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Functional Complementarity between the HMG1-like Yeast Mitochondrial Histone HM and the Bacterial Histone-like Protein HU*

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The mitochondrial histone HM is the major DNA-binding protein in mitochondria and is necessary for maintenance of the mitochondrial genome in the yeast *Saccharomyces cerevisiae* during growth on fermentable sugars. HM and the *Escherichia coli* histone-like protein HU have similar activities *in vitro*, including DNA supercoiling, but share no sequence similarity. We show that HU can functionally complement the respiration deficiency associated with yeast strains lacking HM. Conversely, phenotypes of *E. coli* cells lacking HU protein, including nucleoid loss and a filamentous cell morphology, were alleviated by expression of HM in these cells. The HU protein of bacteria and the HM protein of mitochondria are therefore functionally complementary *in vivo*. Functional similarities among HM, HU, and the nuclear HMG1 proteins are implicated and discussed.

The genomes of organisms invariably exist as chromatin (1). Genomic DNA of prokaryotes and eukaryotes is complexed with proteins such as histones, histone-like proteins, and non-histone proteins to form nucleoprotein structures that serve to package and condense the DNA. In the nucleus, the fundamental unit of chromatin is the nucleosome, in which the DNA is wrapped approximately two turns around the histone core (1). The bacterial genome is likewise assembled into a beaded polynucleosome-like structure (2), and the abundant histone-like protein HU can assemble DNA into nucleosome-like structures *in vitro* (3-5). The chromatin structures present in the mitochondrion, however, are poorly characterized.

The mitochondrial histone HM is an abundant basic nuclear-encoded protein in yeast mitochondria that supercoils DNA *in vitro* (6). HM is not unique to yeast. Homologs have been found in human (7) and amphibian (8) mitochondria. Disruption of the gene encoding HM (*ABF2*, but referred to here as *HIM1*) causes cells to rapidly lose their mitochondrial DNA, resulting in cells that can no longer respire (9). Loss of the HU protein in *Escherichia coli* is not lethal, but a large percentage of cells in a growing population (11-33%) lose their chromosome, resulting in DNA-less cells (10, 11). Thus, HM is required to maintain the mitochondrial chromosome, and HU is required for maintenance of the *E. coli* chromosome. Like HM, HU supercoils DNA *in vitro* in the presence of topoisomerase I, and both proteins are required in a 1:1 mass ratio with DNA to obtain maximal DNA supercoiling (3, 6). Previous studies *in vitro* and *in vivo* indicate that HU is important for various cellular functions including DNA replication, transcription, and transposition (5).

According to the endosymbiont theory, the mitochondrion evolved from a free-living aerobic bacterium that formed an intracellular symbiotic relationship with its host, a primordial eukaryote (12). The phylogenetic arrangement of organisms using comparative analysis of ribosomal RNA sequences places the mitochondria in a direct ancestral relationship with purple photosynthetic bacteria, a result that is compatible with other biochemical data (12). This evolutionary relationship combined with similar properties and activities shared by HM and HU *in vitro* and the requirement for each in chromosome maintenance led us to investigate the functional similarity between the mitochondrial histone HM and the *E. coli* histone-like protein HU *in vivo*. In this study, we show that HU can substitute for HM in yeast mitochondria and that HM can likewise substitute for HU in *E. coli*. Therefore, although the two proteins share no apparent sequence similarity, HM and HU appear to have similar functions.

EXPERIMENTAL PROCEDURES

Isolation of Mitochondria—Yeast mitochondria were prepared from cultures (300 ml) grown to late-log phase by differential centrifugation of a cell lysate using the procedure of Gasser (13), in which mitochondrial buffer was replaced with 0.25 M sucrose, 1 mM EDTA, 10 mM Pipes,[1] pH 6.5, and 0.2 mM phenylmethylsulfonyl fluoride.

Purification of HM Protein and NH_2-terminal Sequencing—The HM protein was isolated from yeast mitochondria as described (6) and further purified by binding to a Bio-Rex 70 cation-exchange column (Bio-Rad). HM was eluted with 0.45 M NaCl, 10 mM Tris, pH 7.5, 0.1 mM EDTA, and 1 mM dithiothreitol after washing the column with the same buffer containing 0.3 M NaCl. The purified protein was sequenced at the NH_2 terminus, and the following 21-amino acid sequence was obtained: KASKRTQLRNE-LIKQGPKRPT.

Cloning of HIM1—The gene encoding the HM protein was cloned from a λZAP yeast genomic library (Stratagene) using an antiserum to the HM protein as a probe. A 2.3-kilobase pair DNA fragment containing the entire coding region of the HM gene was recovered in plasmid form (pHMA1) from the phage clone. This gene was designated *HIM1*, and DNA sequencing showed that it is identical to the yeast *ABF2* gene (9).

Western Blotting—Western blotting was performed essentially as described (14). Antibodies to the *E. coli* HU protein were a generous gift from Josette Rouvière-Yaniv.

Plasmid Construction—To construct pGAL-HM, the *HIM1* gene was amplified from yeast genomic DNA by polymerase chain reaction using primers HMPRE (5′-ATGAACAGTTACAGCCTATTAAC-3′) and HMA1-9 (5′-CAAAAGCTTGAAGACTTACATAG-3′). This DNA fragment was inserted into the *Cla*I site of the pRS314G plasmid shuttle vector (*TRP1*, *CEN6*, and *ARS4*). pRS314G was a gift from Michael Douglas and contains the 650-base pair *GAL1* and *GAL10* *Eco*RI-*Bam*HI promoter fragment inserted into the *Eco*RI-*Bam*HI site of pRS314 (15). Plasmid pGAL-HU was constructed by insertion of a DNA fragment containing the *HUPA* coding sequence (HU2

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[1] The abbreviation used is: Pipes, 1,4-piperazinediethanesulfonic acid.

protein) fused to the *HIM1* presequence into the *Cla*I site of pRS314G as was done for pGAL-HM. The HU2 coding sequence was isolated from genomic *E. coli* DNA and fused to the *HIM1* presequence by polymerase chain reaction using primers HU2-1 (5′-TTTGGCTAGCACCTTGTTGAAGGCTTCCAAGACTCAACTGATTGATG-3′) and HU2-2 (5′-ACTGCTAGCCAATCTTACTTAACTGCGTC-3′).

Plasmid pHIM1 contains the *HUPA* gene promoter (160 base pairs 5′ from the initiator methionine) fused to the coding sequence for the mature HM protein (starting from codon 27) in the pSP72 vector (Promega Biotec).

Yeast Methods—The various growth media used in this work are described in Ref. 16. The growth medium YPGalGE (containing yeast, peptone, galactose (0.1%), glycerol, and ethanol) has not to our knowledge been described elsewhere and consists of replacing the 0.1% glucose (dextrose) present in YPDGE (containing yeast, peptone, dextrose (0.1%), glycerol, and ethanol) (16) with 0.1% galactose. Since galactose is a fermentable sugar, like glucose, this medium distinguishes respiration-competent cells from respiration-deficient (*petite*) cells, as does YPDGE. Disruption of *HIM1* was accomplished by inserting a 1.1-kilobase pair *Hin*dIII fragment containing the *URA3* gene into the *Nhe*I site near the NH_2 terminus of the *HIM1* coding sequence. One copy of the *HIM1* gene was replaced with this construct as a linear fragment by one-step gene replacement in yeast strain W303 (17). A diploid strain with one copy of *HIM1* disrupted (TM144) was sporulated and dissected. Individual asci produced four viable spores, two URA^+ and two URA^-. One haploid segregant that has the *HIM1* gene disrupted (TM144-15d) and one that is wild-type *HIM1* (TM144-15a) were used in this study.

For the determination of galactose-dependent respiration-competent phenotypes, TM144-15d (*him1::URA3*) cells transformed with various plasmids were grown in galactose-containing liquid medium and then streaked out onto YPDGE and YPGalGE plates and grown at 37 °C, the nonpermissive temperature for growth of *him1* mutant cells on nonfermentable substrates. The small amount of galactose in YPGalGE was added to induce genes that have been fused to the *GAL* promoter. The *GAL* promoter is repressed by glucose, but not by glycerol or ethanol. Yeast strain TM179 was created by mating DNY1 (*petite lethal*) to TM144-9 (*him1::URA3*). TM179 was sporulated, and isogenic *petite lethal* (LEU^+ and HIS^+) haploids TM179-5b (*HIM1*) and TM179-12b (*him1::URA3*) differing only at *HIM1* were isolated.

Strains—The yeast and bacterial strains used are described in Table I.

Cell Length Measurements—The average lengths of *E. coli* cells were as determined from negatives of photographs of 4′,6-diamidino-2-phenylindole-stained *E. coli* cells. Several microscope fields and a total of 400 cells/sample were measured using a molecular measurement device (courtesy of Jack Griffith) by projecting the images of cells onto a screen and measuring with a stylus.

TABLE I
Strain list

Strain	Genotype	Source or Ref.[a]
Saccharomyces cerevisiae		
W303	***a***/*α ade2-1/ade2-1 ura3-1/ura3-1 his3-11,15/his3-11,15 trp1-1/trp1-1 leu2-3,112/leu2-3,112*	R. Rothstein
TM144	*W303 him1::URA3/HIM1*	
TM144-15a	*MAT**a** ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 HIM1*	Segregant of TM144
TM144-15d	*MAT**a** ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 him1::URA3*	Segregant of TM144
TM164	TM144-15d, pRS314G	
TM165	TM144-15d, pGAL-HM	
TM167	TM144-15d, pGAL-HU	
TM168	TM144-15d, pGAL-HNS	
DNY1	*MAT**a** aac1::LEU2 aac2::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112*	D. Nelson and M. Douglas
TM144-9	*MATα him1::URA3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112*	
TM179	*DNY1 X TM144-9*	
TM179-5b	*MAT**a** aac1::LEU2 aac2::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 HIM1*	Segregant of TM179
TM179-12b	*MAT**a** aac1::LEU2 aac2::HIS3 ade2-1 ura3-1 his3-11,15 trp1-1 leu2-3,112 him1::URA3*	Segregant of TM179
E. coli		
YK1100	W3110 *trpC9941*	F. Imamoto
YK1340	YK1100 *hupA16(Km′) hupB11(Cm′)*	F. Imamoto
NH279	*F⁻ thr⁻ leu⁻ thi⁻ lacY supE tonA HU1::Kn′ HU2::Cm′*	N. P. Higgins

[a] Strains listed are derived from this work unless otherwise indicated.

RESULTS

We have purified the HM protein from yeast mitochondria and raised antibodies to it. The protein has DNA supercoiling activity, as reported by Caron *et al* (6). Immunocytochemistry showed that HM is principally localized in mitochondria, indicating that we indeed purified the mitochondrial protein.[2] A 2.3-kilobase pair yeast genomic DNA containing the HM coding region was cloned from a yeast genomic library using antibodies as a probe. The deduced amino acid sequence contained the NH_2-terminal peptide sequence we obtained from the purified protein, confirming cloning of the HM gene. The gene sequence turned out to be the same as that of *ABF2* (autonomous replication sequence-binding factor 2) (9). In this study, we refer to the previously described *ABF2* gene as *HIM1* (histone mitochondria 1). The HM coding sequence contains a 26-amino acid presequence that is not found in the mature HM protein. This appears to be the mitochondrial localization signal sequence. The sequence MNSYSLLTRSFHESSKPLFNLASTLL was used in this study to target HU and other proteins to the mitochondria.

HM Is Required for Chromosome Maintenance—The *HIM1* gene was disrupted *in vivo* by gene replacement (17) using the *URA3* marker, and a haploid *him1* (URA^+) mutant was obtained and shown to lack HM protein.[2] The *him1* mutant strain (TM144-15d) was examined for its ability to grow on glycerol, where cell growth requires functional (respiring) mitochondria, and on glucose, where functional mitochondria are not required for growth. TM144-15d can be maintained indefinitely on a nonfermentable carbon source like glycerol at 30 °C and therefore is respiration-competent, but grows at about one-third the rate of wild-type *HIM1* (TM144-15a) cells in liquid glycerol medium at 30 °C. However, at 37 °C, the growth of TM144-15d was completely inhibited in glycerol medium (YPG (containing yeast, peptone, and glycerol)) (Fig. 1*A*). TM144-15d is not temperature-sensitive for growth in YPD (containing yeast, peptone, and dextrose (glucose)),[2] so the growth defect is specific to mitochondrial function.

After growing TM144-15d for multiple generations on fermentable substrates like glucose, where mitochondrial respiration is not required, it would no longer grow in glycerol medium and therefore became respiration-deficient. Yeast cells that have defective mitochondria can be distinguished from respiration-competent cells by growth in medium that distinguishes the mitochondrial mutants as forming small (*petite*) colonies. This medium, YPDGE, contains glycerol and ethanol as the predominant (nonfermentable) carbon source and a small amount (0.1%) of glucose (dextrose), which enables the respiration-deficient cells to grow, although only enough to form *petite* colonies. When TM144-15d cells were grown in YPD to late-log phase and plated onto YPDGE, the cell population was predominantly *petite*, with only a small percentage of cells being capable of forming respiration-competent colonies, as shown in Fig. 1*B*. The respiration-compe-

[2] Megraw, T. L., and Chae, C.-B. (1993) *Proc. Natl. Acad. Sci. U. S. A.*, in press.

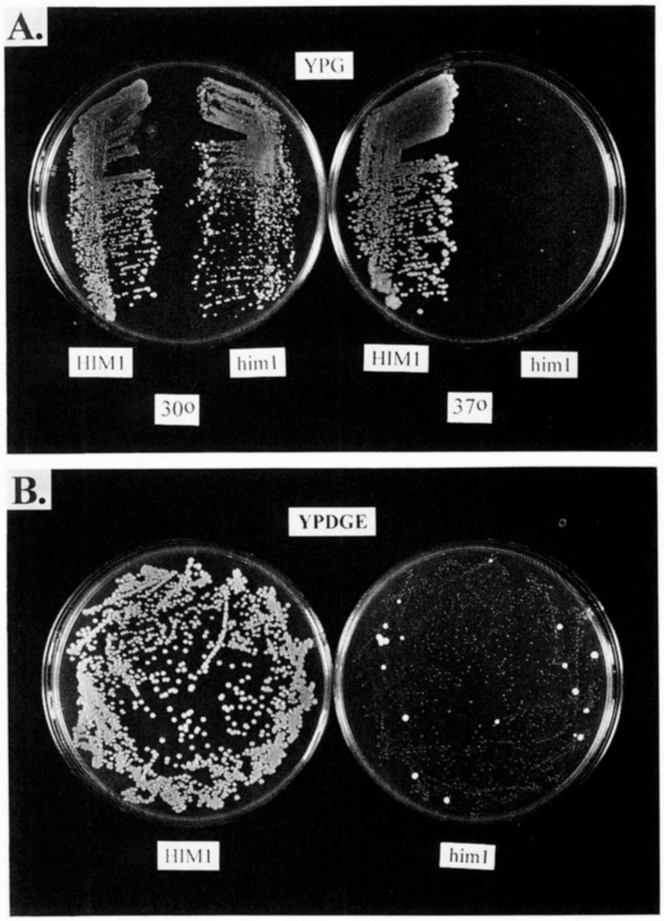

FIG. 1. **Phenotypic consequences of yeast cells lacking HM protein.** *A*, growth of the *him1* disruption mutant is temperature-sensitive for growth on glycerol at 37 °C. Shown are two isogenic haploid strains, one with the wild-type *HIM1* gene (*HIM1*) and one with the disrupted allele (*him1*), corresponding to strains TM144-15a and TM144-15d, respectively. The cells were grown in liquid YPG (glycerol) medium at 30 °C and then streaked onto YPG plates and grown at 30 and 37 °C as indicated. *B*, the loss of HM protein confers a *high frequency of petite* phenotype to yeast cells grown on fermentable sugars. Wild-type and *him1* mutant cells were grown in YPD (glucose) liquid medium (inoculated with a colony from a YPG plate) to late-log phase and plated onto YPDGE medium (see "Experimental Procedures"). Both plates were incubated at 30 °C, and the *HIM1* cells were grown for 3 days, whereas the *him1* cells were grown for 7 days to allow time for the respiration-competent colonies to grow. Cells that have lost mitochondrial function appear as small (*petite*) colonies.

tent colonies formed by TM144-15d are not revertants because these colonies generate *petites* when grown and replated. A growing culture of TM144-15d cells in YPD formed *petites* at a rate of 5–8%/cell doubling at 30 °C. Cells lacking HM therefore have a *high frequency of petite* phenotype and are temperature-sensitive for growth on glycerol. We have recently observed the same phenotypes in a strain that is isogenic to TM144-15d, but has the entire coding sequence of *HIM1* deleted.[2]

The *him1* mutant becomes *petite* at a high rate due to complete loss of the mitochondrial genome when the cells are not maintained on glycerol, generating rho^0 mutants (lacking all mitochondrial DNA).[2] Diffley and Stillman (9) also reported the loss of mitochondrial DNA in *abf2* mutant cells.

Complementation of him1 Mutant by E. coli HU Protein—Complementation of cells lacking HM by the *E. coli* HU protein was investigated by expressing the *E. coli* HU2 protein from the *HUPA* gene in the mitochondria of the *him1* mutant TM144-15d. Fig. 2*A* shows a schematic diagram of plasmids pGAL-HU and pGAL-HM. These plasmids allow the inducible expression of HM and HU by galactose and their repression by glucose. HU was fused to the *HIM1* presequence to direct its import to mitochondria (19).[3] Mitochondria prepared from TM144-15d carrying pGAL-HU contained the HU protein of the correct size when the cells were grown in the presence of galactose (Fig. 2*B*). The introduction of pGAL-HM into TM144-15d resulted in the appearance of a high level of HM protein in the mitochondria in the presence of galactose, as shown in Fig. 2*B*.

To analyze galactose-dependent restoration of respiration competence of the *him1* mutant by pGAL-HU and pGAL-HM, the TM144-15d cells containing these plasmids were first grown in galactose-containing liquid medium and then streaked onto YPDGE (glucose) and YPGalGE (galactose) plates (see "Experimental Procedures") and incubated at 37 °C, the nonpermissive temperature for growth of TM144-15d when supplied with a nonfermentable carbon source. Fig. 2*C* shows a limited growth of TM144-15d cells on either plate due to the small amount of fermentable carbon source present. However, the TM144-15d cells containing pGAL-HU grew much better (formed respiration-competent colonies) on galactose than on glucose. Expression of HM from pGAL-HM likewise permitted TM144-15d to form respiration-competent colonies on galactose and not on glucose, whereas TM144-15d cells containing the vector showed no difference in growth on either plate. The pGAL-HU plasmid was less effective than pGAL-HM in reverting the *him1* growth defect (compared to wild-type cells (labeled *HIM1* in Fig. 2*C*)), although the expression of HU clearly enhanced the growth of the TM144-15d strain in the presence of galactose. This discrepancy may be due in part to the difficulty of achieving an optimal level of protein expression in mitochondria. We found that overproduction of HM in wild-type *HIM1* cells (TM144-15a) or in TM144-15d with pGal-HM results in a *high frequency of petite* phenotype when cells are grown in the presence of a high concentration (2%) of galactose as opposed to the 0.1% galactose added to YPGalGE.[2]

Maintenance of the mitochondrial DNA is usually not essential for yeast to grow in the presence of fermentable carbon sources such as glucose or galactose. However, yeast strains that contain mutations in the *AAC* genes (or *op1* or *pet9*), which encode the ADP/ATP carrier, are *petite lethal*, giving rise to nonviable cytoplasmic rho^- or rho^0 mutants, and therefore must maintain the mitochondrial DNA or die (20, 21). We constructed isogenic *petite lethal* $HIM1^+$ and $him1^-$ strains TM179-5b and TM179-12b, respectively. Neither strain grows on glycerol due to the *aac* mutations, but TM179-5b ($HIM1^+$) grew in YPD at 37 °C, whereas TM179-12b ($him1^-$) did not due to the lack of the HM protein.[2] pGAL-HU rescued the lethal effects of the *him1* mutation in TM179-12b when the cells were grown on galactose at 37 °C.[2] pGAL-HM also rescued the lethality of TM179-12b at 37 °C as expected, but only on low (0.2%) galactose. pGAL-HM is lethal to TM179-12b and TM179-5b on high (2.0%) galactose.[2] The inability of *petite lethal* cells lacking HM to grow at 37 °C and their restoration by HU are consistent with the results obtained with TM144-15d shown in Fig. 2.

Another bacterial histone-like protein, the H-NS (or H1a) protein of *E. coli*, was unable to complement the *him1* temperature-sensitive growth defect in either TM144-15d or TM179-12b.[2]

Complementation of E. coli Cells Lacking HU by HM Pro-

[3] L.-R. Kao, T. L. Megraw, and C.-B. Chae, submitted for publication.

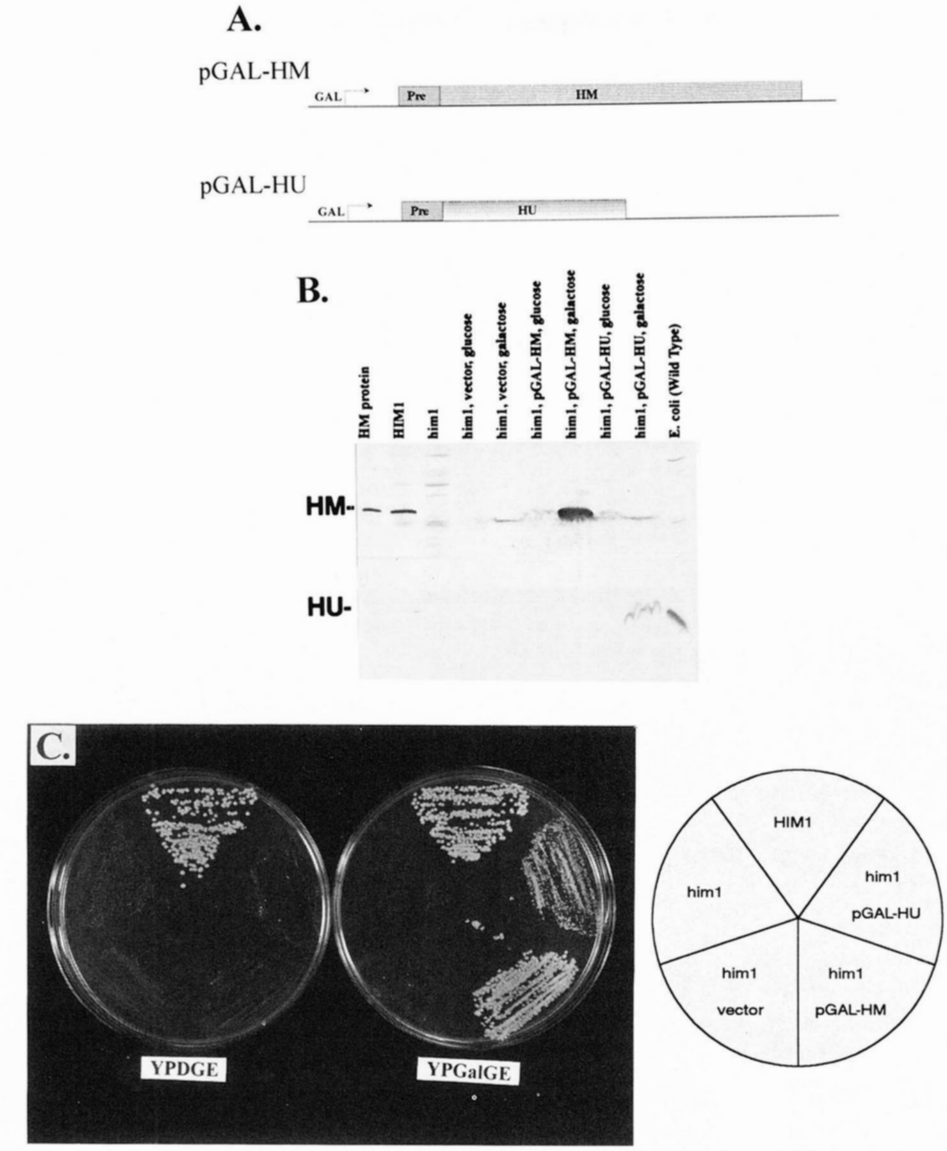

FIG. 2. **Complementation of *him1* respiration-deficient phenotype by *E. coli* HU protein.** *A*, schematic representation of plasmids pGAL-HU and pGAL-HM, which express HU and HM proteins, respectively, in mitochondria when cells are grown on galactose. Both constructs include DNA sequences encoding the *HIM1* gene presequence, the mitochondrial localization sequence (*box* labeled *Pre*). *B*, Western blot analysis of the mitochondrial proteins from the *him1* mutant strains containing pGAL-HU, pGAL-HM, and pRS314G (*vector*). Two blots containing identical samples are shown. One (*top*) was probed with an antiserum to the HM protein, and the other (*bottom*) with antibodies to the HU protein. The *lane* labeled *HM protein* contains 150 ng of purified HM, which migrated on SDS-polyacrylamide gel with an apparent molecular mass of 20 kDa. The *lanes* labeled *HIM1* and *him1* contain total mitochondrial proteins (60 μg of protein/lane). *HIM1* refers to the mitochondria from wild-type strain TM144-15a, and *him1* to the TM144-15d strain with the disrupted *HIM1* gene. Lanes containing mitochondria from strain TM144-15d (*him1*) containing the relevant plasmids and grown in the presence of either glucose or galactose (2%) are indicated. The *lane* labeled *E. coli (Wild Type)* refers to proteins from the *E. coli* strain containing wild-type HU genes (YK1100). *C*, galactose-dependent respiration competence of yeast strains. Strains TM144-15a (*HIM1*) and TM144-15d (*him1*) grown in YPGal liquid medium (containing yeast, peptone, and galactose) and strain TM144-15d transformed with vector, pGAL-HM, and pGAL-HU grown in synthetic complement without tryptophan galactose medium to late-log phase were streaked out onto YPGalGE (galactose) and YPDGE (glucose) plates and grown at 37 °C (the nonpermissive temperature). The key indicates the position of each strain on the plates.

tein—The restoration of respiration competence to *him1* cells by HU led us to investigate the ability of HM to complement an *E. coli* strain lacking HU protein. Mutations in either *HUPA* (HU2) or *HUPB* (HU1) genes in *E. coli* produce no apparent phenotype. However, null mutations of both genes (*hupA,B*) result in cells with an extremely filamentous morphology and a high rate of loss of the nucleoid, resulting in DNA-less cells (10, 11).

We fused the *HIM1* gene sequence coding for the mature HM protein (without the presequence) to the *HUPA* gene promoter, and the resulting plasmid (pHIM1) was used to transform the *E. coli hupA,B* mutant YK1340 (10). The absence of HU and the production of HM in the transformed YK1340 cells were confirmed by immunoblotting (Fig. 3*A*). YK1340 cells that produced HM protein no longer lost the nucleoid and showed a substantial decrease in the filamentous morphology. Fig. 3*B* shows the *E. coli* cells stained with 4′,6-diamidino-2-phenylindole. The field containing YK1340 cells transformed with the vector demonstrates the characteristic filamentous character of HU-deficient cells, and the *arrows* point out some of the DNA-less cells that appear as lightly staining bodies (10, 11). In contrast, the YK1340 cells carrying pHIM1 maintained their DNA and showed a less filamentous morphology. Fig. 3*C* illustrates the reduction of the average

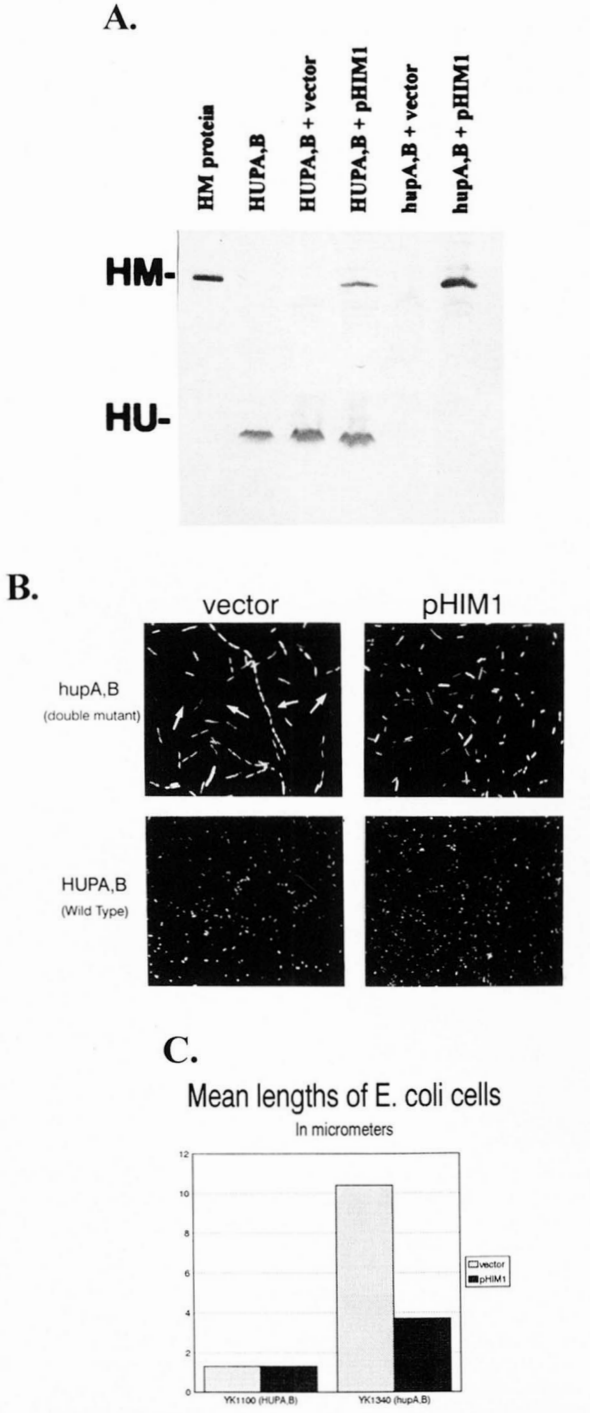

FIG. 3. **Complementation of HU-deficient *E. coli* mutant by HM protein.** *A*, Western blot showing expression of the HM protein in wild-type (*HUPA,B*) or mutant (*hupA,B*) *E. coli* cells from plasmid pHIM1. Shown are two blots containing the same samples as indicated above each lane. One blot was probed with an antiserum to HM (*top*), and the other with anti-HU antibodies (*bottom*). The protein bands corresponding to HM and HU are indicated on the left. The *lane* labeled *HM protein* contains 150 ng of purified HM. *HUPA,B* refers to *E. coli* strain YK1100, and *hupA,B* refers to strain YK1340. Lanes were loaded with total cell proteins from 50 μl of a mid-log phase culture. *vector* refers to the parent plasmid (pSP72) used to construct pHIM1. *B*, 4′,6-diamidino-2-phenylindole-stained wild-type *HUPA,B* and mutant *hupA,B* cells containing either pHIM1 or vector plasmid DNA. *Arrows* in *top left panel* indicate cells that have lost the nucleoid and appear as faintly staining cells. The *hupA,B* cells with pHIM1 contain no nucleoid-less cells and have a less filamentous morphology. *C*, bar graph depicting the average lengths of *HUPA,B* wild-type (YK1100) and *hupA,B* mutant (YK1340) cells containing either vector or pHIM1. The wild-type YK1100 cells that had either the vector or pHIM1 had an average length of 1.3 μm. The mutant YK1340 cells containing the vector had an average cell length of 10.4 μm, and the mutant cells carrying pHIM1 had an average length of 3.7 μm.

length of these *hup* mutant cells from 10.4 μm (8.0 times longer than wild-type cells) to 3.7 μm (2.8 times longer than wild-type cells) due to the expression of HM. The average length of the wild-type cells (YK1100) was 1.3 μm. YK1340 carrying the vector alone produced approximately 11% nucleoid-less cells in the growing population compared to very few DNA-less cells in YK1340 containing pHIM1 (<0.3%).

The complementation of *hupA,B* by pHIM1 was observed in all of the original transformant colonies of YK1340 examined in contrast to the transformants obtained with the vector, which consistently exhibited DNA-less cells. Therefore, the results obtained with pHIM1 appear not to be due to a possible reversion effect (11) that is unrelated to the presence of pHIM1. We also observed complementation of another *hupA,B* strain, NH279 (a gift from N. Patrick Higgins), by pHIM1.[2] Taken together, these results demonstrate substantial complementation of the *hupA,B* mutations by the HM protein and show that the yeast HM protein can substitute for HU in *E. coli* cells with respect to chromosome maintenance. It will be interesting to examine the ability of HM to complement HU-dependent cellular processes such as bacteriophage *Mu* growth, lysogeny, and transposition in the *hupA,B* mutant.

DISCUSSION

We have cloned the gene for the yeast mitochondrial histone HM and have designated the gene *HIM1*, which is identical to a previously described gene called *ABF2* (9). Disruption of *HIM1* results in cells that lose their mitochondrial DNA at a fast rate on fermentable carbon sources, giving rise to rho^0 mutants. Furthermore, *him1* mutants are temperature-sensitive for proper mitochondrial function. In this study, we show that the HM protein and the bacterial histone-like protein HU, which have functional similarities *in vitro*, can substitute for each other *in vivo*. HU can revert *him1* mutants (TM144-15d and TM179-12b) from their temperature-sensitive growth defects and therefore permits mitochondrial function and maintains the mitochondrial DNA despite the absence of HM. Conversely, *E. coli* cells lacking HU no longer show nucleoid loss when HM protein is present. In addition, HM substantially reduces the filamentous morphology that is characteristic of *E. coli* cells lacking HU. At this time, the biological function of neither HM nor HU is clearly known. The only common biochemical properties of the two proteins appears to be the DNA supercoiling and the DNA binding activities (6, 22–24). HM and HU also have in common an essential role in chromosome maintenance *in vivo*.

It is curious that the disruption of *HIM1* results in a temperature-sensitive phenotype. Disruption of the *PIF1* gene, which encodes a mitochondrial helicase, was also shown to cause cells to be thermosensitive for growth on glycerol and to lose their mitochondrial DNA (25, 26). It is interesting that the loss of PIF1, a protein that unwinds DNA, a process that induces positive supercoiling of DNA, results in a phenotype that is very similar to that seen for the loss of HM protein, which confers a negative superhelicity to DNA.

Another abundant histone-like chromatin protein in bacteria, the H-NS protein, does not complement the loss of HM in yeast. However, the function of the H-NS protein is apparently very different from that of HU. H-NS appears to be involved in a general DNA "inactivation," being expressed at higher levels in cells as they reach stationary phase. H-NS is

required for the regulation of genes in response to environmental changes and does so by mediating changes in DNA topology. However, it does not supercoil DNA itself directly (27, 28). In bacteria, some *hns* mutants show increased plasmid supercoiling (29) and increased *Mu* transposition (30), unlike *hup* mutants (HU mutants), which show decreased plasmid supercoiling and decreased *Mu* transposition (11, 31).

The HM protein has a high degree of sequence similarity to the HMG1 family of nuclear non-histone proteins (9). The only apparent structural difference between HM and HMG1 is the lack of the acid tail domain on the HM protein (32). We have recently demonstrated that the yeast HMG1 homolog NHP6A can complement the HM mutant.[3] Furthermore, NHP6A can also supercoil plasmid DNA *in vitro*.[3] These results are significant and indicate that the function of the HM protein in the mitochondrion is equivalent to the function of HMG1 proteins in the nucleus. We are currently investigating the ability of HM to complement the loss of the *NHP6* genes when targeted to the nucleus. The functional homology between HM and HU and between HM and NHP6A indicates that the functions of HU in *E. coli*, of HM in mitochondria, and of HMG1 in the nucleus are all homologous. This raises an interesting possibility. Perhaps the ancestral HU-encoding gene was lost along with most of the genome inherited by the present-day mitochondrion, and HU was replaced by the functionally similar HM protein, which evolved from a nuclear gene that encodes a protein related to HMG1. Nuclear HMG1 proteins may therefore fulfill a role in nuclear chromatin that is similar to the role of HM in mitochondria and of HU in bacteria. Functional similarity between HM and HU is supported by the finding that LEF-1 (a high mobility group box protein) and its cognate DNA-binding site can substitute for the integration host factor (an HU-like protein) and its DNA-binding site in the λ phage integrase reaction (33).

Chloroplasts contain a histone-like protein that cross-reacts with antisera to HU (18) and may therefore be directly related evolutionarily to HU. The chloroplast HU-like protein might therefore still be encoded by the chloroplast genome. Thus, it appears that the chloroplast took an alternative evolutionary path than that taken by the mitochondrion, with the chloroplast retaining the HU protein and mitochondria replacing it with HM, a functional homolog.

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