

# Structure of the Yeast RNA Polymerase II Holoenzyme: Mediator Conformation and Polymerase Interaction

Joshua A. Davis,<sup>1</sup> Yuichiro Takagi,<sup>2</sup>  
Roger D. Kornberg,<sup>2</sup> and Francisco J. Asturias<sup>1,3</sup>

<sup>1</sup>Department of Cell Biology  
The Scripps Research Institute  
10550 North Torrey Pines Road  
La Jolla, California 92037

<sup>2</sup>Department of Structural Biology  
Stanford University School of Medicine  
Stanford, California 94035

## Summary

The holoenzyme formed by RNA polymerase II (RNAPII) and the Mediator complex is the target of transcriptional regulators *in vivo*. A three-dimensional structure of the yeast holoenzyme has been generated from electron microscopic images of single holoenzyme particles. Extensive changes in Mediator conformation required for interaction with RNAPII have been modeled by correlating the polymerase-bound and free Mediator structures. Determination of the precise orientation of the RNAPII in the holoenzyme indicates that Mediator contacts are centered on the RNAPII Rpb3/Rpb11 heterodimer, the eukaryotic homolog of the  $\alpha_2$  homodimer involved in transcription regulation in prokaryotes. Implications for the possible mechanism of transcription regulation by Mediator are discussed.

## Introduction

Synthesis of all mRNA in eukaryotes is carried out by RNA polymerase II (RNAPII), the enzyme that provides the catalytic activity for RNA polymerization. In order to recognize promoter DNA and initiate transcription, RNAPII must associate with at least five general transcription factors (TFIIB, TFIID, TFIIIE, TFIIIF, and TFIIH) which, along with the polymerase, are assembled on a promoter to form the transcription preinitiation complex. Regulation of transcription occurs mostly at initiation, through the action of activator and repressor proteins on the preinitiation complex. Many regulatory factors may bind to DNA upstream of a single promoter and exert their combined effect on the transcription machinery. Another set of protein factors acts as a necessary interface between the basal machinery and activators and repressors. In the yeast *Saccharomyces cerevisiae*, the search for a factor that could mediate activated transcription in a fully defined *in vitro* system resulted in the discovery of the Mediator complex (Flanagan et al., 1991; Kim et al., 1994). Individual subunits of the yeast Mediator complex had been identified as being important in transcriptional regulation by previous genetic screens, but the resolution of Mediator as a stable, well-defined entity revealed the physical association of these multiple regulatory proteins (reviewed in Myers

and Kornberg 2000). The general significance of Mediator in eukaryotic transcription regulation is evidenced by the discovery and isolation of homologs of yeast Mediator in the mouse, *Drosophila*, and human systems (reviewed in Rachez and Freedman 2001).

Mediator plays a complex role in the regulation of transcription in yeast. Besides stimulating basal transcription 10-fold, and providing a 30-fold upregulated response to activators, Mediator also causes a 30- to 50-fold increase in TFIIH-dependent phosphorylation of the carboxy-terminal domain (CTD) of Rpb1, the largest RNAPII subunit (Kim et al., 1994). CTD phosphorylation plays a critical role in orchestrating the interaction between the polymerase and Mediator. Biochemical and structural evidence (Myers et al., 1998; Asturias et al., 1999) indicates that Mediator and RNAPII interact to form a stable complex, known as the RNAPII holoenzyme. Removal of the repetitive CTD region of RNAPII is lethal *in vivo* (Nonet et al., 1987) and, *in vitro*, prevents the formation of the holoenzyme complex (Asturias et al., 1999). While Mediator-associated RNAPII has a hypophosphorylated CTD, actively transcribing polymerases have a heavily phosphorylated CTD and are not associated with Mediator. These findings suggest a model in which CTD phosphorylation dissociates Mediator from RNAPII and allows it to be recycled by binding to hypophosphorylated polymerase to initiate a new round of transcription (Svejstrup et al., 1997). In addition, *in vitro* studies indicate that Mediator and a subset of the general transcription factors are left behind at the promoter during the transition from initiation to elongation (Yudkovsky et al., 1999), suggesting that Mediator may facilitate reinitiation.

Previous investigations of Mediator organization have revealed that it comprises distinct structural domains that undergo an extensive rearrangement upon interaction with RNAPII. In the conformation that it adopts in the holoenzyme, Mediator appears as consisting of three modules (head, middle, and tail) of approximately equal mass (Asturias et al., 1999). The modularity evident in the structure of the Mediator complex most likely reflects the association of subsets of Mediator subunits in a similar fashion. Mediator complexes purified from yeast strains in which individual proteins were deleted led to the characterization of a Sin4 subcomplex containing Sin4, Gal11, Pgd1, and Med2 (Li et al., 1995; Myers et al., 1999). An Srb-subcomplex seems to comprise subunits Srb2, Srb4, Srb5, Srb6, Rox3, Med6, Med8, and Med11, which interact with one another as shown by a variety of *in vitro* experiments (Lee and Kim, 1998; Kang et al., 2001; Koh et al., 1998). At least one of these biochemically defined subcomplexes corresponds roughly to a defined structural domain, as shown by imaging a Sin4-subcomplex-deficient holoenzyme prepared from a *sin4* deletion yeast strain (Dotson et al., 2000).

Information regarding the structure of the RNAPII holoenzyme has so far been limited to a  $\sim 50$  Å resolution 2D projection map (Asturias et al., 1999), and basic questions regarding organization of the complex remain

<sup>3</sup>Correspondence: asturias@scripps.edu

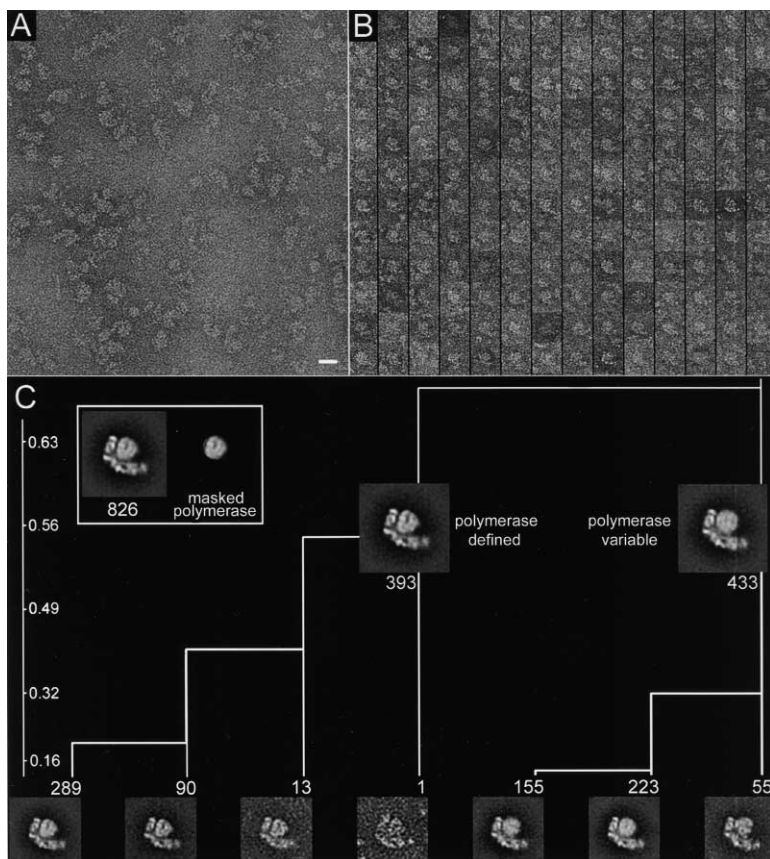


Figure 1. Image Data and Classification

(A) Typical image from negatively stained holoenzyme sample (45,000 $\times$ ) (scale bar = 100 Å).

(B) A gallery of reference-free aligned holoenzyme images before classification but after interactive categorization.

(C) A dendrogram summarizing the results of hierarchical ascendant classification (HAC) of 826 holoenzyme images using a circular mask to define the polymerase density (see inset). Averages of related particles are displayed along the bottom (threshold 0.16). The averages for the two main clusters are shown at the 0.56 threshold level. (Dendrogram generated using WEB).

unanswered. What is the relative orientation of RNAPII and Mediator, and what are the implications for the regulation mechanism? What is the significance of the large structural changes that Mediator must undergo to interact with RNAPII? Despite limited sequence homology between subunit components, low-resolution structures of several eukaryotic Mediator complexes suggest similarities in structural organization (Dotson et al., 2000); and structural characterization of the yeast holoenzyme might serve as a paradigm for the understanding of eukaryotic transcription. We present here a  $\sim 35$  Å resolution 3D reconstruction of the yeast holoenzyme in which the relative orientation of RNAPII and Mediator has been unambiguously determined. A discussion of the significance of the holoenzyme structure for the mechanism of transcription regulation by Mediator is presented.

## Results

### Calculation of 3D Holoenzyme Structure

Examination of holoenzyme preparations by electron microscopy revealed the presence of free RNAPII, free Mediator, holoenzyme, and other unidentified particles (Figure 1A). Holoenzyme particles appeared to adopt a preferred orientation on the grid; therefore, images were collected as tilt pairs at  $-55^\circ$  and  $0^\circ$  angles. After interactive screening,  $\sim 40\%$  (826) of the image pairs initially selected from 60 micrographs were identified as holoenzyme particles and used for further analysis (Figure 1B).

Comparison of the untilted holoenzyme average with the previously determined projection map of the complex (Asturias et al., 1999) indicated that the selected holoenzyme particles showed variability that could not be attributed to deviations from the preferred orientation exhibited by the particles in the samples. Features in the Mediator portion appeared more detailed than in the RNAPII portion, suggesting variability in the polymerase orientation. The holoenzyme images were therefore subjected to principal component analysis and hierarchical ascendant classification (see Frank, 1996) to separate them into homogeneous groups (Figure 1C). Mediator appears well defined in all class averages generated by hierarchical ascendant classification; but features in RNAPII are blurred in some of the class averages, indicating that the orientation of polymerase with respect to Mediator is variable in those particle groups. Therefore, only the tilted counterparts of those untilted holoenzyme particles with a well-defined RNAPII orientation were included in the calculation of the 3D holoenzyme reconstruction (Figure 2A).

### Analysis of Mediator Conformational Changes

To better understand the relation between the conformation of Mediator in its polymerase-bound (holoenzyme) and free forms, the Mediator portion of the holoenzyme 3D reconstruction was parsed into three segments (head, middle, and tail) which were then fitted into a published reconstruction of the free Mediator complex (Dotson et al., 2000) (Figure 2B). A close match

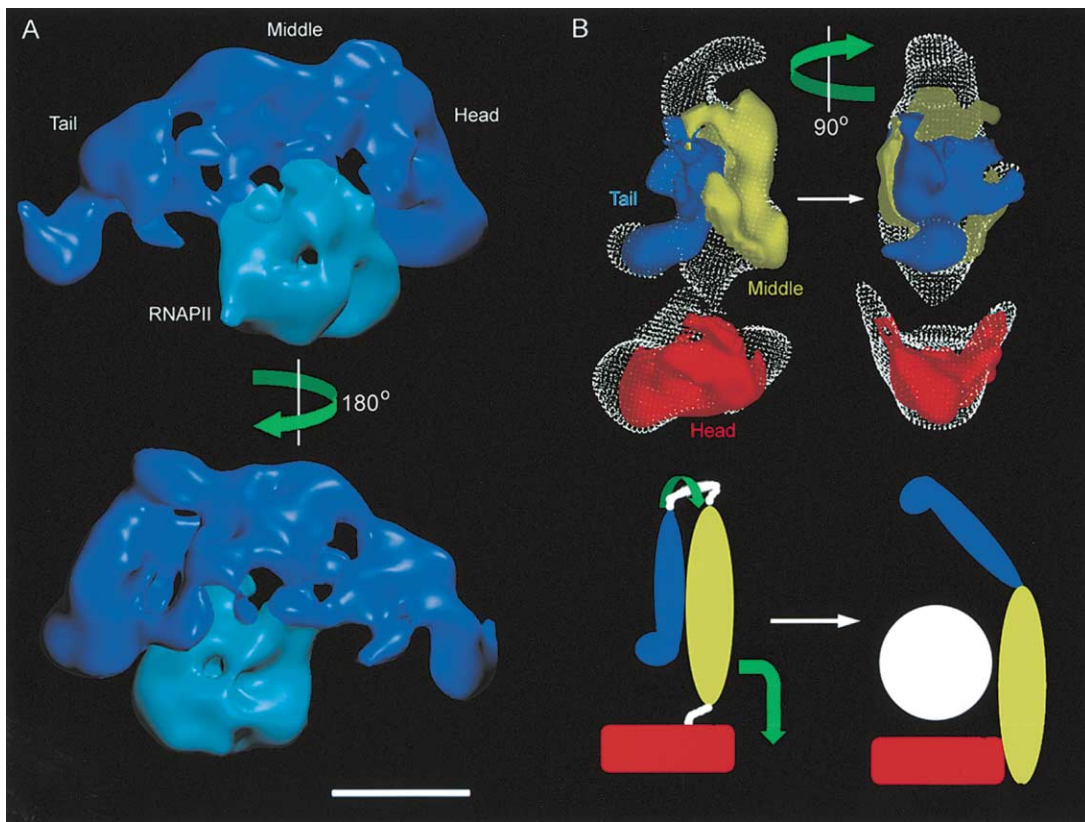


Figure 2. Three-Dimensional Image of the Holoenzyme and Its Relationship to Polymerase-free Mediator

(A) The holoenzyme volume is shown in the preferred grid orientation (top) and from the opposite side (bottom). Scale bar = 100 Å. Different colors have been used to represent the regions of the reconstruction that approximately correspond to either Mediator or RNAPII. (B) Top, Mediator modules extracted from the holoenzyme reconstruction (red/head, yellow/middle, and blue/tail) were fitted to a reconstruction of free Mediator (shown as a dotted surface). Bottom, a model for the conformational changes in Mediator necessary to form the holoenzyme complex. Putative flexible linkers are shown in white. Green arrows indicate the relative movements of Mediator modules. Polymerase is represented by a white circle. The holoenzyme schematized in the bottom right corresponds to the same view as in (A, top) except with a 90° in-plane rotation.

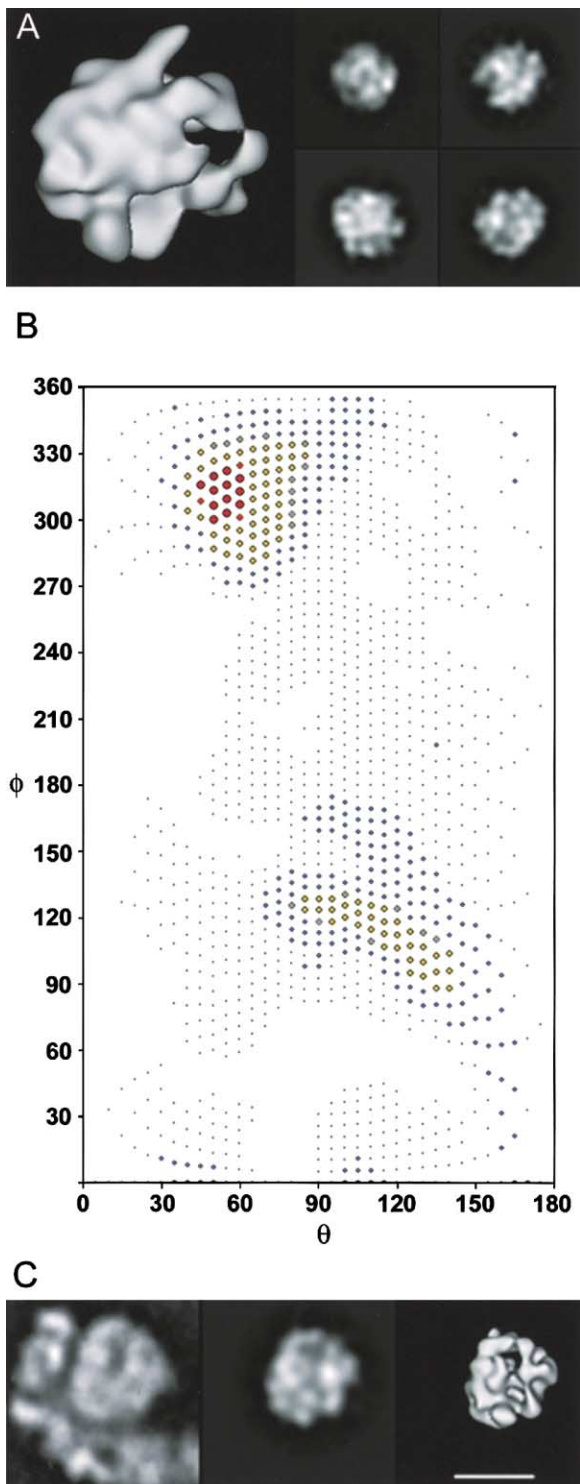
of features between segments of the two Mediator conformations suggests a simple unfolding model in which relative motions of the three modules allow Mediator to adopt the extended conformation apparent in the holoenzyme complex (Figure 2B, bottom). This model is consistent with the appearance of partially unfolded single Mediator particles observed by EM (Asturias et al., 1999). The middle module appears to move from atop the head module to a position behind it. The separation of the middle and tail modules involves not only a hinging motion, but also a rotation that requires an approximately 180° twist of the tail. The overall length between the extreme ends of Mediator in both conformations is relatively constant (~330 Å) and is maintained by the motions described by the model.

#### Determination of the RNAPII Orientation in the Holoenzyme Complex

The use of holoenzyme images related to a single orientation of the complex on the grid results in a slight deformation of the 3D reconstruction (“missing cone” problem). On the other hand, after proper alignment and classification, untilted holoenzyme images can be averaged to obtain a 2D projection map of the holo-

zyme complex that is free of distortions and contains information to higher resolution than the 3D reconstruction. We decided to determine the precise orientation of RNAPII in the holoenzyme by comparing the polymerase portion of such a projection map with a set of projections of an RNAPII 3D reconstruction calculated from EM images obtained under the same conditions as those used for imaging of the holoenzyme complex (Craighead et al., 2002). The RNAPII reconstruction was filtered to match the resolution of the holoenzyme projection map (Figure 3A), and cross-correlation values were calculated between the RNAPII portion of the holoenzyme map and the set of RNAPII projections. A clear maximum in the multireference cross-correlation plot identified a unique RNAPII orientation (Figure 3B). The holoenzyme projection map, the best RNAPII projection match (within 5°; see Experimental Procedures), and the correspondingly oriented RNAPII volume are shown in Figure 3C. The orientation roughly corresponds to a “bottom” view of RNAPII with the enzyme’s active site/DNA binding cleft toward the back of the figure.

Regions of polymerase in direct contact with Mediator were identified (Figure 4A) by placing the RNAPII reconstruction in the appropriate position with respect to the



**Figure 3. Determination of Polymerase Orientation**  
(A) A reconstructed 3D volume of  $\Delta 4/7$  RNAPII (left) was used as the reference object in the calculation of  $\sim 1600$  2D projections. A representative gallery of these projections is shown (right). Projections were calculated from directions  $(\theta, \phi)$  equally spaced about the surface of a sphere with the volume at the center.  
(B) Cross-correlation coefficients calculated between each projection and the holoenzyme 2D average are plotted versus  $\theta$  and  $\phi$ . Several high-scoring projections are clustered in one region of the projection sphere. Red and yellow colored points represent cross-

correlation coefficients  $3\sigma$  and  $2\sigma$  above the mean, respectively.

## Discussion

The yeast RNAPII holoenzyme structure reported here shows an extended Mediator consisting of three separate structural modules that wrap around RNAPII. Multiple contacts between Mediator and polymerase extend from the head Mediator module to the intersection between the middle and tail modules. Despite these extensive contacts, most of the RNAPII surface ( $>75\%$ ) remains accessible for interaction with additional components of the transcription preinitiation complex. Correlating the polymerase-associated (holoenzyme) and free (compact) Mediator conformations suggests a sequence of steps that results in the extensive change that Mediator undergoes upon species-specific interaction with RNAPII (Asturias et al., 1999). The rearrangements of Mediator structure and the variability in the relative orientation of Mediator and RNAPII in the holoenzyme particles are both consistent with the idea that Mediator/RNAPII contacts are established sequentially. The process most likely starts with the separation of the middle and tail Mediator modules, which is required to generate the RNAPII binding area in the extended Mediator conformation (Figure 2B). The subset of holoenzyme images where the relative orientation of Mediator and RNAPII is well defined (those included in the calculation of the 3D holoenzyme reconstruction) would then correspond to the structure of a fully formed, functionally relevant Mediator/RNAPII complex.

The large changes in Mediator structure required for formation of the yeast holoenzyme suggest that the role of activators and repressors in transcription regulation may extend beyond recruitment of Mediator to a promoter. Effects on Mediator conformation could facilitate formation of the RNAPII holoenzyme or alter its structure in a way that affects transcription by polymerase. A study of the structure of the CRSP/ARC mammalian coactivator reported activator-induced conformational changes in the complex (Taatjes et al., 2002). It will be interesting to examine if changes as extensive as those required for formation of the yeast holoenzyme occur in higher organisms and to study the effect of regulatory proteins in the context of the Mediator/RNAPII system.

Determining the relative orientation of RNAPII and Mediator in the holoenzyme is essential for understanding the mechanism of transcription regulation by Mediator. Features in the polymerase portion of the holoenzyme structure can be easily correlated to the structure of RNAPII. We were able to determine the precise orientation of RNAPII by cross-correlation analysis in 2D. The validity of this approach relies on the observation that, at the resolution of our current holoenzyme structure, the conformation of RNAPII in the complex does not

correlation coefficients  $3\sigma$  and  $2\sigma$  above the mean, respectively. (C) The interpolated holoenzyme 2D average (left), the best matching polymerase projection (middle), and the corresponding 3D view of the polymerase (right) are shown. Scale bar = 100 Å.

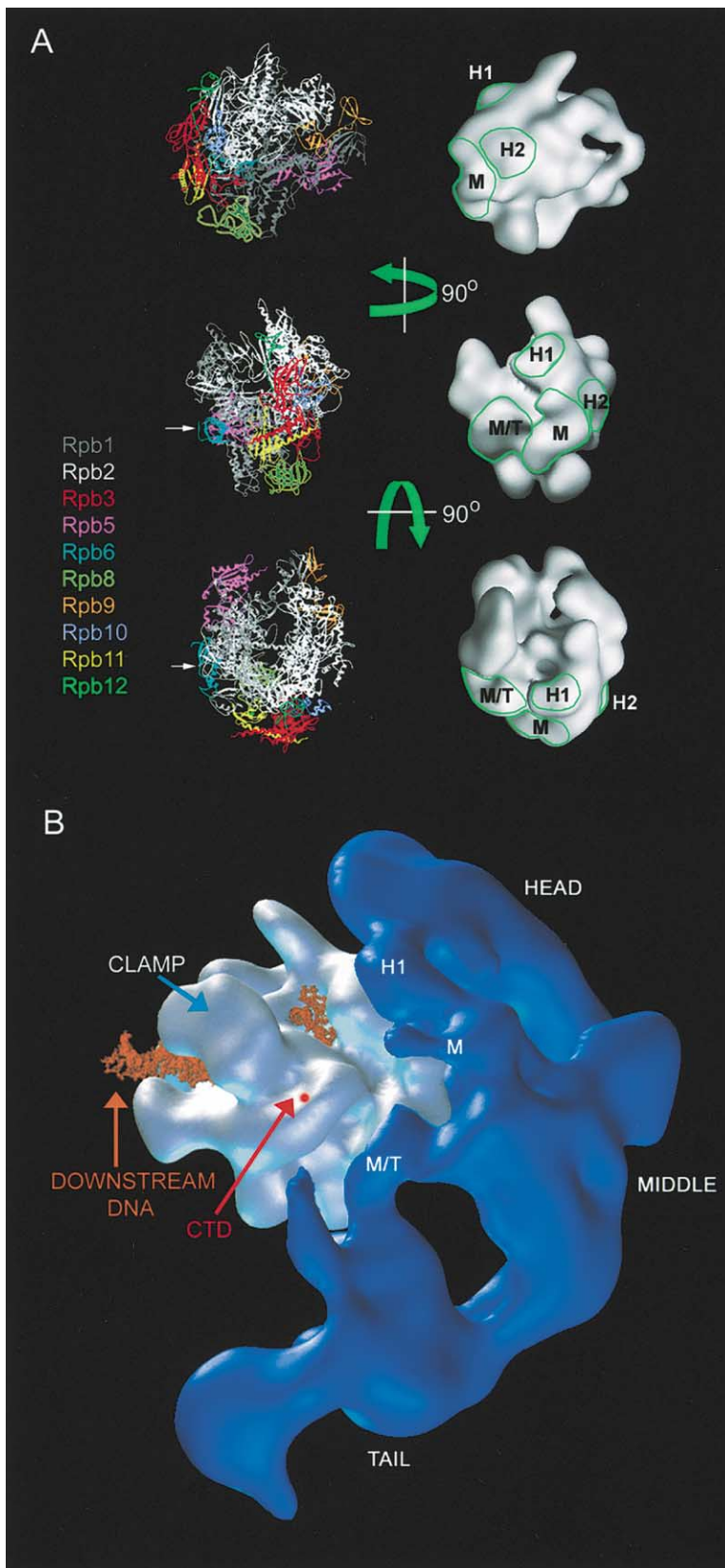


Figure 4. RNAPII/Mediator Interactions in the Holoenzyme

(A) The atomic resolution structure of ( $\Delta 4/7$ ) RNAPII was docked into the holoenzyme envelope based on the projection matching results. Inspection of the surface of polymerase that is contacted by Mediator density reveals that four regions are involved in these interactions.

These patches are indicated as bounded regions on the surface of the  $\Delta 4/7$  RNAPII volume. Mediator regions involved in the interactions are labeled "M/T" (middle/tail), "M" (middle), and "H1" and "H2" (head). The head flaps contribute H1 and H2 with H1 being toward the foreground in (B) and in Figure 2A, bottom. The location of the last ordered residue of the CTD in the X-ray crystal structure (Cramer et al., 2001) is indicated by arrows. (B) A cartoon demonstrating the interaction of polymerase and Mediator. The  $\Delta 4/7$  RNAPII structure is merged with the Mediator in the holoenzyme conformation and is shown from a view that is most similar to Figure 2A, bottom (except that the head module is pushed beneath the plane of the page). The pathway of DNA in the immediate vicinity of polymerase (Gnatt et al., 2001) is unobstructed by the position of Mediator.

differ significantly from that of RNAPII alone. The results of the 2D analysis using RNAPII projections agrees well with the polymerase orientation initially determined through interactive fitting, and the clustering of high cross-correlation values within a small angular range (Figure 3B) reflects the precision of the fit. Examination of the resulting fit reveals the identity of RNAPII subunits involved in contacts with specific Mediator modules.

The tail and middle Mediator modules converge and contact an area of RNAPII that includes a region of Rpb1 (adjacent to the last ordered CTD residue in the RNAPII X-ray crystal structure) and portions of subunits Rpb3, Rpb11, and Rpb6. A second contact patch involves the opposite end of the middle Mediator module (closer to the head) and subunits Rpb3 and Rpb11. Finally, "flaps" previously identified on either side of the Mediator head module (see Figure 2B) contribute two additional contacts. One head flap contacts portions of Rpb2 located on the side of the polymerase structure opposite the DNA clamp. The second head flap contacts Rpb12 and the surrounding region of Rpb2, an area of the polymerase structure that includes the "wall" domain of Rpb2 at the back end of the enzyme's active site cleft. A revised model for interaction of RNAPII and promoter DNA based on an 18 Å resolution cryo-EM reconstruction of wild-type RNAPII (Craighead et al., 2002) that reveals the conformation of the enzyme in solution suggests that this same area (labeled H1 in Figure 4A) of the polymerase surface is involved in RNAPII/DNA contacts at initiation. Our understanding of the significance of these RNAPII/Mediator interactions will undoubtedly increase when a higher resolution structure of the holoenzyme is determined and the locations of Mediator subunits and regulatory protein binding sites are established.

The structure of the RNAPII holoenzyme shows that the DNA binding cleft of polymerase remains fully accessible in the holoenzyme complex (Figure 4B). This is consistent with the idea of RNAPII recruitment to a promoter by Mediator (Cosma et al., 2001) and with the possibility that RNAPII may interact with a Mediator-based reinitiation scaffold left behind at a promoter after the initial round of transcription (Yudkovsky et al., 1999). RNAPII/Mediator interactions largely involve the conformationally stable "core" module identified by comparing different RNAPII conformations characterized by X-ray crystallography (Cramer et al., 2001). Mediator does not appear to interact directly with more flexible RNAPII modules, but our results do not rule out the possibility that the effect of Mediator on transcription regulation might be mediated by changes in polymerase conformation not apparent at the limited resolution of our current holoenzyme reconstruction. Mediator/RNAPII interactions are centered on the Rpb3/Rpb11 heterodimer, the eukaryotic homolog of the prokaryotic  $\alpha_2$  homodimer that mediates activation by cyclic-AMP receptor protein (CRP) in a variety (>100) of *E. coli* promoters (reviewed in Busby and Ebright 1999). At class II CRP-dependent promoters, a well-defined activation target in the  $\alpha_2$  homodimer plays a role in postrecruitment isomerization of the polymerase promoter closed complex to polymerase promoter open complex (Niu et al., 1996). Mutations in the corresponding region of Rpb3 have been found to affect activator-dependent, but not basal transcription

(Tan et al., 2000). The structure of the RNAPII holoenzyme described in this paper has now revealed that the Rpb3/Rpb11 heterodimer plays a central role in interaction of RNAPII with Mediator and provides a structural explanation for the results of the mutation analysis. The apparent conservation from prokaryotes to eukaryotes of the polymerase surface involved in transcription regulation may help us to understand the way in which more intricate mechanisms for regulation in higher cells have evolved.

#### Experimental Procedures

##### Sample Preparation and Electron Microscopy

Growth of yeast strain YPH/TFB1.6HIS (Svejstrup et al., 1994) and preparation of whole-cell extract is described elsewhere (Sayre et al., 1992). Fractionation of whole-cell extract by chromatography on Bio-Rex70 (Bio-Rad), DEAE-Sephacel (Pharmacia), and Bio-Gel HTP hydroxyapatite (Bio-Rad) was as described (Kim et al., 1994). After fractionation on hydroxyapatite, the holoenzyme peak was detected by nonspecific transcription assay and immunoblotting against Med4 and Med6 subunits. Peak fractions were pooled and loaded onto a Mono Q HR 5/5 column (Pharmacia), and holoenzyme was eluted in a potassium acetate gradient at around 800 mM. The peak of holoenzyme was again detected by transcription assay and immunoblotting. Holoenzyme was then loaded onto a TSK-4000 sizing column (TosoHaas, Montgomeryville, PA) equilibrated with 20 mM HEPES-KOH (pH 7.25), 1 mM EDTA, 1 mM DTT, 400 mM potassium acetate, and 5% glycerol. Holoenzyme was detected again by transcription assay and immunoblotting.

An aliquot of the holoenzyme preparation was diluted to a concentration of approximately 50  $\mu$ g/ml with a buffer containing 40 mM HEPES (pH 7.25), 120 mM KOAc, 5 mM MgCl<sub>2</sub>, and 1 mM DTT. Two microliters of this material was applied to a carbon-coated, 400-mesh Cu/Rh grid (Ted Pella, Redding, CA) that was glow discharged in the presence of water vapor. Approximately 30 s after adsorption, the excess buffer was blotted and 3  $\mu$ l of water was used to wash the grid. Several washes with 1% uranyl acetate were followed by application of 3  $\mu$ l uranyl acetate for 30 s. The grid was immersed in uranyl acetate and surfaced under a second layer of carbon, creating a "carbon layer sandwich" to improve particle staining (Tischendorf et al., 1974; Stöffler and Stöffler-Meilicke, 1983). Samples were imaged under low-dose conditions using a CM120 TEM (Philips/FEI) fitted with a LaB<sub>6</sub> filament and operating at an accelerating voltage of 100 kV. Micrographs were recorded at a nominal magnification of 45,000 $\times$ . Most particles in the images showed the holoenzyme complex in one preferred orientation, and well-stained particle fields were imaged at both 0° and -55° angles to provide the images required for a random conical tilt reconstruction (Radermacher et al., 1987). Micrographs were digitized on a SCAI (ZI/Carl Zeiss) scanner using a 7  $\mu$ m sampling. Subsequent 3-fold pixel-averaging resulted in a final sampling of 4.78 Å/pixel (calibrated using images of catalase crystals).

##### Image Processing and Calculation of the Holoenzyme Structure

All image processing was carried out using the SPIDER software package (Frank et al., 1996). Particle images were interactively selected and windowed using WEB. Approximately 3000 particle windows (121  $\times$  121 pixels), found to include a mixture of holoenzyme, free Mediator, and free RNAPII particles, were selected from 60 micrograph pairs, and montaged for interactive screening. Holoenzyme particles were selected as objects with a recognizable polymerase and an associated density of approximately twice the mass of polymerase.

A total of 826 interactively screened and reference-free aligned zero-tilt holoenzyme images were subjected to principal component analysis using a circular mask that isolated the RNAPII portion of the structure, allowing polymerase features to dictate the segregation of the data. Hierarchical ascendant classification indicated that two groups of particles existed in the data set, one in which polymerase is in a consistent orientation with respect to the Mediator density and another in which the polymerase orientation is not as consistent.

Images of 393 tilted particles with a consistent RNAPII orientation were used to calculate a 3D holoenzyme reconstruction by back-projection. The resulting structure was then subjected to refinement by iterative projection matching, and a final volume was rendered using a threshold that resulted in a volume consistent with a molecular weight of 1.6 MDa (assuming a molecular density of  $0.83 \text{ Da}\text{\AA}^{-3}$ ) for the holoenzyme complex.

#### Determination of RNAPII Orientation in the Holoenzyme Complex

The 2D average calculated from the zero-tilt holoenzyme images was interpolated to the same pixel size ( $\sim 3.312 \text{ \AA}/\text{pixel}$ ) as a 3D RNAPII reconstruction (calculated from RNAPII particles preserved in uranyl acetate) and windowed to the same size as the RNAPII reference projections ( $80 \times 80$  pixels). This holoenzyme image was aligned and cross-correlated to a set of 1600 RNAPII projections (separated by  $5^\circ$  increments in  $\theta$ ). Cross-correlation coefficients were used to determine the best match. The RNAPII orientation determined by this analysis was used to dock the X-ray crystal structures of RNAPII (Cramer et al., 2001, Gnatt et al., 2001) into the holoenzyme complex using the program O (Jones et al., 1991) and identify RNAPII subunits involved in contacts with Mediator.

#### Acknowledgments

We gratefully acknowledge members of the Asturias laboratory, especially Wen-hsiang Chung and John L. Craighead for discussion and critical reading of the manuscript. F.J.A. is a Leukemia and Lymphoma Society of America Scholar. This work was supported by NIH grants GM60607 (F.J.A.) and GM36659 (R.D.K.).

Received: May 15, 2002

Revised: June 18, 2002

#### References

Asturias, F.J., Jiang, Y.W., Myers, L.C., Gustafsson, C.M., and Kornberg, R.D. (1999). Conserved structures of mediator and RNA polymerase II holoenzyme. *Science* 283, 985–987.

Busby, S., and Ebright, R.H. (1999). Transcription activation by catabolite activator protein (CAP). *J. Mol. Biol.* 293, 199–213.

Cosma, M.P., Panizza, S., and Nasmyth, K. (2001). Cdk1 triggers association of RNA polymerase to cell cycle promoters only after recruitment of the mediator by SBF. *Mol. Cell* 7, 1213–1220.

Craighead, J.L., Chang, W.-h., and Asturias, F.J. (2002). Structure of yeast RNA polymerase II in solution: implications for enzyme regulation and interaction with promoter DNA. *Structure* 10, 1117–1125.

Cramer, P., Bushnell, D.A., and Kornberg, R.D. (2001). Structural basis of transcription: RNA polymerase II at 2.8 angstrom resolution. *Science* 292, 1863–1876.

Dotson, M.R., Yuan, C.X., Roeder, R.G., Myers, L.C., Gustafsson, C.M., Jiang, Y.W., Li, Y., Kornberg, R.D., and Asturias, F.J. (2000). Structural organization of yeast and mammalian mediator complexes. *Proc. Natl. Acad. Sci. USA* 97, 14307–14310.

Flanagan, P.M., Kelleher, R.J., III, Sayre, M.H., Tschochner, H., and Kornberg, R.D. (1991). A mediator required for activation of RNA polymerase II transcription *in vitro*. *Nature* 350, 436–438.

Frank, J. (1996). *Three-Dimensional Electron Microscopy of Macromolecular Assemblies* (San Diego: Academic Press).

Frank, J., Radermacher, M., Penczek, P., Zhu, J., Li, Y., Ladjadj, M., and Leith, A. (1996). SPIDER and WEB: processing and visualization of images in 3D electron microscopy and related fields. *J. Struct. Biol.* 116, 190–199.

Gnatt, A.L., Cramer, P., Fu, J., Bushnell, D.A., Kornberg, R.D. (2001). Structural basis of transcription: an RNA polymerase II elongation complex at 3.3 Å resolution. *Science* 292, 1844–1846.

Jones, T.A., Zou, J.-Y., Cowan, S.W., and Kjeldgaard, M. (1991). Improved methods for building protein models in electron density maps and the location of errors in these models. *Acta Crystallogr. A* 47, 110–119.

Kang, J.S., Kim, S.H., Hwang, M.S., Han, S.J., Lee, Y.C., and Kim,

Y.J. (2001). The structural and functional organization of the yeast mediator complex. *J. Biol. Chem.* 276, 42003–42010.

Kim, Y.J., Bjorklund, S., Li, Y., Sayre, M.H., and Kornberg, R.D. (1994). A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. *Genes Dev.* 12, 45–54.

Koh, S.S., Ansari, A.Z., Ptashne, M., and Young, R.A. (1998). An activator target in the RNA polymerase II holoenzyme. *Mol. Cell* 1, 895–904.

Lee, C.L., and Kim, Y.J. (1998). Requirement for a functional interaction between Mediator components Med6 and Srb4 in RNA polymerase II transcription. *Mol. Cell. Biol.* 18, 5364–5370.

Li, Y., Bjorklund, S., Jiang, Y.W., Kim, Y.J., Lane, W.S., Stillman, D.J., and Kornberg, R.D. (1995). Yeast global transcriptional regulators Sin4 and Rgr1 are components of mediator complex/RNA polymerase II holoenzyme. *Proc. Natl. Acad. Sci. USA* 92, 10864–10868.

Myers, L.C., and Kornberg, R.D. (2000). Mediator of transcriptional regulation. *Annu. Rev. Biochem.* 69, 729–749.

Myers, L.C., Gustafsson, C.M., Bushnell, D.A., Lui, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R.D. (1998). The Med proteins of yeast and their function through the RNA polymerase II carboxy-terminal domain. *Genes Dev.* 12, 45–54.

Myers, L.C., Gustafsson, C.M., Hayashibara, K.C., Brown, P.O., and Kornberg, R.D. (1999). Mediator protein mutations that selectively abolish activated transcription. *Proc. Natl. Acad. Sci. USA* 96, 61–72.

Niu, W., Kim, Y., Tau, G., Heyduk, T., and Ebright, R.H. (1996). Transcription activation at class II CAP-dependent promoters: two interactions between CAP and RNA polymerase. *Cell* 87, 1123–1134.

Nonet, M., Sweetser, D., and Young, R.A. (1987). Functional redundancy and structural polymorphism in the large subunit of RNA polymerase II. *Cell* 50, 909–915.

Rachez, C., and Freedman, L.P. (2001). Mediator complexes and transcription. *Curr. Opin. Cell Biol.* 13, 274–280.

Radermacher, M., Wagenknecht, T., Verschoor, A., and Frank, J. (1987). Three-dimensional reconstruction from a single-exposure random conical tilt series applied to the 50s ribosomal subunit of *Escherichia coli*. *J. Microsc.* 146, 113–136.

Sayre, M.H., Tschochner, H., and Kornberg, R.D. (1992). Reconstitution of transcription with five purified initiation factors and RNA polymerase II from *Saccharomyces cerevisiae*. *J. Biol. Chem.* 267, 23376–23382.

Stöffler, G., and Stöffler-Meilicke, M. (1983). The ultrastructure of macromolecular complexes studied with antibodies. In *Modern Methods in Protein Chemistry*, H. Tesche, ed. (Berlin: De Gruyter), pp. 409–455.

Svejstrup, J.Q., Feaver, W.J., LaPointe, J., and Kornberg, R.D. (1994). RNA polymerase transcription factor II holoenzyme from yeast. *J. Biol. Chem.* 269, 28044–28048.

Svejstrup, J.Q., Li, Y., Fellows, J., Gnatt, A., Bjorklund, S., and Kornberg, R.D. (1997). Evidence for a mediator cycle at the initiation of transcription. *Proc. Natl. Acad. Sci. USA* 94, 6075–6078.

Taatjes, D.J., Naar, A.M., Andel, F. 3<sup>rd</sup>, Nogales, E., Tjian, R. (2002). Structure, function, and activator-induced conformations of the CRSP coactivator. *Science* 295, 1058–1062.

Tan, Q., Linask, K.L., Ebright, R.H., and Woychik, N.A. (2000). Activation mutants in yeast RNA polymerase II subunit RPB3 provide evidence for a structurally conserved surface required for activation in eukaryotes and bacteria. *Genes Dev.* 14, 339–348.

Tischendorf, G.W., Zeichhardt, H., and Stöffler, G. (1974). Determination of the location of proteins L14, L17, L18, L19, L22, L23 on the surface of the 50S ribosomal subunit of *Escherichia coli* by immune electron microscopy. *Mol. Gen. Genet.* 134, 187–208.

Yudkovsky, N., Ranish, J.A., and Hahn, S. (1999). A transcription reinitiation intermediate that is stabilized by activator. *Nature* 408, 225–229.