ABF2* gene under control of the *GAL1* promoter, was maintained on liquid YPG medium, shifted to YPGal medium, and aliquots removed at the times indicated. The Abf2p content, the fraction of petites in the population, and the mtDNA content were determined as described in materials and methods.

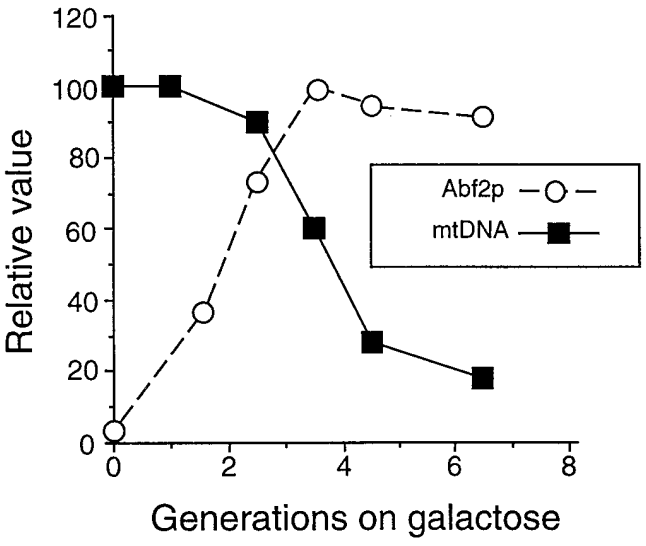

Figure 5.—Instability of HS40 ρ^- mtDNA in cells overexpressing Abf2p. Strain 14WW/GAT(ρ^-HS40), containing the HS40 ρ^- mitochondrial genome and a single integrated copy of the *ABF2* gene under control of the *GAL1* promoter, was maintained in liquid YPD medium. Cultures were shifted to YPGal medium, and aliquots were removed to determine the Abf2p content and the mtDNA content, as described in materials and methods.

growth of the petite strain on galactose. These data suggest that the reduction of mtDNA relative to nuclear DNA is caused by an inhibition of net mtDNA synthesis by the excess Abf2p.

Loss of mtDNA caused by high levels of Abf2p is relieved by mutations of the DNA-binding motifs of the HMG boxes of Abf2p: One can envisage that the loss of mtDNA in cells overproducing Abf2p is caused by some indirect effect on mitochondrial biogenesis. For example, the large increase in Abf2p synthesis may saturate some component of the mitochondrial protein import system, which then could conceivably lead to an imbalance of intramitochondrial components that are necessary for replication and maintenance of mtDNA. Alternatively, a large increase in the amount of Abf2p could have a direct effect on the ability of mtDNA to be propagated. In that case, the deleterious effect of a large dose of Abf2p would be expected to depend directly on its DNA-binding properties. To distinguish between these possibilities, we made site-directed mutations in key residues of both HMG box domains of Abf2p that are expected to compromise the ability of the protein to bind to DNA, and we tested effects of that mutant allele on the stability of mtDNA.

A highly conserved motif near the N terminus of HMG boxes contains a Pro followed by one or two basic residues (Baxevanis and Landsman 1995). Mutating that motif in a model polypeptide derived from the rat *HMG1* gene reduced its binding to four-way junction DNA by 10-fold (Falciola *et al.* 1994). In the *ABF2* gene, that motif (Figure 6A) is Pro-Lys-Arg-Pro (residues 43–46) in box 1 and Pro-Lys-Lys-Pro (residues 116–119) in box 2 (Difley and Stillman 1991). To test whether DNA binding is important for the Abf2p overproduction phenotype of mtDNA instability, we mutated the box 1 and box 2 sites (see Figure 6A) to Pro-Gly-Ala-Pro and Pro-Ala-Gly-Pro, respectively (see materials and methods). This mutant *abf2* allele, which we refer to as *abf2-1$^-$2$^-$*, was transformed into Δ*abf2* ρ$^+$ cells in the *CEN* plasmid pRSabf2-1$^-$2$^-$, and its expression was compared with that of the wild-type *ABF2* allele, which is also expressed in the same strain from the same *CEN* vector. Western blot analysis of extracts from these cells grown on YPG medium shows that the wild-type and mutant proteins accumulate to comparable levels (Figure 6B).

We next determined whether Abf2p-1$^-$2$^-$ complements the mtDNA instability phenotype of Δ*abf2* cells. Derivatives of strain 14WWΔ*abf2* ρ$^+$ with *ABF2* or *abf2-1$^-$2$^-$* on a *CEN* plasmid, as well as a control strain transformed with vector pRS416 and lacking the insert, were constructed. Cells were grown on YNBG+cas medium and then transferred to liquid YNBD+cas medium, grown for ~15 generations, and aliquots were plated directly on YPD medium. After 3 days growth, the colonies were scored as ρ$^+$ or petite using the tetrazolium overlay agar method, in which colonies of respiratory-competent ρ$^+$

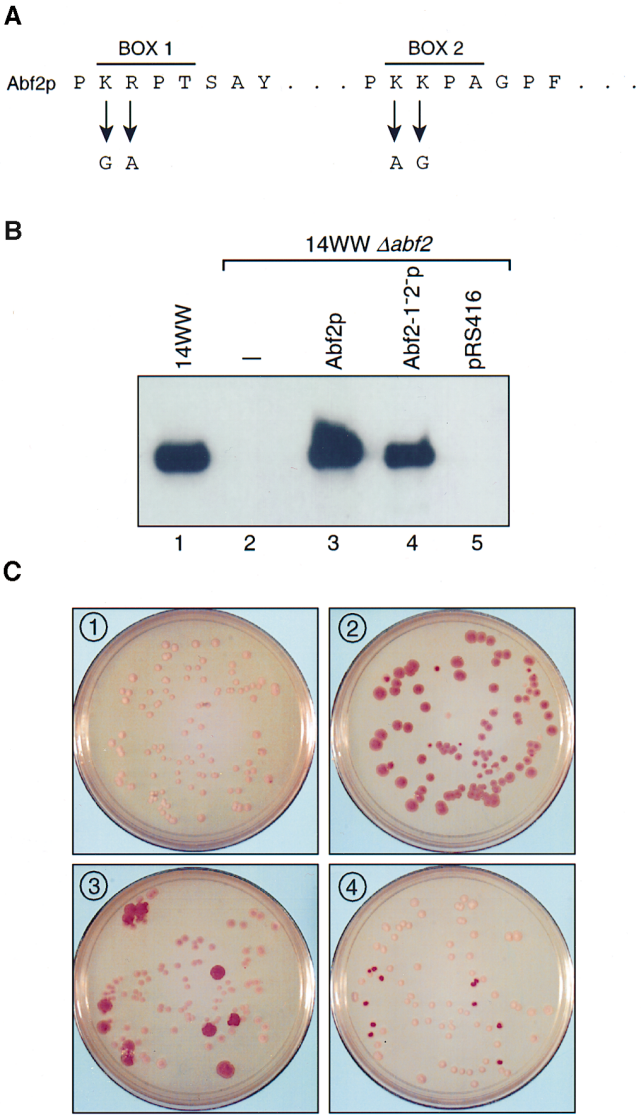

Figure 6.—Analysis of an HMG box mutant of Abf2p. (A) Mutations in the HMG box domains of Abf2p. Shown are amino acid residues 43–50 of the HMG box 1 domain and amino acid residues 116–123 of the HMG box 2 domain. (B) Abf2p and Abf2p-1$^-$2$^-$ are expressed at comparable levels. For Western blot analysis, equal amounts of protein obtained from a NaOH lysate were fractionated on a SDS-15% polyacrylamide gel, transferred to nitrocellulose, and incubated with polyclonal antiserum to Abf2p. (C) Abf2p-1$^-$2$^-$ weakly complements mtDNA instability in Δ*abf2* cells. Cells of strain 14WWΔ*abf2* ρ$^+$ and derivatives transformed with pRSABF2 or pRSabf2-1$^-$2$^-$, grown on YNBGly + cas medium, were innoculated into YNBD + cas medium and grown for ~15 generations. Aliquots of these cultures were plated on YPD medium, grown for 3 days, and then overlayed with tetrazolium agar to stain for respiratory-competent colonies (Ogur *et al.* 1957). Plate 1, 14WWΔ*abf2*; plate 2, 14WWΔ*abf2* transformed with pRSABF2; plate 3, 14WWΔ*abf2* transformed with pRSabf2-1$^-$2$^-$; plate 4, 14WWΔ*abf2* transformed with pRS416 (no insert).

cells stain red, while those of respiratory-deficient petite cells remain white (Ogur *et al.* 1957). Figure 6C shows representative petri dishes from that experiment. As expected, ~100% of the colonies derived from the Δ*abf2* cells were petite (white; plate 1), whereas only ~5% of the colonies derived from Δ*abf2* cells containing the wild-type *ABF2* allele were petite (plate 2). By contrast, ~80% of the colonies derived from cells containing the *abf2-1⁻2⁻* allele were petite (plate 3), comparable to the Δ*abf2* cells transformed with the vector pRS416 alone (plate 4). These results show that Abf2p-1⁻2⁻, although present at a comparable level as Abf2p, is substantially defective because it only weakly complements the mtDNA instability phenotype of Δ*abf2* cells.

Although these HMG box mutations in Abf2p-1⁻2⁻ were chosen on the basis of the reduced *in vitro* DNA-binding capacity of the rat HMG1 polypeptide (Falciola *et al.* 1994), it was conceivable that such mutations in the context of Abf2p would have little effect on the ability of the protein to bind to mtDNA. Gel shift experiments using recombinant Abf2p and Abf2p-1⁻2⁻ showed that these mutations do inhibit binding to double-stranded DNA by at least fivefold (not shown), but these experiments allow no conclusion as to whether binding is affected *in vivo*.

To address this question, we constructed expression plasmids encoding fusion proteins between the C-terminal end of full-length Abf2p, the mutant protein Abf2p-1⁻2⁻, and GFP so that the tagged proteins are expressed from the natural promoter of the *ABF2* gene and targeted to mitochondria. As a control, another construct was made between the promoter and sequences encoding the first 52 amino acids of CS1 (which contains the matrix-targeting presequence) and coding sequences of GFP. Cells of strain 14WW ρ⁺ were transformed with a plasmid carrying each gene fusion, and the intracellular localization of the resulting fusion proteins was examined by epifluorescene microscopy. Figure 7 (top panel) shows a typical result for Abf2p-GFP: this fusion protein shows a bright, punctate staining pattern that colocalizes with the punctate, DAPI staining of mtDNA. In contrast, Abf2p-1⁻2⁻-GFP (middle panel) is localized diffusely throughout the mitochondrial reticulum in a pattern that is more similar to that of CS1-GFP (bottom panel). These results strongly suggest that while most, if not all, of the Abf2p-GFP is associated with mtDNA *in vivo*, the vast majority of Abf2p-1⁻2⁻-GFP is not bound to mtDNA and is probably localized in the mitochondrial matrix.

We next tested whether overexpression of *abf2-1⁻2⁻* causes the loss of ρ⁺ mtDNA. To insure comparable retention of plasmids containing the wild-type *ABF2* and mutant *abf2-1⁻2⁻* alleles under control of the *GAL1* promoter, galactose induction was carried out in selective YNBGal+cas medium. The experiment of Figure 8 shows that, after a shift of cells from glycerol to galactose medium, both the wild-type and mutant proteins are induced with similar kinetics and reach the same maximum level after about three generations. As seen in Figure 8, cells overexpressing wild-type Abf2p rapidly produce petite mutants, such that by two generations only ~5% of the population is ρ⁺. In contrast, at two

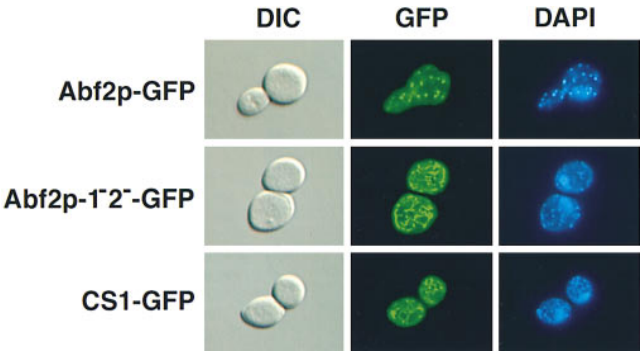

Figure 7.—Abf2p-GFP and Abf2-1⁻2⁻p-GFP localize differently in mitochondria of 14WW ρ⁺ cells. The top panel shows the colocalization of Abf2p-GFP with DAPI-stained mtDNA in cells transformed with the plasmid pRS416/Abf2-GFP. The middle panel shows the more diffuse localization of Abf2-1⁻2⁻p-GFP in cells transformed with the plasmid pRS416/Abf2-1⁻2⁻-GFP. The bottom panel is a control showing the localization of CS1-GFP in 14WW ρ⁺ cells transformed with the plasmid pRS416/CS1-GFP. Shown are the DIC, GFP, and DAPI images, as indicated in the figure.

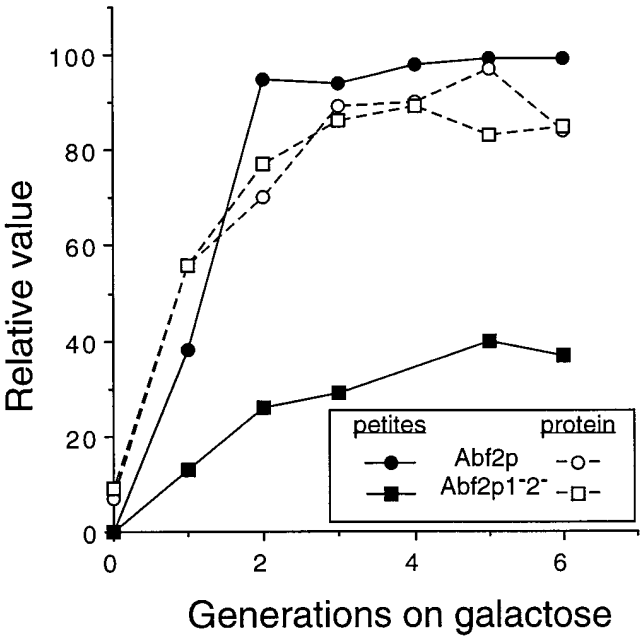

Figure 8.—Overproduction of Abf2-1⁻2⁻p induces fewer petite mutants than does overproduction of wild-type Abf2p. Strain 14WW was transformed with pGalABF2 or pGALabf2-1⁻2⁻. Transformants, maintained on YNBGly + cas medium, were transferred to liquid YNBGal + cas medium to induce expression of Abf2p or Abf2-1⁻2⁻p. Aliquots from each culture were removed after various generations of growth on YNBGal + cas medium, analyzed by Western blotting for the level of Abf2p or Abf2-1⁻2⁻p, and plated on YPD medium to determine the number of petites in the population.

generations, >70% of the cells in the culture overexpressing the mutant protein are still ρ^+, and even by six generations, ~60% of the cells are ρ^+. Thus, despite comparable kinetics and levels of induction of the wild-type and mutant proteins, these mutations in the Abf2p HMG boxes that are involved in DNA binding largely suppress the production of petites when the mutant protein is overexpressed.

DISCUSSION

Abf2p has been suggested to play a structural role in mtDNA metabolism through its ability to bend and wrap DNA (Diffley and Stillman 1992; Fisher *et al.* 1992; Newman *et al.* 1996)—features that are general properties of the HMG family of DNA-binding proteins (Landsman and Bustin 1993). In the present work, we have identified and characterized new phenotypes associated with the deletion or overexpression of the *ABF2* gene in yeast. Our studies illustrate the importance of Abf2p in a variety of mtDNA transactions, and they reveal unexpected differences in the requirements of ρ^+ and ρ^- mtDNAs for Abf2p.

The absence of Abf2p affects mtDNA recombination and the sorting of mitochondrial constituents in crosses: We have previously characterized the pattern of sorting of mitochondrial matrix protein and mtDNA in zygotes derived from parental strains with different mitochondrial genotypes, particularly the mixing of these mitochondrial constituents in ρ^0 by ρ^+ crosses (Azpiroz and Butow 1993). The present results show that the absence of Abf2p changes the sorting pattern in a ρ^0 by ρ^+ cross such that it resembles the sorting pattern previously described for ρ^0 by ρ^- crosses, namely the loss of A-form zygotes and the appearance of the P–D form, in which matrix protein and mtDNA are concentrated in the neck region of the zygote. Those results suggested that the ρ^- mtDNA movement through the zygote was altered with respect to ρ^+ mtDNA. In addition, although we do not yet understand the mechanism accounting for the vectorial movement of matrix protein in ρ^o by ρ^+ crosses that gives rise to the A-form zygotes, the fact that this form is absent in Δ*abf2* zygotes and that the P–D form is detected suggests that the absence of Abf2p may have a similar effect in altering the movement of ρ^+ mtDNA.

We have also shown that the absence of Abf2p markedly reduces the efficiency of mtDNA recombination. Because the extent of mtDNA recombination must clearly depend on the extent to which parental mtDNA molecules mix in the zygote, it is conceivable that the reduced level of recombination observed in the cross between ρ^+ Δ*abf2* parental strains could be caused by some effect on mtDNA mixing. Earlier genetic data showed that the mixing and recombination of mtDNA is restricted largely to the medial portion of the zygote, where the parental mitochondria presumably fuse (Strausberg and Perlman 1978; Zinn *et al.* 1987). This conclusion was recently confirmed by direct visualization of parental mtDNAs in zygotes (Nunnari *et al.* 1997). Although it is possible that the absence of Abf2p somehow affects the mixing of parental mtDNAs in this limited region of the zygote, it is also plausible that Abf2p is likely to play a more direct role in mtDNA recombination (see below).

Variations in the content of Abf2p affect the amount and stability of mtDNA: In Δ*abf2* ρ^+ cells maintained on glycerol medium, the mtDNA content is reduced by roughly 50% compared with *ABF2* cells, whereas in ρ^+ and ρ^- cells carrying several extra copies of the *ABF2* gene, the amount of mtDNA increases typically by 50–150%. These findings imply that the amount of mtDNA could be regulated through regulation of Abf2p levels. Little is known about the regulation of Abf2p levels in mitochondria, although it has been shown that Abf2p mRNA abundance is not repressed by glucose in cells in which there is repression of the ρ^+ mtDNA content (Ulery *et al.* 1994). The amount of mtDNA in wild-type cells is typically in the range of 5–15% of the total cellular DNA (depending on the strain and carbon source), so any control of mtDNA copy number that might be exerted by Abf2p is likely to be within that range and to be limited by other effects of the protein on mtDNA stability. For example, high levels of Abf2p (≥10-fold the wild-type amount) result in a failure of both ρ^+ and ρ^- mtDNAs to be propagated. These findings confirm and extend the observation reported by Megraw and Chae (1993) that there is a high rate of petite formation when the *ABF2* gene is expressed in ρ^+ cells from a galactose-inducible promoter.

Our results strongly suggest that the dramatic instability of mtDNA in cells with high levels of Abf2p depends on the interaction of the protein with mtDNA. We constructed and characterized a new allele of the *ABF2* gene with mutations in a highly conserved motif of both HMG boxes. Analogous mutations in a polypeptide fragment of a mammalian HMG1 protein reduced its binding to cruciform DNA by ~10-fold (Falciola *et al.* 1994). Analysis of the location of the fusion constructs Abf2p-GFP and Abf2-1$^-$2$^-$p-GFP shows clearly that the wild-type protein colocalizes precisely with mtDNA *in vivo*, whereas localization of the mutant protein closely resembles that of a matrix marker, CS1-GFP. While the mutant protein, with or without the GFP tag, accumulates in cells at levels comparable to that of the wild-type protein, it only weakly complements the mtDNA instability phenotype of Δ*abf2* cells. And when overexpressed in ρ^+ cells, Abf2-1$^-$2$^-$p is much less effective than Abf2p in inducing petites.

Functions of Abf2p in the maintenance of mtDNA: One possibility to account for some of the observations described here is that Abf2p functions directly in mtDNA replication as a transcription factor for the generation of RNA primers. This would be analogous to the role of the human mitochondrial homologue of Abf2p, h-mtTFA, in promoter activation of human

mtDNA (Fisher and Clayton 1988; Fisher *et al.* 1987). However, the mechanism of yeast mtDNA replication, specifically whether origin-dependent RNA priming is involved, is unclear. To date, there has been only one study providing evidence for RNA priming of yeast mtDNA replication (Baldacci *et al.* 1984). *In vitro* studies with Abf2p and h-mtTFA show only a modest enhancement of transcription of yeast mtDNA promoter templates by Abf2p, in contrast to a much more robust stimulation of transcription with h-mtTFA (Parisi *et al.* 1993). Moreover, unlike h-mtTFA, Abf2p lacks a C-terminal tail that provides some sequence specificity to DNA-binding and a function in transcription (Parisi and Clayton 1991; Parisi *et al.* 1993). Indeed, when the C-terminal tail of h-mtTFA was fused to the C terminus of Abf2p, the chimeric protein was able to activate transcription from the human mitochondrial light strand-specific promoter *in vitro*, whereas unmodified Abf2p was inactive (Dairaghi *et al.* 1995).

It has also been shown that ρ^- mtDNAs can be maintained in cells lacking the product of the nuclear *RPO41* gene (Fangman *et al.* 1990), which encodes the only known mitochondrial RNA polymerase activity. Although the *RPO41* gene is required for the stability of ρ^+ mtDNA (Greenleaf *et al.* 1986), it is well known that a block of ρ^+ mtDNA gene expression at many different levels results in instability of the mitochondrial genome (Tzagoloff and Meyers 1986; Weislogel and Butow 1970); thus, it is not possible at the present time to pinpoint the requirement for mitochondrial RNA polymerase in the replication or stability of ρ^+ mtDNA. It is conceivable that ρ^- mtDNAs replicate by a mechanism entirely different from that of ρ^+ mtDNA, but definitive evidence supporting that view is lacking. Finally, recent studies showing that hypersuppressivity—the preferential transmission in crosses of ρ^- mtDNAs containing putative origin sequences—is maintained in the absence of the *RPO41* gene product (Lorimer *et al.* 1995), raising further uncertainties as to whether RNA priming is a mechanism for yeast mtDNA replication.

An alternative possibility is that Abf2p affects mtDNA stability and copy number through a function in recombination. This view is consistent with the following observations: (1) HMG box proteins, including Abf2p, bind preferentially to cruciform DNA (Teo *et al.* 1995), which is the equivalent of a four-stranded Holliday junction recombination intermediate; (2) HMG box proteins stimulate a site-specfic, double-strand DNA cleavage reaction *in vitro* catalyzed by the RAG1 and RAG2 proteins, which is the first step in V(D)J recombination (van Gent *et al.* 1997); (3) some fraction of steady-state mtDNA in yeast exists as recombination intermediates, and changes in the level of these intermediates can influence mtDNA transmission (Lockshon *et al.* 1995); and (4) replication of yeast mtDNA may be a recombination-dependent processs (Bendich 1996; Lockshon *et al.* 1995). Although electron microscopic evidence suggesting that yeast mtDNA replication proceeds via a rolling circle mechanism has been presented (Maleszka *et al.* 1991), structures that resemble rolling circle intermediates of DNA replication can also be generated via recombination-driven replication (Asai *et al.* 1993). Finally, it has recently been shown that the replication of mtDNA from the malarial parasite *Plasmodium falciparum* is associated with recombination (Preiser *et al.* 1996).

The yeast mitochondrial genome is very active in recombination, where 1% recombination corresponds to a mtDNA interval of ~100 bp. A significant fraction of yeast mtDNA in wild-type cells is known to exist as larger than unit size molecules (Bendich 1996); these are branched structures that accumulate to very high levels in the mitochondria of cells that lack the product of the *MGT1* gene (Lockshon *et al.* 1995). *MGT1* encodes a mitochondrial enzyme that cleaves four-way DNA junctions, which are the equivalent of Holliday recombination intermediates (Evans and Kolodner 1988; Kleff *et al.* 1992). Recombination-dependent replication, whereby DNA replication is primed from the 3′ end of an invading DNA strand, has been shown to be used for the replication of some plasmid and bacteriophage DNAs (Asai *et al.* 1993; Formosa and Alberts 1986; Luder and Mosig 1982).

An important relationship between recombination intermediates and mtDNA transmission has been established from the studies of Zweifel and Fangman (1991) and Lockshon *et al.* (1995). These investigators showed that deletion of the *MGT1* gene resulted in loss of suppressivity of hypersuppressive petite mitochondrial genomes, as well as an increase in mitotic instability of mtDNA, particularly of ρ^- mtDNA. The mechanism of hypersuppressivity is not known, but it is generally thought to result from outcompetition by hypersuppressive ρ^- mtDNA of ρ^+ mtDNA in zygotes of hypersuppressive $\rho^- \times \rho^+$ crosses. Although the effect of loss of the *MGT1* product on the stability of ρ^+ mtDNA was more subtle than the dramatic loss of hypersuppressivity in hypersuppressive $\rho^- \times \rho^+$ crosses, the overall conclusion of those studies was that the requirement to cleave recombination intermediates in an *MGT1*-dependent reaction affects transmission of mtDNA during mitotic growth and in crosses.

The observed decrease in ρ^+ mtDNA copy number in Δ*abf2* cells grown on glycerol medium may result from a reduction in the steady-state level of recombination intermediates, whereas in cells containing greater than wild-type levels of Abf2p, more mtDNA would be present as recombination structures. By stabilizing recombination intermediates, increased Abf2p levels would effectively increase the recombination-dependent priming of mtDNA replication that leads to higher levels of mtDNA. In the extreme case of cells with ≥10-fold higher levels of Abf2p, however, most of the mtDNA might be trapped as networks of recombination structures. That such structures could lead to a rapid produc-

tion of ρ^o petites is suggested by the studies of Lockshon *et al.* (1995), who showed that a null allele of the *MGT1* gene leads to large mtDNA networks that severly hamper efficient mtDNA transmission. The fact that the *MGT1* null mutation has only a modest effect on the stability and transmission of ρ^+ mtDNA (Lockshon *et al.* 1995) suggests that the relationship between recombination, Abf2p, and mtDNA stability is likely to be complex.

It is possible that the dramatic effect of high levels of Abf2p on mtDNA replication and stability may result from the general DNA-binding activity of Abf2p rather than or in combination with its cruciform-binding activity. For example, large increases in the amount of Abf2p may convert mtDNA to a more bent or twisted structure that is less accessible to the replication apparatus, despite the potential for stabilizing recombination structures. Finally, the finding that ρ^- mtDNAs are stable in the absence of Abf2p could be accounted for by the fact that many ρ^- mitochondrial genomes, such as the VAR1 and HS40 mitochondrial genomes studied here, are organized as short, tandem repeating units of identical sequence that would be expected to enhance greatly the opportunity for homologous recombination. This increased opportunity for recombination could thus override any requirement for Abf2p to stabilize recombination structures.

We thank B. Stillman (Cold Spring Harbor Laboratory) and B. Horozdovsky (University of Texas Southwestern Medical Center) for generous gifts of plasmids. This research was supported by grants GM33510 from the National Institutes of Health and I-0642 from The Robert A. Welch Foundation.

LITERATURE CITED

Asai, T., S. Sommer, A. Bailone and T. Kogoma, 1993 Homologous recombination-dependent initiation of DNA replication from DNA damage-inducible origins in Escherichia coli. EMBO J. **12:** 3287–3295.

Azpiroz, R., and R. A. Butow, 1993 Patterns of mitochondrial sorting in yeast zygotes. Mol. Biol. Cell **4:** 21–36.

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.

Baxevanis, A. D., and D. Landsman, 1995 The HMG-1 box protein family: classification and functional relationships. Nucleic Acids Res. **23:** 1604–1613.

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.

Berben, G., J. Dumont, V. Gilliquet, P. A. Bolle and F. Hilger, 1991 The YDp plasmids: a uniform set of vectors bearing versatile gene disruption cassettes for *Saccharomyces cerevisiae*. Yeast **7:** 475–477.

Caron, F., C. Jacq and J. Rouviere-Yaniv, 1979 Characterization of a histone-like protein extracted from yeast mitochondria. Proc. Natl. Acad. Sci. USA **76:** 4265–4269.

Chen, D.-C., B.-C. Yang and T.-T. Kuo, 1992 One-step transformation of yeast in stationary phase. Curr. Genet. **21:** 83–84.

Conde, J., and G. Fink, 1976 A mutant of *Saccharomyces cerevisiae* defective for nuclear fusion. Proc. Natl. Acad. Sci. USA **73:** 3651–3655.

Dairaghi, D., G. Shadel and D. Clayton, 1995 Addition of a 29 residue carboxyl-terminal tail converts a simple HMG box-containing protein into a transcriptional activator. J. Mol. Biol. **249:** 11–28.

Diffley, J. F., and B. Stillman, 1991 A close relative of the nuclear, chromosomal high-mobility group protein HMG1 in yeast mitochondria. Proc. Natl. Acad. Sci. USA **88:** 7864–7868.

Diffley, J. F. X., 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*, edited by J. N. Strathern, E. W. Jones and J. R. Broach. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Evans, D. H., and R. Kolodner, 1988 Effect of DNA structure and nucleotide sequence on Holliday junction resolution by the *Saccharomyces cerevisiae* endonuclease. J. Mol. Biol. **201:** 69–80.

Falciola, L., A. I. H. Murchie, D. M. J. Lilley and M. E. Bianchi, 1994 Mutational analysis of the DNA binding domain A of chromosomal protein HMG1. Nucleic Acids Res. **22:** 285–292.

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

Fisher, R. P., and D. A. Clayton, 1988 Purification and characterization of human mitochondrial transcription factor 1. Mol. Cell. Biol. **8:** 3496–3509.

Fisher, R. P., J. N. Topper and D. A. Clayton, 1987 Promoter selection in human mitochondria involves binding of a transcription factor to orientation-independent upstream regulatory elements. Cell **50:** 247–258.

Fisher, R. P., T. Lisowsky, M. A. Parisi and D. A. Clayton, 1992 DNA wrapping and bending by a mitochondrial high mobility group-like transcriptional activator protein. J. Biol. Chem. **267:** 3358–3367.

Formosa, T., and B. M. Alberts, 1986 DNA synthesis dependent on genetic recombination: characterization of a reaction catalyzed by purified bacteriophage T4 proteins. Cell **47:** 793–806.

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

Hall, R. M., P. Nagley and A. W. Linnane, 1976 Biogenesis of mitochondria. XLII. Genetic analysis of the control of cellular mitochondrial DNA levels in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **145:** 169–175.

Herskowitz, I., and R. E. Jensen, 1991 Putting the *HO* gene to work: practical uses for mating-type switching. Methods Enzymol. **194:** 132–146.

Hoffman, C. S., and F. Winston, 1987 A ten-minute preparation from yeast efficiently releases autonomous plasmids for transformation of *Escherichia coli*. Gene **57:** 267–272.

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.

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.

Landsman, D., and M. Bustin, 1993 A signature for the HMG-1 box DNA-binding proteins. BioEssays **15:** 1–8.

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.

Lopez, I. C., F. Farelly and R. A. Butow, 1981 Trans action of the *var1* determinant region on yeast mitochondrial DNA. Specific labeling of mitochondrial translation products in zygotes. J. Biol. Chem. **256:** 6496–6501.

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.

Luder, A., and G. Mosig, 1982 Two alternative mechanisms for intitiation of DNA replication forks in bacteriophage T4: priming by RNA polymerase and recombination. Proc. Natl. Acad. Sci. USA **78:** 1101–1105.

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

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.

Myers, A. M., A. Tzagoloff, D. M. Kinney and C. J. Lusty, 1986 Yeast shuttle and integrative vectors with multiple cloning sites suitable for construction of *lacZ* fusions. Gene **45:** 299–310.

Nagley, P., and A. W. Linnane, 1972 Studies on the nature of the mitochondrial genome in yeast, the degenerative effects of ethidium bromide on mitochondrial genetic information in a respiratory competent strain. J. Mol. Biol. **66:** 181–193.

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.

Ng, R., and J. Abelson, 1980 Isolation and sequence of the gene for actin in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **77:** 3912–3916.

Nunnari, J., W. F. Marshall, A. Straight, A. Murray, J. W. Sedat *et al.*, 1997 Mitochondrial transmission during mating in *Saccharomyces cerevisiae* is determined by mitochondrial fusion and fission and the intramitochondrial segregation of mitochondrial DNA. Mol. Biol. Cell **8:** 1233–1242.

Ogur, M., R. St. John and S. Nagai, 1957 Tetrazolium overlay technique for population studies of respiration deficiency in yeast genetics. Science **125:** 928–929.

Parikh, V. S., H. Conrad-Webb, R. Docherty and R. A. Butow, 1989 Interaction between the yeast mitochondrial and nuclear genomes influences the abundance of novel transcripts derived from the spacer region of the nuclear ribosomal DNA repeat. Mol. Cell. Biol. **9:** 1897–1907.

Parisi, M. A., and D. A. Clayton, 1991 Similarity of human mitochondrial trancription factor 1 to high mobility group proteins. Science **252:** 965–969.

Parisi, M. A., B. J. Xu and D. A. Clayton, 1993 A human mitochondrial transcriptional activator can functionally replace a yeast mitochondrial HMG-box protein both *in vivo* and *in vitro*. Mol. Cell. Biol. **13:** 1951–1961.

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

Rose, M. D., F. Winston and P. Heiter, 1988 *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Strausberg, R. L., and P. S. Perlman, 1978 The effect of zygotic bud position on the transmission of mitochondrial genes in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **163:** 131–144.

Teo, S. H., K. D. Grasser, C. H. Hardman, R. W. Broadhurst, E. D. Laue *et al.*, 1995 Two mutations in the HMG-box with very different structural consequences provide insights into the nature of binding to four-way junction DNA. EMBO J. **14:** 3844–3853.

Troitskaya, O., P. S. Perlman and R. A. Butow, 1995 *ILV5* encodes a bifunctional mitochondrial protein involved in branched chain amino acid biosynthesis and maintenance of mitochondrial DNA. EMBO J. **14:** 3268–3276.

Tzagoloff, A., and A. M. Meyers, 1986 Genetics of mitochondrial biogenesis. Annu. Rev. Biochem. **55:** 249–285.

Ulery, T. L., S. H. Jang and J. A. Jaehning, 1994 Glucose repression of yeast mitochondrial transcription: kinetics of derepression and role of nuclear genes. Mol. Cell. Biol. **14:** 1160–1170.

van Gent, D. C., K. Hiom, T. T. Paull and M. Gellert, 1997 Stimulation of V(D)J cleavage by high mobility group proteins. EMBO J. **16:** 2665–2670.

Vida, T. A., T. R. Graham and S. D. Emr, 1990 In vitro reconstitution of intercompartmental protein transport to the yeast vacuole. J. Cell Biol. **111:** 2871–2884.

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.

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

Zinn, A. R., J. K. Pohlman, P. S. Perlman and R. A. Butow, 1987 Kinetic and segregational analysis of mitochondrial DNA recombination in yeast. Plasmid **17:** 248–256.

Zweifel, S. G., and W. L. Fangman, 1991 A nuclear mutation reversing a biased transmission of yeast mitochondrial DNA. Genetics **128:** 241–249.

Communicating editor: K. J. Newton