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Global Regulation of Mitochondrial Biogenesis in *Saccharomyces cerevisiae*: ABF1 and CPF1 Play Opposite Roles in Regulating Expression of the *QCR8* Gene, Which Encodes Subunit VIII of the Mitochondrial Ubiquinol-Cytochrome *c* Oxidoreductase

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The multifunctional DNA-binding proteins ABF1 and CPF1 bind in a mutually exclusive manner to the promoter region of the *QCR8* gene, which encodes 11-kDa subunit VIII of the *Saccharomyces cerevisiae* mitochondrial ubiquinol-cytochrome *c* oxidoreductase (QCR). We investigated the roles that the two factors play in transcriptional regulation of this gene. To this end, the overlapping binding sites for ABF1 and CPF1 were mutated and placed in the chromosomal context of the *QCR8* promoter. The effects on transcription of the *QCR8* gene were analyzed both under steady-state conditions and during nutritional shifts. We found that ABF1 is required for repressed and derepressed transcription levels and for efficient induction of transcription upon escape from catabolite repression, independently of DNA replication. CPF1 acts as a negative regulator, modulating the overall induction response. Alleviation of repression through CPF1 requires passage through the S phase. Implications of these findings for the roles played by ABF1 and CPF1 in global regulation of mitochondrial biogenesis are discussed.

In the yeast *Saccharomyces cerevisiae*, the biogenesis of a functional mitochondrion is controlled mainly by environmental stimuli. Oxygen (heme) and the carbon source exert regulation primarily at the level of transcription (35). In the presence of heme and a nonfermentable carbon source, transcription of many nuclear genes that encode mitochondrial proteins is induced 4- to 10-fold (30). Of the factors responsible for this specific regulation, only HAP1, HAP2, HAP3, and HAP4 (15, 30, 31, 62 and references therein) have been described in detail. Heme-induced binding of HAP1 to the promoter region of several genes triggers transcriptional activation under aerobic conditions. The mode of binding and activation may be regulated through alternative conformations of the factor that are thought to be dependent on the redox state of the cell (15). HAP2, HAP3, and HAP4 form a complex resembling the CCAAT box-binding protein complex of higher eukaryotes (14, 60, 61). Transcriptional activation through this complex may depend on the availability of HAP2 and the activator subunit HAP4, whose synthesis is regulated by the carbon source (31, 63). These specific regulatory proteins are likely to interact with other protein factors, before or while exerting their regulatory effect on a gene of interest. More global regulatory mechanisms are expected to exist to adjust the overall rate of mitochondrial biogenesis in relation to cellular growth.

Biosynthesis of the yeast mitochondrial ubiquinol-cytochrome *c* oxidoreductase (QCR) complex offers a suitable model system to study regulation of mitochondrial biogenesis. The complex consists of one mitochondrially and eight nuclearly encoded subunits (19), whose expression is coordinately regulated (73). For the *QCR8* gene, which encodes 11-kDa subunit VIII of the QCR complex (53), a UAS element responsive to the carbon source has been defined (51). This contains the consensus recognition sequence for the HAP2-HAP3-HAP4 regulatory complex (61). In addition, mobility shift and footprinting assays have revealed two abundant proteins that bind in a mutually exclusive fashion to a site just upstream of this UAS region (28, 51). These factors bind to the 5′ flanks of a large number of genes that encode other mitochondrial proteins, including subunits of the QCR complex, or encode proteins involved in processes important for cell growth, and to elements important for cell division (ARS and CEN; 28, 29). Subsequent analysis revealed these factors to be identical to ARS-binding factor ABF1 and centromere- and promoter-binding factor CPF1, respectively (26).

ABF1 and CPF1 have both been described as multifunctional DNA-binding proteins. ABF1 was originally identified as binding to the ARS1B element (69) and to the silent mating type loci (9). The factor is involved in the initiation of DNA replication (22) and in transcriptional activation (17, 25, 34, 39) and repression (45). The gene that encodes this essential protein has been cloned and sequenced independently by four different groups (23, 32, 38, 64). CPF1 binds to the centromeric core element CDE1 and to CDE1-like recognition sequences in various promoters (7, 11). The protein is not essential, and the corresponding gene has been characterized independently by three different groups (5, 12, 55). A role in transcriptional regulation has not been established.

We have previously suggested that both ABF1 and CPF1 are involved in coupling the rate of mitochondrial biosynthesis to cellular growth (36). To test this hypothesis, we investigated the effect of each factor separately and of both factors together on transcriptional regulation of the *QCR8* gene, both under steady-state growth conditions and during nutritional shifts. We found that ABF1 is required for repressed and derepressed *QCR8* transcription and for efficient induction of transcription during escape from catabolite repression. CPF1 functions as a negative regulator during the induction response. Implications for the effects

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TABLE 1. Yeast strains

Strain	Genotype	Reference
DL1	*MATα his3-11,15 leu2-3,112 ura3-251,328,372*	75
DLH811	Same as DL1 but with *QCR8-11*	This study
DLH821	Same as DL1 but with *QCR8-21*	This study
DLH832	Same as DL1 but with *QCR8-32*	This study
DLH848	Same as DL1 but with *QCR8-48*	This study
DLH854	Same as DL1 but with *QCR8-54*	This study
DLL80	Same as DL1 but with *qcr8::LEU2*	52, 66
DHP80	Same as DLL80 transformed with pCF80 (*FPS-QCR8* on YCp50)	This study
DHP848	Same as DLL80 transformed with pCF848 (*FPS-QCR8-48* on YCp50)	This study
DHP854	Same as DLL80 transformed with pCF854 (*FFS-QCR8-54* on YCp50)	This study
DPH866	Same as DLL80 transformed with pCF866 (*FPS-QCR8-66* on YCp50)	This study
BWG1-7A	*MATa ade1-100 his4-519 leu2-3,112 ura3-52*	37
BWG-H1	Same as BWG1-7A but with *hap1-1*	37
LGW1	Same as BWG1-7A but with *hap2-1*	37
JP60	Same as BWG1-7A but with *hap3-1*	61
BJ1991	*Matα leu2-1 trp1-1 ura3-52 prb1-1122 pep4-3*	44
YPH266	*MATa ade2-101* ochre *leu2-1 lys2-801* amber *ura3-52*	55
YSS90	Same as YPH266 but with *cpf1::URA3*	55
YSS91	Same as YPH266 but with *cpf1::LEU2*	55

that both proteins may have on various promoters and other genetic elements are discussed.

MATERIALS AND METHODS

Strains and media. The yeast strains used in this study are listed in Table 1. Yeast cells were grown in YP medium (1% yeast extract, 2% Bacto-Peptone) containing 4% glucose (YPD), 2% (wt/vol) ethanol (YPE), 2% galactose (YPGal), 2% (wt/vol) glycerol (YPGlyc), or 60 mM sodium acetate (pH 6.0) (YPAc). Selective media contained 0.67% yeast nitrogen base without amino acids and either 2% glucose (MMd) or 0.05% yeast extract, 0.04% glucose, and 2% (wt/vol) ethanol (MMe) and were supplemented with the appropriate amino acids and nucleotides. Solid media contained 2% Bacto Agar, 1% yeast extract, 2% Bacto-Peptone, and 2% glucose (YPDagar) or 2% (wt/vol) ethanol and 2% (wt/vol) glycerol (YPEGagar).

The *Escherichia coli* strains used for DNA manipulations were HB101, JM101, and JM109 (54). Bacterial cultures were grown in 2YT medium (1.6% Bacto-Tryptone, 1% yeast extract, 0.5% NaCl) supplemented with 50 μg of ampicillin per ml when necessary.

Construction of plasmids. A 2.8-kb *Kpn*I-*Eco*RI fragment, containing the 3′ half of the *FPS* gene (2) and the *QCR8* gene, was isolated from pFL1-cIII-11k-1 (74) and inserted into the *Kpn*I and *Eco*RI sites of pUC18, from which the *Hin*dIII-*Sal*I MCS fragment had been removed. The resulting plasmid is named pJH1 (Fig. 1). An 840-bp *Hin*dIII-*Sal*I fragment from pJH1 was inserted into the *Hin*dIII and *Sal*I sites of M13mp10 and pUC18, giving M13-11k and pUC-11k, respectively. Deletions and site-directed mutagenesis (see below) resulted in *QCR8* promoter mutations that were recovered from M13-11k and pUC-11k as *Hin*dIII-*Sal*I fragments and reinserted into the *Hin*dIII and *Sal*I sites of pJH1, giving pJH1-10 (*QCR8-11*), pJH1-20 (*QCR8-21*), pJH1-30 (*QCR8-32*), pJH1-238 (*QCR8-48*), pJH1-224 (*QCR8-54*), and pJH1-206 (*QCR8-66*).

A 1.3-kb *Sph*I-*Kpn*I fragment containing the 5′ half of the *FPS* gene was isolated from pFL1-cIII-11k-1 and inserted into the *Sph*I and *Kpn*I sites of pUC18, giving pJH2. *Kpn*I-*Eco*RI fragments (2,800 bp) from pJH1, pJH1-238, pJH1-224, and pJH1-206 were isolated and inserted into the *Kpn*I and *Eco*RI sites of pJH2, resulting in pJHF80 (wild-type *QCR8*), pJHF848 (*QCR8-48*), pJHF854 (*QCR8-54*), and pJHF866 (*QCR8-66*). *Sph*I-*Eco*RI fragments (4,100 bp) were isolated from the pJHF plasmids and inserted into the *Sph*I and *Eco*RI sites of YCp50 (65) to give pCF80, pCF848, pCF854, and pCF866.

Construction of *QCR8* promoter mutants. To create small deletions in the *QCR8* promoter region, pUC-11k was digested with *Hin*dIII and treated with 0.5 U of *Bal* 31 exonuclease per ml at 26.5°C. The extent of *Bal* 31 digestion in successive time samples was tested by subsequent digestion of aliquots with *Ssp*I and agarose gel electrophoresis. Adequately digested samples were ligated with *Hin*dIII linkers and transformed into HB101, and isolated plasmid DNA sequences were analyzed.

Site-directed mutagenesis was adapted from previously published procedures (47, 77). Single-stranded M13-11k DNA was isolated through transfection of JM101. Single-stranded phosphorylated oligonucleotides containing the *QCR8* promoter mutations indicated in Fig. 2 were independently annealed to the single-stranded *QCR8* template. For convenience, complementary sequences are shown in Fig. 2. Second-strand synthesis was achieved through successive treatment with the DNA polymerase Klenow fragment and T4 DNA ligase. Double-stranded DNA was purified by low-melting-point agarose electrophoresis and transformed into JM101. Bacteriophage plaques were screened for the presence of the desired mutations by plaque lift hybridization with the respective radiolabeled oligonucleotides. Hybridization was performed in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–10× Denhardt's solution–0.1% sodium dodecyl sulfate (SDS)–100 μg of salmon sperm DNA per ml at 30°C. Filters were washed in 3× SSC–0.1% SDS at empirically determined optimal temperatures. Phage particles from positive plaques were used to

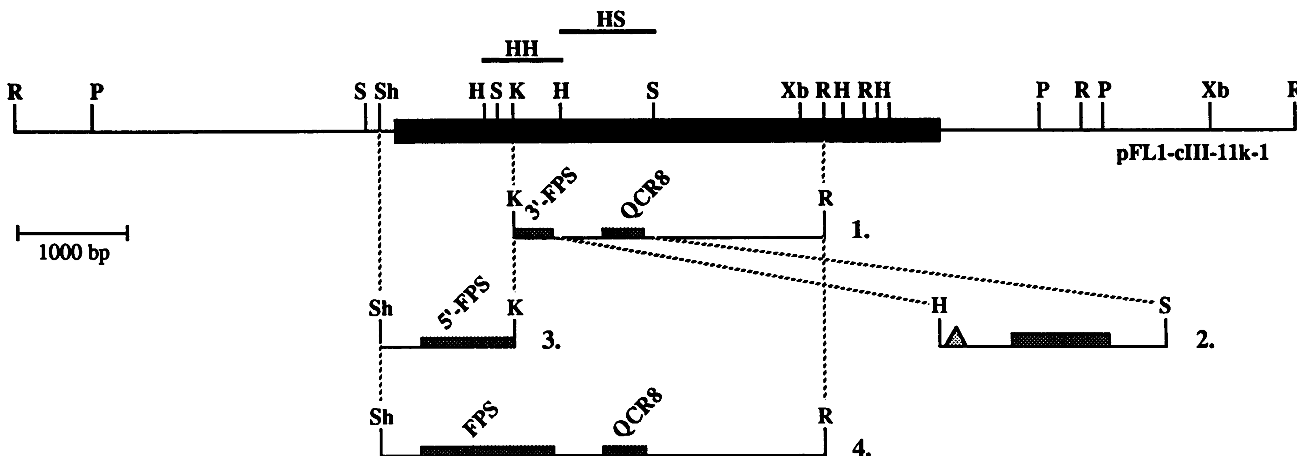

FIG. 1. Construction of subclones and mutants. The upper part represents the original plasmid, pFL1-cIII-11k-1, which contains a yeast genomic insert (thick bar) that carries the *QCR8* gene (74). A 2.8-kb *Kpn*I-*Eco*RI fragment that carries the 3' part of the *FPS* gene and all of the *QCR8* gene (fragment 1.) was subcloned into pUC18ΔHS and used to restore the endogenous *QCR8* locus in DLL80. An 840-bp *Hin*dIII-*Sal*I fragment that carries the *QCR8* gene (fragment 2.) was subcloned into pUC18 and M13mp10 and used to construct *QCR8* promoter mutations (triangle). Mutated fragments were subsequently reinserted into fragment 1. A 1.3-kb *Sph*I-*Kpn*I fragment that carries the 5' part of the *FPS* gene was subcloned into pUC18 (fragment 3.). *Kpn*I-*Eco*RI fragments containing wild-type and mutated *QCR8* alleles were ligated with fragment 3. The resulting 4.1-kb *Sph*I-*Eco*RI fragments were inserted into YCp50. Also indicated are the 734-bp *Hin*dIII fragment (HH) and the 840-bp *Hin*dIII-*Sal*I fragment (HS) used as probes in Northern analyses. Restriction sites are indicated as follows: H, *Hin*dIII; K, *Kpn*I; P, *Pst*I; R, *Eco*RI; S, *Sal*I; Sh, *Sph*I; Xb, *Xba*I.

transfect JM101 and isolate double- and single-stranded DNAs. Sequence analysis confirmed the presence of the *QCR8* promoter mutations. The *QCR8* alleles were recovered as *Hin*dIII-*Sal*I fragments and ligated into pUC18 and pJH1 as described above.

The *QCR8* promoter mutations were introduced into the chromosomal context of the *QCR8* locus by direct gene substitution in *QCR8* disruption strain DLL80. pJH1, pJH1-10, pJH1-20, pJH1-30, pJH1-238, and pJH1-224 were digested with *Kpn*I and *Eco*RI, and 10-μg portions were used to transform DLL80 (46). After transformation, cells were incubated for 3 h in YPD at 30°C and plated on YPEGagar. Restoration of the *QCR8* locus, as indicated by growth on a nonfermentable carbon source, was subsequently confirmed by Southern hybridization (data not shown) and genomic sequencing (58).

RNA isolation and Northern (RNA) analysis. Yeast cultures used for steady-state mRNA analysis were grown to the early log phase (optical density at 600 nm, 1.5 to 2.0) at 29°C. Total RNA was prepared as previously described (49, 76), with minor modifications. RNA was suspended in 10 mM Tris-HCl (pH 7.5)–1 mM EDTA–0.1% SDS and stored at −20°C.

Total yeast RNA was separated on a horizontal 1.2% agarose gel in 1× TBE (0.09 M Tris-HCl–0.09 M boric acid [pH 8.3]–2.5 mM EDTA) without ethidium bromide at 20 μg per lane. The gel was soaked in 200 mM sodium acetate (pH 5.0), and RNA was transferred to Hybond-N (Amersham) by capillary blotting in 200 mM sodium acetate (pH 5.0), UV cross-linked, and soaked in 5× SSC. Prehybridization was performed in 50% formamide–5× SSC–5× Denhardt's solution–50 mM Na_2HPO_4 (pH 6.5)–10 mg of glycine per ml–0.1 mg of salmon sperm DNA (denatured) per ml at 42°C. Hybridization was performed in 50% formamide–5× SSC–5× Denhardt's solution–25 mM Na_2HPO_4 (pH 6.5)–0.1 mg of salmon sperm DNA per ml and the denatured probe fragment of interest at 42°C. Filters were washed three times in 2× SSC–0.1% SDS and twice in 0.5× SSC–0.1% SDS at room temperature and air dried completely. Following autoradiography, probed filters were washed for new probings by incubation in 95% formamide–0.1% SDS–1 mM EDTA–50 mM Tris-Cl (pH 7.5) at 50°C for 2 h and then washed in 2× SSC–0.1% SDS at 50°C and in 0.5% SSC–0.1% SDS at room temperature. Quantitation of hybridization signals was performed by cutting the radiolabeled bands of interest out of the filter and measuring the radioactivity by liquid scintillation counting (Packard).

Probe fragments used in this study included a 734-bp *Hin*dIII-*Hin*dIII fragment containing the 3' part of the *FPS* gene (Fig. 1), an 840-bp *Hin*dIII-*Sal*I fragment containing the *QCR8* gene (Fig. 1), and a 1.6-kb *Bam*HI-*Kpn*I fragment containing the yeast actin gene (59).

Medium shift experiments. For a shift from a nonfermentable carbon source to glucose, yeast cells were grown in YPE or YPAc to the early log phase (optical density at 600 nm, 1.5 to 2.0) at 29°C. Then 0.1 volume of prewarmed 20% glucose was added and the culture was rapidly mixed. Samples (10 ml) were taken at various time points, as indicated. For a shift from glucose to a nonfermentable carbon source, yeast cells were grown in YPD (4% glucose) to the early log phase (optical density at 600 nm, 1.5 to 2.0) at 29°C. Cells were then harvested by centrifugation at 25 to 28°C, washed once with prewarmed double-distilled water, suspended in prewarmed YPE at the zero time point, and incubated at 29°C. Samples (20 ml) were taken at various time points, as indicated. To block DNA replication during nutrient shift, hydroxyurea (HU) was dissolved in the shift medium, prior to suspension of cells, to a final concentration of 100 mM (see the legend to Fig. 10). In all cases, cells were immediately cooled down to 0°C, recovered by centrifugation, quick-frozen in liquid nitrogen, and stored at −20°C. Total RNA was subsequently isolated and analyzed as described above.

Gel retardation assay. Preparation of total-cell lysates and retardation-competition assays were performed as previously described (3, 28).

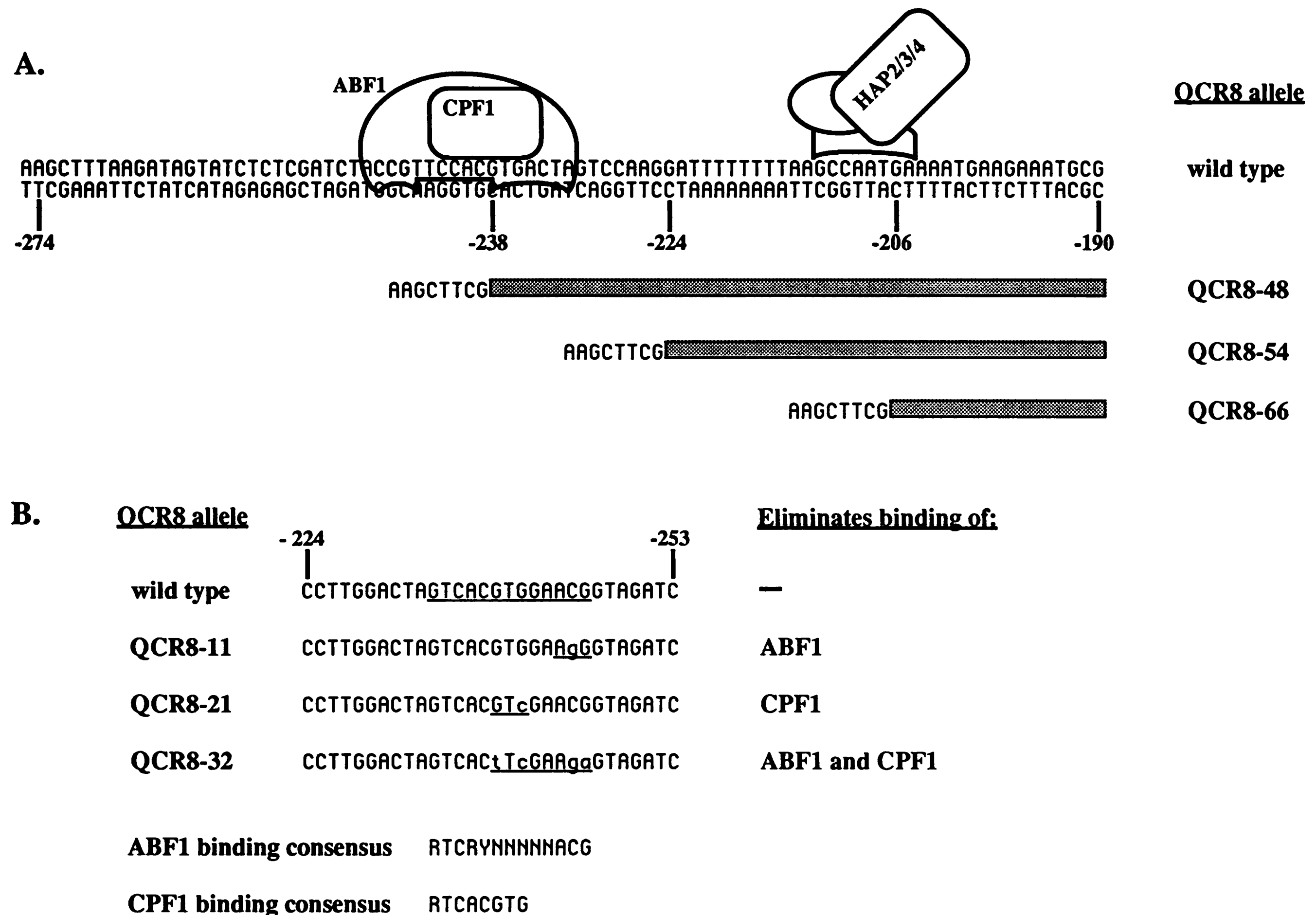

FIG. 2. Organization of wild-type and mutant *QCR8* promoter regions. (A) Primary sequence organization of the *QCR8* promoter. Distances relative to the ATG codon are indicated. Binding of ABF1, CPF1, and the HAP2-HAP3-HAP4 activator complex is depicted schematically. Promoter deletions *QCR8-48*, *QCR8-54*, and *QCR8-66* are indicated by filled bars, coupled to the sequence of the *Hin*dIII linker which is fused to the deletion endpoints. (B) Point mutations in the ABF1-CPF1-binding region. The primary sequence organization is depicted, along with the distance relative to the ATG codon. In the wild-type sequence, the sequence element corresponding to the ABF1- or CPF1-binding consensus is underlined, as are the mutated sequence elements in each of the alleles *QCR8-11*, *QCR8-21*, and *QCR8-32*. Mutated bases are depicted in lowercase.

Miscellaneous. DNA sequence analysis and recombinant DNA techniques were carried out by standard procedures (54 and references therein).

RESULTS

The ABF1-CPF1-binding region is involved in regulation of basal-level transcription of the *QCR8* gene. In a first attempt to clarify the roles played by ABF1 and CPF1 in transcriptional regulation of the *QCR8* gene, we created specific deletions throughout the *QCR8* promoter region (Fig. 2). Alleles *QCR8-48*, *QCR8-54*, and *QCR8-66* (Fig. 2) were inserted into centromeric shuttle vector YCp50 (65; Fig. 1 and Table 1) and transformed into yeast strain DLL80, which lacks the endogenous *QCR8* locus (52). In Fig. 3, steady-state levels of *QCR8* mRNA are shown. Deletion of half (*QCR8-48*) and all (*QCR8-54*) of the overlapping binding sites for ABF1 and CPF1 did not lead to loss of growth on nonfermentable carbon sources (data not shown). This is in line with previous findings that suggest that ABF1 and CPF1 are not absolutely required for carbon source-regulated expression (51). Accordingly, both *QCR8-48* and *QCR8-54* produced wild-type levels of *QCR8* mRNA on nonfermentable carbon sources (Fig. 3, lanes 9 and 10). However, when grown on rich glucose medium, both deletion mutants possessed decreased *QCR8* mRNA levels compared with wild-type *QCR8* on pCF80 (Fig. 3, lanes 4 and 5). These findings suggest that the overlapping binding sites for ABF1 and CPF1 are required for basal-level transcription of the *QCR8* gene. Deletion of consensus HAP2-HAP3-HAP4-binding sequence RCCAATNA (61; *QCR8-66*) caused severely impaired growth on YPE (data not shown), owing to almost undetectable *QCR8* transcription from pCF866 (Fig. 3, lane 11). On glucose, *QCR8* mRNA was also almost absent from DHP866, indicating that the HAP2-HAP3-HAP4-binding site is involved in basal-level transcription. The involvement of the HAP2-HAP3-HAP4 regulatory complex was further established by the experiment whose results are shown in Fig. 4, in which *QCR8* mRNA levels were decreased in *hap2* and *hap3* mutant yeast strains grown on glucose (lanes 3 and 4) or galactose (lanes 7 and 8) compared with those of the

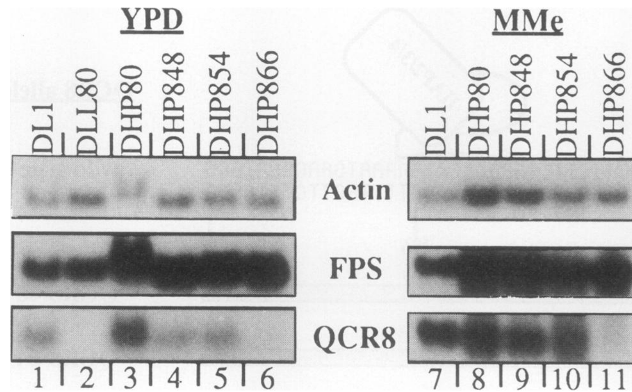

FIG. 3. Northern analysis of *QCR8* transcription from centromeric constructs. Wild-type yeast strain DL1 (lanes 1 and 7), *qcr8* disruption strain DLL80 (lane 2), and centromeric construct-containing strains DHP80 (wild-type *QCR8*; lanes 3 and 8), DHP848 (*QCR8-48*; lanes 4 and 9), DHP854 (*QCR8-54*; lanes 5 and 10), and DHP866 (*QCR8-66*; lanes 6 and 11) were grown in YPD (lanes 1 to 6) and MMe (lanes 7 to 11). Total RNA was hybridized with probes specific for actin mRNA, *FPS* mRNA (probe HH; Fig. 1), and *QCR8* mRNA (probe HS; Fig. 1).

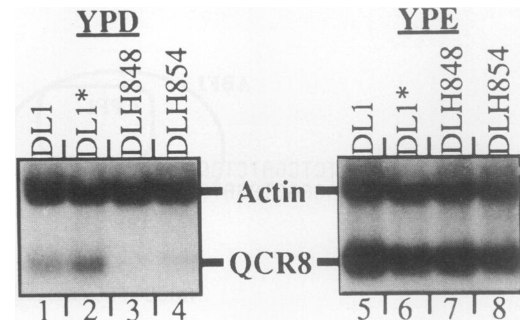

FIG. 5. Northern analysis of *QCR8* promoter deletions in the chromosomal context. Wild-type yeast strain DL1 (lanes 1 and 5) and strains DLH848 (*QCR8-48*; lanes 3 and 7) and DLH854 (*QCR8-54*; lanes 4 and 8) were grown on YPD (lanes 1 to 4) and YPE (lanes 5 to 8). Strain DL1* was obtained by restoration of the *QCR8* locus in DLL80 and is regarded as wild type. Total RNA was hybridized as described in the legend to Fig. 4.

isogenic wild-type strain (lanes 1 and 5, respectively). The *QCR8* gene is not regulated by HAP1 (Fig. 4, lanes 2 and 6), in contrast to other genes that encode respiratory chain proteins (27, 37, 48).

The mRNA levels that originated from the farnesyl diphosphate synthetase gene (*FPS*; 2), located immediately upstream of the *QCR8* gene (50) and also present on the centromeric constructs, were constant in all cases (Fig. 3). Thus, the *QCR8* promoter deletions caused only downstream effects. The elevated mRNA levels for both *FPS* and *QCR8* in all transformants compared with wild-type yeast strain DL1 are explained mainly by a copy number of two to three of the centromeric plasmids (72). In addition, the efficiency of transcription initiation from the plasmid constructs may be somewhat higher than in the chromosomal context (see Discussion).

To study the effects of ABF1 and CPF1 on *QCR8* gene expression further, *QCR8* promoter deletions *QCR8-48* and *QCR8-54* were placed into the chromosomal context of the *QCR8* locus via direct gene substitution in *qcr8* disruption strain DLL80 (Fig. 1). The resulting strains are listed in Table 1. As expected from the results described above, strains DLH848 and DLH854 grew like the wild type on

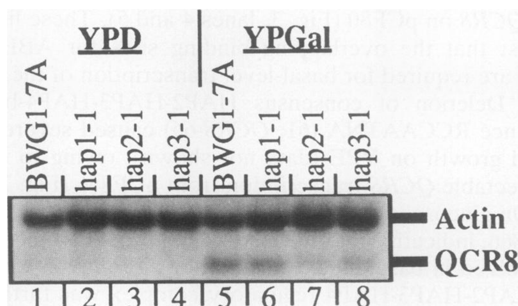

FIG. 4. Northern analysis of *hap* mutants. BWG1-7A and isogenic *hap* mutants were grown in YPD (lanes 1 to 4) and YPGal (lanes 5 to 8). Total RNA was hybridized with probes specific for actin mRNA and *QCR8* mRNA (probe HS; Fig. 1).

medium containing ethanol as the sole carbon source (data not shown). Correspondingly, both strains showed only a minute decrease in steady-state *QCR8* mRNA levels compared with the wild type when grown on a nonfermentable carbon source (Fig. 5, lanes 7 and 8 versus 5 and 6). When both strains were grown on glucose (Fig. 5, lanes 3 and 4), however, steady-state *QCR8* mRNA levels were dramatically lowered compared with the wild type. The decrease in basal-level transcription was more severe than when assayed on centromeric constructs (compare Fig. 3, lanes 4 and 5). We conclude that the overlapping binding sites for ABF1 and CPF1 in the promoter region of the *QCR8* gene are important for basal-level transcription. The role of these sequence elements in regulation of induced levels will be dealt with below.

Point mutations in the overlapping binding sequences at the *QCR8* promoter reveal different functions for ABF1 and CPF1. The effects caused by deletion of the ABF1-CPF1-binding region cannot be attributed only to the absence of both DNA-binding proteins from the *QCR8* promoter. Relatively large deletions can be expected to grossly affect the chromatin structure and context of the *QCR8* promoter region. To further investigate the roles played by ABF1 and CPF1, we introduced into the binding region point mutations that cause loss of binding of either ABF1 or CPF1 individually or of both factors together (Fig. 2). The mutations introduced into alleles *QCR8-11*, *QCR8-21*, and *QCR8-32* (Fig. 2) were derived from previous studies that described binding characteristics and sequence specificity for both ABF1 (9, 10, 29) and CPF1 (4, 11, 41). Figure 6A shows the retarded ABF1 and CPF1 complexes obtained with a wild-type *QCR8* promoter fragment. As shown in Fig. 6B, the mutated binding sequences lost the ability to bind ABF1 (lane 4), CPF1 (lane 6), or both (lane 8) in binding assays in vitro. The *QCR8-21* mutation caused an increase in ABF1 complex formation compared with the wild-type allele (see below). Alleles *QCR8-11*, *QCR8-21*, and *QCR8-32* were placed into the chromosomal context of the *QCR8* gene via direct gene substitution in *qcr8* disruptant strain DLL80. Yeast strains DLH811, DLH821, and DLH832 all showed generation times comparable to that of wild-type DL1 when assayed in the log phase on glucose or ethanol (data not shown). Steady-state mRNA levels, however, showed several interesting features (Fig. 7 and Table 2). In DLH811, the *QCR8* mRNA level was clearly reduced, both on glucose

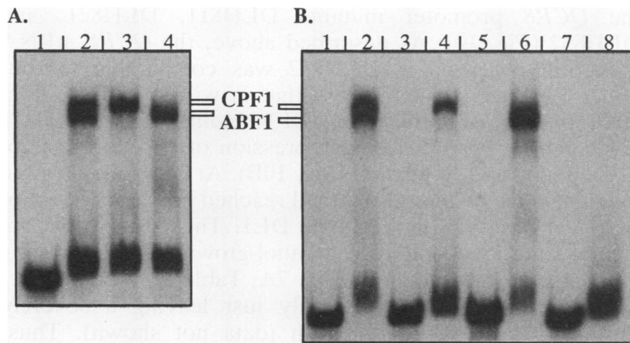

FIG. 6. Band shift analysis of *QCR8* promoter mutations. (A) A 222-bp *Hin*dIII-*Spl*I fragment containing the wild-type *QCR8* promoter region was incubated without (lane 1) and with (lanes 2 to 4) a BJ1991 total-cell lysate. Specific competition for binding of ABF1 (lane 3) or CPF1 (lane 4) was established by coincubation of a 20-fold molar excess of $QCR2_{UAS}$ oligonucleotide (28; lane 3) or a 20-fold molar excess of *CEN6* fragment (28; lane 4). (B) *Hin*dIII-*Spl*I fragments from the wild-type *QCR8* promoter (lanes 1 and 2) and from mutant alleles *QCR8-11* (lanes 3 and 4), *QCR8-21* (lanes 5 and 6), and *QCR8-32* (lanes 7 and 8) were incubated without (lanes 1, 3, 5, and 7) or with (lanes 2, 4, 6, and 8) a BJ1991 total-cell lysate as described previously (28). Complexes specifically formed through binding of ABF1 and CPF1 are indicated.

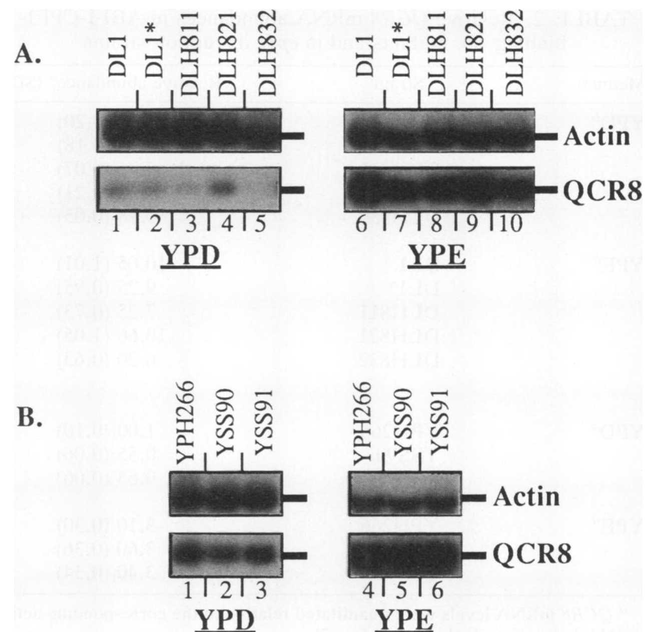

FIG. 7. (A) Northern analysis of chromosomal point mutations in the ABF1-CPF1-binding region of *QCR8*. Wild-type yeast strain DL1 (lanes 1 and 6) and strains DLH811 (*QCR8-11*; lanes 3 and 8), DLH821 (*QCR8-21*; lanes 4 and 9), and DLH832 (*QCR8-32*; lanes 5 and 10) were grown in YPD (lanes 1 to 5) and YPE (lanes 6 to 10). Total RNA was hybridized as described in the legend to Fig. 4. DL1* is described in the legend to Fig. 5. (B) Northern analysis of *cpf1* disruption strains. YPH266 (lanes 1 and 4) and isogenic *cpf1* disruption strains YSS90 (lanes 2 and 5) and YSS91 (lanes 3 and 6) were grown in YPD (lanes 1 to 3) and YPE (lanes 4 to 6). Total RNA was hybridized as described in the legend to Fig. 4. Quantitation of *QCR8* mRNA levels is shown in Table 2.

(Fig. 7A, lane 3) and on ethanol (lane 8). In DLH832, an even more pronounced decrease of the *QCR8* mRNA level was observed after growth on glucose (Fig. 7A, lane 5; Table 2); this is in line with the decreased basal level seen in *QCR8* promoter deletion mutants DLH848 and DLH854 (Fig. 5, lanes 3 and 4). In contrast to DLH848 and DLH854 (Fig. 5, lanes 7 and 8), the *QCR8* mRNA level in ethanol-grown DLH832 was also obviously decreased compared with the wild type (Fig. 7A, lane 10; Table 2). These findings suggest that absence of ABF1 alone (*QCR8-11*; DLH811) or ABF1 and CPF1 together (*QCR8-32*; DLH832) from the *QCR8* promoter results in a strong decrease of basal-level transcription and a reduced level of induced transcription of the *QCR8* gene. Again, *FPS* mRNA levels were unaffected by the *QCR8* promoter mutations (data not shown), confirming that ABF1 and CPF1 control only expression of the *QCR8* gene.

Mutant strain DLH821 displayed *QCR8* mRNA levels comparable to those of the wild type, both on glucose and on ethanol (Fig. 7A, lanes 4 and 9; Table 2). Thus, absence of CPF1 does not interfere with basal and induced transcription from the *QCR8* promoter. Like DLH821, *cpf1* disruptant strains YSS90 and YSS91 (55) displayed *QCR8* mRNA levels comparable to those of isogenic wild-type strain YPH266 when grown on ethanol (Fig. 7B, lanes 5 and 6; Table 2). On glucose, however, *QCR8* mRNA levels in YSS90 and YSS91 were lowered to about 60% compared with the wild type (Fig. 7B, lanes 2 and 3; Table 2). This may result from more pleiotropic effects caused by the complete absence of CPF1. On the other hand, the higher QCR8 mRNA levels in DLH821 may be explained by a higher affinity of ABF1 for its recognition sequence in the *QCR8-21* allele (Fig. 6B). Previous studies have shown that the binding affinity of ABF1 for its recognition sequence is higher when the intervening region between the two conserved sequence elements contains more pyrimidine residues (10, 29). The intensity of the retarded ABF1 complex in Fig. 6B, lane 6, was obviously increased compared with the one formed with the wild-type binding region (Fig. 6B, lane 2). When the CPF1 protein was specifically kept away from the wild-type binding site by competition (Fig. 6A, lane 4), the amount of the ABF1 complex formed was not increased. Thus, the increase cannot be explained by the availability of more ABF1-binding sites in the binding reaction, as a result of mutation of the overlapping CPF1 recognition sequence.

From these findings we conclude that ABF1 functions as a transcriptional activator at the *QCR8* promoter, during both glucose repression and nonfermentable carbon source-mediated derepression. CPF1 does not play an important role under steady-state growth conditions.

ABF1 is required for efficient derepression of *QCR8* transcription. Transcriptional regulation of genes that encode mitochondrial proteins is tightly coupled to the available carbon source. Upon a shift from glucose to a nonfermentable carbon source, mRNA levels are coordinately and rapidly adapted to the increased need for mitochondrial proteins. When yeast cells are transferred from a nonfermentable carbon source to glucose-based medium, transcription of the *QCR8* gene is stopped abruptly and subsequent turnover of the mRNA results in a rapid decrease of its steady-state level, even below the level that is obtained during log-phase growth on glucose (Fig. 8). After 30 min, mRNA levels again matched basal transcription levels. Deletion of the ABF1-CPF1-binding region from the *QCR8* promoter does not affect this response (20), suggesting that neither factor plays an important role.

TABLE 2. Relative *QCR8* mRNA abundances in ABF1-CPF1-binding site mutants and in *cpf1* disruption strains

Medium	Strain	Relative abundance[a] (SD)
YPD[b]	DL1	1.00 (0.20)
	DL1*[c]	0.91 (0.18)
	DLH811	0.35 (0.07)
	DLH821	1.02 (0.21)
	DLH832	0.18 (0.05)
YPE[b]	DL1	10.05 (1.01)
	DL1*	9.25 (0.95)
	DLH811	7.25 (0.73)
	DLH821	10.60 (1.05)
	DLH832	6.20 (0.63)
YPD[d]	YPH266	1.00 (0.10)
	YSS90	0.55 (0.06)
	YSS91	0.63 (0.06)
YPE[d]	YPH266	3.10 (0.30)
	YSS90	3.60 (0.36)
	YSS91	3.40 (0.34)

[a] *QCR8* mRNA levels were quantitated relative to the corresponding actin mRNA levels (see the legend to Fig. 7).
[b] *QCR8* mRNA abundance is standardized to the relative abundance in YPD-grown DL1. Assays were performed with four independent cultures in duplicate experiments for every strain. Values are averages.
[c] DL1* is described in the legend to Fig. 5.
[d] *QCR8* mRNA abundance is standardized to the relative abundance in YPD-grown YPH266. Assays were performed in duplicate experiments for every strain. Values are averages.

Shifting yeast cells from glucose to ethanol resulted in biphasic derepression of *QCR8* transcription (Fig. 9). After an initial rapid derepression during the first hour following medium shift, continued induction of transcription led to a steady-state *QCR8* mRNA level observed normally in ethanol-grown cells. Upon medium shift, cells stopped growing immediately; growth resumed after 7 to 8 h (data not shown). In DLH832 cells, in which both the ABF1 and CPF1 recognition sequences in the *QCR8* promoter were mutated, the overall response was the same. The level of transcription induction was, however, lowered to about 60 to 70% of that of the wild type (Fig. 9). This is in agreement with the steady-state level of *QCR8* mRNA normally observed in ethanol-grown DLH832 (Fig. 7A; Table 2). These findings suggest that in the absence of ABF1 and CPF1 from the *QCR8* promoter, derepression still can take place, albeit less efficiently than from the wild-type promoter.

To analyze the relative contributions of ABF1 and CPF1 to the shift-mediated induction response, medium shift experiments were carried out with wild-type DL1 and each of the *QCR8* promoter mutants DLH811, DLH821, and DLH832 (Fig. 10). As described above, the *QCR8* mRNA induction observed in DLH832 was comparable to, but about 30% less efficient than, that in wild-type DL1 (Fig. 10D). In contrast to this, non-ABF1-binding mutant DLH811 displayed only very slow derepression of *QCR8* transcription upon a shift to ethanol (Fig. 10B). At 24 h following the medium shift, *QCR8* mRNA had reached only about 50% of the level observed in wild-type DL1. This is less than the steady-state level found in ethanol-grown DLH811 during log-phase growth (compare Fig. 7A; Table 2). Indeed, after 24 h, DLH811 cells were only just leaving a severely prolonged lag phase of growth (data not shown). Thus, absence of ABF1 from the *QCR8* promoter region and, presumably, binding of CPF1 severely inhibits derepression of transcription of the *QCR8* gene. Non-CPF1-binding mutant DLH821 is equally interesting in this respect. Upon a shift to ethanol, transcription of the *QCR8* gene was induced more rapidly and efficiently than in wild-type DL1 (Fig. 10C). Absence of CPF1 from the *QCR8* promoter apparently allows for very efficient induction of transcription through ABF1. We therefore propose that on the wild-type *QCR8* promoter ABF1 is required for rapid and efficient derepression of transcription upon a shift to a nonfermentable carbon source.

ABF1 and CPF1 have opposite effects on the induction response at the *QCR8* promoter. We have previously suggested that ABF1 and CPF1 may be involved in coupling the rate of biosynthesis of mitochondrial constituents to the rate of cellular growth. Since ABF1 has been implicated in transcriptional activation and replication, we wished to investigate whether ABF1-mediated derepression of *QCR8* transcription is dependent on DNA replication. To this end, we blocked DNA replication by addition of HU to the nutrient shift medium, causing rapid but reversible inhibition of DNA synthesis (40, 68). When glucose-grown wild-type yeast cells were shifted to ethanol-based medium containing 100 mM HU, induction of *QCR8* transcription was somewhat reduced compared with that under noninhibitory conditions (Fig. 10A), in line with previous reports that total RNA synthesis is lowered by 20 to 25% under these conditions (68). The same phenomenon was observed when non-CPF1-binding mutant DLH821 was shifted to medium containing ethanol and HU (Fig. 10C). Again, induction of *QCR8* transcription was not affected by inhibition of DNA synthesis, indicating that derepression of transcription through ABF1 does not require DNA replication. Surprisingly, inhibition of DNA synthesis in DLH832 during a shift to ethanol also did not affect derepression of *QCR8* transcription (Fig. 10D). Inhibition of DNA synthesis in the mutant that cannot bind CPF1 (DLH811), however, completely blocked the slow derepression of *QCR8* transcription observed in noninhibited cells (Fig. 10B). After several hours, *QCR8* mRNA was no longer detectable and the cells stopped growing (data not shown). Apparently, derepression of *QCR8* transcription, most probably through the HAP2-HAP3-HAP4 complex, is competitive with repressively acting CPF1. Removal of CPF1 and subsequent derepression require DNA replication.

We conclude that on the wild-type *QCR8* promoter, ABF1 and CPF1 are cooperatively active in regulating the overall induction response. ABF1 is required for efficient derepression of *QCR8* transcription, independently of DNA replication. CPF1 acts as a repressor, modulating the overall induction response. Alleviation of direct repression through CPF1 requires progression through the S phase.

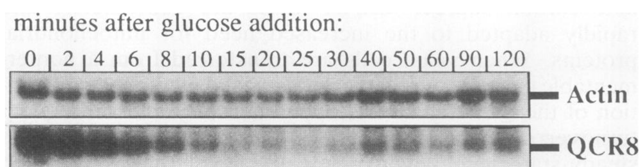

FIG. 8. Catabolite repression of *QCR8* transcription. Wild-type yeast strain DL1 was grown in YPAc. Glucose was added at the zero time point, and samples were taken at the time points indicated at the top. Total RNA from each sample was hybridized with probes specific for actin mRNA and *QCR8* mRNA (probe HS; Fig. 1).

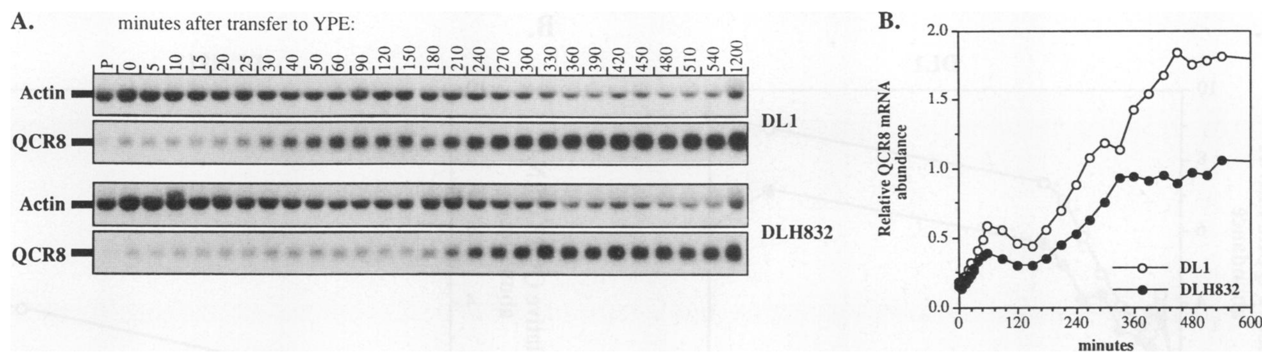

FIG. 9. Derepression of *QCR8* transcription. (A) Wild-type yeast strain DL1 and mutant strain DLH832 (*QCR8-32*) were grown in YPD. Subsequently, cells were harvested (P), washed, and transferred to YPE at the zero time point. Samples were taken at the time points indicated. Total RNA from each sample was hybridized as described in the legend to Fig. 8. *QCR8* mRNA levels are quantitated relative to the corresponding actin mRNA levels (B).

DISCUSSION

In this report, we present an analysis of the roles played by abundant DNA-binding proteins ABF1 and CPF1 in transcriptional regulation of the *QCR8* gene. First we show that mutations in the ABF1-CPF1-binding region cause more obvious and dramatic effects when assayed in the chromosomal context of the *QCR8* gene than when assayed on centromeric constructs. A plasmid-borne promoter region is expected to be localized and associated within chromatin differently from the "native" promoter. In addition, the involvement of ABF1 and CPF1 in activation and repression of transcription, DNA replication, and chromosome segregation suggests that these proteins are associated with some higher-order chromatin structure. For functionally related factor GRF1 or RAP1 (9, 67), such an association has been described (42). Attachment of ARS and CEN sequences to the nuclear scaffold has been demonstrated (1), but association of ABF1 with a nuclear scaffold has not been observed, possibly owing to differences in the nature of the association (24, 33). The involvement of ABF1 at HMR-E in the antagonism between CEN6- and HMR-mediated plasmid segregation also suggests association with a higher-order structure (45).

Basal-level transcription of *QCR8* in glucose-grown yeast cells requires both the ABF1-CPF1-binding region and the HAP2-HAP3-HAP4 activator complex. Mutants deficient in HAP2 or HAP3 show decreased *QCR8* mRNA levels under both repressed and derepressed (galactose) conditions. We constructed mutant strains in which binding of either ABF1 or CPF1 or both factors was abolished by point mutations in the overlapping binding sites in the *QCR8* promoter. Absence of ABF1 resulted in a dramatic decrease in basal-level transcription. Absence of CPF1 from the *QCR8* promoter in mutant DLH821 did not affect *QCR8* transcription during glucose repression. The small difference in basal *QCR8* mRNA levels between non-ABF1-binding mutant DLH811 and mutant DLH832, which can bind neither ABF1 nor CPF1, is also in line with a minor role for CPF1 in basal-level transcription. The ABF1-binding region in the promoter of the *COX6* gene, which encodes subunit VI of the yeast mitochondrial cytochrome *c* oxidase complex, has also been implicated in basal-level transcription, albeit less convincingly (71). Interestingly, this region shows a remarkable sequence similarity to the UAS of the *QCR2* gene (27), which can also bind ABF1. Further analysis is required to establish whether ABF1 is involved in regulation of basal-level transcription of mitochondrial protein genes in general.

ABF1 is also important for wild-type transcription of *QCR8* under derepressed growth conditions. Again, mutating the CPF1-binding site in the *QCR8* promoter does not affect transcription efficiency. Derepressed *QCR8* mRNA levels in *cpf1* disruption strains are even slightly elevated compared with the isogenic wild-type strain, indicating that CPF1 does not play an important role.

The interplay between ABF1 and CPF1 becomes more evident when yeast cells are transferred from medium containing glucose to nonfermentable carbon source-containing medium. In response to the increasing need for respiratory chain constituents, transcription of the *QCR8* gene is rapidly induced during the first hour following medium shift. A second phase of transcription induction subsequently leads to steady-state *QCR8* mRNA levels normally observed in cells growing logarithmically in the presence of ethanol. We showed that ABF1 is required for efficient derepression of the *QCR8* gene. CPF1 acts as a repressor, modulating the overall induction response. As a consequence, a mutant allele which cannot bind CPF1 to the *QCR8* promoter displays an increased rate of transcription induction compared with the wild-type allele. A mutant in which neither ABF1 nor CPF1 can bind to the *QCR8* promoter still displays induction of transcription, but the overall efficiency is lowered to 60 to 70% of the wild-type level. Efficient induction through ABF1 does not require DNA replication, whereas alleviation of repression through CPF1 does require passage through the S phase.

Derepression of many genes that encode mitochondrial proteins is primarily dependent on a functional HAP2-HAP3-HAP4 activator complex (31, 60). Mutants deficient in *hap2*, *hap3*, or *hap4* are not able to grow on a nonfermentable carbon source. Therefore, on the *QCR8* promoter, ABF1 is likely to interact directly or indirectly with the HAP2-HAP3-HAP4 complex, establishing basal and induced transcription levels. Indirect interaction may be based on ABF1, ensuring accessibility of the *QCR8* promoter under all circumstances. We have observed that the ABF1-CPF1-binding region coincides with a nuclease-hypersensitive, nucleosome-free window in the *QCR8* promoter region (21). Point mutations in the ABF1-binding site lead to alterations in the nuclease digestion pattern. Disruption of the recognition sequences for both ABF1 and CPF1 completely abol-

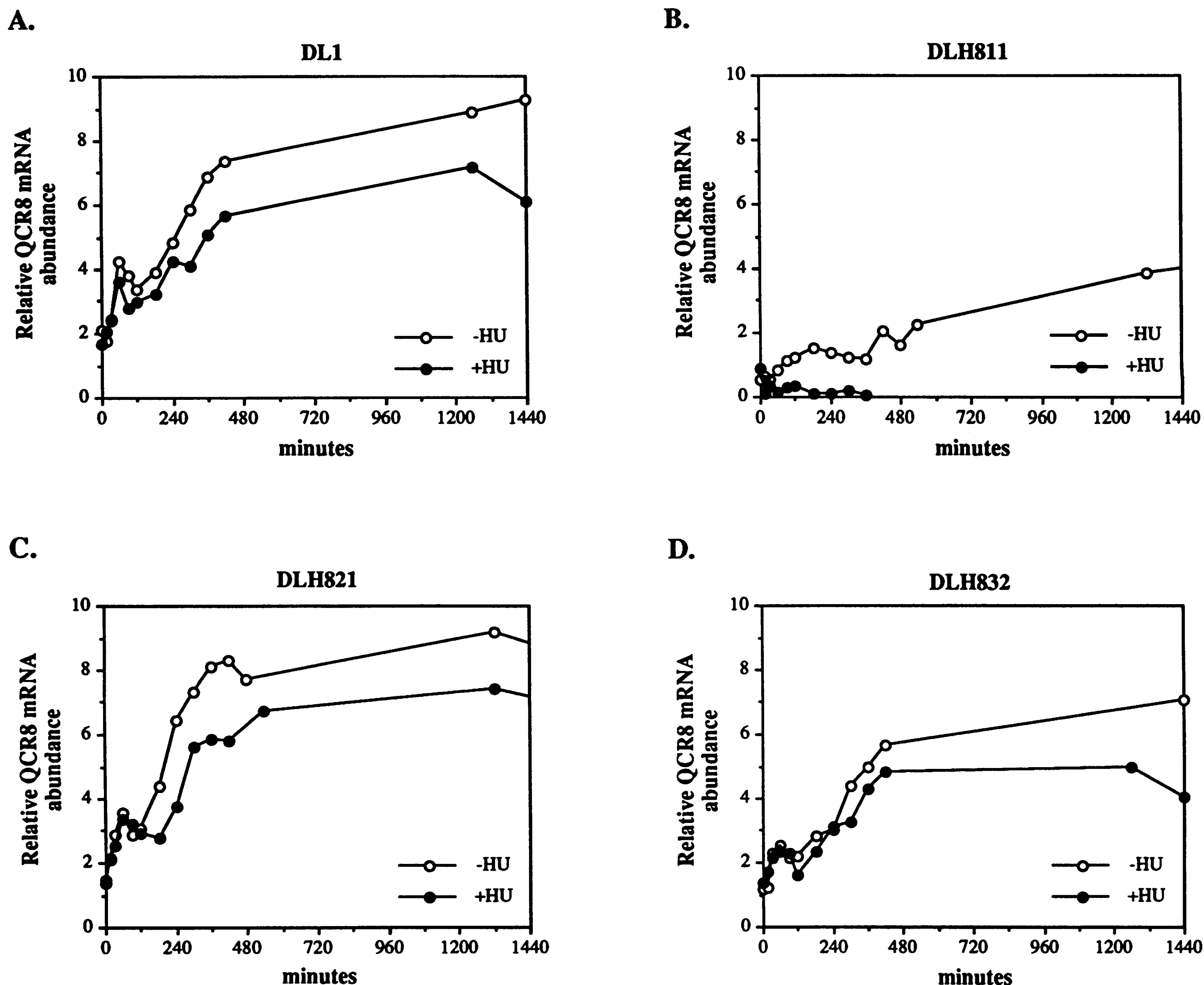

FIG. 10. Derepression of *QCR8* transcription from wild-type and mutated *QCR8* promoters. Medium shift from YPD to YPE was performed as described in the legend to Fig. 9 for wild-type DL1 (panel A) and mutant strains DLH811 (panel B), DLH821 (panel C), and DLH832 (panel D). At the zero time point, cells were transferred to YPE, with or without HU. *QCR8* mRNA levels are quantitated relative to the corresponding actin mRNA levels and standardized to the zero time point level of wild-type DL1.

ished the hypersensitive site, suggesting that the *QCR8* promoter is more densely packaged into nucleosomes. Surprisingly, this mutant can still induce *QCR8* transcription independently of DNA replication, albeit to a lesser extent. Thus, in the absence of both ABF1 and CPF1, the HAP2-HAP3-HAP4 activator complex can still activate transcription under derepressed growth conditions. Upon a shift from a nonfermentable carbon source to glucose, derepressed *QCR8* transcription rapidly ceased, followed by rapid turnover of the remaining mRNA. Only after 0.5 h was a steady-state basal mRNA level established, suggesting that basal-level transcription requires rearrangement of proteins that bind to the promoter region. A mutation that abolishes binding of CPF1 does not affect this response to a shift to glucose (20), indicating that ABF1 is involved.

Alternatively, ABF1 may be directly involved in transcription activation at the *QCR8* promoter. The HAP2-HAP3-HAP4 complex and ABF1, possibly together with additional factors, may synergistically activate transcription. This explains the decrease in steady-state *QCR8* mRNA levels observed in the non-ABF1-binding mutant under repressed and derepressed growth conditions. A synergistic interaction has been observed for heme-regulated transcription factor HAP1 and multifunctional regulator RAP1 (70). The mechanism through which ABF1 functions at the *QCR8* promoter—and hence at other promoters—may thus be a combination of direct and indirect interactions.

CPF1 functions mainly as a repressor at the *QCR8* promoter, modulating the induction response upon derepression of the gene. A repressive function for CPF1 is in line with previous observations. Upon insertion of a CDE1 motif between the UAS and TATA box of a *GAL1-HIS3* fusion, transcription is repressed to 20% of normal levels (7). In a *cpf1* disruption strain, transcription of the *GAL2* gene, which contains a CPF1-binding site in its promoter region (8), is elevated under both repressed and derepressed conditions

(56). For the *RIP1* gene, which encodes the Rieske iron-sulfur subunit of the QCR complex (6), we have also found elevated transcript levels in *cpf1* disruption strains (20). The promoter region of this gene contains a binding site for CPF1 (28).

The *QCR8* promoter is unusual in that the ABF1 and CPF1 recognition sequences overlap. As might be expected, the two proteins interact with their binding sites in a mutually exclusive manner (28, 51). It is interesting that the ABF1-CPF1-binding region does not activate transcription from a heterologous promoter (20), although the relatively weak ABF1-binding site in the *QCR8* promoter is expected to cause weak transcriptional activation (10, 29). Apparently, the overlapping combination with a CPF1-binding site interferes with transcriptional activation in a heterologous promoter context. On the basis of the results presented above, it is tempting to speculate that on the *QCR8* promoter ABF1 binds under steady-state growth conditions, while CPF1 exerts modulating effects only during derepression of transcription. Temporary displacement of either factor may be controlled through binding of additional factors or, alternatively, triggered through protein modifications, dependent on the carbon source or cell cycle phase.

The unique organization of the *QCR8* promoter may be related to the key role played by the 11-kDa protein in biosynthesis of the QCR complex. Together with the product of the *QCR7* gene (14-kDa subunit VII) and cytochrome *b*, the *QCR8* gene product constitutes an initial core complex whose formation is an absolute prerequisite for subsequent assembly of the mature complex (16, 66). Global regulation through ABF1 and CPF1, linking expression of *QCR8* and other mitochondrial constituents to various essential cellular processes, may enable the yeast cell to keep up with the varying needs for mitochondrial biogenesis under all circumstances. Maintenance of basal-level transcription during glucose repression may be essential to prevent total catabolite repression of mitochondrial biogenesis and, thus, inadvertent loss of mitochondrial DNA. The presence of a nonfermentable carbon source must be monitored rapidly, and mitochondrial protein gene expression must be induced rapidly and efficiently, independently of the growth phase of the cell.

Recently, Devlin and coworkers showed that transcription factor RAP1 is required both for basal-level *HIS4* transcription under nonstarvation conditions and for rapid increase of *HIS4* mRNA levels as a response to amino acid starvation (18). Like ABF1, RAP1 has been implicated in transcriptional activation of genes that encode proteins of the glycolytic pathway (13) and in transcriptional activation and repression of ribosomal protein genes (43, 57). At the silent mating type loci, ABF1 and RAP1 cooperate in establishing transcriptional repression through the *SIR* gene products (45). The requirement for RAP1 in GCN4- and BAS1-BAS2-dependent activation of *HIS4* transcription very much resembles the ABF1 requirement for HAP2-HAP3-HAP4-dependent activation of *QCR8* transcription. RAP1 cannot efficiently activate transcription from the *HIS4* promoter by itself, but the factor is involved in formation of a nuclease-sensitive chromatin structure that may ensure accessibility of GCN4- and BAS1-BAS2-binding sites under various conditions (18). Thus, ABF1 and RAP1 may play comparable roles in different promoter contexts.

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