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The Med1 subunit of the yeast mediator complex is involved in both transcriptional activation and repression

(cyclin C/RNA polymerase II/transcription)

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ABSTRACT **The mediator complex is essential for regulated transcription *in vitro*. In the yeast *Saccharomyces cerevisiae*, mediator comprises >15 subunits and interacts with the C-terminal domain of the largest subunit of RNA polymerase II, thus forming an RNA polymerase II holoenzyme. Here we describe the molecular cloning of the *MED1* cDNA encoding the 70-kDa subunit of the mediator complex. Yeast cells lacking the *MED1* gene are viable but show a complex phenotype including partial defects in both repression and induction of the *GAL* genes. Together with results on other mediator subunits, this implies that the mediator is involved in both transcriptional activation and repression. Similar to mutations in the *SRB10* and *SRB11* genes encoding cyclin C and the cyclin C-dependent kinase, a disruption of the *MED1* gene can partially suppress loss of the Snf1 protein kinase. We further found that a lexA-Med1 fusion protein is a strong activator in *srb11* cells, which suggests a functional link between Med1 and the Srb10/11 complex. Finally, we show that the Med2 protein is lost from the mediator on purification from Med1-deficient cells, indicating a physical interaction between Med1 and Med2.**

There is mounting biochemical and genetic evidence identifying the mediator complex as a key factor in regulating RNA polymerase II-dependent transcription. *In vitro* transcription performed with pure, 12-subunit core polymerase and essential general transcription factors does not respond to activator proteins, but addition of the mediator complex to this minimal system both stimulates basal transcription, enhances TFIIH-dependent phosphorylation of the polymerase C-terminal domain (CTD), and enables transcriptional regulation (1–3). Mediator purified to homogeneity is a complex of >15 polypeptides, several of which are encoded by known genes, such as *SRB2*, *-4*, *-5*, *-6*, and *-7*, *GAL11*, *SIN4*, *RGR1*, and *ROX3* (3–5). However, eight mediator polypeptides were not identified previously and were designated Med1–8 according to their apparent molecular weight in SDS/PAGE. Six of them, Pgd1 (Med3) and Med2, -4/5, -6, -7, and -8 recently were identified and cloned (6, 7).

Mediator binds to the CTD of the largest subunit of the RNA polymerase II, thus forming the holo-RNA polymerase II. A similar higher molecular weight form of RNA polymerase II also was identified by Young and coworkers (8) in studies of the CTD-interacting proteins encoded by the suppressor of RNA polymerase B (*SRB*) genes (9, 10). An important difference between the two complexes is the presence in the latter of additional proteins, e.g., the general transcription factors TFIIB, TFIIF, and TFIIH, the SWI/SNF complex, and the Srb8–11 proteins (11–14). TFIIH contains the cyclin H-Kin28 kinase that converts the polymerase into its elongating form by phosphorylating the CTD. According to the mediator cycle model, the mediator then is released from the core polymerase and can participate in another round of initiation (15). Srb11 and Srb10 is another cyclin-kinase complex that also phosphorylates the CTD, but this phosphorylation is thought to occur before binding to the promoter, thus preventing initiation (16).

Several subunits of the yeast mediator complex originally were identified by genetic screens for mutations that would relieve transcriptional repression (17, 18). Thus, *ssn* (19) or *gig* (20) mutations cause a partial relief of glucose repression, *rox* mutations (21) cause a relief of oxygen repression, and *are* mutations (22) cause a relief of mating type repression. Subsequent work revealed that many of the corresponding genes are identical. For example, *SRB10* is identical to *SSN3*, *GIG2*, and *ARE1.* It should be emphasized that most of the corresponding mutations also affect transcriptional activation. For example, *GAL* gene induction, which is reduced in *med2* and *med6*^{ts} cells, is also affected in *srb10* cells (7, 12). Thus, it appears that mutations in these genes may result in either loss of repression or loss of induction, depending on the circumstances. In this paper, we describe the cloning of a cDNA encoding Med1, the 70-kDa subunit of the mediator complex, and functional analysis of the gene in yeast. Our data show that Med1 is required for proper regulation of transcription and is also important for the stability of the RNA polymerase II holoenzyme.

MATERIALS AND METHODS

Yeast Strains. *Saccharomyces cerevisiae* strain BJ926 was used to purify the RNA polymerase II holoenzyme for amino acid sequence determination of Med1. All other experiments were performed in W303–1A congenic strains (23). The *mig1*, *snf1*, and *med2*-Δ*1*::*HIS3* disruptions have been described (7, 24, 25). The *med1*-Δ*2*::*HIS3* disruption was made by cloning the *HIS3 Bam*HI fragment between *Nsi*I and *Xba*I sites in *MED1*. The *med1*-Δ*2*::*LEU2* and *med1*-Δ*2*::*URA3* disruptions have the *LEU2 Hpa*I-*Sal*I fragment and the *URA3 Hin*dIII fragment, respectively, cloned into the same position. The *srb11*-Δ*1*::*LEU2* disruption was made by cloning the *LEU2 Hpa*I-*Sal*I fragment between the *Bsa*BI and *Bgl*II sites of *SRB11*. The *GAL1-lacZ* reporter has an *Eco*RI-*Sau*3AI fragment of the *GAL1* promoter fused to an *Sph*I-*Age*I fragment of pLGΔ312 (26) containing the *CYC1-lacZ* fusion. The *his3*::*(TRP1,GAL1-lacZ)* integrating reporter gene was made by cloning a *Sna*BI-*Nco*I fragment of pJO116 (see below) containing the *TRP1* marker and the *GAL1-lacZ* reporter between the *Msc*I and *Nhe*I sites of *HIS3*. The construction then was targeted to the *HIS3* marker previously

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Abbreviation: CTD, C-terminal domain; HAP, hydroxyapatite.

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used to disrupt the *SNF1* gene (20), thus creating an *snf1-Δ1::his3::(TRP1,GAL1-lacZ)*-integrated marker in the recipient strain.

Plasmids. The *MED1* ORF, including the N-terminal His_6 epitope tag, was excised as a *Pst*I-*Bam*HI fragment from plasmid pET6xhisMed1 (see below) and then was cloned between the *Eco*RI and *Bam*HI sites of the two-hybrid vector pEG202, thus generating pDB176. An *Sph*I fragment of pDB176 encoding the entire lexA-Med1 fusion expressed from the *ADC1* promoter then was cloned into the *Sph*I sites of plasmids pFL45 and pFL39 (27), thus generating plasmids pDB177 and pDB181, respectively. The negative control plasmid pDB185 was made similarly by cloning an *Sph*I fragment of pRFHM1 encoding a lexA-bicoid fusion into the *Sph*I site of pFL39. The positive control plasmid pDB198 was made in two steps. First, the lexA expression cassette from pEG202 was cloned as an *Sph*I fragment into the *Sph*I site of pFL39, thus generating pDB193. pDB198 then was obtained by cloning a *Kpn*I-*Bam*HI fragment encoding the VP16 activating domain into the *Xho*I site of pDB193. pDB187, finally, was made by cloning the same VP16 fragment between the two *Xho*I sites in pDB181. Plasmid pJO116 has the *GAL1-lacZ* reporter (see above) inserted into the polylinker of the *CEN4 TRP1* vector pFL39 (27).

Cloning of the Med1 cDNA, Expression of Recombinant Med1 Protein, and Immunization of Rabbits. An oligonucleotide complementary to nucleotides 1–25 in the Med1 ORF containing an *Nde*I site and six histidine codons in frame with the ORF and a noncoding 3′ oligonucleotide complementary to nucleotides 1,721–1,702 at the end of the Med1 ORF, including a *Bam*HI restriction site to facilitate cloning, were used to amplify the *MED1* ORF in a PCR. The resulting DNA fragment was ligated to the vector pET3a, resulting in the plasmid pET6xhisMed1. Sequencing of the final vector construct revealed no PCR-induced mutations compared with the sequence reported in the database. The pET6xhisMed1 plasmid was used to transform BL21-DE3 cells. Expression of Med1 was induced by addition of isopropyl-β-D-thiogalactopyranoside, and Med1 was purified to >75% homogeneity from the soluble fraction by affinity chromatography on Ni-Agarose (Qiagen, Chatsworth, CA). Approximately 300 μg of purified Med1 was excised from a preparative SDS/PAGE gel and was used to immunize rabbits (Agri Sera AB).

β-Galactosidase Assays. For the galactose induction experiment in Fig. 2, yeast cells were grown to an OD_{600} of 0.5 in a synthetic medium containing 3% raffinose. The cells were harvested at this point and were incubated on ice for at least 30 minutes. Galactose then was added to a final concentration of 2%, and the cells were returned to a 30°C shaking incubator. Samples were removed at indicated time points and were frozen at −70°C. The β-galactosidase assays were performed essentially as described (25).

Protein Purification. Purification of Med1 for amino acid sequencing was done exactly as described (4). Fractionation of whole cell extracts from wild-type and *med1* cells was performed essentially as described (7) except for the for the fourth column, where we used a UNO Q-1 (Bio-Rad) column instead of a Mono Q HR5/5 column. The strains were grown in yeast extract/peptone/dextrose medium containing 1% yeast extract (Merck), 2% Bacto-Peptone (Difco), and 2% glucose.

RESULTS

Identification and Cloning of the *MED1* cDNA and Gene. Holo-RNA polymerase II was purified from *S. cerevisiae* as described (3). The constituent polypeptides were resolved by SDS/PAGE, were transferred to a poly(vinylidiene difluoride) membrane, and were stained with Ponceau S. The protein band migrating at 70 kDa (Med1) was excised and subjected to tryptic digestion *in situ* (28). Four high-confidence peptide sequences, YVETL (peptide I), LVLASNFDNFDYFNQRDGEHEK (peptide II), ESNYTDLIWFPEDFISP (peptide III), and DVSSKPSKPES (peptide IV), were obtained from separate tryptic fragments. A search of the GenBank database by using the BLAST program (36) revealed that all four peptides were derived from ORF YPR070w on chromosome XVI, encoding a protein with a predicted size of 64 kDa that lacks previously known function. A further analysis of the predicted Med1 sequence revealed no obvious features or motifs except for a high content of acidic residues resulting in a net charge of −32.56 at pH 7.

Loss of *MED1* Causes a Complex Phenotype Affecting RNA Polymerase II-Dependent Gene Expression. One-step disruptions of the *MED1* gene were made in W303–1A congenic yeast cells. Tetrad analysis showed that *med1* cells are viable but that the spores are smaller in size than wild-type spores, indicating a moderate growth defect. Because we found evidence that Med1 and Med2 may interact physically (see below), we also disrupted the *MED2* gene in both wild-type and *med1* cells. We found that a *MED2* disruption causes a more severe phenotype than a *MED1* disruption, with a pronounced growth defect, particularly on gluconeogenic carbon sources. As noted (7), the *med2* cells are unable to grow on galactose. Of interest, we further found that *med2* cells are temperature-sensitive at 38°C (Fig. 1*A*) and also cold sensitive at 16°C. However, the latter phenotype is only seen in *leu2* strains such as W303–1A, which already have a reduced growth at 16°C. The *med1 med2* double disrupted cells have a phenotype similar to that of *med2* cells, though the growth defect is slightly more pronounced, particularly at 38°C.

To study the effects of the *MED1* deletion on transcription, plasmids carrying different promoters fused to the *lacZ* reporter gene were transformed into the *med1* strain. Expression was monitored on X-gal indicator plates containing different carbon sources. Promoters tested included *GAL1*, *SUC2*, *FBP1*, *PCK1*, *CYC1*, *MIG1*, and *CAT8*. We found that the *MED1* deletion causes a complex phenotype similar to mutations in *SRB10* and *SRB11*. Thus, expression was reduced below wild-type levels under conditions at which a given promoter normally is expressed. This was particularly evident for the *GAL1* promoter on galactose, but a clear effect also was seen for the *FBP1* promoter on acetate and for the *MIG1* and *CAT8* promoters on nonglucose carbon sources. On the other hand, we also found that several glucose-repressed promoters had an increased expression on glucose in the *med1*-disrupted strain, indicating a partial loss of repression.

Effects of *med1* and *med2* on the *GAL1* Promoter. The effects on the *GAL1* promoter were studied in more detail by using β-galactosidase assays on liquid cultures (Fig. 2). We also included the *med2* strain and an *srb11* strain, as well as double mutant strains for *med1*, *med2*, and *srb11*. We found that *GAL1*

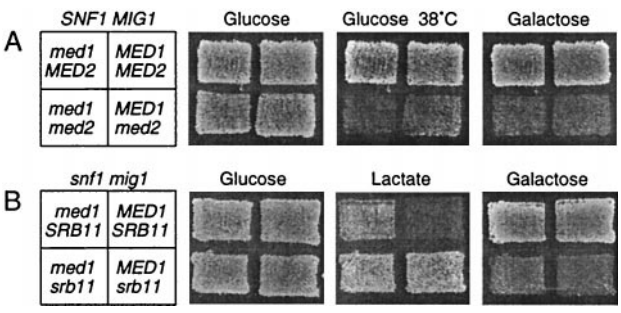

FIG. 1. Growth of wild-type and mutant yeast strains under different conditions. The strains were grown on a yeast extract/peptone/dextrose plate and then were replicated to different synthetic media, as shown in the figure. The relevant genotype of each strain is shown to the left. (*A*) Effects of *med1* and *med2* disruptions in an *SNF1 MIG1* wild-type background. The strains used were W303–1A, H707, H905, and H922 (Table 1). (*B*) Effects of *med1* and *srb11* disruptions in a *snf1 mig1* mutant background. The strains used were H661, H668, H749, and H750 (Table 1).

Table 1. Yeast strains

Strain	Relevant genotype
H661	*mig1-Δ1::LEU2 snf1-Δ1::his3::(TRP1, GAL1-lacZ)*
H668	*mig1-Δ1::LEU2 snf1-Δ1::his3::(TRP1, GAL1-lacZ) srb11-Δ1::HIS3*
H707	*med1-Δ2::HIS3*
H713	*srb11-Δ1::HIS3*
H715	*med1-Δ2::LEU2 srb11-Δ1::HIS3*
H749	*mig1-Δ1::LEU2 snf1-Δ1::his3::(TRP1, GAL1-lacZ) med1-Δ2::URA3*
H750	*mig1-Δ1::LEU2 snf1-Δ1::his3::(TRP1, GAS1-lacZ) med1-Δ2::URA3 srb11-Δ1::HIS3*
H905	*med2-Δ1::HIS3*
H909	*med2-Δ1::HIS3 srb11-Δ1::LEU2*
H922	*med1-Δ2::LEU2 med2-Δ1::HIS3*

All strains are W303-1A congenic and therefore also carry the following genetic markers: *MAT***a** *ade2-1 can1-100 his3-11, 15* leu2-3,112 trp1-1 ura3-1.

induction is severely affected in both the *med1* and *med2* strains, but with different kinetics. The *med1* strain behaves exactly like the *srb11* strain. Both strains express *GAL1* at a significantly lower level (3- to 5-fold) than the wild-type strain but with a similar kinetics (Fig. 2*A*). *GAL1* expression peaks at 4–6 h after induction and then declines. In contrast, the *med2* strain differs from the wild-type also in the kinetics of induction, which is significantly delayed. Thus, expression in the *med2* strain is 30× lower than in the wild-type 1 h after induction but reaches 60% of the wild-type level after 6 h and 92% after 16 h. At this point, *GAL1* expression in the *med2* strains is therefore twice as high as in the *med1* and *srb11* strains. This is surprising in view of the gal$^-$ phenotype of *med2* cells. The *med1 srb11* double mutant did not differ significantly from the *med1* and *srb11* single mutants. This is consistent with previous findings that there is little or no synergism between mutations in *SRB8*, *SRB10*, and *SRB11*, all of which have identical phenotypes (20, 29). The *med1 med2* and *med2 srb11* double mutants, finally, behave like the *med2* single mutant, with a significantly delayed kinetics of induction.

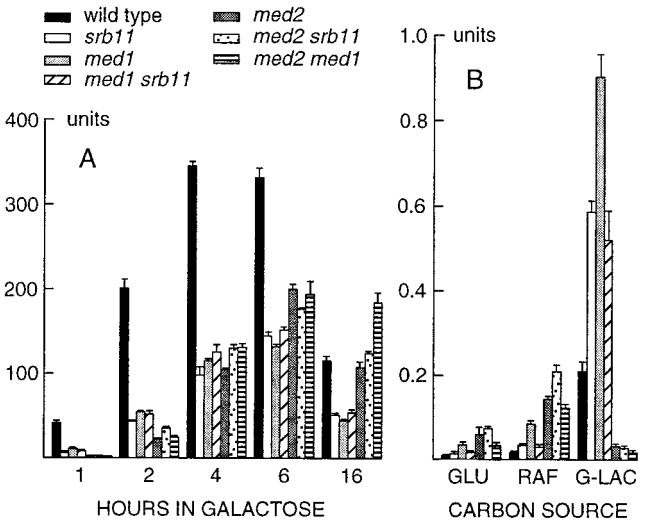

FIG. 2. Effect of *med1*, *med2*, and *srb11* disruptions on *GAL1* expression. (*A*) Single and double disrupted yeast strains as well as a wild-type control were grown to mid-log phase in the presence of 3% raffinose. Galactose then was added to a final concentration of 2%. The level of *GAL1* expression was monitored at indicated timepoints after induction by using a *GAL1-lacZ* reporter gene as described (25). (*B*) *GAL1-lacZ* expression in the same strains when grown in 2% glucose (repressing conditions), 3% raffinose, or 3% glycerol-lactate (nonrepressing, noninducing conditions). The strains used were W303–1A, H707, H713, H715, H905, H909, and H922 (Table 1).

We proceeded to study *GAL1* expression on repressing (2% glucose) and noninducing (3% raffinose or 3% glycerol-lactate) carbon sources in which the *GAL1* promoter normally is silent. All three mutations cause an increased expression under these conditions (Fig. 2*B*). This effect is most pronounced in the *med2* strain (7-fold on glucose and 8-fold on raffinose). On glycerol-lactate, however, there was no detectable expression in the *med2* strain, which hardly grew on this carbon source. The *med1* disruption causes a 4-fold increase in all three cases, and the *srb11* disruption causes a 2-fold increase. The *med1 srb11* double mutant resembles the *srb11* mutant, with a 2-fold effect, whereas the *med1 med2* and *med2 srb11* double mutants most closely resemble the *med2* mutant (Fig. 2*B*). We conclude that Med1, Med2, and Srb11 all contribute to keeping the *GAL1* promoter silent when it should not be expressed and that this effect is seen also in the absence of glucose repression (on glycerol-lactate). However, the levels of expression in the mutant strains are still very low, ≈1,000-fold less than in fully induced cells (2% galactose).

MED1 Is a Suppressor of *snf1*. Several holopolymerase subunits were isolated in genetic screens for mutations that can suppress loss of the Snf1 protein kinase (20, 29, 30). Cells that lack Snf1 have a complex phenotype including inability to grow on all carbon sources except glucose, inability to accumulate storage carbohydrates, temperature sensitivity, sensitivity to nitrogen starvation, and a generally poor growth (31). Some of these phenotypes, such as the inability to grow on galactose and raffinose, are suppressed by a deletion of the *MIG1* gene, but *snf1 mig1* cells are still unable to grow on gluconeogenic carbon sources (32). However, such growth is possible after additional mutations in *SRB8*, *SRB10*, or *SRB11* (20), which also permit *snf1 MIG1* cells to grow on raffinose.

We therefore tested the effect of disrupting *MED1* in both *snf1* and *snf1 mig1* strains. As a control, we included disruptions of *SRB11*. We found that the *med1* disruption partially suppresses the *snf1* phenotype, though to a somewhat lesser extent than the *srb11* disruption. Thus, *snf1 mig1 med1* cells can grow on gluconeogenic carbon sources, but the effect is less pronounced than in *snf1 mig1 srb11* cells (Fig. 1*B*). Similarly, the *med1* disruption permits growth of *snf1 MIG1* cells on raffinose, but not as clearly as the *srb11* disruption (data not shown). Other *snf1* phenotypes are not significantly suppressed by either the *med1* or *srb11* disruption. We conclude that the *med1* disruption strongly resembles the *srb11* disruption in its ability to suppress some *snf1* phenotypes. One clear difference between the *srb11* and *med1* disruptions is that *snf1 mig1 srb11* cells have a reduced growth on galactose, an effect that is not seen in *snf1 mig1 med1* cells. Finally, we tested the effect of a *MED2* disruption in the *snf1* background. We found that *med2 snf1* double disrupted cells have a general growth defect that is much more severe than either of the *med2* or *snf1* cells. It was therefore not possible to determine whether the *med2* disruption modifies any of the *snf1* phenotypes.

LexA-Med1 Is a Strong Activator that Is Negatively Controlled by Cyclin C. Several subunits of the RNA polymerase II holoenzyme can activate transcription when fused to a heterologous DNA-binding domain. Such activation may reflect the *in vivo* function of a given protein as a co-activator, but it also can be a more general consequence of its ability to recruit RNA polymerase II to the target promoter through interactions with other subunits (33). We therefore fused the entire Med1 protein (including an N-terminal His_6 tag) to the lexA DNA-binding domain. The resulting construct was expressed from a single-copy centromeric plasmid and was tested for its ability to activate transcription from the lex operator. A lexA-VP16 fusion was used as a positive control, and a lexA-bicoid fusion (inactive in yeast) was used as a negative control. As shown in Table 2 (experiments 1–3), we found that the lexA-Med1 fusion fails to activate transcription in the wild-type strain.

We proceeded to test the lexA-Med1 fusion in *med1*, *med2*, and *srb11* strains. Surprisingly, we found that it is a strong activator in

Table 2. Ability of lexA fusion proteins to activate transcription

Fusion protein	Wild-type	*med1*	*srb11*	*med2*	Vector
lexA-Bicoid	0.8 (0.1)	0.9 (0.0)	0.9 (0.0)	0.9 (0.0)	CEN6
lexA-Med1 (exp 1)	1.5 (0.0)	4.3 (0.1)	432 (5.5)	4.4 (0.3)	CEN6
lexA-Med1 (exp 2)	1.9 (0.1)	3.7 (0.1)	359 (4.5)	3.1 (0.1)	CEN6
lexA-Bicoid	1.9 (0.5)	1.3 (0.1)	0.9 (0.1)	1.8 (0.3)	2 micron
lexA-Med1	526 (130)	763 (122)	441 (49)	32 (3.5)	2 micron
lexA-VP16	2030 (113)	2477 (21)	2360 (41)	1628 (416)	CEN6
lexA-Med1$_{2-40}$-VP16	1998 (142)	2451 (43)	1644 (102)	2174 (35)	CEN6

Plasmids expressing different lexA fusion proteins (see *Materials and Methods*) were transformed into wild-type (W303-1A), *med1* (H707), *srb1* (H713), and *med2* (H905) yeast strains together with the pSH18-34 reporter, which has several lexA binding sites in front of a *CYC1-lacZ* fusion gene. The values shown are β-galactosidase units with standard errors in parentheses. All cells were grown in synthetic 8% glucose media.

the *srb11* strain, in which the level of expression reaches 432 units (Table 2). This is well above the maximal level observed for the fully induced and derepressed *GAL1* promoter (Fig. 2*A*) but still 6-fold below the activity of lexA-VP16 (Table 2). In contrast, lexA-Med1 showed very low levels of activation in the *med1* and *med2* strains. None of the three mutations had any significant effect on either the positive control (lexA-VP16) or the negative control (lexA-bicoid). Finally, we tested a construct in which lexA is fused to the His_6 epitope tag, followed by residues 2–40 of Med1 and then the VP16 activating domain, to rule out that the observed effect is mediated by the His_6 tag. As shown in Table 2, this construct behaved like the lexA-VP16 fusion protein. Therefore, the effect does not seem to involve the His_6 tag or the N-terminal 40 residues of Med1.

A possible explanation for the observed effect would be if loss of Srb11 significantly increases the amount of lexA-Med1 protein. To check for this, total protein was analyzed in a Western blot with the Med1 antiserum. As a control, we also used an antiserum that reacts with tubulin (34). We found that loss of Srb11 does increase the amount of lexA-Med1, but only 3-fold, i.e. much less than the 300-fold effect on lexA-Med1 activity. Loss of Med2 also has a 3-fold effect on the amount of lexA-Med1, but, in this case, the increase in lexA-Med1 activity is only 3-fold. Neither disruption has a significant effect on the amounts of wild-type Med1 or tubulin. We proceeded to overexpress lexA-Med1 from a 2-μm plasmid. We found that lexA-Med1 under these conditions is a strong activator (300- to 800-fold) also in wild-type and *med1* cells (Table 2). In *med2* cells, its activity is reduced 16-fold as compared with wild-type cells. This indicates that an increase in lexA-Med1 protein can cause a significant increase in its activity and also suggests that Med2 to some extent is required for lexA-Med1 function.

Med1 Is Important for Stability of the Mediator Complex. Holopolymerase was purified from both the *med1* strain and the isogenic wild-type W303–1A by using the previously described procedure (3). Immunoblotting with antibodies specific for Med1 and for the recently described mediator subunits Med2, Med4/5, Med6, Med7, and Med8 showed that all subunits (except Med1 in the *med1* cells) were present in the 550 mM potassium acetate DEAE-Sephacel fraction from both strains (data not shown). In the third purification step on hydroxyapatite (HAP), most mediator subunits and the second peak of the polymerase coeluted at ≈90 mM potassium phosphate in the wild-type strain (Fig. 3*A*). The two peaks of polymerase represent the core and holo-RNA polymerase, respectively, as reported (3). Two distinct changes were seen in the *med1* strain (Fig. 3*B*). First, the Med2 protein is absent from the holoenzyme complex and instead elutes at a much later position in the *med1* strain. Second, the Med6 subunit elutes in two separate peaks. Although most of Med6 coelutes with the holopolymerase, a significant fraction is also found in approximately the same position as the Med2 protein.

Fractions 50–74 from the HAP columns, which correspond to the holo-polymerase complex, were pooled and further purified on a UNO Q-1-column. In both strains, most of the mediator subunits also copurified on this column, but they were clearly separated from the major peak of RNA polymerase II that eluted at a higher concentration of potassium acetate (data not shown). This is similar to what has been reported when purifying the mediator complex from strains that are not protease deficient (7). Of interest, one mediator subunit, Med6, coeluted with the core polymerase rather than with the other mediator subunits in both *med1* and wild-type strains. This suggests that the Med6 protein is more strongly associated with the core polymerase than the other mediator subunits and also that the interaction between Med6 and the other subunits might be particularly sensitive to proteolysis.

To verify these results, one fraction from each UNO Q-1 column containing both the mediator subunits and the core polymerase was fractionated further by gel filtration on a Bio-Sil SEC 400 column. Similar to the elution profile on the UNO Q-1 column, the mediator subunits split in two peaks on Bio-Sil SEC 400: one larger complex comprising Med1 (absent in the *med1* strain), Med2 (also absent in *med1* strain), Med4, Med7, and Med8 and one smaller complex containing Med6 and the core polymerase (Fig. 4). The molecular masses of the two complexes, as estimated from the elution profile of marker proteins on the same column, were 1.100 and 650 kDa, respectively. The fact that Med6, with a predicted molecular mass of 33 kDa, elutes as a

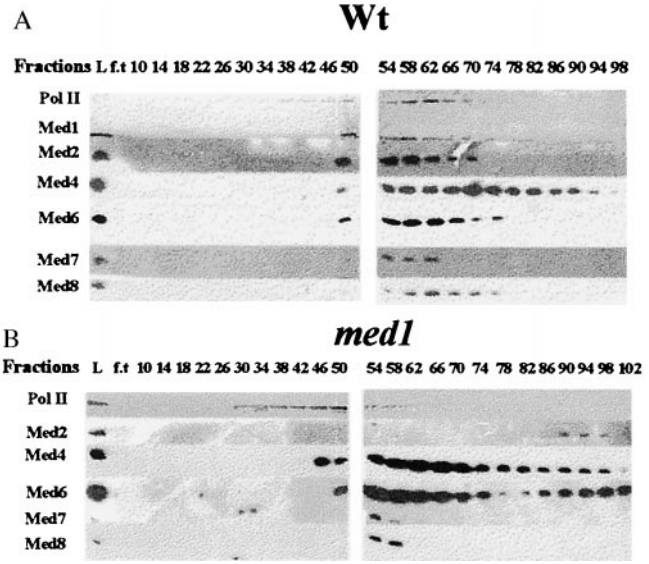

FIG. 3. Elution profiles of RNA polymerase II and mediator subunits during chromatography on hydroxyapatite. Proteins from the wild-type W303–1A (*A*) and *med1* H707 (*B*) strains eluting in the 550 mM potassium acetate fraction from the DEAE-sephacel columns were applied to 10 ml HAP columns and were developed with a 100 ml-gradient of 0.01–0.2 M potassium phosphate. Fractions eluting from the columns were analyzed on a 10% SDS/PAGE gel and were immunoblotted with antibodies specific for different proteins indicated to the left. "L" represents proteins loaded to the HAP column, and "f.t" represents proteins present in the flow-through.

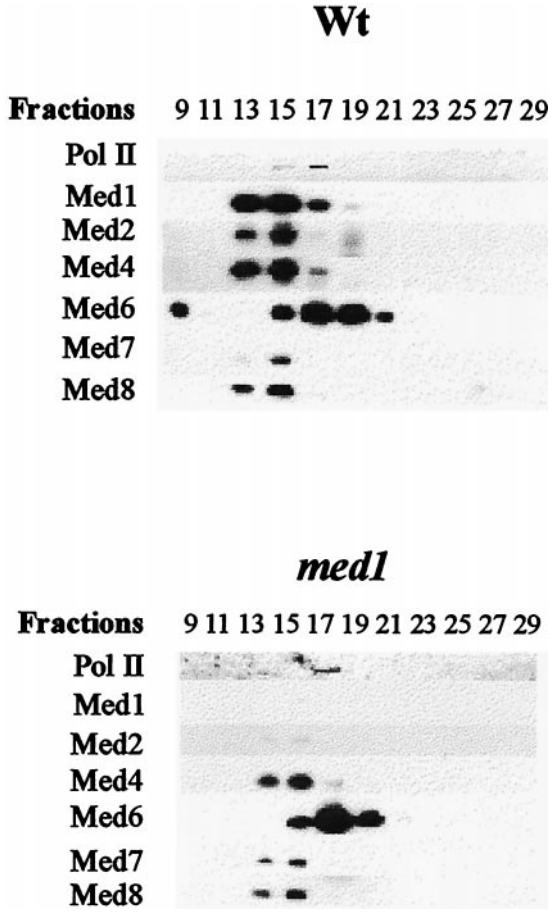

FIG. 4. Dissociation of Med6 and core polymerase from the mediator complex. Shown is an immunoblot of Bio-Sil 400 SEC fractions from wild-type (W303–1A) and *med1* (H707) yeast cells. One fraction from each UNO Q-1 column containing both the mediator subunits and the core polymerase was analyzed by gel filtration on a Bio-Sil 400 SEC column. The fractions from the gradient were separated on a 10% SDS/PAGE gel and were immunoblotted with antibodies directed against individual proteins as indicated to the left.

650-kDa protein complex confirms that the cofractionation of the core polymerase and Med6 on the UNO Q-1 column was not fortuitous.

DISCUSSION

The yeast mediator complex was discovered during biochemical fractionations aimed at identifying factors required for regulated transcription. Several subunits of the mediator complex (Gal11, Sin4, Rgr1, Rox3, Pgd1, and Srb2, -4, -5, -6, and -7) soon were identified as proteins encoded by genes already known to be involved in RNA polymerase II-dependent transcription. In contrast, amino acid sequencing of the remaining subunits (Med1, -2, -4/5, -6, -7, and -8) showed that they are encoded by genes without previously known function (6, 7).

Our analysis of the *med1* disrupted cells revealed that they have a phenotype that strongly resembles that of mutations in *SRB10* and *SRB11*. Like these mutations, a disruption of *MED1* causes both a partial defect in *GAL* gene induction and an increased expression under repressing or noninduced conditions in which the *GAL* genes normally are silent (Fig. 2). It is notable that disrupting *MED1* or *SRB11* causes a similar increase in expression on glucose, raffinose, and glycerol-lactate (4-fold for *MED1* and 2-fold for *SRB11*). This suggests that these genes are not directly involved in glucose repression but, rather, may have a more general role in keeping nonexpressed genes silent. We further

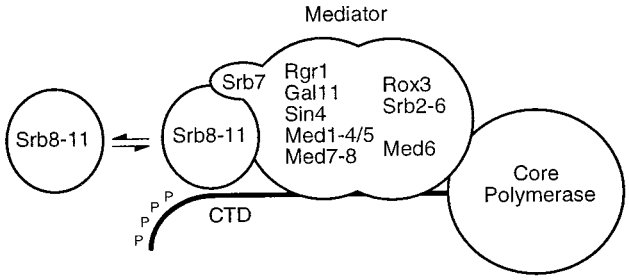

FIG. 5. Mediator and associated protein complexes within the yeast RNA polymerase II holoenzyme.

found that the *med1* disruption suppresses some phenotypes associated with a loss of the Snf1 protein kinase, again similar to disruptions of *SRB10* and *SRB11*. The only qualitative difference is that *snf1 mig1 med1* cells, unlike *snf1 mig1 srb11* cells, can grow on galactose. However, it is conceivable that this also reflects a quantitative difference and that *GAL* gene expression in the *snf1 mig1 med1* strain is just above the level required for growth on galactose.

Given the pronounced similarities in the phenotypes, it seems likely that Med1 somehow is functionally connected to Srb10 and Srb11. Together with Srb8 and Srb9, Srb10 and Srb11 originally were identified in genetic screens for mutations that relieve transcriptional repression but also in a screen for mutations that can suppress the effect of a C-terminal truncation in the large subunit of RNA polymerase II (12, 20, 22, 29). Srb8–11 are present within the holopolymerase complex when purified according to one procedure (11), but they are not found in the holopolymerase preparation used in our work (7). Given these differences, it has been suggested that Srb8–11 form a separate subcomplex within the holopolymerase, which is more or less loosely associated with the mediator complex (17). Recent evidence suggests that Srb10 inhibits transcription by phosphorylating the CTD tail of RNA polymerase II, thus causing dissociation of the preinitiation complex (16). The human mediator subunit hSrb7 has been reported to associate with hSrb10 and hSrb11 (35), possibly indicating that it mediates the interaction between Srb8–11 and the mediator complex (Fig. 5). It is conceivable that Med1 also could be involved in this interaction, which would explain the phenotypic similarities between med1, srb10, and srb11 mutations. However, because the effect of a *med1* disruption in some instances is weaker than that of a *srb10* or *srb11* disruption, proper function of the latter two proteins cannot be completely dependent on Med1.

Further evidence linking Med1 to the Srb10/11 complex comes from our finding that a lexA-Med1 fusion, which lacks activity in wild-type cells, is a strong activator of transcription in *srb11* cells. It should be noted that there is a 3-fold increase in lexA-Med1 protein in *srb11* cells, but we think this effect alone is unlikely to account for the >300-fold increase in activity. Our basis for this conclusion is that we find a similar 3-fold increase in lexA-Med1 protein in the *med2* strain but only a 2- to 3-fold effect on activation. Furthermore, the lexA-VP16 activity is not increased in the *srb11* cells, although the fusion protein is expressed from the same promoter as the lexA-Med1. Rather, we favor the possibility that Srb11 acts directly to inhibit the activity of lexA-Med1 in wild-type cells. One possible mechanism would be if Srb11 (and, presumably, also the associated Srb8, -9, and -10 proteins) bind directly to Med1, thereby blocking its ability to activate transcription. Such physical interaction is consistent with our finding that overexpression of lexA-Med1 can bypass the Srb11-dependent inhibition of its activity in wild-type cells (Table 2). However, because the Srb10/11 complex is a kinase, it is also possible that Med1 is negatively controlled by an Srb11-dependent phosphorylation that is saturated in the lexA-Med1 overproducing strain. Purification of holopolymerase complexes

from wild-type and *med1*-disrupted yeast cells revealed that the latter differ from the former not only in the absence of Med1 but also in the complete absence of Med2. Moreover, a significant amount of the Med6 protein coeluted with Med2 in the *med1* strain. This unexpected finding suggests that one function of Med1 may be to mediate the interaction between Med2 and the other subunits of the holopolymerase complex. In this context, it is noteworthy that we found the activity of lexA-Med1, when overexpressed from a 2-μm plasmid, to depend partially on *MED2* (Table 2). However, this finding should be interpreted with some caution because *med2* cells containing the 2-μm lexA-Med1 plasmid grew more poorly than those containing the centromeric lexA-Med1 plasmid. The effect on Med6 may reflect a further partial dissociation of the mediator complex in the absence of Med1. In agreement with this, both *med1*, *med2*, and *med6*^{ts} cells are defective in *GAL* gene induction (6, 7). The gal$^-$ phenotype and the general effect on growth are most pronounced in the *med6*^{ts} strain and least pronounced in *med1.* This is consistent with our biochemical results that imply interactions between these subunits in the order Med6–Med2–Med1 in which Med6 is located most proximal to the core polymerase.

The phenotypes we found for *med2* are more pronounced than those described previously (7). In addition to the reduced *GAL* gene induction and weak temperature-sensitive phenotype, we found that *med2* cells are also cold-sensitive and show a marked growth defect on gluconeogenic carbon sources. These additional phenotypes could be caused by differences in the genetic backgrounds used. Because loss of Med2 causes a more severe phenotype than loss of Med1, we consider it unlikely that Med2 is lost from the holopolymerase in *med1* cells. The fact that Med2 cofractionates with the polymerase in several steps and dissociates from it only on the HAP column also suggests that it remains associated with the holopolymerase in *med1* cells, though less strongly so than in wild-type cells. Because Med2 causes a severe growth defect, it was difficult to test these cells for the phenotypes associated with loss of *med1* or *srb11*, such as partial suppression of *snf1*. It is, therefore, possible that further investigations will reveal functional links between Med2 and the latter two proteins.

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1. Kelleher, R. J., III, Flanagan, P. M. & Kornberg, R. D. (1990) *Cell* **61,** 1209–1215.
2. Flanagan, P. M., Kelleher, R. J., III, Sayre, M. H., Tschochner, H. & Kornberg, R. D. (1991) *Nature (London)* **350,** 436–438.
3. Kim, Y. J., Björklund, S., Li, Y., Sayre, M. H. & Kornberg, R. D. (1994) *Cell* **77,** 599–608.
4. Li, Y., Björklund, S., Jiang, Y. W., Kim, Y. J., Lane, W. S., Stillman, D. J. & Kornberg, R. D. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 10864–10868.
5. Gustafsson, C. M., Myers, L. C., Li, Y., Redd, M. J., Lui, M., Erdjument-Bromage, H., Tempst, P. & Kornberg, R. D. (1997) *J. Biol. Chem.* **272,** 48–50.
6. Lee, Y. C., Min, S., Gim, B. S. & Kim, Y. J. (1997) *Mol. Cell. Biol.* **17,** 4622–4632.
7. Myers, L. C., Gustafsson, C. M., Bushnell, D. A., Lui, M., Erdjument-Bromage, H., Tempst, P. & Kornberg, R. D. (1998) *Genes Dev.* **12,** 45–54.
8. Koleske, A. J. & Young, R. A (1994) *Nature (London)* **368,** 466–469.
9. Nonet, M. L. & Young, R. A. (1989) *Genetics* **123,** 715–724.
10. Thompson, C. M., Koleske, A. J., Chao, D. M. & Young, R. A. (1993) *Cell* **73,** 1361–1375.
11. Hengartner, C. J., Thompson, C. M., Zhang, J., Chao, D. M., Liao, S. M., Koleske, A. J., Okamura, S. & Young. R. A (1995) *Genes Dev.* **9,** 897–910.
12. Liao, S. M., Zhang, J., Jeffery, D. A., Koleske, A. J., Thompson, C. M., Chao, D. M., Viljoen, M., van Vuuren, H. J. & Young, R. A. (1995) *Nature (London)* **374,** 193–196.
13. Wilson, C. J., Chao, D. M., Imbalzano, A. N., Schnitzler, G. R., Kingston, R. E. & Young, R. A (1996) *Cell* **84,** 235–244.
14. Myer, V. E. & Young, R. A. (1998) *J. Biol. Chem.* **273,** 27757–27760.
15. Svejstrup, J. Q., Li, Y., Fellows, J., Gnatt, A., Björklund, S. & Kornberg, R. D. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 6075–6078.
16. Hengartner, C. J., Myer, V. E., Liao, S.-M., Wilson, C. J., Koh, S. S. & Young, R. A. (1998) *Mol. Cell* **2,** 45–53.
17. Carlson, M. (1997) *Annu. Rev. Cell. Dev. Biol.* **13,** 1–23.
18. Gancedo, J. M. (1998) *Microbiol. Mol. Biol. Rev.* **62,** 334–361.
19. Carlson, M., Osmond, B. C., Neigeborn, L. & Botstein, D. (1984) *Genetics* **107,** 19–32.
20. Balciunas, D. & Ronne, H. (1995) *Nucleic Acids Res.* **23,** 4421–4425.
21. Lowry, C. W. & Zitomer, R. S. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6129–6123.
22. Wahi, M. & Johnson, A. D. (1995) *Genetics* **140,** 79–90.
23. Thomas, B. J. & Rothstein, R. J. (1989) *Cell* **56,** 619–630.
24. Nehlin, J. O. & Ronne, H. (1990) *EMBO J.* **9,** 2891–2898.
25. Östling, J., Carlberg, M. & Ronne, H. (1996) *Mol. Cell. Biol.* **16,** 753–761.
26. Guarente, L. & Mason, T. (1983) *Cell* **32,** 1279–1286.
27. Kern, L., deMontigny, J., Jund, R. & Lacroute, F. (1990) *Gene* **88,** 149–157.
28. Fernandez, J., DeMott, M., Atherton, D. & Mische, S. M. (1992) *Anal. Biochem.* **201,** 255–264.
29. Kuchin, S., Yeghiayan, P. & Carlson, M. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 4006–4010.
30. Song, W., Treich, I., Qian, N., Kuchin, S. & Carlson, M. (1996) *Mol. Cell. Biol.* **16,** 115–120.
31. Thompson-Jaeger, S., Francois, J., Gaughran, J. P. & Tatchell, K. (1991) *Genetics* **129,** 697–706.
32. Ronne, H. (1995) *Trends Genet.* **11,** 12–17.
33. Ptashne, M. & Gann, A. (1997) *Nature (London)* **386,** 569–577.
34. Kilmartin, J. V., Wright, B. & Milstein, C. (1982) *J. Cell Biol.* **93,** 576–582.
35. Cho, H., Orphanides, G., Sun, X., Yang, X.-J., Ogryzko, V., Lees, E., Nakatani, Y. & Reinberg, D. (1998) *Mol. Cell. Biol.* **18,** 5355–5363.
36. Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997) *Nucleic Acids Res.* **25,** 3389–3402.