Med9/Cse2 and Gal11 Modules Are Required for Transcriptional Repression of Distinct Group of Genes*

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The yeast Mediator is composed of two subcomplexes, Rgr1 and Srb4, known to be required for diverse aspects of transcriptional regulation; however, their structural and functional organizations have not yet been deciphered in detail. Biochemical analyses designed to determine the subunit composition of the Rgr1 subcomplex revealed that the regulator-interacting subcomplex has a modular structure and is composed of the Gal11, Med9/Cse2, and Med10/Nut2 modules. Genome-wide gene expression and Northern analyses performed in the presence or absence of the various Mediator modules revealed a distinct requirement for the Gal11, Med9/Cse2, and Med10/Nut2 modules in transcriptional repression as well as activation. GST pull-down analysis revealed that the transcriptional repressor Tup1 binds to distinct but overlapping regions of the Gal11 module that were shown previously to be transcriptional activator binding sites. These data suggest that competition between transcriptional activators and repressors for a common binding site in the Mediator and distinct conformational changes in the Mediator induced by repressor binding may underlie the mechanism of transcriptional repression in eukaryotes.

The Mediator complex was identified first as a coactivator essential for transcriptional activation in the yeast Saccharomyces cerevisiae (1, 2). Mediator associates tightly with RNA polymerase II (Pol II) to form a Pol II holoenzyme (h-Pol II) and plays a pivotal role in diverse aspects of transcriptional regulation. The search for homologous Mediator complexes in the mammalian system identified a number of protein complexes that contain several yeast Mediator homologs that function as both positive and negative regulators of transcription in vitro (3–7).

The Mediator complex is required in general for the regulation of Pol II transcription. However, certain Mediator subunits function in an activator-specific manner to modulate the expression of distinct subsets of genes (8–10), suggesting the presence of functional modules within the Mediator complex.

Differential dissociation of Mediator components by high urea treatment (11) and compositional analysis of mutant h-Pol II complexes (8, 12, 13) revealed that Mediator subunits with similar genetic properties form distinct modular subassemblies. Recently, the modular structure of Mediator was visualized by low resolution structural analysis (14). Electron microscopy and image processing of single particles of Mediator and h-Pol II revealed that Mediator alone assumes a compact structure; however, at high pH or in the presence of Pol II, Mediator displays an extended conformation with three domains (head, middle, and tail) forming an envelope that wraps around Pol II (14).

Because purified yeast h-Pol II in conjunction with basal transcription factors can support activated transcription in a well defined yeast transcription system (1, 2), it was hypothesized that gene-specific activators communicate either directly or indirectly with Mediator to recruit Pol II to the promoter. This hypothesis was substantiated by the following observations. First, h-Pol II was shown to interact with the functional activation domains of the VP16 and Gcn4 transcriptional activators. GST pull-down analysis revealed that the transcriptional repressor Tup1 binds to distinct but overlapping regions of the Gal11 module that were shown previously to be transcriptional activator binding sites. These data suggest that competition between transcriptional activators and repressors for a common binding site in the Mediator and distinct conformational changes in the Mediator induced by repressor binding may underlie the mechanism of transcriptional repression in eukaryotes.

In addition to the essential role of Mediator complex in transcriptional activation, several lines of evidence indicate the involvement of Mediator in transcriptional repression as well. Genetic analyses revealed that several Mediator genes (such as S1N4, RGR1, and GAL11) play a role in the transcriptional repression of the HO, SUC2, and IME1 genes (17–19). Alleles of S1N4 and GAL11 were identified as suppressors of (i) an NC2 (Dr1/DRAP1) repressor mutation and (ii) defective silencing at the HMR mating-type locus in yeast (20). In addition, yeast cells lacking the MEDI1 gene, which encodes a subunit of the Rgr1 subcomplex, are viable but show a complex phenotype that includes partial defects in both repression and induction of GAL gene transcription (21). Finally, deletion of the HRS1 gene, which encodes a subunit of the Gal11 module, causes derepression of the GAL1, PHO5, and HSP26 genes (22), and Hrs1 protein was shown to interact with Tup1 protein in vitro (23). Taken together, these results suggest that several proteins in the Rgr1 subcomplex are required for transcriptional repression.

To decipher the modular structure of Mediator and identify the functions associated with each module, we used a urea-induced disruption assay and reconstitution experiment with recombinant proteins to analyze the protein-protein interactions among the Mediator subunits and examined the role of each subunit in transcriptional regulation with genome-wide expression analysis.

EXPERIMENTAL PROCEDURES

Construction of Repressor Expression System—The copper-inducible LexA-tagged yeast expression vector was constructed as follows. The
CUP1 promoter region from -1 to -1000 (translation initiation site is +1) was amplified using the polymerase chain reaction (PCR) (Xhol and HindIII sites are in the 5' and 3' end primers, respectively). A DNA fragment containing the LexA DNA binding domain, a multimeric site, and the ADH1 terminator was isolated from pEG202 (CLONTech, Palo Alto, CA) by using HindIII and XbaI. These fragments were inserted into the XhoI and XbaI sites of pRS313 (24) and named pCE1. The SSN6 and SBR10 coding regions were cloned by in vivo gap repair (25) and inserted in-frame into the multimeric site of pCE1 to create pCESSN6 and pCESSR10, respectively. pAJ201 (26), which had four LexA binding sites in the upstream region of the CYC1 promoter, was used as a reporter construct. The TUP1 open reading frame was cloned by in vivo gap repair and then introduced into pGEX-4T-1 in-frame to construct the GST-Tup1 fusion construct. GST-Tup1 was expressed in bacterial strain DH5α and then purified. The TUP1 open reading frame was also cloned into pHAFASTBACHTb to construct an N-terminal hemagglutinin (HA) epitope-tagged TUP1 protein, and the virus expressing the HA-tagged TUP1 was screened as described in the baculovirus expression system manual (Life Technologies, Inc.). The HA-tagged TUP1 was purified with Q-Sepharose FF and HA.11 Ab matrix (BabCo, Richmond, CA).

**RNA Preparation and Northern Analysis**—Various Mediator mutant cells (19, 27) containing the copper-inducible repressor expression system and the reporter construct (Table I) were grown in an appropriate medium to early exponential phase at 37 °C (A 600 nm = 0.5), divided into two aliquots, and then allowed to grow for an additional 2.5 h in the presence or absence of 0.1 mM Cu 2+ ion. After the copper induction, the cells were shifted to 37 °C and then allowed to grow for an additional 2.5 h. Total RNA was prepared as described previously (28). To prepare the probe for Northern blot analysis, the BamHI-SacI DNA fragment of LacZ and the LexA DNA binding region were isolated and 32P-labeled with T4 polynucleotide kinase and poly(adenosine-5'-diphosphate) (dATP)30 (52 trp1-101 ura3-101, pAJ201, pCESSN6, and pCESSR10, respectively). pAJ201 (26), which had four LexA binding sites in the upstream region of the CYC1 promoter, was used as a reporter construct. The TUP1 open reading frame was cloned by in vivo gap repair and then introduced into pGEX-4T-1 in-frame to construct the GST-Tup1 fusion construct. GST-Tup1 was expressed in bacterial strain DH5α and then purified. The TUP1 open reading frame was also cloned into pHAFASTBACHTb (Life Technologies, Inc.) to construct an N-terminal hemagglutinin (HA) epitope-tagged TUP1 protein, and the virus expressing the HA-tagged TUP1 was screened as described in the baculovirus expression system manual (Life Technologies, Inc.). The HA-tagged TUP1 was purified with Q-Sepharose FF and HA.11 Ab matrix (BabCo, Richmond, CA).

| Strain        | Genotype          | Yeast strains used in this study |
|---------------|-------------------|---------------------------------|
| YSJW10        | Mata ade2-1 ura3-52 trp1-363 lys2-801 his3-3200 leu2-Δ1, pAJ201(URA3), pCESSR10(HIS3) |
| YSJ910        | Mata ade2-1 ura3-52 trp1-363 lys2-801 his3-3200 leu2-Δ1 med9Δ. TRP1, pAJ201(URA3), pCESSR10(HIS3) |
| YSJG1110      | Mata ade2-1 ura3-52 trp1-363 lys2-801 his3-3200 leu2-Δ1 gal11Δ. LUE2, pAJ201(URA3), pCESSR10(HIS3) |
| YSJ1010       | Mata ade2-1 ura3-52 trp1-363 lys2-801 his3-3200 leu2-Δ1 med10Δ. TRP1, ts mutant med10 on pRS315 pAJ201(URA3), pCESSR10(HIS3) |
| YSJW8s        | Mata ade2-1 ura3-52 trp1-363 lys2-801 his3-3200 leu2-Δ1, pAJ201(URA3), pCESSR10(HIS3) |
| YSJ98s        | Mata ade2-1 ura3-52 trp1-363 lys2-801 his3-3200 leu2-Δ1, pAJ201(URA3), pCESSR10(HIS3) |
| YSJG118s      | Mata ade2-1 ura3-52 trp1-363 lys2-801 his3-3200 leu2-Δ1, pAJ201(URA3), pCESSR10(HIS3) |
| YSJ1085s      | Mata ade2-1 ura3-52 trp1-363 lys2-801 his3-3200 leu2-Δ1 med10Δ. TRP1, ts mutant med10 on pRS315 pAJ201(URA3), pCESSR10(HIS3) |

**RESULTS**

**Modular Structure of the Mediator Complex**—The observations that the Mediator has a structure that is composed of two functionally distinct subcomplexes (Srb4 and Rgr1) (11) and that the Gal11, Med2, Hrs1, and Sin4 proteins form a functional module that bind to the C-terminal region of Rgr1 (12) prompted us to examine whether Med9/Cse2 and Med10/Nut2 also form modular structures with distinct functions. To this end, we first purified h-Pol II from wild-type yeast and mutant yeast strains that carried either the med9Δ (MED9/CSE2 deletion) or med10ts (temperature-sensitive) mutations. Subsequent immunoblot analysis of h-Pol II purified from the wild-type and mutant yeast strains revealed that the composition of h-Pol II from the med10ts strain was indistinguishable from that of wild type (data not shown). However, the h-Pol II from the med9Δ strain was completely devoid of Med9/Cse2 and Med1, deficient in Med4, and retained all the other Mediator components but at a slightly reduced level (Fig. 1A). This result indicates that Med9/Cse2, Med1, and probably Med4 associate to form a modular structure.

To confirm the above result, we immobilized the Mediator...
complex with anti-Med9/Cse2 Ab-coupled agarose beads and analyzed the polypeptides retained after a urea wash. The 1 M urea wash removed mainly the polymerase subunits associated with the Mediator complex, whereas a 2 M urea wash removed the Srb4 subcomplex from the beads without affecting the association of the Rgr1 subcomplex with the beads (Fig. 1B). When the beads were washed extensively at an even higher urea concentration (3 M), only Med1 and Med4 (in addition to Med9/Cse2) were retained on the beads.

To determine whether direct interactions exist among Med9/Cse2, Med1, and Med4, Sf9 cells were co-infected with baculoviruses that contained Med4, Med9/Cse2, and HA-tagged Med1 expression constructs. The Sf9 lysate was then analyzed by immunoprecipitation with anti-HA monoclonal Ab-coupled agarose beads. Immunoblot and silver-staining analysis of the immunoprecipitate showed that Med4 and Med9/Cse2 proteins co-precipitated with the HA-Med1 protein via direct interaction, whereas GST protein added to the extracts remained in the supernatant (Fig. 2A and data not shown). These observations demonstrate that Med9/Cse2, Med1, and Med4 also form a stable modular structure, as do the components of the Gal11 module.

Although the existence of the Gal11 and Med9/Cse2 modules was demonstrated with several experimental techniques, we were unable to observe a Med10/Nut2 containing module either by compositional analysis of med10ts/nut2ts h-Pol II or using the urea dissociation method (because of the relatively low binding affinity of anti-Med10/Nut2 Ab; data not shown). Therefore, we examined the physical interactions in Sf9 cells of Med10/Nut2 with the rest of the polypeptides (Med7 and Srb7) in the Rgr1 subcomplex. Immunoprecipitation of Sf9 cell extracts in which Med7, Med10/Nut2, and Srb7 were coexpressed with anti-Med10 Ab showed that Med7 and Med10/Nut2 associated directly with Srb7 in the absence of other Mediator proteins, thus demonstrating the presence of a Med10/Nut2 module (Fig. 2B). Therefore, Mediator appears to contain at least three modular structures (the Gal11, Med9/Cse2, and Med10/Nut2 modules) that are tethered independently to the Rgr1 protein in the Rgr1 subcomplex.

**Differential Requirement for Each Mediator Subunit in Transcriptional Activation and Repression**—To identify the distinct roles of the Med9/Cse2 and Med10/Nut2 modules in transcriptional regulation, we analyzed the genome-wide gene expression patterns in yeast strains that contained mutations in the Med9/Cse2 and Med10/Nut2 genes using HDAs for 6181 yeast genes. Total RNA was isolated from med9Δ/cse2Δ and med10ts/nut2ts mutant yeast strains and their wild-type counterparts 45 min after a shift to the nonpermissive temperature, and these RNA preparations were hybridized to the HDAs. Among the 5376 yeast genes, the expression levels of which were determined for the med10ts mutant strain, 2899 genes (54%) were down-regulated more than 2-fold under restrictive conditions, whereas only 56 genes were up-regulated (1%) (Table II). In contrast, the number of genes, the expression of which was affected in the med9Δ mutant strain was quite small (384 of 4806 genes down- or up-regulated); however, a significant number of genes (209 of 384 genes affected) were up-regulated (Tables II and III). Therefore, Med10/Nut2 seems to be required for the transcriptional activation of a large number of genes, whereas Med9/Cse2 is generally dispensable for transcriptional activation but required for the mediation of transcriptional repression for a subset of genes.

To examine whether the Med9/Cse2 module is involved in transcriptional repression as suggested by the HDA analysis, we used Northern analysis to examine the effect of med9Δ mutation on the expression of genes that are repressed under a rich growth condition (FLO1, SUC2, SOLA, and HXX1) and compared their effect with that of the gal11Δ mutation. When the cells were grown in YPD, expressions of the SOLA, SUC2, and HXX1 genes were repressed completely (SUC2) or at least several fold (SOL4 and HXX1) in wild-type cells. However, the med9Δ/cse2Δ mutant was defective in transcriptional repression of SOL4 and HXX1 (4- and 3.5-fold increases in transcripts, respectively), whereas gal11Δ mutants showed defects in the transcriptional repression of the SOL4 and SUC2 genes (12- and 10-fold increases, respectively; Fig. 3). In addition, Med9 is required for the transcriptional repression of FLO1, but Gal11 is dispensable for the FLO1 repression (Fig. 3). Therefore, Gal11 and Med9 proteins are required for transcriptional repression of distinct group of genes and seem to constitute repressor interaction modules of the Mediator complex.

In addition to the Med9/Cse2 and Gal11 results shown here, certain alleles of other Mediator components (SIN4 and RGR1) have been implicated in transcriptional repression (18, 19, 22, 31, 32). However, whether the Mediator proteins are involved directly in transcriptional repression or indirectly by controlling the expression of a specific transcriptional repressor protein has not yet been demonstrated clearly. To determin...
mine whether specific Mediator subunits are involved directly in transcriptional repression, we measured the transcriptional repression of a reporter gene (LacZ) that contained a binding site for the LexA repressor protein. We expressed LexA-Srb10 or LexA-Srb10 repressor fusion proteins in various Mediator mutant backgrounds and monitored reporter gene expression (Fig. 4A). In the absence of copper, LexA-Srb10 and LexA-Srb10 were expressed at a barely detectable level from the CUP1 promoter; therefore a moderate level of basal expression of the reporter gene (LacZ) was detected in all of the strains tested (Fig. 4B). Upon induction with copper, the highly expressed repressor fusion proteins were recruited to the reporter gene promoter (CYC1 promoter fused to LacZ), which resulted in a 9-fold repression of LacZ transcription in wild-type cells. The inactivation of MEDI10/NUT2 activity did not affect the transcriptional repression of LacZ by either of the repressor fusion proteins. However, when the transcriptional repressors were overexpressed in the absence of the Med9/Cse2 or Gal11 proteins, neither LexA-Srb10 nor LexA-Srb10 was able to repress LacZ expression. These results suggest that Med9/Cse2 and Gal11, but not Med10/Nut2, are required directly for transfer of the repression signal from gene-specific transcriptional repressor proteins to the basal transcription machinery. Therefore, the Med9/Cse2 and Med10/Nut2 modules seem to play major roles in the transcriptional repression and activation processes, respectively, whereas the Gal11 module is required for both types of gene regulation.

Transcriptional Repressor Binding Module of Mediator—Our previous studies (8, 10) and the genome-wide gene expression analysis presented here show that two Mediator modules (the Gal11 and Med10/Med2) are required for distinct aspects of transcriptional activation. The Gal11 module functions as binding sites for transcriptional activator proteins, whereas the Med10/Nut2 module functions at the post-activator binding stages to modify and relay the activation signal to the basal transcription machinery (8). The observation that transcriptional repression also requires two different Mediator modules (the Gal11 and Med9/Cse2 modules) suggests that a similar mechanism may work to mediate the signal between repressors and the basal transcription machinery. To test this idea, we analyzed the physical interactions between transcriptional repressor Tup1 and Mediator modules using a GST pull-down assay. The Med9/Cse2 and Med10/Nut2 modules and the Srb4 subcomplex were purified with anti-HA Ab-coupled beads, and the HA-agarose eluates were applied to GST- or GST-Tup1-agarose beads. The protein bound to the GST and GST-Tup1 beads were subjected to immunoblot analysis with anti-HA antibodies that recognize the HA-tagged versions of Med1, Srb7, and Srb4 in the Med9/Cse2 and Med10/Nut2 modules and the Srb4 subcomplex, respectively. This analysis revealed that none of the Mediator components tested bound to the Tup1 protein (Fig. 5A). However, when we tested Rgr1 and each of the Gal11 module proteins for Tup1 interaction using the GST pull-down assay, we detected strong Tup1 binding to the Rgr1 and Med2 in addition to the previously reported Hrs1 (23) but not to the Sin4 and Gal11 proteins (Fig. 5B). Therefore, Tup1 interacts with the Mediator mainly through the Rgr1 and Gal11 module proteins, as do certain transcriptional activator proteins (VP16, Gal4, and Gcn4).

Although some of the individual Mediator subunits required for Tup1 interaction (Rgr1 and Med2) are distinct from those for the transcriptional activator proteins we tested (VP16, Gal4, and Gcn4), the fact that both types of transcriptional regulators interact with Mediator through the Gal11 module is intriguing. In particular, the interaction of Hrs1 both with Gcn4 and Tup1 suggests that certain transcriptional activator and repressor proteins may share Mediator binding sites. To investigate this possibility, we deciphered the transcriptional regulator binding regions of Hrs1 using a series of GST-fusion proteins that contained various Hrs1 fragments in GST pull-down experiments. The GST pull-down assays revealed amino acids 180–343 of Hrs1 interact specifically with Gcn4, and amino acids 83–179 interact specifically with Tup1. The N-terminal region of Hrs1 that includes amino acids 1–82 interacted with both Gcn4 and Tup1 proteins (Fig. 5C). These results demonstrate that Gcn4 and Tup1 each utilize distinct but overlapping regions of Hrs1 as the Mediator binding targets.

Disruption of Mediator Complex by a Transcriptional Repressor Protein—The observation that the Mediator binding sites for a transcriptional activator (Gcn4) and a transcriptional repressor (Tup1) partially overlap suggests that competition between the two types of transcriptional regulators for a common binding site in the Mediator complex may play a significant role in mechanisms of transcriptional regulation. To test this hypothesis, we challenged the Gcn4-Mediator interaction with increasing amounts of Tup1 protein or bovine serum albumin as follows. Purified Mediator complex was first bound to GST-Gcn4 fusion protein beads. After extensive washing, increasing amounts of Tup1 or bovine serum albumin protein were added to the beads, and the amount of Mediator complex retained in the beads or released to the supernatant was monitored by immunoblot assay with anti-Srb4 Ab. No detectable change in the amount of Mediator bound to GST-Gcn4 was observed by the addition of bovine serum albumin protein (Fig. 6). In contrast, the addition of a 10-fold excess of Tup1 protein caused a significant reduction in the amount of Mediator bound to the GST-Gcn4 beads along with a significant increase in the amount of Mediator proteins released to the supernatant. This result suggests a mechanism in which a transcriptional repressor blocks the access of a transcriptional activator protein to Mediator complex to repress transcription.

**DISCUSSION**

Modular Structure of Mediator Complex—Mediator was identified first as a coactivator that enables the basal transcription machinery to respond to traditional gene-specific transcriptional activator proteins (33, 34). Subsequent biochemical purification of the Mediator complex revealed its role as an intermediary molecule between transcriptional regulators and Pol II (1). The intermediary role of Mediator was also shown by genetic studies that revealed the existence of two classes of Mediator genes: one that encodes Pol II-interacting proteins and another that interacts with gene-specific transcriptional factors (11, 18, 19, 31, 35).

The structural organization of the Mediator complex also reflects the two aspects of Mediator function, as suggested by several experiments, some of which are in this study. First, the co-disappearance of several polypeptides from the Mediator complex in med9Δ/cse2Δ, and gal11Δ strains indicates that

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**DNA chip analysis of Mediator mutants**

mRNA isolated from med10ts/mut2ts and med9Δ/cse2 Δ mutant yeast strains and their wild-type counterparts 45 min after a shift to the nonpermissive temperature were hybridized to four Affymetrix GeneChip arrays that contain probes for 6181 yeast genes. The number of genes that are up-regulated or down-regulated in each Mediator mutant strain was scored.

| Table II |
|-----------------|-----------------|
| Number of scored genesa | Number of genesb |
| med10ts | Down 2-fold or more | Up 2-fold or more |
| 5376 | 2899 (54.0 %) | 56 (1.0 %) |
| med9Δ | 4806 | 175 (3.6 %) | 209 (4.3 %) |

a Number of genes that gave significant hybridization signals above background.

b The number of genes that are up-regulated or down-regulated in each Mediator mutant strain was scored.
these subunits are connected to the rest of the complex via the Med9/Cse2 or Gal11 proteins. Second, high concentration urea treatment of Mediator complex immobilized on beads revealed not only the Srb4 and Rgr1 subcomplexes but also the Med5/Cse2 module. Third, coimmunoprecipitation from Sf9 cells of Mediator subunits that belong to the same module demonstrates that the Mediator modules or subcomplexes can be assembled with recombinant proteins. Lastly, electron microscopy and image processing of Mediator revealed the modular structure of the complex, which is composed of head, middle, and tail domains (14). In particular, the absence of the tail domain in the sin4Δ Mediator indicates that the tail domain corresponds to the Gal11 module. Because the Gal11 module is connected to the C-terminal region of Rgr1, the tail and middle domains must constitute the Rgr1 subcomplex. Therefore, the Srb4 subcomplex appears to form the head structure, which is connected to Pol II. An extensive genome-wide two-hybrid screen in yeast (36) also identified physical interactions among the subunits of each Mediator subcomplex (Srb5-Med8 and Srb6-Med11 interactions for the Srb4 subcomplex; Med7-Med4, Med7-Srb7, and Med9/Cse2-Med4 interactions for the Rgr1 subcomplex), which are in good agreement with the physical interactions identified in this study.

Among the modular structures of the Mediator complex, only two components of the middle domain (the Rgr1 and Med10/Nut2 module proteins) and Med6 of the head domain are conserved evolutionarily (37). Even in the Rgr1 protein, only the N-terminal region of the protein is conserved. The C-terminal region, where the species-specific tail domain is connected, shows a high sequence divergence (5). Therefore, the head and tail domains of the Mediator complex seems to have evolved to accommodate the diversity and specificity of higher eukaryotic transcriptional regulators and Pol II, whereas the conserved middle domain contains the basic architecture of the Mediator complex.

**Function of Repressor-specific Modules of Mediator**—The modular structure of the Mediator complex suggests that each module is responsible for distinct functions. Transcriptional activation requires the Gal11 and Med10/Nut2 modules for the activator binding and post-activator binding processes, respectively. Similarly, the Tup1 repressor also requires two separate modules, one for binding to Mediator and the other for post-repressor binding processes (transfer of the repressive signal to inhibit Pol II transcription of target genes). Intriguingly, the Gal11 module seems to be a common binding site for transcriptional activator and repressor proteins. Therefore, a simple competition for Mediator-binding sites may occur between transcriptional activator and repressor proteins and may ultimately determine the mode of transcriptional regulation. In addition to the Tup1-Mediator interaction shown here, biochemical analysis showed that Sfl1 coimmunoprecipitates from yeast cell extracts with Srb9, Srb11, Sin4, and Rox3 (38). Sfl1 is a known transcriptional repressor and regulates SUC2 transcription through a repressor-binding site located immediately 5′ to the TATA box. Therefore, the direct interaction between

**TABLE III**

Negative and positive requirement of Med9 for transcription

| Gene                | Description                          | Fold up/down |
|---------------------|--------------------------------------|--------------|
| YPL110L             | Similar to Pho1p                       | 18           |
| FLO1                |                                      | 16           |
| HSP26               | Heat shock protein 26 kDa             | 14           |
| PGM2                | Phosphoglucomutase                    | 13           |
| MRS4                | Splicing protein and member of the mitochondria carrier family | 10           |
| NDH1                | NADH-ubiquinone oxidoreductase        | 10           |
| HSP12               | Heat shock protein 12 kDa             | 9            |
| FKH2                | Homolog of Drosophila forhead protein | 6            |
| PUT4                | Proline and γ-aminobutyrate (GABA) permease | 5            |
| YMR250W             | Similar to glutamate decarboxylase     | 4            |
| HSP30               | Heat shock protein 30 kDa             | 5            |
| PUR5                | Inosine-5′-monophosphosphate dehydrogenase | 5            |
| MAL33               | Malotase fermentation regulatory protein | 5            |
| YFR022W             | Similar to Rhd1p                       | 4            |
| PCT1                | Cholinephosphate cytidyltransferase   | 4            |
| YNL227C             | Similar to Escherichia coli DnaJ and other DnaJ-like proteins | 4            |
| YLR269C             | Protein of unknown function           | 3            |
| PRP38               | Required for pre-mRNA splicing         | 3            |
| STB2                | Sin3p-binding protein                 | 3            |
| YDR111C             | Similar to alanine aminotransferase    | 3            |

*a* The fold change in gene expression in the mutant as compared to its isogenic wild-type strain.
Sfl1 and Mediator may be the main mechanism of transcriptional repression for SUC2.

If both transcriptional activator and repressor proteins interact with Mediator through the Gal11 module, how can activators and repressors regulate transcription in opposite directions? One of the mechanisms by which regulatory proteins activate or inhibit transcription is through their interaction with additional coactivator or corepressor complexes. In addition to its interaction with Mediator, the Ssn6-Tup1 repressor complex interacts with histones H3 and H4 in chromatin to form a repressive chromatin structure over the promoter region, and the interaction of Ssn6-Tup1 with Mediator may block the access of transcriptional activator proteins to the promoter (39). Therefore, it would be interesting to determine whether this repressor-Mediator complex interacts with additional repressor complexes such as the histone deacetylation complex to repress target gene expression.

Our results suggest another mechanism for transcriptional repression. Although transcriptional activator and repressor proteins may share some binding sites in the Mediator complex, the precise collection of Mediator binding surfaces required for each type of regulatory protein are distinct. Therefore, in addition to the competition between activator and repressor proteins for binding to Mediator, the binding of a transcriptional repressor to a distinct surface of the Gal11 module may cause a specific conformational change in the Mediator complex that results in transcriptional repression. This may result in the dissociation of the Mediator complex from other transcriptional factors, which in turn destabilizes the preinitiation complex formation at the promoter region as shown by the decreased occupancy of TATA-binding protein and h-Pol II at the Tup1-bound promoters (40).

Contrary to the most common function of the Gal11 module in the binding of both types of transcriptional regulators, the Med9/Cse2 module seems to be involved mainly in transcriptional repression. Because transcriptional repressor proteins we tested do not interact directly with the Med9/Cse2 module, we hypothesized that it functions at the post-repressor binding step of transcriptional repression. Therefore, we tested do not interact directly with the Med9/Cse2 module, we hypothesized that it functions at the post-repressor binding step of transcriptional repression.
stages, as does the Med10/Nut2 module during transcriptional activation, or Med9/Cse2 may serve as a binding site for a different type of transcriptional repressor protein. Identification of the precise function of the Med9/Cse2 module will be necessary to determine the mechanism by which Mediator complex participates in the transcriptional repression process.

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