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Cdc73p and Paf1p Are Found in a Novel RNA Polymerase II-Containing Complex Distinct from the Srbp-Containing Holoenzyme

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The products of the yeast *CDC73* and *PAF1* genes were originally identified as RNA polymerase II-associated proteins. Paf1p is a nuclear protein important for cell growth and transcriptional regulation of a subset of yeast genes. In this study we demonstrate that the product of *CDC73* is a nuclear protein that interacts directly with purified RNA polymerase II in vitro. Deletion of *CDC73* confers a temperature-sensitive phenotype. Combination of the *cdc73* mutation with the more severe *paf1* mutation does not result in an enhanced phenotype, indicating that the two proteins may function in the same cellular processes. To determine the relationship between Cdc73p and Paf1p and the recently described holoenzyme form of RNA polymerase II, we created yeast strains containing glutathione *S*-transferase (GST)-tagged forms of *CDC73*, *PAF1*, and *TFG2* functionally replacing the chromosomal copies of the genes. Isolation of GST-tagged Cdc73p and Paf1p complexes has revealed a unique form of RNA polymerase II that contains both Cdc73p and Paf1p but lacks the Srbps found in the holoenzyme. The Cdc73p-Paf1p-RNA polymerase II-containing complex also includes Gal11p, and the general initiation factors TFIIB and TFIIF, but lacks TBP, TFIIH, and transcription elongation factor TFIIS as well as the Srbps. The Srbp-containing holoenzyme does not include either Paf1p or Cdc73p, demonstrating that these two forms of RNA polymerase II are distinct. In confirmation of the hypothesis that the two forms coexist in yeast cells, we found that a TFIIF-containing complex isolated via the GST-tagged Tfg2p construct contains both (i) the Srbps and (ii) Cdc73p and Paf1p. The Srbps and Cdc73p-Paf1p therefore appear to define two complexes with partially redundant, essential functions in the yeast cell. Using the technique of differential display, we have identified several genes whose transcripts require Cdc73p and/or Paf1p for normal levels of expression. Our analysis suggests that there are multiple RNA polymerase II-containing complexes involved in the expression of different classes of protein-coding genes.

A minimal set of transcription factors (RNA polymerase II plus TATA-binding protein [TBP], TFIIB, TFIIE, TFIIF, and TFIIH) are necessary for mRNA promoter-specific transcription initiation in vitro (for reviews, see references 7 and 12). Regulated transcription requires, in addition to these basal factors, many accessory proteins responsible for conveying regulatory signals to the general transcriptional machinery (68). There are at least two different classes of accessory factors that have been well characterized. One class includes the TBP-associated factors (TAFs) (for a review, see reference 58). In vitro reconstitution experiments strongly implicate the TAFs in the process of transcriptional activation (10). Another class of accessory factors exists in the mediator complex associated with the C-terminal repeat domain (CTD) of the largest subunit of RNA polymerase II (for a review, see reference 33). In the yeast *Saccharomyces cerevisiae*, the mediator can associate with RNA polymerase II and several general initiation factors to form a large protein complex termed the holoenzyme (30, 32). Most components of the holoenzyme, including the Srbps, Gal11p, Sin4p, Rgr1p, and Swi/Snfps, were originally identified by mutations that caused transcriptional alterations in yeast (24, 27, 34, 39, 43, 53, 64). Although mutations in some of these gene products affect the expression of only subsets of yeast genes, an analysis of temperature-sensitive mutations of *SRB4* and *SRB6* revealed transcription defects at all class II promoters assayed (57). Mammalian RNA polymerase II-containing complexes that include Srbp homologs, and many of the general transcription factors as well as DNA-repair factors, have recently been described (36, 42).

The reported complex forms of RNA polymerase II vary widely in terms of composition. In particular, some of the general initiation factors (TBP, TFIIE, and TFIIH) are present in some complexes but not others (30, 32, 36, 42). In addition, some of the factors, including the Srbps and Gal11p, can be found in dissociable subcomplexes (30, 34). Although it is probable that some of these differences reflect the different purification protocols used to isolate these extremely large complexes from widely differing cell types, it is also possible that RNA polymerase II exists in multiple functional forms in vivo. We have recently described the isolation of a collection 20 or more RNA polymerase II-associated proteins (RAPs) from a transcriptionally active yeast whole-cell extract (62). Our isolation technique required immunoaffinity interactions with the CTD of RNA polymerase II; the Srbps, which are known to interact directly with the CTD, were not found in this collec-

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tion of RAPs. Factors TBP and TFIIH were also absent; however, some of the general factors were identified: TFIIB, all three subunits of TFIIF, and elongation factor TFIIS. Two novel factors, Paf1p and Cdc73p, were also identified in the RNA polymerase-associated fraction. We have recently reported the initial characterization of Paf1p (polymerase-associated factor 1 [50]). Paf1p is a low-abundance nuclear protein required for normal growth and morphology of yeast cells. Consistent with a function in RNA polymerase II transcription, expression of some yeast mRNAs, including those from the galactose-inducible genes, is altered in a *paf1*Δ mutant strain. We have also found a dramatic enhancement of the *paf1*Δ mutant phenotype when it is combined with a *gal11*Δ mutation (50), demonstrating a genetic interaction between Paf1p and at least one component of the RNA polymerase II holoenzyme.

CDC73 was previously identified as a gene involved in the pheromone response pathway (45), but the function of Cdc73p has not been determined. The identification of Cdc73p as an RNA polymerase II-associated factor prompted us to investigate its potential involvement in transcriptional regulation. We present both biochemical and genetic evidence in support of the idea that Cdc73p and Paf1p define a novel form of RNA polymerase II, distinct from the Srbp containing holoenzyme, and important for expression of a subset of yeast genes.

MATERIALS AND METHODS

Strains and media. The *S. cerevisiae* strains used in this study were YJJ453 (*MAT***a**/α *leu2Δ1/leu2Δ1 his3Δ200 /his3Δ200 ura3-52/ura3-52*), YJJ564 (*MAT***a** *leu2Δ1 his3Δ200 ura3-52 gal11Δ::LEU2*), YJJ577 (*MAT*α *leu2Δ1 his3Δ200 ura3-52 paf1Δ::HIS3*), YJJ662 (*MAT***a** *leu2Δ1 his3Δ200 ura3-52*), YJJ663 (*MAT***a** *leu2Δ1 his3Δ200 ura3-52 cdc73::LEU2*), YJJ664 (*MAT***a** *leu2Δ1 ura3-52 his3Δ200 paf1Δ::HIS3*), YJJ665 (*MAT***a** *leu2Δ1 ura3-52 his3Δ200 cdc73Δ::HIS3*), YJJ674 (*MAT***a** *leu2Δ1 ura3-52 his3Δ200 paf1Δ::HIS3* with pEGST), YJJ676 (*MAT***a** *leu2Δ1 ura3-52 his3Δ200 paf1Δ::HIS3* with pEGST-*PAF1*), YJJ691 (*MAT***a** *leu2Δ1 ura3-52 his3Δ200 cdc73Δ::HIS3* with pEGST-*CDC73*), YJJ693 (*MAT***a** *leu2Δ1 ura3-52 his3Δ200 cdc73Δ::HIS3* with pEGST), YJJ854 (*MAT*α *leu2Δ1 ura3-52 his3Δ200 tfg2Δ::HIS3* with pEGST-*TFG2*), and YJJ855 (*MAT***a** *leu2Δ1 ura3-52 his3Δ200* with pEGST). These strains were all derived from the homozygous diploid YJJ453 (59) and are therefore isogenic. Yeast strains were grown in YPD or synthetic medium prepared according to standard methods (20).

Identification of Cdc73p. Isolation of Cdc73p and peptide sequencing were done as described previously (62). The following peptide sequence was obtained: KDPIILIPSAASSIL. Initial identification of the gene encoding this peptide as *CDC73* (45) was through a private database maintained by M. Goebl with confirmation from S. Reed (44). The *CDC73* gene was subsequently sequenced in the yeast genome project (28). The GenBank accession number for *CDC73* is U20162 (gene 9931.10).

Disruption of *CDC73*. A PCR-based strategy was used to entirely delete one copy of *CDC73* in the diploid YJJ453. Four PCR primers were used (from 5′ to 3′): P1 (ATTAATGAAAGCAATTGCGATG), P2 (ATCGCGGATCCTTTTC ACCAGTTTCTTGCTC), P3 (CATACCCGCTCGAGGATGTGGAAGTCGT GCG), and P4 (GTAATAAATGCATGGTTTGATTACC. *Bam*HI and *Xho*I sites (underlined) were added to the 5′ ends of P2 and P3, respectively. After amplification of 310 bp of the 5′ flanking sequence of *CDC73* with P1 and P2, and 260 bp of 3′ flanking sequence with P3 and P4, the PCR products were gel purified and digested with *Bam*HI (5′ flanking sequence) and *Xho*I (3′ flanking sequence), purified, and ligated to the *Bam*HI-*Xho*I *HIS3* fragment (1.4 kb) from Sc2676 (52). The ligation product was amplified by PCR using P1 and P4, and the 2-kb product was used to transform YJJ453 (46). The disruption was confirmed by Southern analysis, the disruptant was sporulated, and tetrads were dissected (20). To make a truncation disruption, *CDC73* was amplified by PCR from yeast genomic DNA, using primers 5′CGCGGATCCATGGCGAACTCATTAGAC AG3′ and 5′CGGAATTCGGATCCTTATGGAGGTATTAC3′. Introduced restriction sites of *Bam*HI, *Nco*I, *Eco*RI, and *Bam*HI are underlined. The PCR fragment was cloned into the pGEM-T vector (Promega). The *Bam*HI-*CDC73* fragment was then released and ligated to pJJ536. The resulting plasmid was linearized at the unique *Sal*I site, and a *Sal*I-*Xho*I *LEU2* fragment from pJJ606 was ligated to the linearized plasmid. The *Bam*HI *CDC73::LEU2* fragment (truncated at amino acid [aa] 143 in a total of 393 aa) was used to transform yeast YJJ662. The disruption was confirmed by Southern analysis.

Production of recombinant Cdc73p and anti-Cdc73p antibody. Recombinant Cdc73p and the anti-Cdc73p antibody were produced as follows. Full-length, recombinant Cdc73p was produced in *Escherichia coli*. The *CDC73* gene was amplified from yeast genomic DNA by using the following primers in a standard PCR reaction: 5′CATATGGCGAACTCATTAGACAGACTGAGAG3′ and 5′GCGGCCGCACGGTATCCTCTTGAAATAAGTTCC3′. This primer set introduces an *Nde*I site at the 5′ end (concurrent with the initiator methionine residue; underlined in the primer sequence) and an *Not*I site at the 3′ end (underlined). The resulting *Nde*I-*Not*I fragment was cloned into pET21a (40). The Cdc73p-producing plasmid was introduced into *E. coli* BL21(DE3). Growth, induction with isopropylthiogalactopyranoside (IPTG), lysis, and purification by Ni^{2+}-nitrilotriacetic acid chromatography were performed as described previously (40). Antibodies were raised against Cdc73p in rabbits as follows. Initial immunization was accomplished by subcutaneous injection of 350 μg of Cdc73p (full-length recombinant protein) emulsified in 0.5 ml of Titer-Max adjuvant (Vaxcel, Inc.). Boosts were at 4, 6, and 8 weeks following immunization with 1 mg of Cdc73p (inclusion bodies) emulsified in 1 ml of phosphate-buffered saline. Immune serum was collected at 9 weeks.

Subcellular localization of Cdc73p. Cellular fractionation of *S. cerevisiae* whole-cell extracts was described previously (50). Proteins from fractions (total, cytoplasmic, and nuclear) were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) and transferred to Immobilon P membranes (Millipore) as instructed by the manufacturer.

Cdc73p and RNA polymerase II interaction assay. For assessing interaction with RNA polymerase II, a threefold molar excess of Cdc73p was mixed with purified RNA polymerase II in buffer A (20 mM HEPES [pH 7.9], 20% glycerol, 0.2 mM EDTA, 2 mM dithiothreitol [DTT]) containing 0.5 M KCl. Samples were then concentrated to a volume less than 200 μl and dialyzed overnight versus buffer A containing 0.1 M KCl at 4°C. The dialyzed samples were then briefly centrifuged and chromatographed on a Superdex 200 HR 10/30 gel filtration column (Pharmacia) equilibrated in buffer A containing 0.1 M KCl. Absorbance peaks (280 nm) corresponding to RNA polymerase II and bound proteins were collected, concentrated, and analyzed by SDS-PAGE followed by Coomassie blue staining.

Construction of CDC73 wild-type and mutant plasmids. To prepare a construct expressing the C terminus of Cdc73p (aa 143 to 393), the *Sal*I-*Bam*HI fragment from a *CDC73* clone (pJJ702) was ligated in frame to the pYES2HISC vector (Invitrogen). To prepare the *GST-CDC73* yeast expression plasmid, the *Bam*HI-*CDC73* fragment was ligated into the pEGST vector to make an in-frame glutathione S-transferase (GST)–Cdc73p fusion under the control of the *GAL* promoter. The vector is a 2μm plasmid. Similarly, the *PAF1* coding sequence was amplified by PCR from yeast genomic DNA by using the following primers: 5′ATCGCGGATCCATGTCCAAAAAACAGGAATATATTGC3′ and 5′ATC GCGGATCCCTATTCTTCTTGTAAAGTTTCC3′. Both primers are flanked by *Bam*HI sites (underlined). The PCR product was digested with *Bam*HI and ligated in frame to pEGST. The ligation product was found to be toxic to *E. coli* and could not be cloned. Thus, the ligation mix was directly used to transform YJJ664 (*paf1Δ::HIS3*), and the transformants were tested for complementation of the *paf1* mutant phenotypic traits. The expression of GST-Cdc73p and GST-Paf1p in yeast was confirmed by Western blot analysis using both anti-Cdc73p or anti-Paf1p and anti-GST antibodies.

Construction of the *paf1 cdc73* double mutant. To construct the *paf1 cdc73* mutant, strain YJJ577 (a *paf1Δ::HIS3* mutant) was crossed to strain YJJ663 (a *cdc73::LEU2* truncation mutant). The resultant diploid was sporulated, and tetrads were dissected. The spores were then tested for their corresponding *paf1* or *cdc73* genotypes by checking *HIS3* and *LEU2* markers. In some cases, the double and single mutants were also confirmed by PCR analysis, using the same sets of primers as used to disrupt *PAF1* (50) and *CDC73*.

Protein-protein interaction assays. Transcriptionally active whole-cell extracts were prepared from yeast strains YJJ674, YJJ676, YJJ691, and YJJ693 as described previously (65). The protein concentrations of the whole-cell extracts were measured by using reagents from Bio-Rad. Equal amounts of the extracts were mixed with glutathione-agarose beads in SK(20) (20 mM HEPES [pH 7.9], 20% glycerol, 10 mM $MgSO_4$, 10 mM EGTA, 5 mM DTT, 20 mM potassium acetate, 1 mM phenylmethylsulfonyl fluoride, 0.5 μg of leupeptin per ml, 0.4 μg of bestatin per ml, 0.35 μg of pepstatin A per ml), incubated at 4°C for 2 to 4 h, and washed once with SK(20) and several times with SK(200) (30 mM HEPES [pH 7.9], 10% glycerol, 1 mM EDTA, 1 mM DTT, 200 mM potassium acetate, protease inhibitors as specified above). The glutathione-agarose beads were spun down briefly and eluted with the sample buffer. The samples were resolved on an SDS–10% polyacrylamide gel (21). Western blot analysis was performed as described elsewhere (21).

Differential display and yeast mRNA analysis. Total yeast RNA was isolated as described previously (15). RNA was used for reverse transcription (RT)-PCR differential display (35), using a kit from Display Systems. Differentially expressed bands were reamplified by PCR, cloned, and sequenced by standard methods (47). RNA samples for Northern blots 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 according to standard methods (47). [α-^{32}P]dATP-labeled probes were prepared by PCR (47). An *ACT1* probe was prepared by random priming (47) an *Eco*RI-*Bam*HI fragment from pJJ157 (59). Northern blots were quantitated by PhosphorImager analysis.

RESULTS

Identification of Cdc73p as a nuclear, RNA polymerase II-associated factor. RAPs were isolated from a transcriptionally active yeast whole cell extract by anti-CTD affinity chromatography as described previously (62). The RAPs eluted from the column under moderate salt conditions where RNA polymerase II remained associated with the anti-CTD monoclonal antibody but were not present in fractions eluted from control columns containing monoclonal antibodies directed against the σ^{70} or β subunit of *E. coli* RNA polymerase. The peptide sequence -KDPIILIPSAASSI- identified the RAP of 50 kDa as the product of the *CDC73* gene (44), originally identified in a suppressor screen for mutations that would allow cells bearing a defective *STE2*-encoded α-factor receptor to mate (45). The complete sequence of *CDC73* has been determined in the yeast genome sequencing project (accession number U20162, gene 9931.10. [28]). The predicted amino acid sequence of Cdc73p has no obvious similarities to those of any other genes in the current database. A relatively high percentage (24%) of the amino acids are charged, which may explain the discrepancy between the observed (50 kDa) and predicted (43 kDa) molecular masses. The low codon bias of 0.049 for Cdc73p would predict that this is a low-abundance protein, which is consistent with our observation that the *CDC73* mRNA is very low abundance (49).

Identification of Cdc73p as a RAP suggested that it might function as a transcription factor. To begin to analyze the role of Cdc73p in transcription, we produced a recombinant form of the protein and used it to elicit antibodies (Materials and Methods). The antibodies were used to confirm the presence of Cdc73p in the RNA polymerase-associated fraction and its absence from the fraction eluted from the control column (Fig. 1A). The antibodies were also used to determine the localization of Cdc73p within the cell. Figure 1B demonstrates that Cdc73p is localized to the soluble nuclear fraction, with very little signal seen in the cytoplasm (Fig. 1B; compare lanes 1 and 2), consistent with a role in transcription. As controls, the general initiation factor TBP was detected only in the nuclear fraction, and Sup35p, a ribosome-associated protein (55), was found only in the cytoplasm.

Cdc73p interacts directly with purified RNA polymerase II. Given that Cdc73p and Paf1p were isolated as RNA polymerase II-associated factors, we investigated the possibility that Cdc73p and Paf1p can bind directly to RNA polymerase II. Recombinant proteins were mixed with purified RNA polymerase II, and association was analyzed by gel filtration chromatography. RNA polymerase II alone elutes from the column in the high-molecular-weight fractions with a peak at 16 to 17 min (data not shown). When recombinant Cdc73p was fractionated alone, it was found exclusively in low-molecular-weight fractions at around 40 min, consistent with it behaving as a monomer in solution (Fig. 2A). In contrast, when excess Cdc73p was first mixed with RNA polymerase II, a significant portion shifted into the high-molecular-weight fractions with RNA polymerase II (Fig. 2B). As shown in Fig. 2C, Cdc73p is recovered from these high-molecular-weight fractions in approximately stoichiometric amounts with RNA polymerase II subunits (Fig. 2C, lane 2). Similar experiments performed with recombinant Paf1p did not reveal any selective interaction with RNA polymerase II (data not shown). Cdc73p therefore appears to interact directly with RNA polymerase II, explaining its presence in the original RAP fraction. Paf1p may require interactions with another of the RAPs for its association with RNA polymerase II.

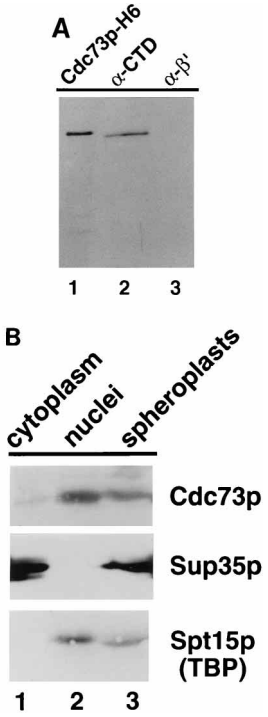

FIG. 1. Cdc73p is found in the RAP fraction and in yeast cell nuclei. (A) Cdc73p is present in the RAP fraction but not in the control fraction. The RAP and control fractions were obtained as described previously (62). The 0.5 M KCl eluates from the anti-CTD column (α-CTD) and the negative control column (α-β′) (80 μl of each) were analyzed by SDS-PAGE (10% gel) and Western blotting using the anti-Cdc73p antibody. Recombinant His6-tagged Cdc73p (100 ng) was used as a reference standard. (B) Nuclear localization of Cdc73p. Subcellular fractions were prepared and analyzed as described in Materials and Methods. Protein fractions were resolved by SDS-PAGE (10% gel), blotted, and probed with the appropriate antibodies. The following amounts of each fraction were used: cytoplasm, 200 μg of protein (lane 1); nuclei, 50 μg of protein (lane 2); and spheroplasts, 300 μg of protein (lane 3). Blots were probed with anti-Cdc73p antibody (1:300), anti-TBP antibody (1:1,000), and anti-Sup35p antibody (1:1,000).

Disruption-deletion analysis of *CDC73*. It was previously reported that a truncation of *CDC73* led to a temperature-sensitive phenotype, and a complete disruption of the *CDC73* gene was lethal (45). We have repeated these experiments with different results. To create isogenic mutants in our strain background, we first created a truncation mutation of *cdc73* identical to that previously described (truncated at aa 143 [45]) (Fig. 3A) and found the mutant strain to be temperature sensitive (Fig. 3B). We also created a total deletion of *CDC73* as described in Materials and Methods (Fig. 3A). When a heterozygous diploid carrying the deletion was sporulated, all of the tetrads contained four viable spores, and the *HIS3* gene used for the disruption segregated 2:2 in the 23 tetrads dissected (49). We confirmed that the *CDC73* gene was absent from the His^+ spores by PCR and Southern blotting (49). Spores from the dissection of the deletion strain were mated to strains from other commonly used genetic backgrounds (SC288c and D273-10B), and all spores from the resulting diploids were viable (49). Although we cannot rule out a tightly linked suppressor of the *cdc73* null mutant, our results indicate that *CDC73* is not an essential gene in several common laboratory strains.

Deletion of *CDC73* conveys a modest but measurable growth defect at 30°C (a doubling time of 2 h, compared with 1.6 h for an isogenic wild-type strain) and a temperature-

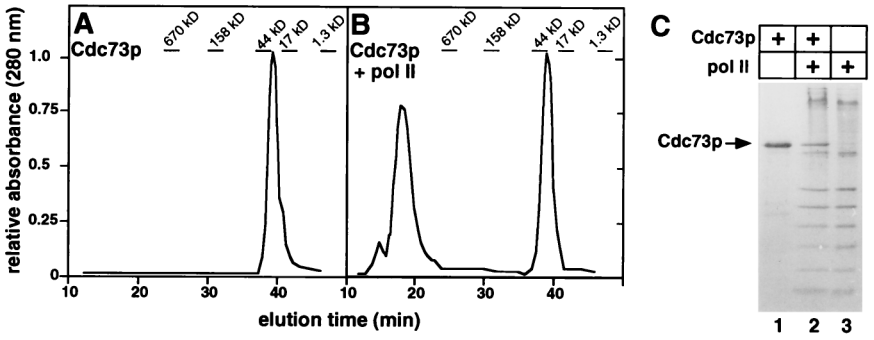

FIG. 2. Cdc73p associates directly with RNA polymerase II. (A and B) Gel filtration chromatography of RNA polymerase II and Cdc73p. Free Cdc73p (225 pmol) (A) and a mixture of Cdc73p and RNA polymerase (pol) II (72 pmol of RNA polymerase II plus 225 pmol of Cdc73p) (B) were resolved by gel filtration chromatography as described in Materials and Methods. (C) Coelution of RNA polymerase II and Cdc73p. The high-molecular-weight protein peak from the mixture of RNA polymerase II and Cdc73p (B) was analyzed by SDS-PAGE (lane 2). Purified RNA polymerase II (lane 3, 2.5 μg) and recombinant Cdc73p (lane 1, 2 μg) were included as markers. The standards for the gel filtration experiment were thyroglobulin (670 kDa), 25 min; bovine gamma globulin (158 kDa), 31 min; chicken ovalbumin (44 kDa), 37 min; equine myoglobin (17 kDa), 41 min; and vitamin B_{12} (1.35 kDa), 48 min.

sensitive phenotype at 38°C (Fig. 3B). There are no obvious morphological changes associated with the mutation (Fig. 3C). The *cdc73* truncation mutation was found to have a growth phenotype indistinguishable from that of the deletion mutant under the growth conditions tested (Fig. 3B). Both mutations can be rescued by a low-copy-number plasmid bearing a wild-type copy of *CDC73* (49). Since *CDC73* was originally identified genetically as a gene involved in the mating response pathway, we assessed whether the *cdc73* mutations conferred a mating defect. In a quantitative mating assay, we did not detect significant differences in mating efficiency between the *cdc73* mutants and an isogenic wild-type strain (49). This result indicates that *CDC73* itself does not directly contribute to the mating response. The differences between these results and those reported earlier (45) for the phenotype of *CDC73* mutations may be due to the presence of other mutations in the original mutagenized strain.

We expressed the C terminus (aa 143 to 393) of Cdc73p to test whether it could rescue the *cdc73* truncation or null mutations. This portion of Cdc73p was able to fully rescue both the truncation mutation and the total deletion mutation of *CDC73* at 30°C (49). Although the C terminus can rescue the temperature-sensitive phenotype of a *cdc73* deletion, at 38°C, based on growth rates, it does not function as well as the full-length protein. The N-terminal 143-aa segment, when expressed from a plasmid vector, has no effect on the growth of the *cdc73* null mutant (49). These results indicate that although the N-terminal portion is dispensable for growth at 30°C, it is required for full activity of Cdc73p at 38°C.

Cdc73p and Paf1p may function in the same cellular processes. Both Cdc73p and Paf1p were recovered as proteins that specifically associate with RNA polymerase II in the RAP fraction. Although neither gene is essential, null mutations of either gene give rise to pleiotropic phenotypes, including slow growth and temperature-sensitive lethality. The *paf1* mutant phenotype is more severe, with a slower growth rate than the *cdc73* mutation and an enlarged cell morphology (Fig. 3C) (50). To determine the potential functional relationship between Paf1p and Cdc73p, we constructed a *paf1 cdc73* double mutant. If the products of these two genes function in the same processes, a double mutant should not exhibit phenotypes more severe than those of either of the single mutants. This was shown to be the case. The *paf1 cdc73* cells had about the same growth rate as the *paf1* mutant (49), and the morphology of the double mutant was comparable to that of the *paf1* mutant (Fig. 3C). This result suggests that Paf1p and Cdc73p are functionally related and that they function in similar cellular processes. The fact that the *cdc73p* mutation is less severe indicates that it may affect only a subset of the processes controlled by Paf1p.

Paf1p and Cdc73p associate with each other and with RNA polymerase II in vivo. To explore the possibility that Cdc73p and Paf1p may reside in the same complex, and to determine the relationship of this complex to the previously described holoenzyme (30, 32), we created recombinant constructs to express GST-tagged Cdc73p and Paf1p and used these to transform the strains deleted for the *CDC73* and *PAF1* genes. Both the GST-Cdc73p and GST-Paf1p fusion proteins complemented their respective null mutations, restoring growth at 38°C (Fig. 4A) and correcting the slow growth and abnormal morphology of the *paf1* mutant (Fig. 4B) (49). We also created a GST-tagged form of Tfg2p, the second subunit of yeast general initiation factor TFIIF (23). This construct was used to transform a heterozygous diploid with one copy of the essential *TFG2* gene deleted. The diploid was sporulated, and a haploid strain bearing the chromosomal *TFG2* deletion fully complemented by the GST-tagged construct was isolated (Materials and Methods). These results demonstrate that the Cdc73p, Paf1p, and Tfg2p tagged constructs participate in the normal spectrum of protein-protein interactions.

To begin to identify some of these interactions, GST-tagged Cdc73p, Paf1p, and Tfg2p and their associated proteins were isolated from whole-cell transcription extracts by glutathione-agarose chromatography (Materials and Methods). In a large-scale version of this isolation, the GST-tagged Cdc73p complex was further subjected to gel filtration chromatography, where a significant percentage of the tagged Cdc73p was found in a high-molecular-weight complex which also contained RNA polymerase II, Paf1p, TFIIB, and subunits of TFIIF (8). Smaller-scale isolations were used to prepare complexes for multiple analyses by Western blotting using a variety of antibodies directed against known transcription factors (Fig. 5). All of the results for complex composition were compared to results for unfused GST vectors in the same (*cdc73*Δ *paf1*Δ) or comparable (*TFG2*) strains. As shown in Fig. 5A (lane 5), the GST-Cdc73p complex contained Rpb1p, the largest subunit of RNA polymerase II (also known as Rpo21p [2]), and TFIIB, but not TBP, elongation factor TFIIS, or Ssl1p, a subunit of TFIIH (63). The complex also included Anc1p (8), the smallest subunit of TFIIF (23). The GST-Paf1p complex contained RNA

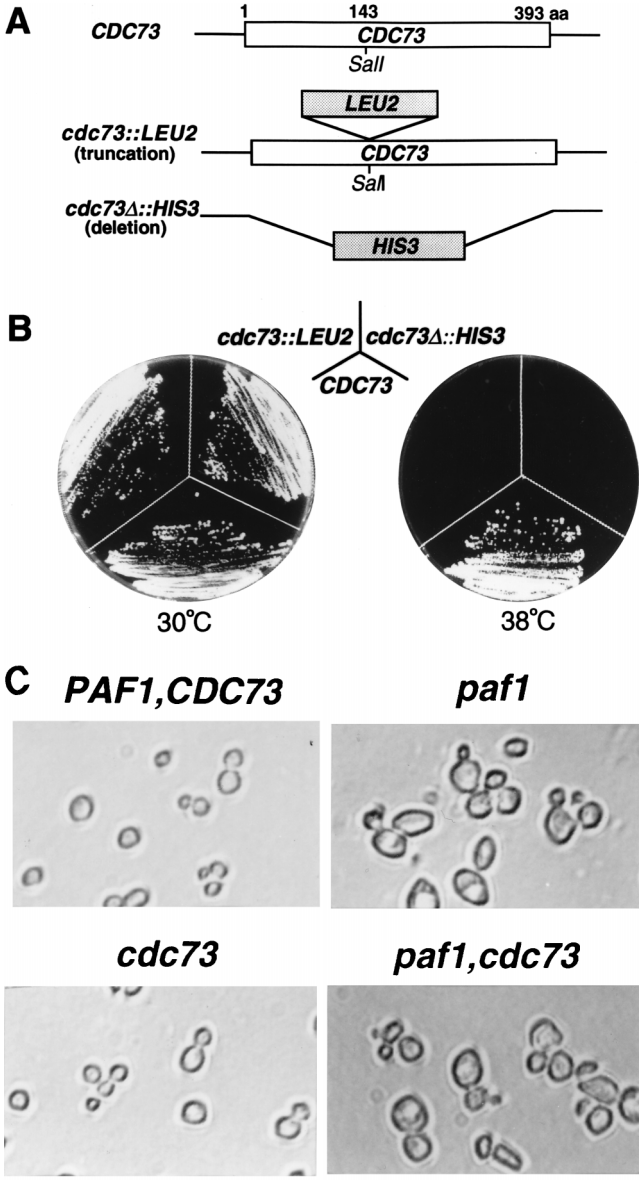

FIG. 3. Deletion or truncation of *CDC73* results in a temperature-sensitive phenotype which neither suppresses nor enhances a *paf1*Δ mutation. (A) Schematic representation of *CDC73* truncation and disruption constructs. *CDC73* was either truncated at a unique *Sal*I site (aa 143 of *CDC73* coding sequence) by insertion of *LEU2* or entirely deleted and replaced with a *HIS3* marker. The disruptions were confirmed by Southern blot analysis (49). (B) The phenotypes of the *cdc73* truncation and deletion are indistinguishable. Isogenic *CDC73* (YJJ662), *cdc73::LEU2* (YJJ663), and *cdc73*Δ*::HIS3* (YJJ665) strains were streaked onto YPD plates and grown at 30 or 38°C for 3 days. (C) The morphology of *paf1 cdc73* cells is similar to that of *paf1* cells. The pictures were taken at the same magnification.

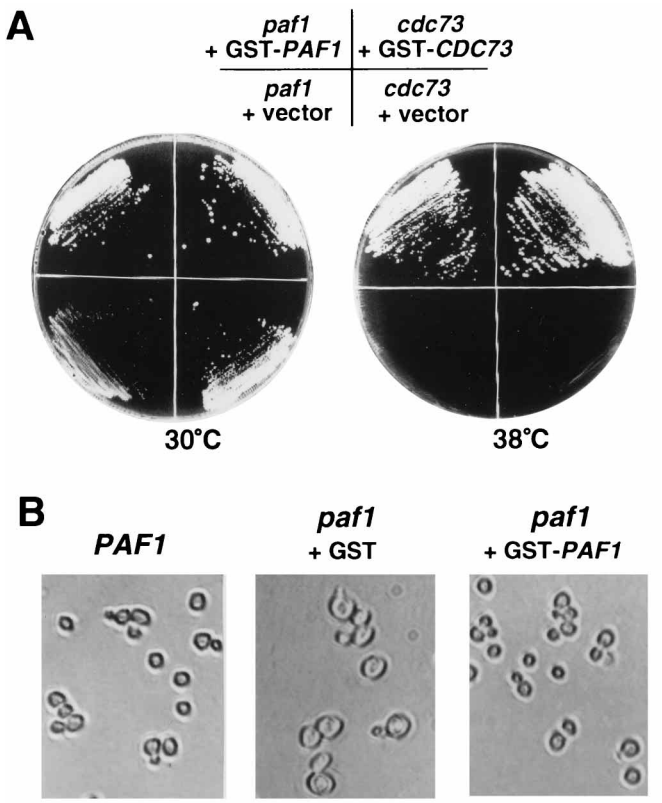

FIG. 4. The *cdc73*Δ and *paf1*Δ mutations can be rescued by Cdc73p and Paf1p GST-fusion constructs. (A) The GST constructs allow growth at high temperature. Isogenic *cdc73*Δ*::HIS3* (YJJ665) and *paf1*Δ*::HIS3* (YJJ664) strains were transformed with a vector expressing GST-Cdc73p (pJJ692) and the ligation product of pEGST and PAF1 (the GST-PAF1 plasmid is toxic to *E. coli*, and it cannot be propagated through *E. coli* cells), or the corresponding vectors, respectively. Cells were then grown on synthetic dropout plates at 30 and 38°C. (B) The GST-Paf1p construct corrects the abnormal morphology of the *paf1* mutant. Pictures were taken at the same magnification.

polymerase II (Fig. 5B, lane 2) and Anc1p (49), but not TBP (Fig. 5B, lane 2). The association between TFIIF, Cdc73p, and Paf1p is further confirmed in Fig. 5C (lane 5) and Fig. 5D (lane 4), where Cdc73p and Paf1p are readily apparent in the GST-Tfg2p complex. These results are consistent with the original composition of the RAP fraction isolated by affinity chromatography of RNA polymerase II from similar transcription extracts (62).

In addition to RNA polymerase II and a subset of the general transcription factors, we found that a large fraction of Paf1p was found in the GST-Cdc73p complex (Fig. 5C, lane 9) and that nearly all of the cellular Cdc73p was found associated with GST-Paf1p (Fig. 5B, lane 2). Therefore, based on the genetic interactions described above and the coisolation of Cdc73p and Paf1p, these factors appear to exist in the same complex with RNA polymerase II. In several repetitions of these experiments, and in direct analyses of extracts prepared from a variety of strains, we have found that while overexpression of either Cdc73p or Paf1p did not result in any obvious deleterious phenotypes, it did lead to increased levels of the other polymerase-associated factor (compare the abundance of Paf1p and Cdc73p in the control GST lanes to the abundance in the GST-Cdc73p and GST-Paf1p lanes in Fig. 5B and C). This effect was not due to alterations of the respective mRNAs (8) but instead was probably due to stabilization of the proteins within the complex. Only a small fraction of RNA polymerase II was found in the GST-Cdc73p and Paf1p complexes (Fig. 5A and B). Although we have not been able to accurately quantitate the number of RNA polymerase II and general transcription factor molecules in this complex, it was significantly lower than those found associated with TFIIF (compare the abundance of Rpb1p in the GST-Cdc73p and GST-Tfg2p complexes in Fig. 5A, lanes 5 and 9). This result is consistent with the predicted low abundance of the Cdc73p and Paf1p proteins and the fact that expression of only a small

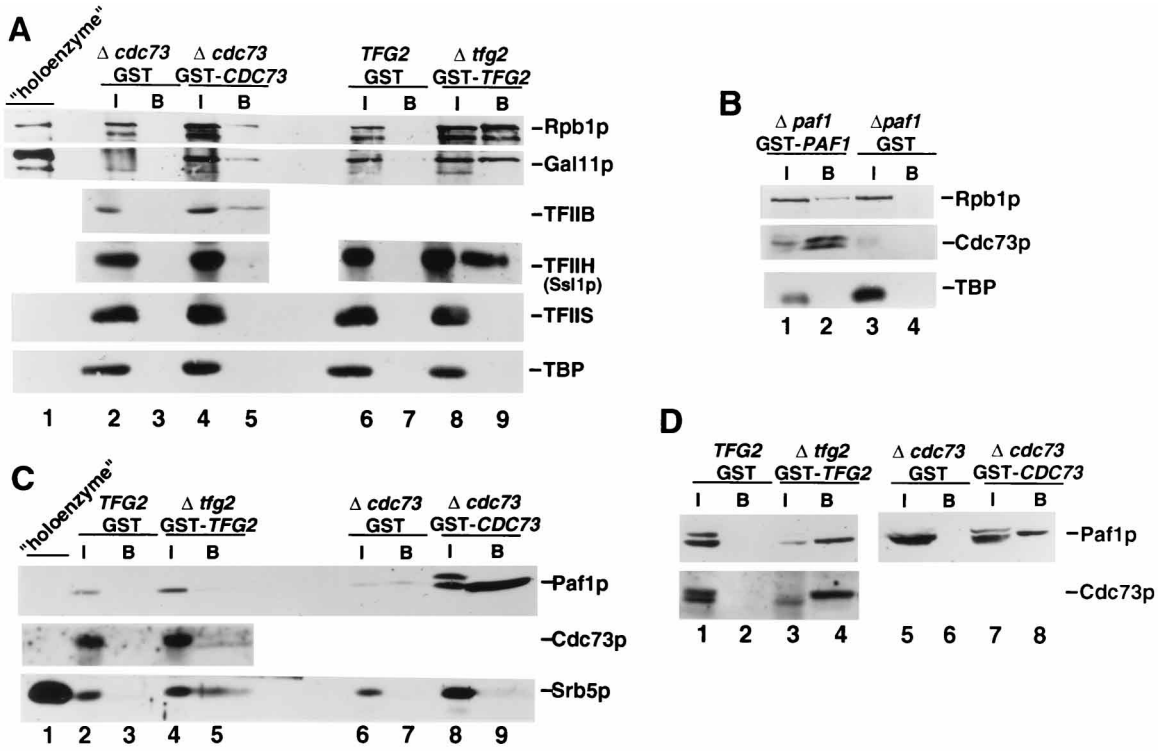

FIG. 5. Cdc73p and Paf1p are found in a novel complex with RNA polymerase II. GST-tagged complexes were isolated from yeast whole-cell transcription extracts and analyzed for protein content as described in Materials and Methods. For all panels, I indicates input and B indicates fraction bound to the glutathione-agarose beads. The antibodies used were anti-Cdc73p (1:300 dilution), anti-Rpb1p, -TBP, and -TFIIB (61) (1:1,000 dilution), anti-TFIIS (62) (1:1,000), anti-Gal11p (from T. Fukasawa) (1:1,000), anti-Ssl1p (from K. Gulyas) (1:1,000), anti-Srb5p (from R. Young) (1:1,000), and anti-Paf1p (50) (1:1,000). (A) The GST-Cdc73p complex includes RNA polymerase II, Gal11p, and TFIIB, but not TFIIH, TFIIS, or TBP. The GST-Cdc73p and GST control fractions were isolated from whole-cell extracts of cells deleted for the *CDC73* gene (YJJ691 and YJJ693); the GST-Tfg2p complex was isolated from cells deleted for the *TFG2* gene (YJJ854); the GST control was from a strain with the chromosomal copy of this essential gene (YJJ855). Five milligrams of whole-cell extract protein for each sample was subjected to glutathione-agarose chromatography. One-tenth of the input protein and one-fourth of the eluate were separated by SDS-PAGE and processed for Western blotting as described in Materials and Methods. The yeast "holoenzyme" sample was obtained from R. Young; a sufficient quantity was loaded to result in a similar signal intensity for the large subunit of RNA polymerase II (Rpb1p). Strips from the blotted gel were probed with antibodies directed against the indicated proteins. Strips that include lanes 1 to 9 (Rpb1p, Gal11p, TFIIS, and TBP) are from the same gel for direct comparison. The strips probed with antibodies against TFIIB and Ssl1p are from separate experiments using similar amounts of the GST-tagged complexes. (B) The GST-Paf1p complex includes RNA polymerase II and Cdc73p but not TBP. The GST-Paf1p and GST control fractions were isolated from whole-cell extracts of YJJ676 and YJJ674. One-fiftieth of the input and 1/10 of the eluate were resolved by SDS-PAGE. All strips shown are from the same gel for direct comparison. (C) The GST-Cdc73p complex includes Paf1p but not Srb5p. Complexes were isolated and analyzed as described for panel A. The strips probed for Paf1p and Cdc73p as well as the strip probed with Srb5p are from the same gel, using the same amounts of materials as the strips in panel A for direct comparison. The double bands seen in the strips probed for Paf1p are due to posttranslational processing of the protein (8). (D) The GST-Tfg2p complex includes both Paf1p and Cdc73p. The experiment shown is a repetition of the experiment shown in panel B, using similar amounts of material.

fraction of yeast genes is affected by mutations in *CDC73* and *PAF1* (50; see also below).

Cdc73p and Paf1p define a novel form of RNA polymerase II distinct from the holoenzyme. Is the complex containing Cdc73p and Paf1p the same as the recently identified holoenzyme? To answer this question, we probed the GST-tagged complexes with antibodies directed against components of the holoenzyme, including Gal11p and Srb5p. We previously reported that Gal11p and the Srbps were not found in the initial RAP fraction (62); however, using a different anti-Gal11p antibody and comparing it to an extract from *gal11*Δ cells, we have been able to detect Gal11p in the RAP fraction (8). The fact that the Srbps were not present in the RAP fraction was consistent with their known association with the RNA polymerase II CTD, because the CTD had to be accessible to antibody for the affinity isolation of the RAPs. Did the affinity isolation of the RAPs disrupt the interactions between RNA polymerase II and the Srbps, or did it identify a unique form of RNA polymerase II? Based on the results shown in Fig. 5, it appears that the RAPs define forms of RNA polymerase II distinct from the holoenzyme. We compared the composition of a sample of holoenzyme (obtained from R. Young) to that of the GST-Cdc73p and Tfg2p complexes. Using an amount of the holoenzyme that gave comparable signals for RNA polymerase II, we found that all three complexes contained Gal11p (Fig. 5A, lanes 1, 5, and 9). The observed ratios of Rpb1p to Gal11p suggest that the holoenzyme contains relatively more Gal11p than does the GST-Cdc73p complex.

TFIIH was clearly present in the GST-Tfg2p complex but not in the GST-Cdc73p complex (Fig. 5A; compare lanes 5 and 9). Some preparations of the holoenzyme from yeast are reported to contain TFIIH (32), while others apparently do not (30); we did not directly measure the abundance of TFIIH in the holoenzyme sample. TBP was not present in any of the three samples, consistent with previous reports (30, 32), and TFIIS, which has not been previously analyzed in the holoenzyme, was not present in any complex including the GST-Tfg2p complex (Fig. 5A).

In contrast to the similarities between complexes revealed in Fig. 5A, the results in Fig. 5C and D clearly demonstrate that these complexes have different compositions. Although Cdc73p and Paf1p were found in the GST-Tfg2p complex, they

were not present in the holoenzyme sample (Fig. 5C; compare lanes 1 and 5). These results have been confirmed by Young, using anti-Paf1p and Cdc73p antibodies to probe holoenzyme samples (66). Srb5p is present in the holoenzyme and the GST-Tfg2p complex but not in the GST-Cdc73p complex (Fig. 5C; compare lanes 1, 5, and 9). Srb5p was also not detected in a sample of the GST-Paf1p complex, and we did not detect signals for Srb2p or Srb6p in the GST-Cdc73p complex (8). Based on the lack of Srbps in the Cdc73p-Paf1p complex and the fact that no Cdc73p or Paf1p could be detected in the holoenzyme, these two complexes appear to represent distinct forms of RNA polymerase II.

Mutations in *CDC73* or *PAF1* lead to altered expression of some yeast genes. We have previously shown that the *paf1*Δ mutation leads to a five- to eightfold reduction in expression of the galactose-regulated genes (50). In contrast, we found that the *cdc73*Δ mutation has no effect on galactose induction (49). If Paf1p and Cdc73p do function in the same processes, we would predict that expression of at least a subset of yeast genes would be similarly affected in *paf1*Δ and *cdc73*Δ strains. Since the *paf1*Δ mutation results in a more severe phenotype, we would also expect that some genes dependent on Paf1p would not require Cdc73p. It is also possible that some genes could be affected by mutations in *CDC73* but not by mutations in *PAF1*. We have screened transcripts for a large number of yeast genes, including *SPT13* (TBP), *ACT1*, and 17 genes on chromosome I (50), and found that the expression of most genes is not affected in the *paf1*Δ or *cdc73*Δ mutant strains. This is in marked contrast to the phenotype of mutants in the *SRB* genes, where transcription of the majority of genes is affected (57). To more rapidly identify the small subset of affected transcripts, we have used the technique of differential display (35) to isolate genes with altered expression patterns in *cdc73*Δ, *paf1*Δ, and *gal11*Δ strains.

In Fig. 6, the expression patterns of five genes identified by differential display are presented. In the left-hand panel of Fig. 6A is a sample of the output of the differential display analysis. Total RNA isolated from isogenic wild-type, *paf1*Δ, and *cdc73*Δ strains was subjected to RT-PCR using one set of primer pairs (Materials and Methods). In the small section of the gel shown, it is clear that most transcripts had unaltered abundance, but in this example one transcript (marked with the double-headed arrow) was dramatically increased in abundance in the *paf1*Δ strain relative to the wild type and to the *cdc73*Δ strain. The center panel of Fig. 6A shows that the same pattern of altered expression was observed when the differentially displayed DNA fragment was cloned and used to probe total yeast RNA. Sequence analysis of the clone revealed that it encodes an uncharacterized open reading frame (ORF) (O5492) on yeast chromosome XV (14). Quantitation of the O5492 transcript relative to *ACT1* (Fig. 6B) showed that expression was more than fourfold higher in the *paf1*Δ strain relative to the wild type.

In the right panel of Fig. 6A, the RNA expression patterns of four additional genes identified by differential display are shown; the normalized abundance of each transcript is presented in Fig. 6B. The expression of each gene was analyzed in RNA isolated from isogenic wild-type, *paf1*Δ, *cdc73*Δ, and *gal11*Δ strains. Two of these genes, *YHB1*, encoding a putative flavohemoglobin (69), and *CMK2*, encoding a calmodulin-dependent protein kinase (41), have been previously identified. Two other genes, L8083.14 and YIL094C, were identified as ORFs of unknown function in the yeast genome sequencing project (29). In some cases, as for O5492, only a single mutation affected transcript abundance. This is true for the transcript from YIL094C, which was elevated twofold in the *gal11*Δ strain but is relatively unaffected by the *paf1*Δ and *cdc73*Δ mutations. In another case, all three mutations have similar effects, reducing expression of the *CMK2* transcript two- to threefold. Consistent with previous reports (18, 50), we observed that deletion of either *PAF1* or *GAL11* could lead to both increased and decreased transcript abundance of different genes (e.g., expression of O5492 was increased whereas *CMK2* expression was decreased in the *paf1*Δ strain, and L8083.14 expression was increased and *CMK2* expression was decreased in the *gal11*Δ strain).

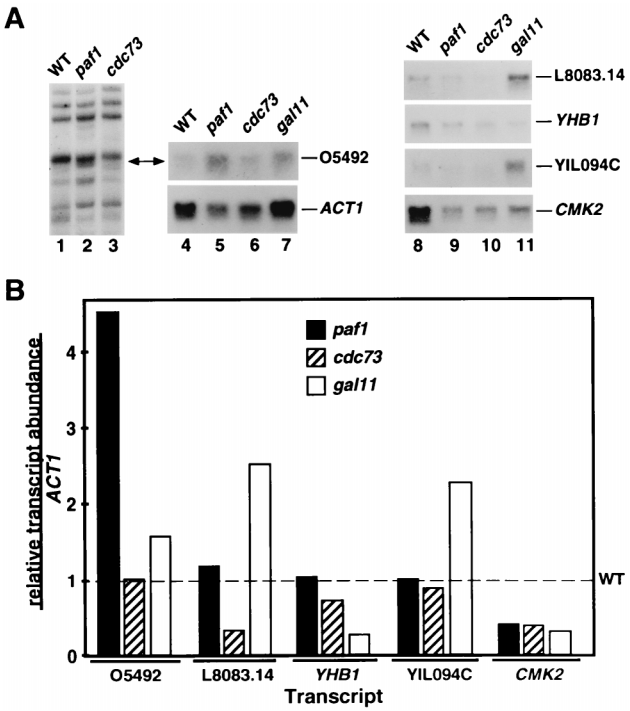

FIG. 6. Identification of genes whose expression is altered in *paf1*, *cdc73*, and *gal11* mutant strains. (A) Differential display detects genes altered in expression between the isogenic yeast strains. Equal amounts of total RNA isolated from strains YJJ662 (wild type [WT]), YJJ664 (*paf1*), and YJJ665 (*cdc73*) were analyzed by differential display as described in Materials and Methods. Lanes 1 to 3 show an example of a portion of a gel displaying the products of an RT-PCR amplification. The double-headed arrow indicates the position of an RNA differentially expressed in the *paf1* strain relative to the wild-type and *cdc73* strains. The band indicated by the double-headed arrow was PCR amplified, tested for differential expression by Northern analysis, cloned, retested for differential expression, and sequenced. The sequenced clone encoding part of ORF O5492 from chromosome XII was used to probe RNA isolated from isogenic strains YJJ662 (wild type [WT]), YJJ664 (*paf1*), YJJ665 (*cdc73*), and YJJ564 (*gal11*) (lanes 4 to 7). In lanes 8 to 11, total RNA isolated from isogenic strains YJJ662 (wild type [WT]), YJJ664 (*paf1*), YJJ665 (*cdc73*), and YJJ564 (*gal11*) was probed with the cloned differentially displayed fragments encoding parts of L8083.14, *YHB1*, YIL094C, and *CMK2* as described in Materials and Methods. (B) Normalization of differentially expressed transcripts. The abundance of the RNA shown in the blots in panel A was measured with a PhosphorImager and normalized to the abundance of *ACT1* from the same blots. For each gene probed, the abundance in the wild-type strain (WT) was set to 1. The numbers reflect data from a single experiment; similar numbers were obtained with several repetitions of the Northern analysis with different RNA samples.

The data in Fig. 6 include examples where the *cdc73*Δ mutation resulted in lowered expression (L8083.14 and *CMK2*). Although we have not yet observed an example in which expression in this strain is increased, we think it likely that Cdc73p is also involved in both positive and negative regulation. Although mutations in each of the genes encoding the RNA polymerase-associated factors affect the expression of some of the genes analyzed in Fig. 6, none of the patterns are

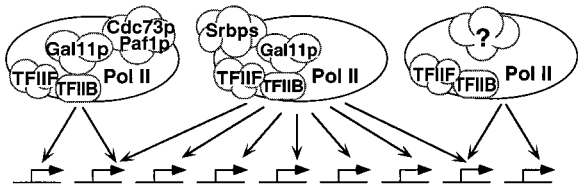

FIG. 7. Multiple RNA polymerase II-containing complexes may transcribe overlapping subsets of yeast genes. Details of the model are described in the text.

identical. These results are consistent with these RAPs playing individually unique but in some cases overlapping roles in transcription of subsets of yeast genes.

DISCUSSION

The general transcription factors required for initiation by RNA polymerase II were originally characterized by biochemical fractionation and reconstitution experiments. Careful order-of-addition experiments using purified components led to a view of sequential assembly of factors at the promoter (reviewed in references 12 and 68). With the knowledge of the complete complement of essential factors has come the ability to analyze the spectrum of interactions between these factors in vitro and in vivo. This has led to the recent identification of very large complexes containing RNA polymerase II, subsets of the general initiation factors, and many cofactors with predicted roles in transcriptional regulation (9, 30, 32, 36, 42). A hallmark of the complexes identified in yeast and in mammalian cells has been the presence of the Srbp family of proteins. The genes encoding these proteins were originally identified in yeast by using a screen for suppression of mutations in the CTD of RNA polymerase II (39). Mutations in the yeast *SRB* genes clearly affect the expression of the majority of transcription units (57). However, some of the *SRB* genes are not essential (56), indicating that there may be some functional redundancy in the yeast cell. In this work, we have identified a novel RNA polymerase II-containing complex that, like the holoenzyme, contains general initiation factors TFIIF and TFIIB but, unlike the previously characterized complexes, lacks the Srbps. Instead of the Srbps, we find that this complex contains at least two unique proteins encoded by the *CDC73* and *PAF1* genes. Because the products of these genes are not found in the holoenzyme, these two complexes appear to be physically distinct (model in Fig. 7). The different compositions of the two complexes are not the result of different isolation procedures based on the fact that using the same isolation procedure (glutathione-agarose chromatography) to isolate RNA polymerase II complexes via a tagged form of TFIIF, we can detect both the Srbps and Cdc73p-Paf1p. These complexes are therefore present simultaneously in yeast cells, where they apparently carry out different functions.

The composition of the Cdc73p-Paf1p-RNA polymerase II complex is similar to that of the yeast holoenzyme in that it lacks TBP and TFIIH (30). Although Young and coworkers did detect some TFIIH and variable but low amounts of TBP in their holoenzyme sample, the addition of TFIIH as well as TBP is required for optimal transcription with the complex in vitro, indicating that TFIIH is substoichiometric (32, 56). In contrast, a recently described human holoenzyme complex contains TFIIH, again in apparently substoichiometric amounts, but requires the addition of both TBP and TFIIB for transcription in vitro (36), while Ossipow et al. (42) have reported a fully functional mammalian holoenzyme containing all of the required general factors. Although we have carried out transcription reconstitution reactions with the Cdc73p-Paf1p complex by adding back the general factors not found in the complex, we have not yet determined the requirements for promoter selective in vitro transcription. Based on the fact that only a small fraction of yeast promoters appear to require this complex in vivo, and the fact that the model promoter constructs that we have tested in vitro are unaffected by the *paf1*Δ mutation (50), it is probable that we first need to identify the unique features of Cdc73p-Paf1p-dependent promoters before we can define the full complement of general factors required for initiation by this complex.

Is it possible that the Cdc73p-Paf1p complex is involved in elongation rather than initiation of transcription? Neither the holoenzyme nor the Cdc73p-Paf1p complex appears to contain the transcription elongation factor TFIIS. Somewhat surprisingly, the tagged TFIIF complex also lacks TFIIS. Both TFIIF and TFIIS were originally identified as RAPs (51), and both appear to play roles in elongation (5, 19); therefore, it is possible that the two could be associated with the same RNA polymerase molecules. Zawel et al. have shown that TFIIF appears to dissociate from RNA polymerase II shortly after initiation but can reassociate with polymerase stalled at internal pause sites (67). It may be that TFIIF and TFIIS define different elongating forms of the RNA polymerase, or it may be that the interactions between TFIIS and RNA polymerase, reported to be weaker than those with TFIIF (5), have been disrupted by our isolation procedures. This second possibility seems unlikely since we have found abundant amounts of TFIIS in the RNA polymerase II-associated fraction of RAPs isolated by a similar affinity technique from the same whole-cell transcription extracts (62). The lack of TFIIS in the Cdc73p-Paf1p complex implies that this complex is involved in the initiation rather than the elongation stage of transcription. This idea is consistent with our previous observation that the *paf1*Δ mutation affects expression of a reporter construct containing a *GAL* upstream activation sequence element to the same extent that it does a natural galactose-induced transcription unit (50).

Gal11p is found both in the holoenzyme mediator complex (30) and in the Cdc73p-Paf1p complex (Fig. 7). The role of Gal11p in transcription has been difficult to define. Mutations in this gene lead to little or no overt effect on yeast cell growth in many laboratory strains but do result in significant increases and decreases in the expression of a subset of genes; a *gal11* mutation does affect cell growth in some yeast strains (18, 38, 53). Although Gal11p has not been shown to directly contact activators and repressors, the artificial creation of a protein-protein contact between a DNA binding protein and a mutant form of Gal11p is sufficient to establish activation (3, 24). We have previously shown that the phenotype of *paf1*Δ mutant cells is dramatically enhanced by a mutation in *GAL11* (50). The enhanced phenotype includes very slow growth (a doubling time of greater than 8 h on rich medium) and extreme morphological changes. This could be interpreted as the additive effect of creating mutations in two distinct RNA polymerase complexes or as the result of inactivating two distinct functions of the complex that contains Cdc73p, Paf1p, and Gal11p. Our identification of genes whose transcription patterns are altered by mutations in these three RNA polymerase-associated factors does not immediately distinguish between these two models. We have found complex expression patterns with some genes affected in similar ways by all three mutations, while other genes are affected by only one or two of the mutations. These differences clearly establish that each of these RNA polymerase-associated factors is involved in the expres-

sion of different but overlapping subsets of yeast genes but do not elucidate whether the differences are due to altered properties of one or multiple RNA polymerase II-containing complexes.

Many genes encoding mediator components of the RNA polymerase II holoenzyme, including some of the *SRB* genes (56), *GAL11* (18, 53), *SIN4* (27), and the *SWI/SNF* genes (43), are not essential for yeast cell growth. However, mutations in these genes often have global effects on transcription and can give rise to slow growth, sensitivity to high and low temperatures, and other defects. Mutations in *CDC73* and *PAF1* have very similar properties. We interpret the pleiotropic but nonessential character of these mutations to reflect functional redundancy that has been built into this important aspect of transcriptional regulation. We have recently found that a mutation in *SRB5* is synthetically lethal with a mutation in *PAF1* and that the phenotype of a *cdc73*Δ mutant strain is dramatically enhanced by the loss of functional Srb5p (8). This is additional compelling evidence for functional redundancy of these two biochemically distinct complexes. As shown in the model in Fig. 7, the Srbp-containing holoenzyme and the Cdc73p-Paf1p form of RNA polymerase appear to represent major and minor subforms involved in expression of partially overlapping subsets of genes.

It is likely that other RNA polymerase II complexes distinct from these two also exist and participate in the expression of large or small fractions of yeast genes (Fig. 7). The Ccr4p-containing complex described by Denis et al. (13), the Ada2,3p-Gcn5p complex described by Berger and Guarente and coworkers (4, 25, 37), the Not1,2,3,4p complex described by Collart and Struhl (11), and the Spt4,5,6p complex identified by Swanson and Winston (54) may represent other examples of specialized complexes. Since the holoenzyme has been compared to the sigma factor-containing, initiation-competent form of the prokaryotic RNA polymerase (42), perhaps these other, more minor forms of RNA polymerase II will be shown to play roles similar to those of the minor sigma factors (22). Finding multiple forms of RNA polymerase II potentially responsive to different classes of activators and repressors is consistent with the discovery that the TAFs of TFIID also appear to exist in different forms. Novel forms of both activator- and repressor-responsive TFIID complexes which appear to carry out different functional roles have recently been described (6, 26, 60).

Without a more global analysis of gene expression patterns in yeast, it is not yet possible to identify the nature of the subsets of genes that these different RNA polymerase II complexes may transcribe. Promoter structure is critical for responsiveness to Gal11p, but the precise DNA sequence elements have not been identified (38). The different RNA polymerase-associated factors may communicate with different classes of activators and repressors as described for the different complex forms of TBP, or they may be involved in interactions with promoter-specific factors like proteins bound to the TATA box, the start site of transcription, or the unique elements present in the snRNA genes. Another possible role for different RNA polymerase II complexes is in coupling of transcription to other cellular processes. The essential transcription factor TFIIH also plays a role in the complex process of DNA repair (48), and very large forms of RNA polymerase II that contain many factors involved in DNA repair as well as the general transcription factors have been identified (36). A link between transcription and recombination was recently established with the genetic identification of interactions between general transcription factors, including a subunit of RNA polymerase II, TFIIB, and *HPR1*, a gene involved in the regulation of recombination (1, 16, 17). Mutations in the general transcription factor genes were identified as suppressors of the hyperrecombination phenotype of an *hpr1* mutation. In this regard, it is very interesting that overexpression of Cdc73p suppresses a mutation in *SOH7*, one of the genes discovered in this screen, and that a mutation in *CDC73* has an enhanced phenotype in combination with a mutation in *HPR1* (31). Perhaps one of the functions of the Cdc73p-Paf1p complex is in regulating the expression of genes involved in recombination. Further biochemical and genetic analysis of this interesting complex will be required to define its role in the yeast cell.

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The first two authors contributed equally to this work.

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