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Paf1p, an RNA Polymerase II-Associated Factor in *Saccharomyces cerevisiae*, May Have both Positive and Negative Roles in Transcription

XIAOMING SHI,[1] ANN FINKELSTEIN,[2] ALEXANDER J. WOLF,[1] PAUL A. WADE,[1] ZACHARY F. BURTON,[2] AND JUDITH A. JAEHNING[1]*

Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Denver, Colorado 80262,[1] and Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824[2]

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Regulated transcription initiation requires, in addition to RNA polymerase II and the general transcription factors, accessory factors termed mediators or adapters. We have used affinity chromatography to identify a collection of factors that associate with *Saccharomyces cerevisiae* RNA polymerase II (P. A. Wade, W. Werel, R. C. Fentzke, N. E. Thompson, J. F. Leykam, R. R. Burgess, J. A. Jaehning, and Z. F. Burton, submitted for publication). Here we report identification and characterization of a gene encoding one of these factors, *PAF1* (for RNA polymerase-associated factor 1). *PAF1* encodes a novel, highly charged protein of 445 amino acids. Disruption of *PAF1* in *S. cerevisiae* leads to pleiotropic phenotypic traits, including slow growth, temperature sensitivity, and abnormal cell morphology. Consistent with a possible role in transcription, Paf1p is localized to the nucleus. By comparing the abundances of many yeast transcripts in isogenic wild-type and *paf1* mutant strains, we have identified genes whose expression is affected by *PAF1*. In particular, disruption of *PAF1* decreases the induction of the galactose-regulated genes three- to fivefold. In contrast, the transcript level of *MAK16*, an essential gene involved in cell cycle regulation, is greatly increased in the *paf1* mutant strain. Paf1p may therefore be required for both positive and negative regulation of subsets of yeast genes. Like Paf1p, the *GAL11* gene product is found associated with RNA polymerase II and is required for regulated expression of many yeast genes including those controlled by galactose. We have found that a *gal11 paf1* double mutant has a much more severe growth defect than either of the single mutants, indicating that these two proteins may function in parallel pathways to communicate signals from regulatory factors to RNA polymerase II.

Promoter-specific transcription initiation from protein-coding genes in eukaryotic systems requires the concerted action of a complex array of factors, including the general factors (TFIIA, -B, -E, -F, and -H and TBP) and RNA polymerase II (for reviews, see references 11 and 72). These factors are sufficient for basal transcription in vitro. Transcription initiation is also subject to regulation by promoter-specific activators and repressors (57). In vitro studies using reconstituted systems have revealed that an additional class of factors, coactivators or adapters, are required to mediate communication between promoter-specific regulatory factors and the general transcription machinery (8, 32, 33).

Transcriptional coactivators can be divided into several subclasses, probably corresponding to different pathways of communication between regulatory factors and the general machinery. The TFIID complex, which mediates signals from some activators, consists of TBP and TBP-associated factors (8). Individual TBP-associated factors have been shown to make direct contacts with different classes of activators (8). A second subclass of coactivator in *Saccharomyces cerevisiae* is the mediator complex found associated with the C-terminal domain (CTD) of the largest subunit of RNA polymerase II (32, 33). Independently isolated by two different laboratories, the mediator consists of the products of the *SRB* genes (suppressor of RNA polymerase B), Gal11p, Sug1p, and additional unidentified polypeptides (32, 33). The *SRB* genes were initially identified by virtue of their ability to suppress the cold-sensitive phenotypes conferred by truncation mutations in the RNA polymerase II CTD (40). All the *SRB* genes are essential for normal growth of yeast cells, suggesting their importance in transcriptional regulation (33). Previous biochemical and genetic evidence also implicates *GAL11* and *SUG1* as transcriptional coactivators (17, 24, 39, 46, 53, 54). A third class of accessory factors is represented by the yeast *SWI-SNF* complex, which affects transcription by counteracting the repressive effects imposed by chromatin structure (41, 67).

To identify additional factors which interact with RNA polymerase II, we used a monoclonal antibody directed against the RNA polymerase II CTD to immobilize the enzyme and associated factors from a transcriptionally active yeast whole-cell extract (60). The collection of factors eluted from the immobilized RNA polymerase included TFIIB, TFIIS, and all three subunits of TFIIF but not other factors found in the CTD-interacting mediator complex, including the Srb proteins, Gal11p, and Sug1p (32, 33, 60). We also identified additional factors with interesting connections to the regulation of gene expression (48, 60). Here we report analysis of one of these factors, Paf1p (RNA polymerase-associated factor 1). Using peptide sequence derived from one of the RNA polymerase-associated proteins, we have cloned the *PAF1* gene and found that its disruption leads to pleiotropic phenotypic traits including slow growth, temperature sensitivity, and enlarged cells. Consistent with a role in transcription, we have also localized Paf1p to the yeast cell nucleus. On the basis of our analysis of expression of many yeast genes in the *PAF1* mutant cells, we

* Corresponding author. Mailing address: Department of Biochemistry, Biophysics and Genetics, University of Colorado Health Sciences Center, Box B121, 4200 East Ninth Ave., Denver, CO 80262. Phone: (303) 270-3004. Fax: (303) 270-3326.

postulate that Paf1p may act as a cofactor important for transcriptional activation and repression from diverse promoters. The combination of mutations in *PAF1* and *GAL11* leads to severe growth defects consistent with the possibility that these factors are involved in duplication of essential communication functions between regulatory factors and RNA polymerase II.

MATERIALS AND METHODS

Strains and media. The *S. cerevisiae* strains used in this study were YJJ453 (*MAT***a**/*MAT*α *leu2*Δ*1*/*leu2*Δ*1 his3*Δ*200*/*his3*Δ*200 ura3-52*/*ura3-52*), YJJ564 (*MAT***a** *leu2*Δ*1 his3*Δ*200 ura3-52 gal11*Δ::*LEU2*), YJJ662 (*MAT***a** *leu2*Δ*1 his3*Δ*200 ura3-52*), YJJ664 (*MAT***a** *leu2*Δ*1 ura3-52 his3*Δ*200 paf1*Δ::*HIS3*), YJJ699 (*MAT***a** *leu2*Δ*1 gal11*Δ::*LEU2 ura3-52 his3*Δ*200 paf1*Δ::*HIS3*), YJJ575 (*MAT***a** *leu2*Δ*1 his3*Δ*200 ura3-52*), and YJJ577 (*MAT*α *leu2*Δ*1 his3*Δ*200 ura3-52 paf1*Δ::*HIS3*). These strains were all derived from the homozygous diploid YJJ453 (58) and are therefore isogenic. Yeast strains were grown in YPD or synthetic medium prepared by standard methods (21).

Isolation of the *PAF1* gene. Isolation of Paf1p and sequencing of peptides were done as described previously (60). Two peptide sequences were obtained: QEYIAPI and AADTPETS. A BLAST search (1) indicated that these two peptides were a perfect match to an open reading frame on chromosome II. The following PCR primers were designed to amplify the *PAF1* coding sequence from the yeast genomic DNA: 5′ATCGCGGATCCATGTCCAAAAAACAGGAATATATTG C3′ and 5′ATCGCGGATCCCTATTCTTCTTGTAAAGTTTCC3′. Both primers are flanked by a *Bam*HI site (underlined) and extra bases to facilitate cloning.

Deletion and disruption of *PAF1*. The one-step gene replacement method (44) was used to disrupt *PAF1*. A novel PCR-based strategy was used to make a disruption construct with the entire coding sequence of *PAF1* deleted. First, flanking sequences of *PAF1* were amplified by PCR. The 5′-flanking sequence of *PAF1* was amplified with the primers P1 (5′CTTAGCACAACTGAATTCGA AAGG3′) and P2 (5′ATCGCGGATCCGTGACCCTTTATACTATTATGAG C3′). The 3′-flanking sequence of *PAF1* was amplified with primers P3 (5′CAT-ACCCGCTCGAGGAATATTGAGAAGTGAAGGGAGATTG3′) and P4 (5′AT ACGAATGATGTTAATGGAGACTCCAGGATTGTCGACT3′). *Bam*HI and *Xho*I restriction sites were added at the 5′ ends of P2 and P3 (underlined), respectively. The PCR products were gel purified, cut with *Bam*HI and *Xho*I, and ligated to the *Xho*I-*Bam*HI fragment of *HIS3* from Sc2676 (52). The ligated products were amplified by PCR with primers P1 and P4, and the expected PCR product (3 kb) was used to transform diploid yeast cells (YJJ453) (21). The diploid cells were sporulated, and tetrads were dissected (21). Deletion of the entire coding sequence of *PAF1* was confirmed by Southern blot analysis (47).

Preparation of anti-Paf1p antibody. Paf1p was produced as a recombinant protein by PCR amplification of the *PAF1* coding sequence from total yeast DNA by using 5′TCCATATGTCCAAAAAACAGGAATATA3′ and 5′GCCG GATCCTTATTCTTCTTGTAAAGTTTCC3′ as primers. The underlined sequences are the *Nde*I and *Bam*HI sites included for subsequent cloning steps; the PCR product was cleaved with these restriction enzymes, gel purified, and ligated to pET11a (Novagen) cut with the same enzymes (47). The resulting plasmid was used to transform *Escherichia coli* BL21(DE3), and Paf1p was produced and partially purified from the transformed strain as previously described (62). Two hundred micrograms of an insoluble suspension of Paf1p was mixed with an equal volume of Titer Max Adjuvant (Vaxcel Inc.) and used to raise antibodies in rabbits (22). The resulting antibody was affinity purified by using Paf1p purified by chromatography on Mono Q (Pharmacia HR5/5) (63). Purified Paf1p was coupled to Affi-Gel 10 (Bio-Rad) according to the manufacturer's instructions. The anti-Paf1p antiserum was applied to the column, washed with a solution containing 20 mM HEPES (*N*-2-hydroxylethylpiperazine-*N*′-2-ethanesulfonic acid) (pH 7.9), 150 mM NaCl, and 1 mM EDTA and eluted with 0.1 M glycine (pH 2.5). One-milliliter fractions were collected into tubes containing 200 μl of 1.0 M HEPES, pH 7.9. Peak fractions were determined spectrophotometrically, pooled, and dialyzed against the column wash buffer.

Subcellular localization of Paf1p. Yeast strain YJJ575 was grown in YPD medium (21) at 30°C to an A_{600} of 1. Cells were harvested by centrifugation, washed with water, and resuspended in 0.1 M Tris-SO_4 (pH 9.3) containing 10 mM dithiothreitol (0.1 to 0.3 g of cells per ml of buffer), and gently shaken at room temperature for 20 min. Cells were collected, washed once in spheroplasting buffer (1.2 M sorbitol, 20 mM potassium phosphate; pH 7.4), and resuspended in spheroplasting buffer at 0.1 g of cells per ml. Yeast lytic enzyme (ICN Biochemicals) was added (7.5 mg of enzyme per g of cells), and the resulting suspension was shaken gently at 30°C until 80 to 90% of the yeast cells were spheroplasts. The progress of the spheroplasting reaction was monitored by diluting aliquots of the suspension into 9 volumes of water and checking for lysis by light microscopy. The resulting spheroplasts were washed three times in spheroplasting buffer and resuspended in MIB buffer (0.6 M mannitol, 20 mM HEPES [pH 7.4], 1 mM phenylmethylsulfonyl fluoride [PMSF]) at 0.5 g of cells per ml. Cells were subsequently lysed in a precooled Dounce homogenizer with 20 strokes by using the B pestle and diluted twofold with MIB buffer. The resulting suspension was then centrifuged for 5 min at 3,000 × *g* (4°C). Supernatants from this spin are the cytoplasmic fraction and were frozen and stored at −70°C. The pellets were resuspended in a solution (4°C) containing 18% Ficoll, 50 mM Tris-acetate (pH 7.9), 0.1 M potassium acetate, and 1 mM PMSF. Nuclei were isolated by centrifuging this suspension at 3,000 × *g* for 5 min. The supernatants were decanted and recentrifuged until they were a uniform white color. Nuclei were recovered from the supernatant by centrifugation at 25,000 × *g* for 1 h. The nuclear pellet was resuspended in TNE (10 mM Tris-HCl [pH 7.9], 1 mM EDTA, 0.1 M NaCl) at 0.3 ml per g of cells. To this suspension, 4 M ammonium sulfate (pH 7.9) was added, while stirring, to a final concentration of 0.9 M. The suspension was then stirred for an additional 30 min at 4°C. Insoluble protein was removed by centrifugation at 35,000 rpm in an SW-40 rotor (Beckman) at 4°C. Supernatant protein (soluble nuclear fraction) was precipitated by the addition of dry ammonium sulfate (0.35 g per ml), collected by centrifugation, resuspended in a minimal volume of TNE, frozen, and stored at −70°C. Protein concentrations were measured by using reagents from Bio-Rad. For Western blot (immunoblot) analysis, 300 μg of protein from spheroplasts, 200 μg of protein from the cytoplasmic fraction, and 50 μg of protein from the nuclear fraction were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10% polyacrylamide) (22). Affinity-purified anti-Paf1p antibody was used to detect immobilized Paf1p (22).

Genomic DNA analysis. Yeast strain YJJ453 was grown in YPD medium to mid-log phase, and DNA was isolated from 1.5 ml by the glass bead method (16). Genomic DNA was digested overnight with the appropriate restriction enzymes, electrophoresed on a 1% agarose gel, transferred to a nitrocellulose membrane, cross-linked with UV light, and probed with the *PAF1* PCR product prepared by random priming (47).

Yeast mRNA analysis. Total yeast RNA was isolated as described previously (16). For the galactose induction experiment, cells were grown in synthetic glucose dropout medium to mid-log phase, pelleted, and resuspended in synthetic galactose dropout medium (2% galactose). RNA samples were quantitated by measuring the optical density at 260 nm, equal amounts of RNA were run on 1% agarose-formaldehyde gels, and RNA blots were prepared by the standard methods (47). All probes were prepared by random priming. The probes were the *Sal*I fragment from pJJ58 (to detect the *GAL7* and *GAL10* transcripts [2]), an *Eco*RI-*Bam*HI fragment from pJJ157 (58) (to detect *ACT1* transcripts), PCR-amplified *FUN38* from pBluescript KS+ subclone 159 (6) by using the universal and reverse sequencing primers, and PCR-amplified *MAK16* from pBluescript KS+ subclone 162 (6) by using the reverse and universal sequencing primers. RNA blots were quantitated by PhosphorImager analysis.

β-Galactosidase assays. Reporter plasmids used in β-galactosidase assays were as previously described (19, 20). pLGSD5 contains a 365-bp *GAL10* upstream activating sequence (UAS) fragment; pSV14 has one *GAL4* 17-mer binding site. These 2μm plasmids have the indicated UAS elements inserted 5′ to the *CYC1* TATA region and transcription initiation site. Plasmids were transformed into strains YJJ662 and YJJ664 by the standard methods (21). Growth and induction of transformants were the same as described for Northern (RNA) blot analyses except that uracil was omitted for selection of the plasmids. The induction on galactose was allowed to proceed for 5 h. Extract preparation and β-galactosidase assays were performed as described previously (38).

Construction of the *gal11 paf1* double mutant. Strain YJJ564 was crossed to YJJ577, and diploids were selected on plates lacking both uracil and leucine. The diploid was sporulated, and tetrads were dissected by standard methods (21).

In vitro transcription. The preparation of yeast whole-cell transcription extracts and the transcription reaction conditions were as described previously (69). G-less cassette transcription templates lacking (pJJ460) or containing a UAS_{HSE} (pJJ468) were used (69). Each reaction mixture contained 5 mg of WCE protein per ml and 10 μg of each template per ml. Transcription reactions were performed at 26, 33, and 37°C. Labeled transcripts were isolated, resolved, visualized, and quantitated as described previously (69).

RESULTS

Cloning and sequence analysis of the yeast *PAF1* gene. We have isolated RNA polymerase-associated proteins using a monoclonal antibody directed against the CTD to capture associated factors from a transcriptionally active whole-cell extract (60). Two peptide sequences were obtained from the RNA polymerase-associated protein with an apparent molecular mass of 65 kDa: QEYIAPI and AADTPETS (60). The genes encoding these two peptides exactly match an open reading frame identified in the yeast genome sequencing project (Fig. 1). This sequence encodes a novel gene which resides on the right arm of chromosome II between the known *RIF1* and *SNF5* genes. From the sequence of the *PAF1* (polymerase-associated factor) gene, one can predict a protein of 445 amino acids with a calculated molecular mass of 49 kDa (Fig. 1), lacking significant homology to any sequence in the current database. The protein sequence encoded by *PAF1* has several

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  1 MSKKQEYIAPIKYQNSLPVPQLPPKLLVYPESPETNADSS  40
 41 QLINSLYIKTNVTNLIQQDEDLGMPVDLMKFPGLLNKLDS  80
 81 KLLYGFDNVKLDKDDRILLRDPRIDRLTKTDISKVTFLRR 120
121 TEYVSNTIAAHDNTSLKRKRRLDDGDSDDENLDVNHIISR 160
161 VEGTFNTDKWQHPVKKGVKMVKKWDLLPDTASMDQVYFI  200
201 LKFMGSASLDTKEKKSLNTGIFRPVELEEDEWISMYATDH 240
241 KDSAILENELEKGMDEMDDDSHEGKIYKFKRIRDYDMKQV 280
281 AEKPMTELAIRLNDKDGIAYYKPLRSKIELRRRRVNDIIK 320
321 PLVKEHDIDQLNVTLRNPSTKEANIRDKLRMKFDPINFAT 360
361 VDEEDDEDEEQPEDVKKESEGDSKTEGSEQEGENEKDEEI 400
401 KQEKENEQDEENKQDENRAADTPETSDAVHTEQKPEEEKE 440
441 TLQEE                                     445
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FIG. 1. Deduced amino acid sequence of Paf1p. The *PAF1* gene is encoded by open reading frame YBR2016 on the right arm of chromosome II (25). The sequenced peptides used to identify the gene (QEYIAPI and AADTPETS) are found in positions 5 to 11 and 419 to 426. All charged amino acids (negative [D and E] and positive [R and K]) are underlined. The putative PEST sequence (amino acids 25 to 49) and a potential bipartite nuclear localization motif (amino acids 119 to 141) are underlined twice.

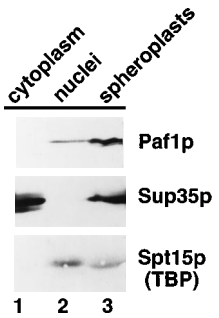

FIG. 2. Paf1p is a nuclear protein. Cellular fractionation was performed to obtain cytoplasmic and nuclear fractions as described in Materials and Methods. Protein samples (300 μg of protein from whole spheroplasts, 200 μg of protein from the cytoplasmic fraction, and 50 μg of protein from the nuclear fraction) were subjected to SDS-PAGE (10% polyacrylamide) and analyzed with polyclonal antibodies against Paf1p, Sup35p, and TBP, as described in Materials and Methods.

interesting features. In particular, Paf1p is highly charged; 40% of the amino acids are charged residues (D, E, R, or K), and its net charge at pH 7 is −23. The carboxyl terminus of the protein is extremely acidic, with glutamic and aspartic acid representing 40 of 80 residues. This high charge density may explain the discrepancy between the calculated and apparent molecular masses (49 versus 65 kDa). Paf1p contains a potential bipartite nuclear localization signal (Fig. 1, amino acids 119 to 141) (13) and several potential casein kinase phosphorylation sites (14). The amino terminus of Paf1p contains a potential PEST sequence (Fig. 1, amino acids 25 to 49) (43), which often signifies rapid turnover of proteins in vivo (43). Analysis of yeast RNA and genomic DNA indicated that *PAF1* is transcribed into a single RNA species of 1.6 kb from a single-copy gene (data not shown).

Paf1p is a nuclear protein. We produced a recombinant form of Paf1p, and this material was used to elicit antibodies. Affinity-purified antibodies were used to probe the fractions from the RNA polymerase II affinity chromatography (60). We confirmed that Paf1p was present in the anti-CTD eluate but not in control column fractions (data not shown). In addition, this antibody failed to detect cross-reacting material of the correct size in a whole-cell extract from a *paf1*Δ strain (data not shown). To determine the intracellular location of Paf1p, we fractionated lysed spheroplasts into cytoplasmic and nuclear fractions and resolved the proteins by gel electrophoresis. Using the anti-Paf1p antibody to probe the immobilized proteins, we found that Paf1p could be detected only in the nuclear fraction (Fig. 2). For controls for the cell fractionation, we found that TBP, a general transcription factor (72), was found exclusively in the nuclear fraction, while Sup35p, known to be associated with ribosomes (12), was detected only in the cytoplasmic fraction. These results indicate that Paf1p is a nuclear protein, consistent with its identification as an RNA polymerase II-associated protein and the presence of a nuclear localization signal.

Pleiotropic phenotypic traits of the *paf1* deletion-disruption mutant. To determine possible functions of Paf1p, we disrupted the *PAF1* gene in a homozygous diploid yeast strain, deleting the entire coding sequence and replacing it with a *HIS3* marker (Fig. 3A; see Materials and Methods). The deletion was confirmed by genomic DNA analysis (data not shown), the diploid was sporulated, and tetrads were dissected (21). At 30°C, all intact tetrads exhibited a pattern of two large His^- and two small His^+ colonies (Fig. 3B). Genomic DNA analysis confirmed deletion of *PAF1* in the His^+ spores but not in the His^- spores (Fig. 3C). Complete rescue of the *paf1* null mutant by a centromere plasmid containing the *PAF1* gene demonstrated that the observed phenotypic traits result from loss of Paf1p function and are not due to the effects of disrupting flanking genes (data not shown). Consistent with the small size of the *paf1* colonies, the doubling time of the *paf1* mutant strain increased to 3 to 4 h from the 2-h generation time of the isogenic wild-type strain (Fig. 4A). Disruption of *PAF1* also resulted in a temperature-sensitive phenotype at 36 or 38°C (Fig. 4B). Although the *paf1* cells are not cold sensitive, they grow more slowly at 14 or 25°C than the cells of the isogenic *PAF1* strain. The *paf1* mutant cells had an abnormal morphology; the cells were enlarged (two- to threefold larger than wild-type cells) and rounded (Fig. 4C).

The expression levels of most yeast genes are not affected by the *paf1* mutation. If Paf1p is important for the transcription of some yeast genes, transcript abundance from the temperature-sensitive null mutation may be altered for subsets of genes at both the permissive and restrictive temperatures. We have screened over 20 yeast transcription units and found several transcripts that begin to fulfill this prediction. We first analyzed transcription patterns after a shift to the nonpermissive temperature. Cells were grown at the permissive temperature, 30°C, and were then shifted to 38°C, and samples were isolated for RNA analysis. The *paf1* cells go through 1 or 2 further doublings at 38°C, and no further growth was observed 5 h after the shift to the nonpermissive temperature. Most transcripts monitored for 5 h after the shift, including those from *ACT1*, *EGD1*, *DED1*, *CDC73*, and *SPT15*, were unaffected by the *paf1* mutation (data not shown). We did observe a two- to threefold-lower abundance of the transcript from the *EGD2* gene (49) in the *paf1* strain within 1 h after the shift to the nonpermissive temperature (data not shown). Consistent with these results, by differential display analysis (35), abundance of approximately 95% of the transcripts was not altered in the *paf1* mutant, while the abundance of the remaining 5% of the transcripts was increased or decreased significantly (48).

The *paf1* mutation impairs the induction of galactose-inducible genes. The *paf1* mutant strain exhibited slow growth on all carbon sources tested, including glycerol and galactose. Since slow growth on these carbon sources has been observed for

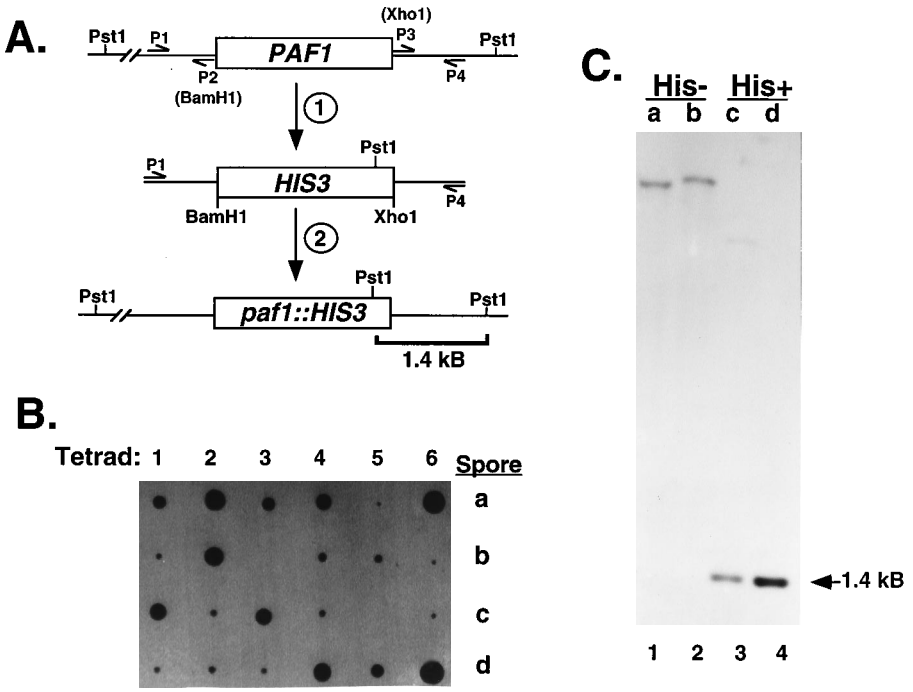

FIG. 3. Disruption of *PAF1* in *S. cerevisiae*. (A) Schematic representation of the PCR-based disruption strategy. The 5′- and 3′-flanking sequences of *PAF1* were amplified from yeast genomic DNA by PCR (Materials and Methods). Restriction sites for *Bam*HI and *Xho*I, absent in the flanking sequences, were added to primers P2 and P3 to facilitate ligation to the *HIS3 Bam*HI-*Xho*I fragment (52). The resulting ligation product (*PAF1* 5′-flanking sequence–*HIS3*–*PAF1* 3′-flanking sequence) was amplified by PCR with primers P1 and P4. The amplified fragment was used to transform the diploid yeast strain YJJ453. (B) Tetrad analysis of the *paf1* deletion-disruption mutant. The heterozygous *paf1*/*PAF1* disruption strain was sporulated, and tetrads were dissected. Six dissected asci are shown. (C) Confirmation of the *paf1* disruption. Genomic DNA was prepared from the four spores of one tetrad, digested with *Pst*I, and resolved on a 1% agarose gel. The hybridization probe was a random-primed PCR product of the 3′-flanking sequence of *PAF1*, as shown in panel A.

strains with mutations in genes important for induction of the galactose-regulated genes (29), we analyzed the effect of the *paf1* mutation on galactose induction. Wild-type and isogenic *paf1* mutant strains were grown to the mid-log phase in glucose-containing synthetic medium and shifted to galactose-containing medium, and samples were collected for RNA analysis (Fig. 5A). Although the *GAL7* and *GAL10* mRNAs are induced in the *paf1* mutant, their transcript levels were significantly lower (three- to fivefold) than those of the wild-type strain (Fig. 5A). To confirm that this result was due to alterations in transcription, we introduced reporter plasmids containing the UAS from a galactose-regulated promoter (UAS_{GAL}), into the wild-type and *paf1* mutant strains. As shown in Fig. 5B, β-galactosidase activity from the UAS_{GAL}-*lacZ* fusion was reduced five- to sixfold in the *paf1* mutant, comparable to the reduction seen by Northern blot analysis. Although mutations in many genes affect induction of the galactose-regulated genes (29), the phenotype conferred by the *paf1* mutation is not a secondary effect of slow growth. An isogenic *cdc73* mutant strain, also slowly growing and temperature sensitive (42, 48), has no defect in galactose induction (48). The reduced galactose induction also does not appear to be due to changes in the expression of *GAL4*, since the *paf1* mutation has no obvious effect on the level of *GAL4* mRNA (68). These results strongly suggest that the alteration in UAS_{GAL}-driven expression seen in the *paf1* strain occurs at the transcriptional level between Gal4p and the general transcription apparatus.

Paf1p negatively regulates *MAK16* gene expression. To identify additional transcriptional defects in the *paf1* mutant, we surveyed transcripts from 17 genes on a 42-kb segment of chromosome I (4). The RNA levels from all but one of the genes were not affected by the *paf1* mutant; one example, the 4.8-kb transcript from the *FUN38* gene, is shown in Fig. 5C. However, the RNA level from the *MAK16* gene was dramatically higher in the *paf1* mutant (five- to eightfold) than in the wild-type strain (Fig. 5C). *MAK16* is an essential gene in *S. cerevisiae* (66), and the amino acid sequence encoded by *MAK16* is similar to those of casein kinases (6). Although the exact function of *MAK16* is not known, the gene is probably involved in cell cycle control, since a temperature-sensitive mutant of *MAK16* causes cells to arrest in G_1 phase at the restrictive temperature (65). In addition, mutations in *MAK16* result in the loss of M1 double-stranded RNA virus-like particles in *S. cerevisiae* (66).

Paf1p is not required for transcription of a model promoter in vitro. To test the possible involvement of Paf1p in transcription in vitro, whole-cell transcription extracts were prepared in parallel from wild-type and isogenic *paf1* mutant strains. As shown in Fig. 6, basal transcription from the *CYC1* core promoter (lacking a UAS [UAS−]) in a G-less cassette template, activated transcription from a similar template containing a heat shock transcription factor-responsive UAS_{HSE}, and the ratios of transcription from the two templates for wild-type and mutant extracts were essentially indistinguishable at the three temperatures tested. The lack of an obvious defect is consistent with the results of our in vivo experiments demonstrating that mutation of Paf1p affects expression from a small subset of promoters; the model templates used in this experiment may not be appropriate to reveal Paf1p function.

Synthetic phenotype of the *gal11 paf1* double mutant. Gal11p, a component of the RNA polymerase II holoenzyme (33), is postulated to play a role in communicating between the

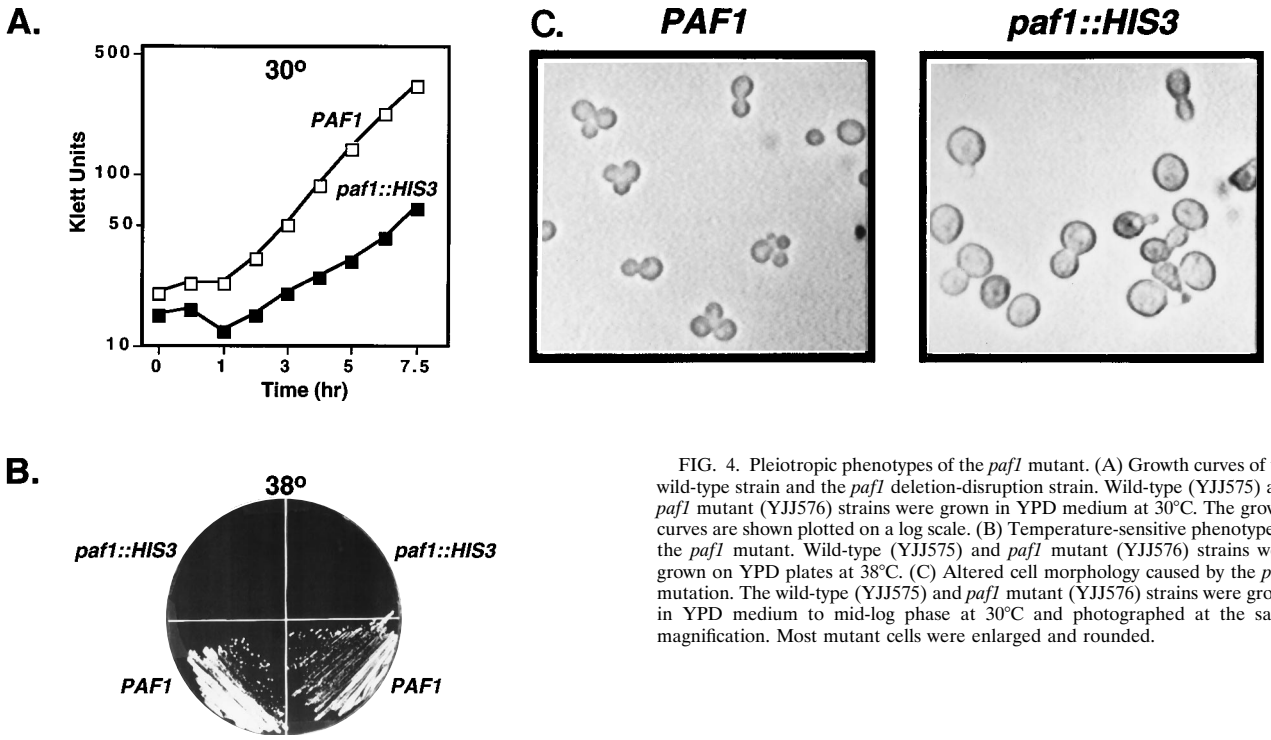

FIG. 4. Pleiotropic phenotypes of the *paf1* mutant. (A) Growth curves of the wild-type strain and the *paf1* deletion-disruption strain. Wild-type (YJJ575) and *paf1* mutant (YJJ576) strains were grown in YPD medium at 30°C. The growth curves are shown plotted on a log scale. (B) Temperature-sensitive phenotype of the *paf1* mutant. Wild-type (YJJ575) and *paf1* mutant (YJJ576) strains were grown on YPD plates at 38°C. (C) Altered cell morphology caused by the *paf1* mutation. The wild-type (YJJ575) and *paf1* mutant (YJJ576) strains were grown in YPD medium to mid-log phase at 30°C and photographed at the same magnification. Most mutant cells were enlarged and rounded.

RNA polymerase and upstream activators and repressors (3, 24, 39, 46, 53). The phenotype conferred by a *gal11* mutation is similar to that of *paf1* resulting in a three- to fivefold reduction in expression of the galactose-regulated genes (53) and in increased and decreased transcript abundance from many yeast genes (17, 39). To determine if these two RNA polymerase II-associated factors functioned in the same pathway or in nonoverlapping pathways, we constructed a *gal11 paf1* double mutant. If Gal11p and Paf1p function in the same pathway, the phenotype of the double mutant may not be more severe than the phenotype of either single mutant. If, however, Gal11p and Paf1p function in distinct pathways, or if they are partially functionally redundant, we would expect a more severe phenotype in the double mutant. We first constructed a *gal11* deletion mutation (*gal11*Δ::*LEU2*) in a strain isogenic to the one used for disruption of *paf1* and observed that, as previously seen in many other yeast strains (53), this mutation impaired galactose induction three- to fivefold and displayed a modest growth defect on YPD medium (data not shown). We used this strain to create a *GAL11/gal11 PAF1/paf1* heterozygous diploid which was sporulated, and from which 15 tetrads were dissected as described in Materials and Methods. All tetrads showed a 2 His^+:2 His^- and a 2 Leu^+:2 Leu^- segregation pattern. However, the double *gal11*Δ::*LEU2 paf1*Δ::*HIS3* mutants grew much more slowly at 30°C (doubling time of >8 h) than either of the single mutants (Fig. 7). Like the *paf1* mutant, the double mutant was inviable at elevated temperatures. The severe growth defect of the double mutant indicates that Paf1p and Gal11p function in two distinct pathways, one or the other of which is required for normal yeast cell growth.

DISCUSSION

In this work we have identified a novel factor, Paf1p, which appears to be involved in the expression of a subset of yeast genes. The identification of the protein as an RNA polymerase II-associated factor, the nuclear localization of Paf1p, the pleiotropic phenotype conferred by a null allele of *PAF1*, and the identification of positive and negative effects on several apparently unrelated genes support the hypothesis that Paf1p is a general transcription factor or mediator for a subset of yeast genes. Paf1p joins a growing list of yeast transcriptional regulators with both positive and negative roles in gene transcription. These regulators include Rap1p (Grf1p or Tuf1p [5, 26, 50, 51]), Mcm1p (Prtf or Grm [5, 31]), Gal11p (Spt13p [17, 53]), Sin4p (Tsf1p [9, 10, 27, 28]), Sin3p (Rpd1p or Gam2p [59, 63, 71]), Spt16p (Cdc68p [37]), Reb1p (Grf2p [7, 64]), and Rgr1p (27). A feature common to all these factors is promoter-specific function, with negative effects on certain promoters and positive effects on others. Two general mechanisms have been invoked to explain the functions of these genes. One involves interaction with chromatin; this mechanism has been suggested for Sin4p (27, 28) and Spt16p (37, 45, 70). The other mechanism envisions direct interaction with the basal transcriptional machinery. In this regard, Gal11p encodes a component of the RNA polymerase II holoenzyme (32). The isolation of Paf1p as an RNA polymerase-associated factor would link it more closely to the second proposed mechanism, although there is no reason to assume that these mechanisms are mutually exclusive.

A striking feature of the *PAF1* coding sequence is its highly charged and acidic nature. Much of the negative charge is localized to the carboxyl terminus. High charge content and acidity are common features of many transcription factors (30). For example, the products of several of the *SPT* genes, including Spt5p (56), Spt6p (55), Spt7p (18), Spt8p (15), and Spt16p (Cdc68p) (45) also encode very acidic proteins. The proteins encoded by the *SPT6*, *-8*, and *-16* genes all have extremely acidic N or C termini, a feature shared by Paf1p. The products of these *SPT* genes, which affect transcription from a subset of yeast promoters, and the *PAF1* product may define a new family of promoter-specific transcription factors.

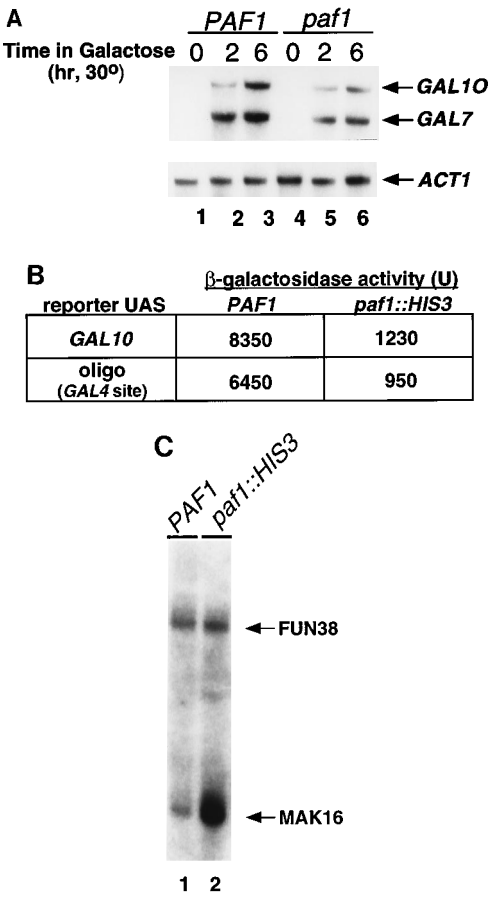

FIG. 5. The *paf1* mutation causes altered transcription in vivo. (A) The *paf1* mutation reduces induction of *GAL* gene transcripts. Wild-type (YJJ662) and *paf1* mutant (YJJ664) strains were grown in synthetic medium containing glucose, pelleted, and resuspended in synthetic medium containing 2% galactose. At the indicated times, aliquots of cells were frozen at −70°C and RNA was prepared and fractionated as described in Materials and Methods. After hybridization with the *GAL7-GAL10* probe, the blot was stripped and rehybridized with an *ACT1* probe as an internal control. (B) The *paf1* mutation reduces induction of a UAS_{GAL} reporter construct. The β-galactosidase assays were performed as described in Materials and Methods. For each indicated reporter plasmid, at least two independent clones were assayed, and each experiment was repeated at least three times. Mean values are presented. Standard errors were typically less than 25% of the reported value. Units of activity were obtained from the following formula: (optical density at 420 nm × 1,000)/(milliliters assayed × time [in minutes] × protein concentration [in milligrams per milliliter]). (C) The *MAK16* transcript is elevated in the *paf1* mutant. Wild-type (YJJ575) and *paf1* mutant (YJJ576) strains were grown in YPD medium, and RNA was prepared and analyzed as outlined above for panels A and B. The probes were prepared by random priming of PCR-amplified *MAK16* DNA and *FUN38* DNA (4) (generous gifts from D. Kaback).

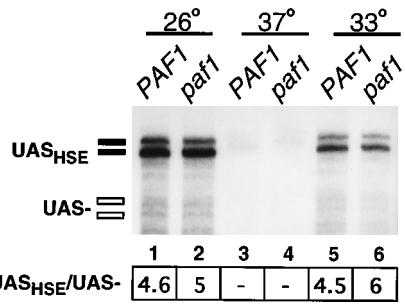

FIG. 6. Paf1p is not required for transcription of a model template in vitro. Preparation of whole-cell transcription extracts and in vitro transcription of G-less cassette templates are described in Materials and Methods. Transcription reactions were performed at 26, 33, and 37°C as indicated. The white bars indicate transcripts from a basal template lacking a UAS (UAS−), and the black bars are transcripts from a template containing the UAS_{HSE} which is activated by the heat shock transcription factor in the transcription extract (69). At the bottom of the figure are ratios of incorporation into the templates containing UAS_{HSE} and the templates lacking UAS (UAS−) as calculated by scanning the indicated bands from the autoradiogram on a densitometer.

The pleiotropic phenotypic traits of the *paf1* mutant suggest that Paf1p is a global transcriptional regulator. This is consistent with our identification of several functionally unrelated targets of Paf1p, including the *GAL* genes, *EGD2*, and *MAK16*. *MAK16* (maintenance of killer) was originally identified as one of the genes necessary for maintaining the L-A double-stranded RNA virus and its killer toxin-encoding satellite, M1 in *S. cerevisiae* (66). *MAK16* is essential for viability; a temperature-sensitive mutant causes a G_1 cell cycle arrest with large unbudded cells as the terminal phenotype (65, 66). The terminal morphology of *paf1* mutant cells arrested at 38°C also showed a high percentage of large, unbudded cells (data not shown), although the functional significance of this observation and the possible connection to *MAK16* remain to be investigated. The requirement for functional Paf1p at high temperatures could be due to decreased expression of an as yet unidentified essential gene, as seen for the *EGD2* transcript, or to altered ratios of expression of multiple genes whose products are dependent on precise stoichiometries for correct function.

The Srb protein-containing mediator complex from *S. cerevisiae* has been shown to mediate transcriptional response by several activators in vitro and in vivo (32, 33). Recently, two genes encoding components of the mediator, *SRB10* and *SRB11*, have been cloned (36). Srb10p and Srb11p are involved not only in transcriptional activation (34, 36) but also in transcriptional repression of both the **a**-specific genes in α cells (61) and of many yeast genes in response to glucose (34). Another component of the mediator, Gal11p, also has both positive and negative regulatory roles in transcription (17, 46). The isolation of Paf1p defines a fourth RNA polymerase II-associated factor with both positive and negative effects on transcription. Paf1p is unlikely to be a component of the mediator complex on the basis of the strategy used for its isolation. Immobilization of the largest RNA polymerase II subunit through an antibody directed against the CTD has been shown to exclude the interaction between the mediator and RNA polymerase II (23, 32). The initial fraction of RNA polymerase-associated factors which included Paf1p did not contain the mediator factors (60). In addition, using a tagged

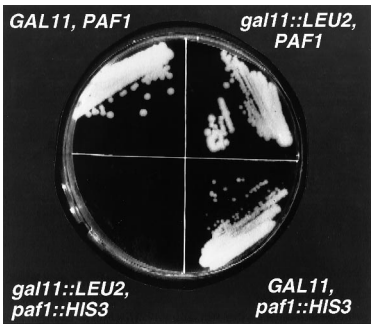

FIG. 7. Synthetic growth defect of the *gal11 paf1* double mutant. Isogenic wild-type (YJJ575), *paf1* (YJJ576), *gal11* (YJJ564), and *gal11 paf1* (YJJ699) strains were streaked onto a YPD plate and grown at 30°C for 3 days.

version of Paf1p, we have identified complexes containing Paf1p and RNA polymerase II that do not contain the Srb proteins (49). Paf1p therefore potentially interacts directly or indirectly with a region(s) of RNA polymerase II other than the CTD.

It is increasingly clear that gene-specific factors (activators or repressors) can utilize multiple pathways to communicate with the basal transcription apparatus. In fact, the artificial creation of an interaction between a DNA-binding factor and Gal11p in the mediator complex is sufficient to trigger activation of transcription (3). For naturally occurring activators and repressors, the different pathways connecting the factors to the transcription apparatus can be totally independent or functionally redundant. A possible interpretation of the severe phenotype of the *paf1 gal11* double mutant is that it reflects the consequence of destruction of two different but partially compensatory pathways of communication with RNA polymerase II. Regulatory proteins like Gal4p may require both Paf1p and Gal11p for full levels of activation or repression. In the absence of either RNA polymerase-associated factor, regulation is reduced but still occurs; when both factors are missing, cell growth is severely impaired.

Many yeast transcription factors were originally identified genetically. Paf1p and another RNA polymerase II-associated factor, Cdc73p (42), were identified by a biochemical screen of factors associated with RNA polymerase II (48, 60). We have found similarities and differences in the altered patterns of transcription in isogenic strains bearing disruptions in either *PAF1* or *CDC73* (48). Both mutations lead to an increased abundance of *MAK16* and *DBP2* transcripts (Fig. 5C [48]); however, unlike the *gal11* and *paf1* defects, the *cdc73* mutation has no effect on galactose induction. In contrast to the severe phenotype conferred by the *gal11 paf1* double mutation, the phenotype conferred by a *cdc73 paf1* mutation resembles that conferred by the *paf1* single mutation (48). We have also found that the phenotype conferred by a *cdc73 gal11* double mutation is similar to that conferred by the *cdc73* single mutation (48). These facts are consistent with the possibility that Paf1p and Cdc73p have distinct functions in a single novel pathway of communication with RNA polymerase II. In support of this idea, we have found complexes which contain RNA polymerase II and both Paf1p and Cdc73p (48). Further characterization of *PAF1*, *CDC73*, and other genes encoding these polymerase-associated factors will allow us to resolve their role in eukaryotic transcriptional regulation.

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