The DNA binding properties of ABF2, an abundant protein found in the mitochondria of the yeast *Saccharomyces cerevisiae* have been examined in detail. ABF2 is closely related to the vertebrate high mobility group protein HMG1 and like HMG1, ABF2 will introduce negative supercoils into a relaxed, double-stranded circular DNA molecule in cooperation with a DNA topoisomerase. Additionally, ABF2 binds approximately 5–10 times more tightly to negatively supercoiled DNA than to relaxed circular or linear DNA. Although ABF2 binds to most random double-stranded sequences with roughly equal affinity, its binding within certain key regulatory regions is qualitatively quite different. First, ABF2 binding induces a distinct pattern of DNA bending within the chromosomal origin of DNA replication, ARS1. Second, ABF2 binding to all nuclear replication origins tested, in addition to a critical mitochondrial promoter and replication origin, is clearly nonrandom as visualized by DNase1 footprinting. Analysis of the sequences found within these regions as well as competition experiments with synthetic DNA molecules suggest that site-specific DNA binding may be accomplished by the phased distribution of short stretches of poly(dA), which exclude ABF2 binding. These patterns of ABF2 DNA binding suggest a role for the protein in genome organization and site-specific regulation of transcription or DNA replication.

Virtually all large genomic DNA molecules are found associated with a diverse group of abundant, basic, low molecular weight, DNA binding proteins whose primary function is to compact these DNA molecules and to neutralize their negative charge, allowing them to fit into relatively small volumes. The elemental packaging unit in eucaryotic nucleosomes is the nucleosome octamer, composed of two copies each of the four core histones H2A, H2B, H3, and H4 linked into higher order structures by the fifth histone, H1 (1). In addition to the histones, other abundant chromosomal proteins known as the high mobility group (HMG)1 proteins are found, although the roles of these proteins is less clear (2). In procaryotes, the prototypical packaging protein is the *Escherichia coli* HU protein, which binds to DNA as a dimer. Additionally, several other, less well characterized, proteins appear to be involved (3, 4).

The proteins that package the genomic DNA of eucaryotic organelles have been less well characterized. Recently, we described ABF2, a protein found in the mitochondria of the yeast *Saccharomyces cerevisiae* (5). The nuclear encoded ABF2 gene was cloned and shown to be required for the efficient maintenance of the mitochondrial genome. This, taken with the abundance of ABF2 (roughly one copy for every 15–30 bp of mitochondrial DNA) argued that ABF2 is involved in packaging the yeast mitochondrial genome. Remarkably, the predicted amino acid sequence of ABF2 is closely related to the vertebrate nuclear high mobility group protein HMG1. Since mitochondria are widely believed to have descended from eubacteria via endosymbiosis (6, 7), this suggests that the packaging function of a primordial HU-related protein was, at some point during evolution, usurped by a nuclear-encoded HMG1 protein. Such an event may have been critical in ultimately establishing the control of the nuclear genome over the mitochondrial genome.

The packaging of DNA poses a general problem: How can the accessibility of key regulatory DNA sequences within the genome to specific trans-acting factors be guaranteed against the overwhelming backdrop of nonspecific DNA binding by the packaging proteins? In this report, we show that ABF2 appears to bind to DNA by wrapping and, therefore, appears capable of compacting DNA. Furthermore, although ABF2 binds to most DNA sequences with similar affinity, its binding to key regulatory sequences from both the nucleus and the mitochondria is strongly phased. We propose that such phased binding could provide access for other trans-acting factors involved in DNA replication and gene expression.

Interestingly, a mitochondrial protein that is related to ABF2 was recently identified as the mitochondrial transcriptional activator protein mtTF1 from human cells (8) and a related transcriptional activator has been identified in *S. cerevisiae* (9). Indeed, it is probable that ABF2 is the *S. cerevisiae* mtTF1 protein. Consistent with this possibility, Fisher et al. (10) report in the accompanying paper that mtTF1 from both human and yeast mitochondria has DNA binding properties similar to those reported herein for ABF2.

**MATERIALS AND METHODS**

**Plasmids and Strains** — ABF2 was purified from the yeast strains BJ 1991 (*Mata leu2 ura3 trpl prb1-1122 pep4*) or BJ 405 (*Mata trpl prb1 pro1 pep4-3*). The plasmid p4ARS1.4.1 has been previously described (11). This plasmid contains ARS1 sequences from the *Real* site at nucleotide 927 to the *HinfI* site at nucleotide 734 (12) blunt-ended and cloned into the *SalI* site of *UC19* such that domain A of ARS1 (13) lies on the EcoRI site of the polynucleotid and domain B (13) lies on the *HindIII* site of the polynucleotid. As previously noted (11), this places domain B of ARS1 between the *BamHI* site of the
pUC19 polylinker and the internal BglII site (12). Domain B was containing 16 head-to-tail copies of domain B (see Fig. 4) by a previously described method (14, 15). pARS2 contains the 627-bp XhoI fragment from ARS2 (16) derived from the plasmid YRpl4/CEN4/ARS2 (17) cloned into the SmaI site of pC2G1. pARS1 contains the 260-bp XmnI-Ssp1 fragment containing the 2 μm origin of DNA replication (18) cloned into the Smal site of pUC118 such that the Spac site lies on the HindIII site and the XmaI site lies on the EcoRI site of the polylinker. The plasmid pC2G1 was derived from C2G1-S200, a gift from Virginia Van Houten and Carol Newlon (University of Medicine and Dentistry of New Jersey), which contains the C2G1 200-bp ARS (19, 20) cloned into M13 mp18. The EcoRI-HindIII fragment from this M13 was subcloned into the polylinker of pUC119. Note that the C2G1 ARS is now called ARS 307.

Purification of ABF2—Yeast was grown in YPD in a 10-liter fermenter to late log phase yielding approximately 700 g of wet weight of cell paste containing approximately 7.8 × 1017 cells. The preparation of yeast extracts, heparin-agarose, and Blue-Sepharose CL-4B chromatography of ABF1 and ABF2 have been previously described (11). The ABF2 activity was assayed by a DNA binding assay using ARS1 DNA as described below. ABF2 was eluted from Blue-Sepharose in 50 mM Pipes pH 6.5, 10 mM sodium metabsulfite, 1 mM phenylmethylsulfonyl fluoride, 10 mM diethylthiocarbamate, 1 mM EDTA, 1 mM diethiothreitol, 1 mM EGTA, 10% (v/v) glycerol, final pH 6.5 containing 2.5 M NaCl. The column was washed successively with 3 column volumes each of buffer X containing 0.5 mM NaCl and buffer X without NaCl. ABF2 was eluted from the column with buffer X containing 5% Triton X-100. This fraction was concentrated by loading it directly onto a 10-ml column of heparin-agarose equilibrated in buffer X and eluting ABF2 with buffer X containing 2 M NaCl. The concentrated ABF2 was loaded directly onto a Sephacryl S-200 column (2 × 100 cm) equilibrated in buffer X containing 0.5 M NaCl. Active fractions were dialyzed against buffer X containing 0.1 M NaCl with buffer X and loaded onto a Mono S HR 5/5 column equilibrated in buffer X containing 0.1 M NaCl. The column was developed with a 5-ml gradient from 0.1 to 0.6 M NaCl in buffer X. Active fractions were pooled and stored at −70 °C.

One such preparation yielded 1.6 mg of purified ABF2. ABF2 DNA binding activity cannot be quantified in crude extracts due to the fact that substantial amounts of competitor DNA are required to see the ABF2 specific signal which, nonetheless, inhibit ABF2 binding substantially. However, an estimate of recovery can be made as follows: 1.6 mg of a 20-kDa protein corresponds to approximately 4.6 × 1010 molecules based on there being approximately 2500 molecules of ABF2 per cell (see below), there were 250,000 × 7.8 × 1011 or 1.95 × 1017 molecules of ABF2 in the starting cell mass. Therefore, the recovery in this purification was approximately 4.6 × 1010/1.95 × 1017 = 0.236 or 23.6%. It should be noted that, because of the dual subcellular location of ABF2, it is not possible at this point to know whether both sources are represented in the purified protein or if one pool is preferentially extracted under the conditions used for this preparation.

DNA Binding and Competitions—DNA fragments were 5'-end-labeled with [γ-32P]ATP by standard procedures (21). DNA binding reactions for band-shift assays and DNase I footprinting were essentially as described (11). The standard competitor DNA used in binding reactions was a mixture of poly(dA)-oligo(dT) and poly(dC)-oligo(dG) prepared in 10 mM Tris-HCl, pH 7.5, and 200 mM KCl as previously described (15). Other competitor DNAs were prepared as follows: poly(dA·dT), poly(dA) and poly(dT) were dissolved at 1 mM nucleotide and treated the same as the other synthetic DNAs (15). Hybridization of equimolar amounts of poly(dA) and poly(dT) was as described for other synthetic DNAs (15) to yield poly(dA)·poly(dT). Plasmid DNAs were purified by alkaline lysis and CsCl gradients (21). These plasmids, digested with restriction enzymes according to the manufacturer's instructions.

Supercoiling Reactions—Plasmid DNA (10 μg) was relaxed with empirically determined amounts of calf thymus topoisomerase I in 50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 0.5 mM diethylthioctol, 0.5 mM EDTA, and 0.02% BSA in a 10-μl reaction at 37 °C for 30 min. Relaxed plasmid was ethanol precipitated with ethanol and resuspended at a concentration of 1 mg/ml. Relaxed plasmid was incubated with the indicated amounts of ABF2 under the standard DNA binding conditions for 30 min at 30 °C in the presence of an empirically determined large excess of calf thymus topoisomerase I. Reactions were then terminated by adding an equal volume (10 μl) of 0.2 mg/ml proteinase K, 20 mM EDTA, and 2% SDS. After 30 min at 37 °C, 1 μg E. coli tRNA was added and reactions were heated to 100 °C for 2 min. DNA was then precipitated with ethanol and NaCl to 100 μl, 0.1 M NaCl and precipitated with ethanol. Samples were resuspended in 10 mM Tris, pH 7.4, 1 mM EDTA (TE) and subjected to electrophoresis in 1% agarose gels in Tris-Borate EDTA (21) buffer containing the indicated amounts of chloroquine.

RESULTS

Purification of ABF2—ABF1 and ABF2 were initially described as proteins from whole cell yeast extracts that interacted specifically with a labeled DNA probe containing the yeast origin of chromosomal DNA replication, ARS1, in a band-shift assay (11). We have used this assay to follow these binding activities during their purification. Our purification of ABF1 has been previously described (11), and in this report we describe the purification and characterization of ABF2.

ABF1 and ABF2 eluted together from heparin-agarose at approximately 300 mM (NH4)2SO4, and were separated from each other on agarose columns containing the blue dye Cibacron Blue 3GA. ABF2 bound extremely tightly to this dye column, requiring at least 1.5 M NaCl for elution. Subsequent experiments showed that dye binding was competitive with DNA binding, suggesting that the dye and DNA interact with the same site on the protein (data not shown). ABF2 was subsequently purified through a series of chromatographic steps (see “Materials and Methods”) culminating in a Mono S FPLC column. The DNA binding activity and polypeptide profile eluting from one such Mono S column is shown in Fig. 1, A and B. A polypeptide of 20 kDa coeluted with DNA binding activity from this column as well as several other columns (data not shown). This, taken with the fact that this 20-kDa polypeptide was absent from strains in which the ABF2 gene has been deleted (5), indicate that it is ABF2.

To carefully quantify the amount of ABF2 per cell, immunoblots were performed. Cells grown to mid-log phase in medium containing either glucose (YPD) or glycerol (YPG) as the sole carbon source were counted, and extracts from 107 cells were made by disruption with glass beads to nearly 100% lysis. Lysed cells were boiled in SDS sample buffer to insure that all of the cellular ABF2 was extracted. Different amounts of these extracts were subjected to electrophoresis alongside different amounts of purified ABF2, proteins were transferred to nitrocellulose, and blots were probed with anti-ABF2 antibody. The results in Fig. 1C reveal several features. First, cells grown in either glucose or glycerol have virtually identical amounts of ABF2. Thus, ABF2 levels are not dictated by mitochondrial activity or total amounts of mitochondrial DNA, which increase when cells are grown on a nonfermentable carbon source such as glycerol. And second, the amount of ABF2 per cell can be estimated quite accurately by comparing the amount of immunoreactive material from a given number of cells with the standards. 300,000 cells (e.g. lanes 3 and 6) contains approximately the same amount of immuno-reactive material at 20 kDa as does 2.5 ng of purified ABF2 (lane 7). Given a molecular weight of approximately 27 kDa, this corresponds to 1.25 × 1012 mol or 7.5 × 1012 molecules and, therefore, represents 250,000 molecules of ABF2 per cell. Since haploid yeast cells contain approximately 50 molecules of mtDNA of approximately 75,000 bp each (34), there are 50 × 75,000/250,000, or 15 bp of mtDNA per molecule of ABF2. We note that this estimate of the amount of ABF2 per cell is in agreement with that of Caron et al. (25) for the HM protein.

Relative Affinity of ABF2 for DNA Sequences—Early in our analysis, we realized that ABF2 would bind many labeled
ABF2 was purified as described under “Materials and Methods.” 0.1 µl of the indicated fractions from the Mono S HR 5/5 column was assayed for DNA binding activity as described under “Materials and Methods.” B, SDS-polyacrylamide gel electrophoresis. 10 µl of the same fractions were subjected to electrophoresis through a 15% SDS-polyacrylamide gel as described. The gel was stained with 0.1% Coomassie Brilliant Blue R-250 in 50% methanol, 10% acetic acid and destained in 5% methanol, 7.5% acetic acid. The apparent molecular weights of the marker proteins are indicated. C, quantitation of intracellular ABF2 levels. BJ405 was grown to mid-log phase with different linear plasmids. Fig. 3 shows that both pUC19 and pARS1.4.1 (pUC19 containing domains A and B of ARS1) inserted in the polylinker (11) were equally effective as competitors for ABF2 bound to either ARS1 DNA or pBR322 DNA. From these and other experiments we conclude that ABF2 has a similar affinity for most DNA (although, see Fig. 7). DNA Bending by ABF2—The band-shift patterns produced with the different probes in Fig. 2 were strikingly different; two different pBR322 probes each produced an orderly ladder of shifted bands with increasing amounts of protein, while the two ARS1 probes produced a more complex pattern of shifted bands. The relative positions of these shifted bands in the case of ARS1 was dependent upon where ARS1 lies within the fragment, suggesting that ABF2 causes the induction of specific bends at ARS1.

Preliminary band shifts with different regions of ARS1 suggested that domain B was primarily responsible for this anomalous behavior (data not shown). Therefore, we constructed a plasmid in which domain B was reiterated in head-to-tail fashion 16 times. From this plasmid, DNA fragments of identical size can be excised using different restriction enzymes that each normally cut just once within domain B. Each of these excised fragments, therefore, contain different circular permutations of the identical sequence. This strategy is outlined in Fig. 4A.

These fragments were then 5'-end-labeled with 32P and used in band-shift assays with different amounts of purified ABF2 (Fig. 4B). Although the molecular weight of each fragment was identical, the relative mobility of these fragments without added protein was slightly different because of a static bend within domain B of ARS1 (22). Because the relative mobility of bend DNA is dependent upon the position of the bend within the fragment, we could infer the effect of ABF2 on bending by examining the relative positions of the band shifts produced by the binding of one, two, three etc. molecules of ABF2. For example, although the P and H fragments...
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Fig. 4. ARS1 bending by ABF1. The plasmid pARS1.4.16, containing 16 head-to-tail copies of domain B (thick black line) (13) was constructed as described under "Materials and Methods" and is shown, in part, in A. The striped box is domain A (13) and the dotted line represents the vector sequence. For the purpose of illustration, only two complete and two partial copies (interrupted by wavy lines) of domain B are shown. Fragments excised with Sau3A (S), HgiAI (H), and PstI (P) all contain identical, circularly permuted DNA sequence. These fragments were 5′-end-labeled and used in band-shift assays with the indicated amounts of ABF2. Different complexes formed with increasing amounts of ABF2, which are referred to in the text, are designated by number at the right of the figure along with the position of unbound DNA, designated U.

migrated together in the absence of ABF2, the first band-shifted product with ABF2 (designated 1 in Fig. 4B and presumably representing a monomer of ABF2 bound) caused the P fragment to migrate slower than the H fragment. This indicated that the ABF2-induced bend center in this complex was different from that in the naked DNA fragment. Another transition in bend center could be seen between the 1 and 2 complexes, at which point the P fragment became the slowest migrating fragment. Again, in the 3 complex, the H fragment became the slowest migrating fragment. The results presented in Figs. 2 and 4 indicate that ABF2 induces a specific and complex pattern of DNA bending at ARS1.

Specific, Phased Binding of ABF2—The specific pattern of DNA bending demonstrated in Figs. 2 and 4 implied that ABF2 must be binding nonrandomly across DNA fragments containing ARS1. Data presented in Figs. 2 and 3, however, suggested that the overall affinity of ABF2 for ARS1- and pBR322-containing DNA fragments was roughly equal.

To resolve this apparent paradox, we have analyzed ABF2 binding to various DNA molecules by DNaseI footprinting. Using DNA fragments for pBR322 as labeled probe, high ABF2 concentrations protected virtually the entire fragment from DNaseI digestion (Fig. 5A). We interpret this result as indicating that ABF2 could bind at multiple random locations across this fragment. At high ABF2 concentrations, each molecule was essentially coated with ABF2, however, the register of ABF2 binding on each fragment was random. Thus, the entire population produces what appears to be random protection which we term "unphased" binding.

In contrast, DNaseI footprints at ARS1 (Fig. 5B) as well as several other nuclear ARS elements including ARS2 (Fig. 5C) (16), the C2G1 ARS (ARS307, Fig. 5D) (20) and the 2 μM origin of DNA replication (Fig. 5E) (18) as well as regulatory sequences from the yeast mitochondrial genome (5) were distinctly nonrandom. In each case, even at the highest ABF2 concentrations, multiple, very clearly defined regions of DNaseI protection of between 25 and 30 bp could be discerned, separated by very short regions of absolutely no protection, often containing distinct DNaseI hypersensitive sites. Thus, with these DNA fragments, ABF2 binding is virtually every molecule in the population occurred at the same site(s). We term this mode of binding as being "phased."

The data presented in Figs. 2, 3 and 5 indicate that phased binding could not be due to higher affinity of ABF2 for specific sites within ARS1. A clue to understanding how phased binding occurred could be found by looking at the sequences in and around the phased binding sites. We noticed that, although we could find no consensus sequence within the footprinted regions, in nearly all cases, the 11-bp ARS consensus sequence (A/TTTTATpTTT^TA/pT) and near matches of this sequence (9/11 and 10/11 matches) were found at the borders of the phased sites (Fig. 6, A-D). This suggested that phased binding may be accomplished by the exclusion of ABF2 from these A+T-rich sequences. The most striking example of this was seen with the mitochondrial sequence p64. This sequence is a 64-bp multiple direct repeat derived from the REP2 origin of mitochondrial DNA replication containing only A and T residues (23). Most of this sequence, consisting primarily of alternating A and T residues, is found entirely within the phased binding sites. The sequence deviates from alternating A and T residues at the borders of the phased binding sites, where a stretch of 9 of 11 T residues in one strand is found (Fig. 6E).

To test the hypothesis that phased binding is due to exclusion of ABF2 by stretches of poly(dA) [or poly(dT)], we have looked at the ability of synthetic and natural DNA molecules to act as competitors for ABF2 binding to labeled DNA fragments containing ARS1. In this experiment, increasing amounts of linear pUC19 (Fig. 7A), the alternating copolymer, poly(d[AT]) (Fig. 7B), and the double-stranded homopolymer, poly(dA)·poly(dT) (Fig. 7C) were used as competitors in band shifts using subsaturating amounts of ABF2. It is clear from this experiment that while both pUC19 and poly(d[AT]) were effective competitors, poly(dA)·poly(dT) was completely ineffective.

Thus, we conclude that ABF2 is unable to bind to stretches of poly(dA) in duplex DNA and that the phased location of even very short stretches of poly(dA) at approximately 25–30-bp intervals is probably responsible for the phased binding seen at nuclear and mitochondrial regulatory sequences.

Negative Supercoiling by ABF2—A common feature of many abundant, small DNA binding proteins including HMG1 is the ability to induce the formation of supercoils in relaxed, closed circular plasmid molecules in cooperation with topoisomerase I. This reflects the fact that, in binding, these proteins induce a superturn in the DNA molecule, presumably by constraining a superturn of the opposite modality (wrapping). Topoisomerase then removes the unconstrained superturn, altering the linking number in the closed circle which, upon deproteinization, can be visualized by agarose gel electrophoresis.

We tested the ability of ABF2 to induce supercoiling under such conditions. Fig. 8A shows that increasing amounts of ABF2 in the presence of a constant amount of calf thymus topoisomerase I generated increasing amounts of a product that comigrated with form I supercoiled plasmid DNA. Although the plasmid used in this experiment contained ARS1, the presence of ARS sequences or, in fact, any yeast sequences, was not required for supercoiling (data not shown) consistent with our finding that ABF2 binds efficiently to
FIG. 5. DNasel footprints with ABF2. DNasel footprints were performed with purified ABF2 essentially as described (11). Reactions contained 32P-5'-end-labeled DNA probes as follows: A, the BamHI-SalI fragment from pBR322 labeled at the SalI site; B, the EcoRI-HindIII fragment from pARS1.2 labeled at the EcoRI site; C, the EcoRI-HindIII fragment from pARS2 labeled at the HindIII site; D, the EcoRI-HindIII fragment from pC2G1 labeled at the EcoRI site; and E, the EcoRI-HindIII fragment from pUC118/2 µM origin labeled at the HindIII site. For B-E reactions in lanes 1-5 contained 0, 0.03, 0.06, 0.09, and 0 µg of purified ABF2. For A, reactions in lanes 1-4 contained 0, 0.05, 0.1, and 0 µg of purified ABF2.

To determine the direction of the supercoiling induced by ABF2, aliquots of the reaction products shown in Fig. 8A were subjected to electrophoresis through agarose gels containing different amounts of chloroquine. Chloroquine intercalates into DNA and unwinds it, introducing positive superhelical twists into covalently closed DNA. Therefore, with increasing concentrations of chloroquine, negatively supercoiled DNA will transiently appear to relax, and then become supercoiled again (positively), while positively supercoiled DNA will be unaffected by any concentration of chloroquine. The results of this experiment are shown in Fig. 8B and C. From this experiment, it is evident, particularly at intermediate concentrations of chloroquine, that the supercoiled product appeared to be relaxed by low concentrations of chloroquine. Therefore, we conclude that ABF2 induces the formation of negatively supercoiled DNA in this assay.

In addition to inducing the formation of negatively supercoiled DNA, competition analysis has shown that ABF2 bound approximately 10 times more tightly to negatively
ABF2 as the Mitochondrial DNA Packaging Protein—Several lines of evidence argue that ABF2 is an important structural element of the yeast mitochondrial nucleoid.

First, ABF2 is an extremely abundant protein. There are approximately 250,000 ABF2 molecules in each yeast cell, at least half of which reside in the mitochondria. Thus, even if only half of the intracellular ABF2 is mitochondrial, there is one ABF2 molecule for every 30 bp of mitochondrial DNA assuming that ABF2 binds as a monomer. Indeed, it exists in solution as a monomer. Since 25–30 bp of DNA are protected in each of the phased footprints, this suggests that there is enough ABF2 to coat all of the mitochondrial DNA. Second, although ABF2 null mutants can be maintained on nonfermentable carbon sources, they grow quite poorly and lose their mitochondrial DNA at an extremely high rate, suggesting a dysfunction in mitochondrial DNA metabolism. Third, as shown in Fig. 8, ABF2 can induce the formation of negative supercoils in DNA in cooperation with DNA topoisomerase. Both the eukaryotic nucleosome and the E. coli HU protein exhibit a similar activity. In the case of the nucleosome and probably the HU protein (although, see Ref. 24), supercoiling is generated by DNA wrapping around a central protein core. Therefore, ABF2, at least potentially, has one of the most important activities of packaging proteins: the ability to compact the DNA molecule. A previously described protein, HM, has some properties that are related to ABF2 and may, in fact, be the same protein (25).

We note that ABF2 is a close relative of the vertebrate HMG1 protein, and it has been long known that HMG1 proteins exhibit a similar supercoiling activity. Based primarily on thermal denaturation studies (26, 27) and the ability of HMG1 to bind single-stranded DNA (29, 29), it has been concluded that this supercoiling activity is not due to wrapping, but rather, to DNA unwinding by HMG1. We believe that, at least with ABF2, this is not the case primarily because ABF2 does not bind well to single-stranded DNA either in the band-shift or competition assays (data not shown).

Dunaway (30) has shown that the RNA polymerase I transcription factor TFIS, also known at UBF, appears to wrap DNA in its binding to the promoter and enhancer of the rRNA gene based, not on the supercoiling assay, but on the periodicity of DNase1 cleavage sites. UBF has been cloned and sequenced and shown to contain at least three HMG boxes (31) suggesting that DNA wrapping may be a general property of HMG1-related proteins.

ABF2 in Mitochondrial Transcription and Replication—Specific trans-acting factors involved in transcription and replication must somehow gain access to key regulatory sequences despite the presence of large amounts of abundant, nonspecific packaging proteins. The DNA-binding properties of ABF2 may provide a mechanism whereby most of the mitochondrial DNA can be packaged by ABF2 without interfering with the binding of these key regulatory proteins. We have demonstrated that ABF2 can be specifically excluded from binding to certain simple DNA sequences [poly(dA)] and have provided evidence that very short runs of this sequence, as few as 9/11 A residues, can exclude ABF2 binding (5). When these short sequences are phased at 25–30 bp intervals, we suggest that ABF2 binding becomes phased by virtue of this exclusion phenomenon. Furthermore, we suggest that ABF2 binding requires a region of at least 25 bp free of these exclusion sequences and that if these exclusion sequences occur more frequently than once every 25 bp, large regions of DNA can exclude ABF2 binding. Footprints on REP2, a putative replication origin from the mitochondrial DNA demonstrate that long DNA sequences can exclude ABF2 binding (5).

In fact, within REP2, there are only two regions of significant ABF2 binding. One of these is the sequence from which p64 was derived, which can presumably function as a minimal replication origin, and the other is a single site immediately adjacent to the promoter for the mitochondrial RNA polym-
erase (32). This is probably not coincidental as Parisi and Clayton (8) have recently shown that the activator of mitochondrial transcription in vitro, mtTF1, is similar to ABF2. Human mtTF1, which is also related to HMG1, binds to promoters present in human and murine mtDNA and activates transcription. In the accompanying paper, Fisher et al. (10) show that mtTF1 from human cell mitochondria has similar DNA binding properties as ABF2. Thus, in addition to being a structural component of the mitochondrial nucleoid, ABF2 may also be an activator of transcription and replication. We note in this regard, the recent identification of HMG1 and/or 2 as a candidate for the general transcription factor IIB (33).

ABF2 in the Nucleus—Subtleties in the distribution of ABF2 within the cell remain unclear. Both indirect immunofluorescence and subcellular fractionation agree that a large fraction of ABF2 is mitochondrial (5). This fact is also supported by the phenotype of abf2- null mutants. By indirect immunofluorescence, however, ABF2 was not detected in the nucleus, while by subcellular fractionation, at least half of the intracellular ABF2 cofractionated with nuclei. This was not due to contamination of the nuclear fraction with mitochondria, as p32, a mitochondrial integral membrane protein (34) was absent in these fractions (5). We cannot exclude the possibility that the nuclear ABF2 was due to leakage out of the mitochondria and into the nuclei, however, we consider this unlikely since, in the same experiments, there was virtually no ABF2 in the cytosolic fraction. Thus, at present, we believe that some ABF2 may be present in the nucleus in a form that is inaccessible to the antibody in the immunofluorescence experiments, although we cannot exclude the possibility that the nuclear localization may be an artifact of the subcellular fractionation.

We have not been able to detect any deficiencies in the abf2- null mutants that cannot be explained by its role in mitochondrial genome function. Its presence in the mitochondria, together with the finding that the possible human homologue of ABF2 is a specific factor for in vitro mitochondrial transcription suggests that at least part of the function of ABF2 is executed in the mitochondria.

None of this, however, excludes the possibility that ABF2 executes some of its functions in the nucleus. It is, for example, tempting to speculate that nuclear localization of ABF2 may function as part of a feedback mechanism whereby the nucleus can monitor the amount of mitochondrial DNA and regulate the expression of the mitochondrial replication machinery accordingly. In this regard, we note that various $\rho^-$ mutants of yeast, in which gross alterations in and deletions of the mitochondrial genome have occurred, all have approximately the same overall amount of mitochondrial DNA (35, 36), as if the nucleus monitors the total amount of mitochondrial DNA rather than the levels of specific sequences within the genome. Furthermore, we note that the mitochondrial genotype ($\rho^+$, $\rho^-$, $\rho^0$, or mit) can alter the level of transcription of several nuclear genes (37).

If ABF2 has any function in the nucleus outside of its role in maintaining the mitochondrial genome, that function must either be dispensable or redundant with another gene product. A true homologue of the vertebrate HMG1 has not yet been identified in yeast, although several genes with regions of HMG1 homology have been identified (38-40). It will be interesting to see if any of these nuclear proteins exhibits the same phased binding to nuclear ARSs.

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