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RESEARCH COMMUNICATION

Structure–system correlation identifies a gene regulatory Mediator submodule

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A combination of crystallography, biochemistry, and gene expression analysis identifies the coactivator subcomplex Med8C/18/20 as a functionally distinct submodule of the Mediator head module. Med8C forms a conserved α-helix that tethers Med18/20 to the Mediator. Deletion of Med8C in vivo results in dissociation of Med18/20 from Mediator and in loss of transcription activity of extracts. Deletion of med8C, med18, or med20 causes similar changes in the yeast transcriptome, establishing Med8C/18/20 as a predominantly positive, genespecific submodule required for low transcription levels of nonactivated genes, including conjugation genes. The presented structure-based system perturbation is superior to gene deletion analysis of gene regulation.

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Many different transcriptional activators and repressors regulate RNA polymerase (Pol) II transcription in eukaryotic cells. These regulatory factors generally bind to upstream DNA sites and recruit large coregulatory complexes such as the Mediator (Malik and Roeder 2000; Naar et al. 2001; Bjorklund and Gustafsson 2005; Kornberg 2005). Mediator complexes were isolated from fungi, metazoans, and a plant (Boube et al. 2002; Backstrom et al. 2007). Mediator complexes from the yeast Saccharomyces cerevisiae (Sc) is a 1-MDa complex that comprises 25 subunits, of which 11 are essential for viability and 22 are at least partially conserved among eukaryotes. Mediator promotes initiation complex assembly through contacts with activators, Pol II, and general transcription factors.

Mediator subunits reside in different modules named head, middle, tail, and kinase modules (Dotson et al. 2000; Kang et al. 2001). Apparently the Mediator modules are required for the regulation of different subsets of genes. Gene deletion studies implicated the middle module in regulating HSP genes and low-iron response genes, the tail module in regulating HSP and OXPHOS genes, and the kinase module in regulating genes required during nutrient starvation (Holstege et al. 1998; Beve et al. 2005; van de Peppel et al. 2005; Singh et al. 2006).

The Mediator head module is important for initiation complex assembly, stimulates basal transcription, and is necessary for activated transcription (van de Peppel et al. 2005). The head module contains subunits Med6, Med8, Med11, Med17, Med18, Med20, and Med22, which are conserved from yeast to human. Head subunits are essential for yeast viability, except for Med18 and Med20 (Koleske et al. 1992; Thompson et al. 1993, Larivièere et al. 2006). In vitro, Med18 and Med20 are required for formation of a stable initiation complex, for efficient basal transcription, and for activated transcription (Thompson et al. 1993; Lee et al. 1999; Ranish et al. 1999). In vivo, Med18 and Med20 regulate transcription of the same subset of genes and have a mainly positive function (van de Peppel et al. 2005).

Based on structural analysis, we proposed previously that the trimeric subcomplex of the C-terminal domain of Med8 (Med8C), Med18, and Med20 (the Med8C/18/20 subcomplex) forms a conserved functional submodule of the Mediator head (Larivièere et al. 2006). Here, we confirm this proposal with a combination of X-ray analysis, yeast genetics, biochemistry, and transcriptomics. Our results indicate that Mediator contains functionally distinct submodules within its previously defined modules, and show how gene regulatory submodules can be identified by a combination of structural and functional studies on the molecular level and gene expression analysis on the systems level.

Results and Discussion

Med8C/18/20 is a subcomplex of the Mediator head

Our previous analysis revealed that Sc Med8 contains an essential N-terminal domain [Med8N, residues 1–137], followed by a nonessential linker (residues 138–189) and a C-terminal region that includes a α-helix [Med8C, residues 190–223] [Fig. 1A, Larivièere et al. 2006]. We proposed that Med8C tethers the Med18/20 heterodimer to the essential part of the Mediator head [Fig. 1A]. To test this, we asked whether Med8C tethers the Med18/20 heterodimer to Mediator in vivo. We isolated Mediator by tandem affinity purification [TAP] from yeast strains expressing a TAP-tagged head subunit, Med17, and identified the copurifying Mediator subunits by mass spectrometry [Fig. 1B]. The same purification from a strain expressing a truncated version of Med8 that lacked Med8C [med8CΔ] resulted in a very similar pattern of protein bands, except that Med18 and Med20 were missing [Fig. 1B]. Therefore, retention of Med18 and Med20 in the Mediator requires Med8C. The truncated Med8 variant was, however, present in the preparation, showing that Med8C is not required to retain Med8 in the Mediator. These data are consistent with interaction data
Figure 1. (A) Overview of the Mediator architecture with the four modules head, tail, and kinase. The head module is separated in the core head, constituted of Med6, Med6N, Med11, Med17, Med22, and the nonessential subcomplex of Med8C, Med18, and Med20 (in orange, blue, and magenta, respectively). (B) Deletion of Med8C in vivo leads to loss of Med18/20 after Mediator purification. N-terminally TAP-tagged Med17 was purified from wild-type yeast or from cells expressing Med8ΔC. The EGTA eluate after purification was separated using 4%–12% discontinuous SDS-PAGE and was analyzed by mass spectrometry after Coomassie staining. The sequenced copurifying proteins are labeled. Arrows mark Med8, Med8CΔ, Med18, and Med20. An asterisk indicates TAP-tagged Med17.

Conserved Med8C/18/20–core head interface

Because of the low sequence homologies between Mediator subunits from different species, the architecture of the Sc head could be a species-specific feature. To investigate this, we solved the crystal structure of the Med8C/18 complex from Schizosaccharomyces pombe (Sp) [Materials and Methods]. We coexpressed Sp Med18 with a hexahistidine-tagged Sp Med8C fragment corresponding to the Sc Med8C fragment used previously (Lariviere et al. 2006) from a bicistronic vector in Escherichia coli. The Sp Med8C fragment was sufficient for interaction with Sp Med18 [data not shown]. The resulting stoichiometric Med8C/18 complex was crystallized and the structure solved [Materials and Methods, Supplemental Table 1]. Sp Med18 adopts a fold similar to its Sc ortholog [Fig. 2A], with a root mean square deviation of 1.7 Å over 173 Ca atoms. Sp Med8C forms an α-helix, followed by a glycine-containing turn, and binds Sp Med18 across its central β-barrel as observed for its Sc counterpart [Fig. 2A]. Key contact residues in the Med8C–Med18 interface are conserved between Sc and Sp [Fig. 2B]. Given the large phylogenetic distance between these two fungi, the Med8C/18 interface is apparently also conserved in Mediator complexes of higher eukaryotes. Indeed, modeling of the human Med8C–Med18 interface showed that key contacts are conserved [data not shown]. Thus, the structural tethering of the Med18/20 heterodimer to the core head module through Med8C is conserved among eukaryotes.

Med8C/18/20 is required for activated transcription in vitro

To investigate whether the structural subcomplex Med8C/18/20 is also a functional subcomplex of the Mediator, we conducted in vitro transcription assays. We prepared nuclear extracts from yeast strains carrying a deletion of the gene for Med18 [med18Δ] or lacking the part of the Med8 gene coding for Med8C [med8CΔ]. Consistent with previous data [Thompson et al. 1993; Ranish et al. 1999], med18Δ nuclear extract did not support activated transcription [Fig. 3, lane 1], apparently since Mediator in this mutant lacks both Med18 and Med20. The transcription defect could indeed be rescued by addition of recombinant Med8C/18/20 [Fig. 3, lanes 5, 6]. This is consistent with the model that Med8C, which is present in the extract, tethers Med18/20 to the Mediator. Recombinant Med8C/18/20 subcomplex was far less efficient in rescue [Fig. 3, lanes 3, 4], likely because endogenous Med8C fails to replace recombinant Med8C for tethering Med18/20. Consistently, a nuclear extract from the med8CΔ strain was inactive [Fig. 3, lane 1], apparently since its Mediator complex lacks Med18 and Med20. Even a large excess of Med18/20 could not rescue the defect [Fig. 3, lanes 5, 6], but recombinant Med8C/18/20 could partially restore transcription [Fig. 3, lane 4]. Thus, Med8C is essential for activated transcription in these assays. Since providing Med8C in trans can partially rescue the defect, Med8C apparently also interacts nonco-
those, 117 were down-regulated, and 48 were up-regulated. Analysis of the expression profiles revealed that a total of 874 genes reside in the same functional module. The expression profiles for these deletion strains of the Med8C/18/20 submodule. Of these, 117 were down-regulated, and 48 were up-regulated, showing that the module predominantly acts as a positive factor in transcription, but can also act as a negative factor for certain genes. Of the down- and upregulated genes, 44% and 37%, respectively, were not annotated in the gene ontology (GO) database [Fig. 4C; Ashburner et al. 2000]. Since we had shown previously that Med8C/18/20 binds to the TATA-box-binding protein (TBP) in vitro [Lariviére et al. 2006], we were encouraged to unravel a molecular basis for Med8C/18/20 function by searching for reoccurring promoter elements or upstream motifs in the deregulated genes, and for common transcription factors known to regulate these genes. This search was, however, unsuccessful, suggesting a complex context-dependent mechanism of Med8C/18/20 function.

We next analyzed the cellular function of genes regulated by Med8C/18/20 and by Med2 or Med3 with the GO Slim Mapper [Materials and Methods; Supplemental Table 2]. Many of the affected biological processes, including amino acid derivative metabolism, carbohydrate metabolic process, and vitamin metabolic process, were overrepresented in both the Med8C/18/20-regulated and Med2/3-regulated genes [Fig. 4D; Supplemental Table 2], reflecting the partial overlap of the expression profiles [Fig. 4A]. However, some biological processes were overrepresented only among Med8C/18/20- or Med2/ Med3-regulated genes. In particular, genes involved in conjugation were specifically Med8C/18/20-regulated, whereas genes involved in sporulation and cell wall organization and biogenesis were specifically Med2/Med3-regulated [Fig. 4E]. These findings are consistent with the previously reported involvement of Med18 in conjugation [Holstege et al. 1998] and with a large-scale functional genomics analysis of sporulation efficiency [Ensenihi and Saunders 2003].

**Down-regulation of nonactivated genes and basal promoter activity**

Down-regulation of conjugation genes in med8CΔ, med18Δ, and med20Δ deletion strains was surprising, since their transcription was expected to be repressed under our experimental conditions. However, the same phenomenon was observed for other genes that should be repressed under optimal growth conditions. The GAL4 gene is further down-regulated in the med8CΔ, med18Δ, and med20Δ deletion strains, but not in the control strains lacking med2 or med3. Genes involved in the catabolism of serine and glycine are further down-regulated in all deletion strains, including CHA1, GCV1, GCV2, and GCV3. CHA1 shows the most down-regulated transcript levels, although it is expected to be expressed only under nitrogen-limiting conditions. Thus, Med8C/18/20 is required for low levels of transcription of nonactivated genes.

Taken together, many genes that are repressed under our growth conditions were apparently transcribed at a low level in a Med8C/18/20-dependent manner. The requirement of Med8C/18/20 for low-level transcription of nonactivated genes is consistent with the observation that even low levels of transcription require Mediator [Kornberg 2005; Takagi and Kornberg 2006]. Although an indirect effect cannot be ruled out, our data suggest that Med8C/18/20 is important for basal promoter activity, and are consistent with a possible TBP interaction in vivo. A role of Mediator in basal transcription may be further investigated by similar analyses of other Media-
The Mediator head module is essential for cell viability, and a temperature-sensitive point mutation in the head leads to a global defect in transcription (Holstege et al. 1998). Here, we show that the head module contains the distinct conserved Med8C/18/20 submodule that is not essential for viability and regulates only a subset of genes. Identification and characterization of the Med8C/18/20 submodule required a combination of structural biology, yeast genetics, biochemistry, and transcriptome analysis. Our results support the idea that the known Mediator modules head, middle, and tail contain distinct submodules with different functions that are involved in the regulation of different subsets of genes.

Figure 4. Transcriptome profiling analysis. [A] Diagram of genes exhibiting significantly altered mRNA levels (vertical axis) for different Mediator deletion strains (horizontal axis), clustered alongside the med18Δ, med20Δ, and med8CΔ expression profiles (indicated by black bar). Changes in mRNA levels compared with the wild-type strain are depicted in red (up), green (down), or black (no change). [B] Pearson’s correlation matrix for expression profiles of strains med8CΔ, med18Δ, and med20Δ, and tail subunits deletion strains med2Δ and med3Δ. [C] Number of significantly altered genes in all three deletion strains of the Med8C/18/20 submodule. Genes not annotated in the GO database are depicted in gray, up-regulated genes are indicated in red, and down-regulated genes are shown in green. [D] Percentage of genes for which expression was significantly changed in all deletion mutants of the Med8C/18/20 [blue] submodule or Med2 and Med3 [yellow] compared with the percentage of the genome [black]. All 32 biological processes from GO Slim Mapper are shown as follows: amino acid derivative metabolic process (1), anatomical structure morphogenesis (2), carbohydrate metabolic process (3), cell budding (4), cell cycle (5), cell wall organization and biogenesis (6), cellular homeostasis (7), cellular respiration (8), conjugation (9), cytokinesis (10), cytoskeleton organization and biogenesis (11), DNA metabolic process (12), electron transport (13), generation of precursor metabolites and energy (14), lipid metabolic process (15), meiosis (16), membrane organization and biogenesis (17), nucleosome organization and biogenesis (18), organelle organization and biogenesis (19), protein catabolic process (20), protein modification process (21), pseudohyphal growth (22), response to stress (23), ribosome biogenesis and assembly (24), RNA metabolic process (25), signal transduction (26), sporulation (27), transcription (28), translation (29), vesicle-mediated transport (31), and vitamin metabolic process (32). Overrepresented processes are marked with a circle. [E] Percentage of genes involved in conjugation, sporulation, and vitamin metabolic process for which expression was significantly changed in all deletion mutants of the Med8C/18/20 submodule or Med2 and Med3. Red and green histograms correspond to up- and down-regulated genes, respectively. The dotted line represents the percentage of the genome involved in the respective process.
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Structure-based system perturbation analysis of gene regulation

More generally, we demonstrate how structural and functional information obtained on the molecular level in vitro can be correlated with changes on the systems level in vivo. In particular, the structure-guided design of mutant yeast strains enabled a precise disruption of molecular interactions and their functional analysis in vitro and in vivo. This approach is superior to the generally used gene deletion analysis, which does not take into account the consequences of such deletions for native protein complex structures. Most proteins reside in complexes (Gavin et al. 2002, 2006; Krogan et al. 2006), and gene deletion will often result in complex disintegration and malfunction, and thus in complicated changes of the transcriptome, which renders correlations between the molecular and the systems level difficult or impossible.

In contrast, the structure-based systems perturbation analysis conducted here reduces the complexity of differential expression patterns, and facilitates the dissection of transcriptional coregulatory complexes into distinct functional submodules. In the future, this approach may be used for a reliable analysis of gene regulatory molecular networks. In particular, structure-based perturbation of cooperative molecular interactions could elucidate combinatorial and context-dependent gene regulatory mechanisms on the system level.

Materials and methods

TAP was performed essentially as described (Puig et al. 2001). Recombinant proteins were expressed in E. coli. For protein coexpression, multicistronic vectors were constructed as described (Larivière et al. 2006). Proteins were purified by affinity chromatography, eventually followed by anion exchange chromatography and gel filtration for crystallization purposes. For Sc Med18/20, affinity purification was replaced by a precipitation with 30% saturated ammonium sulfate. Crystals of Med18 were grown at 20°C by air-lift hanging drops and of the Med8C/18 heterodimer were grown at 20°C by vapour diffusion with 30% saturated ammonium sulfate. Crystals of Med18 were of the p6322 form and of the Med8C/18 heterodimer were grown at 20°C by vapour diffusion with 30% saturated ammonium sulfate. Crystals of the Med8C/18 heterodimer were grown at 20°C in hanging drops over reservoirs containing 100 mM Tris [pH 8.5], 2 M sodium acetate, and 2 M sodium formate. The Med18 structure was solved by the single-wavelength anomalous dispersion method using selenomethionine-labeled protein crystals. The Med8C/Med18 structure was solved by molecular replacement using the Med18 structure as a model. For crystallographic details, see Supplemental Table 1. The Sc Med8C/18 structure was deposited in the Protein Data Bank under accession code 3C0T. Nuclear extracts were purified from yeast cultures as described (http://www.hicrc.org/labs/ahahn). Plasmid transcription and analysis by primer extension were performed essentially as described (Ranish and Hahn 1991). Instead of the 32P-labeled lacI-oligo, 0.125 pmol fluorescently labeled 5'-FAM-oligo was used. For gene expression profiling, all strains were isogenic to the S288c strain. Truncation variant was prepared by introducing the lacI-H9004med8C cassette into the yeast chromosome by two-step transformation into the MED8 array. Instead of the32P-labeled lacI-oligo, 0.125 pmol fluorescently labeled 5'-FAM-oligo was used. For gene expression profiling, all strains were transformed with the pUG28 plasmid that expresses the lacI-oligo at a high concentration.

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