The Structural and Functional Organization of the Yeast Mediator Complex

The Mediator complex of Saccharomyces cerevisiae is required for diverse aspects of transcription by RNA polymerase II (pol II). Mediator is composed of two functionally distinct subcomplexes, Rgr1 and Srb4. To identify the structures and functions of each subcomplex, we expressed recombinant proteins for each subunit and assayed their interactions with each other and with basal transcription proteins. The Rgr1 subcomplex is composed of the Gal11 module, which binds activators, and the Med9/10 module. The Med9/10 module is required for both transcriptional activation and repression, and these activities appear to be carried out by two submodules. Proteins in the Med9 submodule interact physically and genetically with Srb10/11, suggesting that the Med9 submodule mediates the repression of pol II. Puriﬁed recombinant Srb4 subcomplex stimulated basal transcription of pol II but had little effect on activated transcription and phosphorylation of the C-terminal domain of the largest subunit of pol II (Rpb1) (6). To accommodate these diverse activities, the Mediator complex is composed of more than 20 polypeptides, including Srb subunits (Srb2, -4, -5, -6, and -7), Med subunits (Med1, -2, -4, -6, -7, -8, -9, -10, and -11), and several transcriptional coregulator subunits (Gal11, Rgr1, Sin4, Hrs1, and Rox3) (10–17). In addition, one form of Mediator contains additional subunits (Srb8–11) that are involved in transcriptional repression (18, 19).

Genetic and biochemical studies have suggested that the Mediator complex can be divided into two subcomplexes, the Rgr1 and Srb4 subcomplexes (13). The Rgr1 subcomplex contains more than 9 polypeptides, including many genetically identiﬁed coregulator proteins. Among them, Gal11, Sin4, Hrs1, and Med2 have similar mutant phenotypes and form a distinct module, termed the Gal11 module, which binds to the C-terminal region of the Rgr1 (14, 20, 21). The pol II holoenzyme (h-pol II) isolated from a gal11 null mutant is devoid of the entire Gal11 module, and is functionally defective for activated, but not basal transcription (21, 22). In vitro binding assays have identiﬁed the Gal11 module as the activator binding target of the Mediator complex. These results suggest that the Gal11 module is required for efﬁcient recruitment of h-pol II to a promoter via activator-speciﬁc interaction during transcriptional activation.

The Srb4 subcomplex is composed of proteins known to interact genetically with pol II. In vitro reconstitution experiments have shown that Srb2, Srb5, Srb6, and Med6 associate with Srb4, which plays a role as a scaffold in complex formation (23, 24). Genetic and genome-wide expression analyses showed that Srb4 and its associated proteins are generally required for pol II transcription, thus suggesting that the function of Srb4 is to modulate the basic activities of pol II (25).

The modular structure of the Mediator complex has also been shown by electron crystal analysis of the complex. This analysis revealed that Mediator consists of distinct head, middle, and tail domains. The head and tail domains appear to correspond to the Srb4 subcomplex and the Gal11 module, respectively. It was also shown that both the middle and the head domains make contact with pol II, suggesting that there may be multiple contact points between polymerase and Mediator (26, 27).

Despite progress in the structural and functional analysis of yeast Mediator complex, many parts of its structural architecture, and the functions associated with each part, are not yet understood. For example, the Gal11 module is recognized as an activator-binding module with the function of receiving signals from activators (22). Whether the remaining polypeptides of the Rgr1 subcomplex form another modular structure with a speciﬁc function remains to be determined. In addition, the biochemical function of the Srb4 subcomplex and the points of mRNA synthesis requires pol I and a set of general transcription factors (GTFs) including TFIIA, TFIIB, TFIID, TFIIF, and TFIIF. The regulation of this process requires a number of coactivator complexes involved in chromatin remodeling and recruitment of transcriptional machinery to the promoter (1–5). In particular, the yeast Mediator complex is required for diverse aspects of transcriptional regulation, including activated transcription and transcriptional repression (7–9). In addition, Mediator improves the efﬁciency of basal transcription and the phosphorylation of the C-terminal domain (CTD) of the largest subunit of pol II (Rpb1) (6). To accommodate these diverse activities, the Mediator complex is composed of more than 20 polypeptides, including Srb subunits (Srb2, -4, -5, -6, and -7), Med subunits (Med1, -2, -4, -6, -7, -8, -9, -10, and -11), and several transcriptional coregulator subunits (Gal11, Rgr1, Sin4, Hrs1, and Rox3) (10–17). In addition, one form of Mediator contains additional subunits (Srb8–11) that are involved in transcriptional repression (18, 19).

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contact where Mediator binds to GTFs and pol II are still unknown.

To decipher the interactions among the various components, we generated recombinant baculoviruses for each of the Mediator subunit polypeptides and performed in vitro reconstitution experiments. Based on these results, we deduced the modular structure of the Mediator complex. Here we show the physical interactions among the Mediator subunits and basal transcription factors. These interactions underlie the transfer of regulatory signals from activator proteins to pol II. In addition to the activator-interacting Gal11 module, the Rgr1 subcomplex contains an additional module (the Med9/10 module) that interacts with Srb10/11 and GTFs. The reconstituted Srb4 subcomplex also interacts with GTFs and enhances basal transcription without major effect on activated transcription and CTD phosphorylation. These results demonstrate that Mediator is composed of functionally distinct modules required for specific aspects of transcriptional regulation.

EXPERIMENTAL PROCEDURES

Construction of Recombinant Baculoviruses—Recombinant baculoviruses expressing each of the Mediator subunits were created using the Bac-To-Bac baculovirus expression system (Invitrogen, Carlsbad, CA). To construct baculoviruses that express an N-terminal hemagglutinin (HA) epitope, the HA epitope sequence (YPYDYPDY; 5 amino acids) was inserted into the NcoI and EcoRI sites in pFASTBAChtb (Invitrogen, Carlsbad, CA) to construct pHA-FASTBAChtb. All of the Mediator genes were cloned by in vivo gap repair, verified by nucleic acid sequencing, and subcloned in-frame into pFASTBAChtb or pHA-FASTBAChtb expression vectors. Recombinant baculoviral clones for each of the Mediator proteins were isolated and amplified to a titer of >1 × 10⁸ plaque-forming units/ml. Baculoviruses expressing Flag-Srb10 and Srb11 were provided by S. S. Koh and R. Young.

Expression and Purification of Mediator Proteins—Each recombinant virus was used for infection at a multiplicity of infection of 2. In cocleavage experiments, recombinant viruses were infected together at the same multiplicity of infection. Sf9 cells infected with baculoviruses, either alone or in combination, were harvested after 48 h of incubation, washed once with a phosphate-buffered saline solution, and then resuspended in 2.5 ml of IP300 buffer (20 mM Tris acetate (pH 7.9), 0.1 mM EDTA, 0.2% Nonidet P-40, 300 mM potassium acetate) and incubated at 4°C with whole cell extracts (200 μl) derived from Sf9 cells infected with baculoviruses, each expressing Mediator subunits of interest. After binding, the beads were washed three times with binding buffer (400 μl) and boiled in SDS sample buffer. Bound proteins were analyzed by immunoblotting.

In Vitro Transcription—A reconstituted in vitro transcription assay was performed as described (10). To deplete the Mediator complex from the h-pol II fraction, 200 μl of h-pol II (MonoQ fraction) were incubated for 2 h at 4°C with 10 μl of anti-Rgr1 antibody-conjugated beads in buffer Q900 (same as buffer Q150 but with 900 mM potassium acetate) and centrifuged at 100,000 × g for 1 h at 4°C. The supernatant was used for immunoprecipitations.

For the purification of the reconstituted Srb4 subcomplex, supernatant was applied to a Q-Sepharose column equilibrated with buffer Q150 (20 mM Tris acetate (pH 7.9), 0.1 mM EDTA, 0.2% Nonidet P-40, 150 mM potassium acetate, 1 mM dithiothreitol, and protease inhibitors (10 μM phenylmethylsulfonyl fluoride, 20 mM protean A, 6 mM leupeptin, and 20 μM bisbenzamidine)). The resin was washed with buffer Q150, and bound proteins were eluted with buffer Q600 (same as buffer Q150 but with 600 mM potassium acetate). The eluted proteins were applied to a heparin-Sepharose column. The flow-through proteins were equilibrated into IP150 buffer, washed three times with binding buffer (400 μl) and boiled in SDS sample buffer. Bound proteins were analyzed by immunoblotting.

Urea Dissociation—Rat anti-Rgr1 antisera (200 μl) was conjugated with protein G-agarose beads (200 μl) as described elsewhere (28). Each aliquot of antibody beads (20 μl) was incubated for 6 to 12 h at 4°C with 10 μg of h-pol II (MonoQ fraction) in IP150 buffer containing 1 to 3 μg urea, and finally washed with IP150 buffer. The bound proteins were eluted with 100 mM glycine (pH 2.5), precipitated with 10% trichloroacetate, and subjected to SDS-polyacrylamide gel electrophoresis (PAGE), followed by silver staining and immunoblotting with specific antibodies against each Mediator subunit.

Precise Compositional Analysis of the Mediator Subcomplex—Using differential dissociation by urea, we previously demonstrated that Mediator can be dissociated into two tightly associated Rgr1 and Srb4 subcomplexes (28). Although we previously identified several components of each subcomplex (Rgr1, Gal11, Sin4, Hrs1, and Srb7 for Rgr1 subcomplex; and Srb4, Srb2, Srb5, Srb6, Med6, and Rox3 for Srb4 subcomplex), the exact composition of each subcomplex was not known. In particular, the association of the newly identified Mediator components, Med9, Med10, and Med11, was unknown (10).

To address these questions, h-pol II was immobilized on anti-Rgr1 antibody agarose beads, and the proteins retained on the beads after extensive washing with urea-containing buffer were examined by silver staining and immunoblot analyses with specific antibodies against each Mediator component. One molar urea treatment removed completely the core pol II from the immobilized Mediator complex (Fig. 1, lane 2). After 2 mM urea treatment, Med8 and Med11 were removed from Rgr1-anchored beads together with other Srb4 subcomplex components (Fig. 1, lane 3). When this 2 mM urea eluate from Rgr1-anchored h-pol II was immunoprecipitated with anti-Med6 antibody beads, all of the dissociated proteins were immuno-

Fig. 1. Dif ferential dissociation of Mediator components by urea. h-pol II (MonoQ fraction) was immobilized with anti-Rgr1 antibody beads, washed with buffer containing no urea (lane 1) or 1 mM (lane 2), 2 mM (lane 3), and 3 M urea (lane 4). The remaining Mediator polypeptides on the antibody beads were subjected to SDS-PAGE followed by silver staining (left panel) and Western blot analysis (right panel).
precipitated as a complex in the presence of high salt, indicating their physical association within a stable subcomplex (data not shown). On the other hand, Med1, Med2, Med4, Med7, Med9, and Med10 were retained on the antibody beads along with the previously identified components of the Rgr1 subcomplex. Taken together with the previous results, these findings suggested that the Rgr1 subcomplex contains Rgr1 plus the Gal11 module (Gal11, Sin4, Hrs1, and Med2), Med1, Med4, Med7, Med9, Med10, and Srb7, whereas the Srb4 subcomplex contains the remaining Mediator subunits, Srb2, Srb4, Srb5, Srb6, Rox3, Med6, Med8, and Med11.

**Med9/10 Module Formation and Its Structural Organization**—After identifying all of the components of each Mediator subcomplex, we examined the physical interactions among them. We know from electron microscopic structure analysis of h-pol II that the Mediator complex has a modular structure. Compositional analysis of h-pol II in several Mediator mutants showed that Gal11, Hrs1, Med2, and Sin4 form a module attached at the C-terminal region of the Rgr1 subunit (14). Therefore, we examined whether the other components of the Rgr1 subcomplex (Med1, Med4, Med7, Med9, Srb7, and Med10) also form additional modules. To this end, we constructed baculoviruses expressing these Mediator components, expressed them in pairs, and examined their interactions by coimmunoprecipitation. For each pair, one of the subunits was tagged with HA epitope, and the reaction was immunoprecipitated using anti-HA antibody.

We found that Med1 interacted with Med4 and Med7 (Fig. 2A). In turn, Med4 interacted with Med9, and Med7 interacted with Srb7. In addition, Med10 interacted only with Srb7. No other interactions were observed from other pairs of the Mediator proteins examined (Fig. 2A and B; Table I). When Sf9 cells were coinfectected with Med4, Med7, Med9, Med10, and Srb7, and HA-Med1, all six Mediator components were communoprecipitated by anti-HA antibody (Fig. 2C). The immunoprecipitations appear to be specific; the anti-HA antibody did not precipitate the Mediator proteins in the absence of the HA-Med1, nor did it precipitate the nonspecific GST protein added to the extracts (Fig. 2B and data not shown). Therefore, these Mediator subunits appear to form a distinct module (hereafter called the Med9/10 module) that interacts with Rgr1 through regions other than its C-terminal region (Fig. 2D).

Although it was difficult to produce enough recombinant Rgr1 to study its interaction with other Mediator proteins, the urea dissociation experiments showed that at least Med4 can interact with Rgr1. When h-pol II immobilized on Rgr1 antibody beads was washed with 3 M urea, all of the core pol II and Mediator subunits except Rgr1 and Med4 were dissociated from the beads (Fig. 1, lane 4). Med4 may not be the only subunit interacting with Rgr1, but it appears to have the strongest affinity for Rgr1. These results suggest that two distinct modules (Gal11 and Med9/10) were associated with Rgr1. The Gal11 module subunits are known to function as specific binding sites for different transcriptional regulators. The detailed function of the Med9/10 module is not yet known. However, med9 and med10 conditional mutants have distinct phenotypes in transcriptional repression and activation (10), and Med9 and Med10 form distinct substructures within the Med9/10 module (Fig. 2D). These facts suggest that there are distinct functions for submodules within the Rgr1 subcomplex.

**Srb4 Subcomplex Formation and Its Structural Organization**—Previous studies revealed the interactions between some subunits of the Srb4 subcomplex, specifically Srb2, Srb4, Srb5, Srb6, and Med6 (23, 24). To reveal the overall structural features of the Srb4 subcomplex, we extended the mapping of interactions to include the additional components, Med8, Med11, and Rox3. To this end, we constructed baculoviruses for all of the Srb4 subcomplex subunits and examined their pairwise physical interactions by communoprecipitation. Flag- or HA-tagged Srb4 specifically precipitated Med11 and Rox3 from the corresponding cell extracts (Fig. 3A). However, no detectable interaction between Srb4 and Med8 was observed. Instead, Med8 interacted only with Srb5. Other than these interactions, Med8, Med11, and Rox3 showed no detectable interactions with other components of the Srb4 subcomplex (Fig. 3A; data not shown). When extracts from Sf9 cells coinfectected with Flag-tagged Srb4 and the other untagged subunits of the Srb4 subcomplex were immunoprecipitated with anti-Flag antibody, all of the Srb4 subcomplex subunits were precipitated together as

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*S.J. Han and Y.-J. Kim, unpublished data.*

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**Fig. 2. Med9/10 module formation.** A and B, pairwise interactions between subunits of the Med9/10 module. The recombinant Mediator baculoviruses used are indicated at the top of each panel. Extracts from the infected Sf9 cells were immunoprecipitated with anti-HA antibody. A fraction (1/100) of the input and a fraction (1/3) of the pellet (IP:HA) were analyzed by immunoblotting with specific antibodies indicated at the left of the panel. C, Med9/10 module formation. The insect extracts containing all six Mediator components indicated at the top of the panel were immunoprecipitated with anti-HA antibody. D, a model of the predicted structural organization of the Med9/10 module, based on the communoprecipitation results.
a single complex only in the presence of the Flag-tagged version of Srb4 protein (Fig. 3B). Med10, which belongs to the Rgr1 subcomplex, remained in the supernatant. Therefore, a stable Srb4 subcomplex can be assembled in the absence of the Rgr1 subcomplex. The putative structure of the Srb4 subcomplex can be deduced from pairwise interactions (Fig. 3C).

The Mediator core complex, which is composed of the Med9/10 module and part of the Srb4 subcomplex, was recently purified (19). We examined the interaction between the Med9/10 module and the Srb4 subcomplex in the absence of Rgr1. Extracts from Sf9 cells infected with all the components of the Srb4 subcomplex were incubated with equivalent amounts of extracts from Sf9 cells infected with the Med9/10 module components, and Flag-Srb4 was immunoprecipitated with anti-Flag antibody. The result showed that not only the whole Srb4 subcomplex but also the Med9/10 module was precipitated (Fig. 4). Therefore, we know that the Med9/10 module is able to interact with the Srb4 subcomplex.

**Interaction of Mediator with the Basal Transcription Machinery**—The identification of the modular structures of the Mediator complex and their compositional analysis suggested that each module might be involved in different aspects of transcription. To test this hypothesis, we first examined the interaction between the distinct modules of Mediator and the basal transcription machinery. The Srb genes were identified as suppressors of Rpb1 CTD truncation mutations, and most but not all of their products are assembled in the Srb4 subcomplex. For this reason, the Srb4 subcomplex has been suggested to interact with the CTD. However, whether it really serves as the only CTD binding site is not known. To address this question, subcomplexes and modules reconstituted from individual Mediator polypeptides were examined for CTD interaction. To test whether the Mediator subcomplexes/modules bound to the CTD fragment sequence-specifically, we also examined the interaction with a mutant CTD fragment that contains five copies of scrambled CTD sequence (STYPSS). When GST, GST-CTD, or GST-scrambled CTD were incubated with Med9/10 module or Srb4 subcomplex immobilized on anti-HA antibody agarose, only the GST-CTD bound specifically both to the Med9/10 module and the Srb4 subcomplex, whereas GST and GST-scrambled CTD protein did not (Fig. 5A). This result suggests that the Mediator complex interacts specifically with the CTD of pol II at multiple contact points.

We next investigated the interaction between parts of the Mediator complex and GTFs, specifically TBP, TFIIH, TFIIIE, and TFIIH. GST pull-down analysis was used to assay reconstituted Med9/10 module or Srb4 subcomplex and GST-tagged GTFs. TBP and TFIIH bound specifically to both the Srb4 subcomplex and the Med9/10 module (Fig. 5B). The TFIIIE large subunit interacted only with the Med9/10 module. There was no detectable interaction between purified TFIIH and either the Med9/10 module or the Srb4 subcomplex (Fig. 5C). Therefore, TFIIH may associate with Mediator via TFIIIE bound to the Med9/10 module. These results indicated that specific subunits of Mediator complex interact not only with pol II but also with several GTFs.

**Interaction of Med9/10 Module with Srb10/11 Complex**—Although the Srb8–11 proteins were not identified as tightly associated subunits of Mediator under stringent purification conditions (10, 13, 15), their association with Mediator complex under different purification conditions has been reported (18, 19). Additionally, these genes were initially isolated, along with the other Srb genes, on the basis of their genetic interaction with pol II (18). Therefore, it has been suggested that the Srb8–11 subunits may form a separate complex that is loosely
of genes. Therefore, the specific interaction of Srb10/11 with mutant is defective for transcriptional repression of a number of regulatory signals. Subunits of the Rgr1 subcomplex interact specifically with transcriptional regulatory proteins, and defects in these genes produce phenotypes that resemble those of transcriptional regulatory proteins (20, 22). On the other hand, the inclusion of many Srb proteins in the Srb4 subcomplex suggested that its function might be to modulate basal transcription activity. Although the biochemical activities of several mutant Mediator complexes that are deficient for different parts of the Rgr1 subcomplex confirmed the distinct activities associated with the Rgr1 subcomplex (10), studies on the Srb4 subcomplex have been lacking.

To elucidate the biochemical activities of the Srb4 subcomplex, we purified recombinant Srb4 subcomplex and examined its activity in an in vitro transcription system. Sf9 cells were coinfected with recombinant baculoviruses encoding the components of the Srb4 subcomplex together with HA-Srb4. Following the enrichment on Q-Sepharose and heparin chromatographic columns, the Srb4 subcomplex was eluted with HA peptide from an anti-HA antibody column. Silver staining and immunoblot analysis of the eluates revealed that the purified recombinant Srb4 subcomplex contained all of its subunits at approximately equimolar levels (Fig. 7A).

To identify the biochemical activities of the reconstituted
Srb4 subcomplex, we examined whether the recombinant Srb4 subcomplex enhances CTD phosphorylation efficiency by TFIIH as the Mediator complex does. The addition of the Srb4 subcomplex to the phosphorylation reaction caused no detectable enhancement of phosphorylation by TFIIH (data not shown). However, when we added the Srb4 subcomplex to an in vitro transcription system reconstituted with pure transcription factors and h-pol II, it stimulated basal transcription 2–3-fold, without a major effect on activated transcription (less than 1.2-fold) (Fig. 7B). This result indicated that additional Srb4 subcomplex enhances basal transcription activity even in the presence of the endogenous Mediator complex.

To examine the role of the Srb4 subcomplex in the absence of intact Mediator, we depleted endogenous Mediator complex from h-pol II (MonoQ fraction) by immunodepletion using anti-Rgr1 antibody under high salt conditions (900 mM). Immunoblot analysis confirmed that the core pol II and its associated TFIIF in the supernatant did not contain a detectable amount of Mediator (Fig. 8A). Depletion of Mediator complex from h-pol II diminished both the basal and activated transcription to less than 5% of their activities before the Mediator depletion (Fig. 8B). This residual transcriptional activation may have resulted from a small amount of Mediator complex left after the immunodepletion. When we added an increasing amount of recombinant Srb4 subcomplex components are indicated at the right. A, effect of recombinant Srb4 subcomplex on transcription in an in vitro reconstituted transcription system. Purified recombinant Srb4 subcomplex was added to an in vitro transcription system reconstituted with purified general transcription factors with (+) or without (−) transcriptional activators, Gal4-VP16 and Gcn4. The G-less templates containing binding sites for Gcn4 (Gcn4:G−) or Gal4 (Gal4:G+) DNA-binding sites were used to measure basal and activated transcription. IP, immunoprecipitation.
biant Srb4 subcomplex back to the Mediator-depleted transcription system, we observed a gradual increase in both the basal and activated transcription (Fig. 8C). However, the addition of the Srb4 subcomplex did not affect activation itself; the enhancement of activated transcription by the Srb4 subcomplex appeared to result from the increase in basal transcription activity (Fig. 8D). Taken together, these results suggest that Srb4 subcomplex may have activity that enhances basal transcription.

**DISCUSSION**

The Mediator complex is required for diverse aspects of the transcription process, such as activation, repression, and stimulation of basal transcription. The diversity of these functions has raised the possibility that the Mediator complex contains functionally distinct modules. We previously demonstrated the existence of Rgr1 and Srb4 subcomplexes and identified the Gal11 module within the Rgr1 subcomplex as an activator-binding module. Here we show biochemical evidence to demonstrate another distinct module (Med9/10) within the Rgr1 subcomplex, its physical interaction with Srb10/11 proteins, and the biochemical activity of the Srb4 subcomplex in stimulating basal transcription.

**Modular Organization of Mediator Complex**—Our urea-dissociation experiment confirmed the previous observation that the Mediator complex is composed of two distinct subcomplexes, Rgr1 and Srb4, and further defined the components belonging to each of them. The Srb4 subcomplex contains Srb4 plus 8 Mediator components (Srb2, Srb5, Srb6, Srb8, Rox3, Med6, Med8, and Med11). The interaction study with recombinant mediator proteins revealed that Srb4 plays a critical role as a scaffold for Srb4 subcomplex assembly (Fig. 3C). In fact, the subcomplex was not formed in the absence of Srb4 (data not shown). The importance of Srb4 as a structural component may account for the global effect of the srb4 temperature-sensitive mutation on pol II transcription, as shown by whole genome expression analysis (25). Although h-pol II lacking Srb5 or Srb2 displays defects in both basal and activated transcription in a reconstituted in vitro system, the recruitment of the h-pol II to promoter was not affected by a srb5 mutation (11, 21). Therefore, Srb4 and its associated components appear to play a critical role in the modulation of basal transcription machinery rather than act in the reception of gene-specific activator signals. Here, we demonstrate that a highly purified recombinant Srb4 subcomplex is able to stimulate basal transcription activity of pol II in an in vitro transcription assay. This activity is unlikely to be derived from the enhancement of TFIH activity by the Srb4 subcomplex because we could not observe an increase in CTD phosphorylation efficiency by the addition of the recombinant Srb4 subcomplex to an in vitro transcription system. This finding may have resulted from an interaction of the Srb4 subcomplex with other general transcription factors (TBP, TFIIB, and CTD).

Our study also showed that the Rgr1 subcomplex contains Rgr1 plus 10 additional mediator components. The Rgr1 subcomplex may play an important role as a core scaffold upon which the other components are assembled to form a stable subcomplex as Srb4 does in the Srb4 subcomplex. Previous genetic and biochemical analyses demonstrated that the Rgr1 subcomplex contains at least one activator-binding module (Gal11, Sin4, Hrb1, and Med2) that associates with the C-terminal domain of Rgr1 (20, 21, 27). Our studies demonstrated that the Rgr1 subcomplex contains another distinct module, the Med9/10 module. The Med9/10 module contains six Mediator subunits, which are divided into the Med9 and Med10 submodules. These submodules probably interact with Rgr1 through regions other than the Rgr1 C-terminal domain. In particular, the similar phenotype of Med1 mutants to those of Srb10/11 mutants, and the physical interaction of Srb10/11 with Med4 and Med1, indicate the involvement of the Med9 submodule in transcriptional repression mediated by Srb10/11 proteins. Recently, we observed that the deletion of Med9 derepresses transcription from a subset of genes in yeast (33), and we identified a weak genetic interaction between Med9 and repressor proteins such as Rpd3 histone deacetylase.2 These observations suggest that the Med9 submodule may function in modulating the effect of repressors on pol II transcription.

**Interaction of Mediator Subcomplexes (or Modules) with General Transcription Factors**—Because Srb components were originally identified as dominant suppressors for CTD truncation, it has been suggested that Srb components may interact physically with the CTD of pol II. Hence, our observation of physical interaction between CTD and the Srb4 subcomplex demonstrated that the Srb4 subcomplex interacts functionally and physically with CTD. In addition, it is also interesting that the Med9/10 module within the Rgr1 subcomplex interacts physically with CTD. The electron microscopic structural analysis of h-poli II also shows two distinct contact points between Mediator complex and pol II (26, 27). Thus, the Mediator complex has multiple contact points for pol II.

Besides the physical interaction with pol II, Mediator interacts physically with several GTFs. The Srb4 subcomplex interacts with TBP and TFIIB, whereas the Med9/10 module interacts with TBP, TFIIB, and TFIIE\(_{\lambda}\). This result suggests that these interactions may accelerate and stabilize the formation of the preinitiation complex (PIC). This notion is supported by the finding that recruitment of GTFs such as TBP, TFIIB, and TFIIE\(_{\lambda}\) to active promoters requires the function of Mediator (34–35). In addition, these interactions may account for the previous observation that, after initiation, Mediator, along with TFIIB, TFIID, TFIIE\(_{\lambda}\), and TFIIF, remains at the promoter forming a scaffold for assembly of a second PIC (36, 37). However, the mechanism by which Mediator and GTFs are involved in the formation of PIC or formation of a functional reinitiation complex remains to be further analyzed.

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