Analysis of *Schizosaccharomyces pombe* Mediator reveals a set of essential subunits conserved between yeast and metazoan cells

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Edited by Roger D. Kornberg, Stanford University School of Medicine, Stanford, CA, and approved July 24, 2001 (received for review May 22, 2001)

With the identification of eight new polypeptides, we here complete the subunit characterization of the *Schizosaccharomyces pombe* RNA polymerase II holoenzyme. The complex contains homologs to all 10 essential gene products present in the *Saccharomyces cerevisiae* Mediator, but lacks clear homologs to any of the 10 *S. cerevisiae* components encoded by nonessential genes. *S. pombe* Mediator instead contains three unique components (Pmc2, -3, and -6), which lack homologs in other cell types. Presently, *pmc2*$^+$ and *pmc3*$^+$ have been shown to be nonessential genes. The data suggest that *S. pombe* and *S. cerevisiae* share an essential protein module, which associates with nonessential species-specific subunits. In support of this view, sequence analysis of the conserved yeast Mediator components Med4 and Med8 reveals sequence homology to the metazoan Mediator components Trap36 and Arc32. Therefore, 8 of 10 essential genes conserved between *S. pombe* and *S. cerevisiae* also have a metazoan homolog, indicating that an evolutionary conserved Mediator core is present in all eukaryotic cells. Our data suggest a closer functional relationship between yeast and metazoan Mediator than previously anticipated.

RNA polymerase II | transcription

The Mediator complex is essential for basal and regulated expression of nearly all RNA polymerase II-dependent genes in the *Saccharomyces cerevisiae* genome (1). Mediator acts as a bridge, conveying regulatory information from enhancers and other control elements to the promoter (2). The functional activities identified for Mediator include stimulation of basal transcription, support of activated transcription, and enhancement of phosphorylation of the C-terminal domain of pol II by the transcription factor IIH kinase (3, 4). In addition, another possible mechanism for Mediator function was recently suggested by the finding that the Nut1 subunit of *S. cerevisiae* Mediator is a histone acetyltransferase (5).

Mediator-like complexes have also been isolated from *Caenorhabditis elegans*, mouse, and human cells (6–13). Thus far, only 6 of the 20 subunits in *S. cerevisiae* Mediator have been shown to have homologous counterparts in these metazoan complexes (14, 15). The low degree of conservation at the primary sequence level has raised the possibility that Mediator-like complexes in metazoan cells may be significantly different both in structure and in function from yeast Mediator (8). Arguing against this notion, single-particle analysis by electron microscopy and image processing has demonstrated striking structural similarities between Mediator isolated from yeast, mouse, and human cells (16, 17).

S. pombe and *S. cerevisiae* are highly diverged from one another in evolution. Cross-species comparisons between the two yeasts have proven, for example, a valuable tool in analyzing cell cycle control (18). We have initiated a project aiming to compare Mediator structure and function between *S. cerevisiae* and *S. pombe*. Earlier, we reported on the isolation of a *S. pombe* RNA polymerase II holoenzyme (19). We now present a complete subunit characterization of this multiprotein complex. Our analysis extends the number of subunits conserved in all Mediator complexes studied to date and suggests a closer relationship between yeast and metazoan Mediators than previously assumed.

Materials and Methods

Abs and Immunoblot Analyses. Synthetic polypeptides corresponding to amino acids 1–15 of Pmc3 (MSLEEQRTRDELKHK) and amino acids 59–75 of Pmc6 (CSNIESHDETDTEWSK) were used to immunize rabbits. The antisera used in this study were taken 10 days after the second booster injection (Innovagen AB, Lund, Sweden). Recombinant Med10 (full-length cDNA) fused to 6 histidine residues was overproduced in *Escherichia coli* BL21 (DE3) pLysS strain (Stratagene), purified by using nickel column (Probond, Invitrogen), and used to immunize rabbits. The antisera used in this study correspond to the final bleeding (Eurogentec, Brussels).

Protein Identification. RNA polymerase II holoenzyme was purified as described (19). Pmc3, Pmc6, spMed8, and spSrb6 were identified as described earlier (19). For identification of spMed4, spMed6, spRox3, spNut2, and spSrb7, holoenzyme fractions from the Heparin stage were separated on SDS/PAGE and revealed by staining with silver. Matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF) analysis of in-gel digested proteins was carried out with a Reflex III mass spectrometer from Bruker (Billerica, MA). The samples were prepared by using sequencing grade-modified trypsin from Promega and were analyzed essentially as described (20). Database searches were done with the MS BIOTOOLS software from Bruker by using the Mascot search engine (www.matrixscience.com/cgi/index.pl?page =../home.html).

Glutathione *S*-Transferase (GST) Pull-Down Assay. The GST fusion proteins were expressed in *E. coli* BL21-CodonPlus-RIL (Stratagene) and bound to Glutathione-Sepharose as described by the supplier (Amersham Pharmacia Biotech). Beads corresponding to about 5 μg of GST fusion protein were incubated with 5–10 μl of holoenzyme purified to the MonoQ stage (19). The beads were rotated for 3 h at 4°C with 200 μl of binding buffer (20 mM

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This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: GST, glutathione *S*-transferase.

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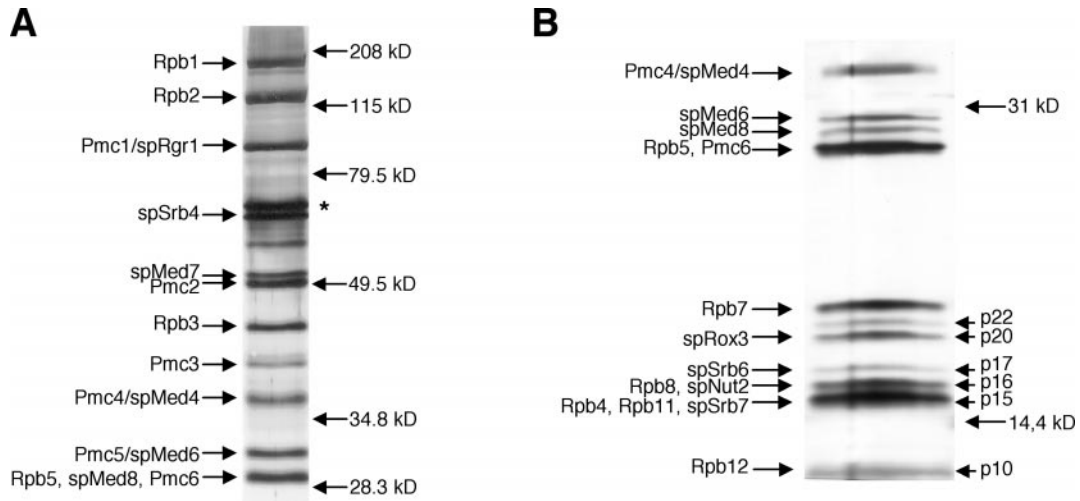

Fig. 1. Polypeptide composition of the *S. pombe* RNA polymerase II holoenzyme. (*A*) Peak fractions from the Superose 12 stage were pooled and analyzed by SDS/PAGE in a 12% gel. The individual RNA polymerase II holoenzyme subunits are indicated on the left. The band just above Srb4 (*) is a contaminant corresponding to the fingerprint region. (*B*) To obtain a clearer visualization of polypeptides in the low molecular mass range, RNA polymerase II holoenzyme was analyzed in an 8–16% gel. Staining with silver revealed proteins. The positions of specific subunits are shown on the left. Arrowheads on the right indicate individual bands referred to in the text as p22, p20, etc. [*A* is Reproduced with permission from ref. 19 (Copyright 2000, American Society for Biochemistry and Molecular Biology).]

Tris-acetate, pH 7.8/1 mM EDTA/10% glycerol/1 mM DTT/0.8 M potassium acetate) supplemented with protease inhibitors (Complete, Roche Molecular Biochemicals). After 4 washes with the binding buffer, the beads were dried under vacuum, resuspended into SDS loading buffer, and analyzed on SDS/PAGE gel. Immunoblot analysis was carried out by using anti-Srb4 polyclonal Ab and IgG-purified Med10 Ab.

Genetic Manipulation. Yeast transformation used lithium acetate as described (21). Yeast strains used in this study are listed in Table 2, which is published as supporting information on the PNAS web site, www.pnas.org. Deletions of the entire coding regions of the *med7*$^+$, *med4*$^+$, *srb4*$^+$, *pmc2*$^+$, and *pmc3*$^+$ genes were performed by the single-step gene disruption technique, using PCR amplification of selectable markers with long tracts of flanking homology (21). For each gene, two sets of primers were used (Table 3, which is published as supporting information on the PNAS web site). The gene-targeting product was cloned by using the pGEM-T Easy vector system I (Promega), and the identity of the flanking regions was verified by DNA sequencing. The final DNA fragments used for yeast transformation were amplified with *Pfu* polymerase with these plasmids as template. All PCR knockout products were genetically marked with G418 resistance. In the cases of *med7*$^+$, *med4*$^+$, and *srb4*$^+$, these PCR products were transformed into the *S. pombe* diploid strain PG1141/SP837; *pmc2*$^+$ and *pmc3*$^+$ knockouts were done in the diploid strain CHP428/CHP429 (Table 2). Yeast genomic DNA was isolated and PCR analysis verified that the kanMX cassette had recombined correctly into the target gene. Southern blot analysis was used to confirm the molecular structure of the integration event and demonstrated that in each case, no additional copy of the kanMX cassette was present in the genome. The obtained G418-resistant diploids were sporulated and asci (>15) dissected on either MSA (minimal) plates supplemented with appropriate amino acids (22) or YES (rich) plates (BIO101).

Dissection of tetrads of the *med4*$^+$/*med4*::*G418*^{R} (strain TP1), *med7*$^+$/*med7*::*G418*^{R} (TP2), and *srb4*$^+$/*srb4*::*G418*^{R} (TP5) diploids resulted in two viable spores. All viable spores were G418-sensitive, showing that all three genes are essential. All four spores from the tetrads from the *pmc2*$^+$/*pmc2*::*G418*^{R} (TP9) and *pmc3*$^+$/*pmc3*::*G418*^{R} (TP14) germinated and scored 2:2 for G418 resistance, showing that *pmc2*$^+$ and *pmc3*$^+$ are nonessential genes.

Results

Identification of spMed4, spMed6, spMed8, Pmc3, and Pmc6. In previous work, *S. pombe* holoenzyme was purified and 15 polypeptides in the size range between 20–200 kDa were described (Fig. 1*A*; ref. 19). Six of the polypeptides corresponded to subunits of RNA polymerase II. Four proteins were the *S. pombe* homologs to *S. cerevisiae* Mediator components Rgr1, Srb4, Med7, and Nut2. One protein, Pmc2, was a novel gene product, which displayed no significant homology to any entries in the protein databases. The remaining four bands, denoted Pmc3, -4, -5, and -6, were characterized in the present work as described in what follows.

Purified RNA polymerase II holoenzyme was separated on SDS/PAGE and stained with Coomassie brilliant blue (data not shown) or silver (Fig. 1*B*). The Pmc3, -4, -5 and -6 bands were cut out and identified through trypsin cleavage and mass fingerprinting by using matrix-assisted laser desorption ionization–time-of-flight mass spectrometry. All four proteins generated mass fingerprinting data compatible with hypothetical ORFs in the *S. pombe* database (Table 1). The amino acid sequences were used for BLAST searches of nucleotide databases. Pmc3 did not demonstrate sequence homology with any entries in the databases. Pmc4 is homologous to the *S. cerevisiae* Mediator component Med4. The sequences exhibited 28% identity and 51% similarity ($E = 2.6 \times 10^{-08}$) in a region spanning amino acids 73 and 193 in Med4. Pmc4 will from now on be referred to as spMed4. Similarly, the Pmc5 sequence exhibited 27% identity and 48% similarity ($E = 8.4 \times 10^{-28}$) to a region spanning amino acids 103 and 228 in *S. cerevisiae* Med6. Therefore, Pmc5 will from now on be referred to as spMed6.

The Pmc6 bands corresponded to three individual proteins, one of which was the RNA pol II subunit Rpb5. The second protein exhibited 30% identity and 52% similarity ($E = 2.7 \times 10^{-19}$) to a region spanning amino acids 31 and 210 in *S. cerevisiae* Med8, and will from now on be referred to as spMed8. The third protein did not demonstrate sequence homology with

Table 1. *S. pombe* Mediator subunit composition

Protein	No. of peaks identified	Sequence coverage, %	Deletion phenotype	*S. cerevisiae* homolog	Human homolog	Apparent mass,‡ kD	Accession no. (NCBI)
Pmc1/Rgr1	13	25	ND	+	+	90	CAA20115
Srb4*	10	25	Inviable	+	+	62	CAB10081
Med7*	8	19	Inviable	+	+	51	CAA19326
Pmc2*	9	30	Conditional			49	Q09696
Pmc3*	5	21	Conditional			40	Q10477
Pmc4/Med4	8	37	Inviable	+	+	38	T39283
Pmc5/Med6†	7	49	ND	+	+	30	CAB65615
Pmc6*	5	29	ND			29.5	O14198
Med8	5	46	Inviable	+	+	29	CAB36884
Rox3	3	26	ND	+		21	T40987
Srb6	6	45	ND	+		17.5	O14010
Nut2*	6	40	ND	+	+	16	CAB10086
Srb7†	4	20	ND	+	+	15.5	CAA22343

*Verified with immunoblotting. ND, not determined; NCBI, National Center for Biotechnology Information.
†Verified with matrix-assisted laser desorption ionization–MS post-source decay analysis (33).
‡As judged by SDS/PAGE analysis with respect to molecular mass standards.

any entries in the databases and will from now on be referred to as the Pmc6 protein.

Thus, the Pmc3 and Pmc6 proteins fall into the family of Mediator subunits with no known homologs in other eukaryotic cells. To confirm their presence in the *S. pombe* Mediator, Abs were raised against Pmc3 and Pmc6. Immunoblot analysis of heparin fractions demonstrated coelution of Pmc3 and Pmc6 with spSrb4 and Rpb1 (Fig. 2*A*). Association of Pmc3 and Pmc6 with the holoenzyme was demonstrated further with gel filtration performed at high ionic strength to minimize nonspecific protein–protein interactions. Immunoblot analysis showed coelution from a Superose 12 column of Rpb1, spSrb4, Pmc3, and Pmc6 (Fig. 2*B*). We conclude that Pmc3 and Pmc6 are bona fide members of the *S. pombe* Mediator complex.

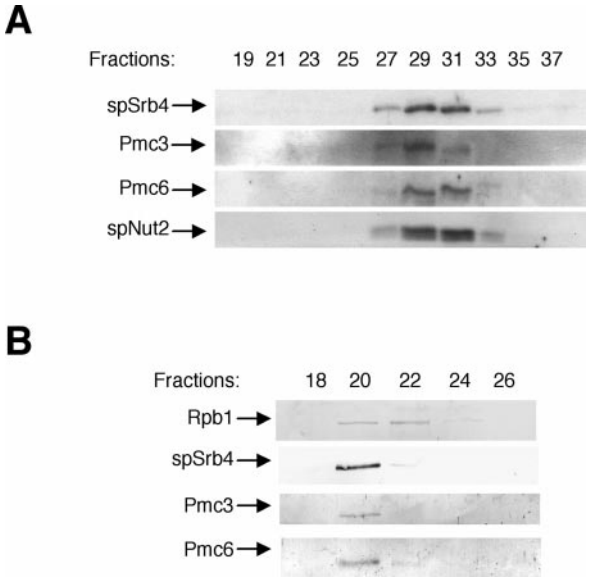

Fig. 2. Mediator proteins comigrate during heparin chromatography and gel filtration. (*A*) Holoenzyme was eluted from the 1-ml heparin HiTrap column with 12 ml of gradient of 0.1–1.0 M potassium acetate. Fractions were separated on 12% SDS/PAGE and immunoblotted with Abs directed against the proteins indicated. (*B*) The mediator peak from heparin was subjected to gel filtration through Superose 12. Fractions were analyzed by SDS/PAGE in a 10% gel and immunoblotted with Abs directed against the proteins indicated.

Identification of spRox3, spSrb6, spNut2, and spSrb7. To investigate the presence of additional subunits in the low molecular mass range below 21 kDa, purified RNA polymerase II holoenzyme was analyzed by SDS/PAGE in a 8–16% gradient gel and staining with silver (Fig. 1*B*). Six bands identified, ranging from 10 to 22 kDa, were cut out and identified with mass fingerprinting and post source decay analysis. The band of apparent mass of 20 kDa (denoted p20 in Fig. 1*B*) exhibited 38% identity and 61% similarity ($E = 2.6 \times 10^{-08}$) to a region spanning amino acids 14 and 83 in the *S. cerevisiae* Mediator protein Rox3. Sequences from p17 showed 25% identity and 43% similarity ($E = 8.5 \times 10^{-04}$) with a region spanning amino acids 1 and 107 in the *S. cerevisiae* Mediator protein Srb6. The p16 band corresponded to two individual polypeptides. One was the pol II subunit Rpb8. The other was spNut2, which has been identified as a subunit of *S. pombe* Mediator (19). The p15 band contained three individual polypeptides. Two were the pol II subunits Rpb4 and Rpb11. The third was the gene product of a previously uncharacterized ORF, which demonstrated 31% identity and 51% similarity ($E = 9.6 \times 10^{-15}$) to a region spanning amino acids 1 and 129 in *S. cerevisiae* Srb7. Finally, the p10 band contained the pol II subunit Rpb12. Except for the p22 band, which contained only keratin, no additional proteins could be identified in our RNA polymerase II holoenzyme preparation. We therefore conclude that *S. pombe* Mediator contains 13 individual subunits with a combined molecular mass of about 490 kDa.

Requirement of *S. pombe* Mediator Subunits for Cell Viability. We noted that the Mediator components conserved between *S. cerevisiae* and *S. pombe* Mediator were all encoded for by genes needed for *S. cerevisiae* cell viability (Table 1). In contrast, the nonessential gene products in *S. cerevisiae* Mediator all lacked a homolog in the *S. pombe* complex. We decided to investigate whether the *S. pombe* genes encoding Mediator components displayed the same genetic pattern, with conserved subunits being essential for viability and species-specific subunits being nonessential. To this end, *S. pombe* diploid strains were constructed heterozygous for Δ*srb4*, Δ*med4*, Δ*med7*, Δ*pmc2*, and Δ*pmc3*, respectively (Tables 2 and 3, which are published as supporting information on the PNAS web site, www.pnas.org). After sporulation and tetrad analysis, viability segregated 2:2 for the Δ*srb4*, Δ*med4*, and Δ*med7* deletions. Tetrad analysis of the Δpmc2 and Δpmc3 deletion strain yielded four viable spores. Therefore, gene products conserved between *S. pombe* and *S.*

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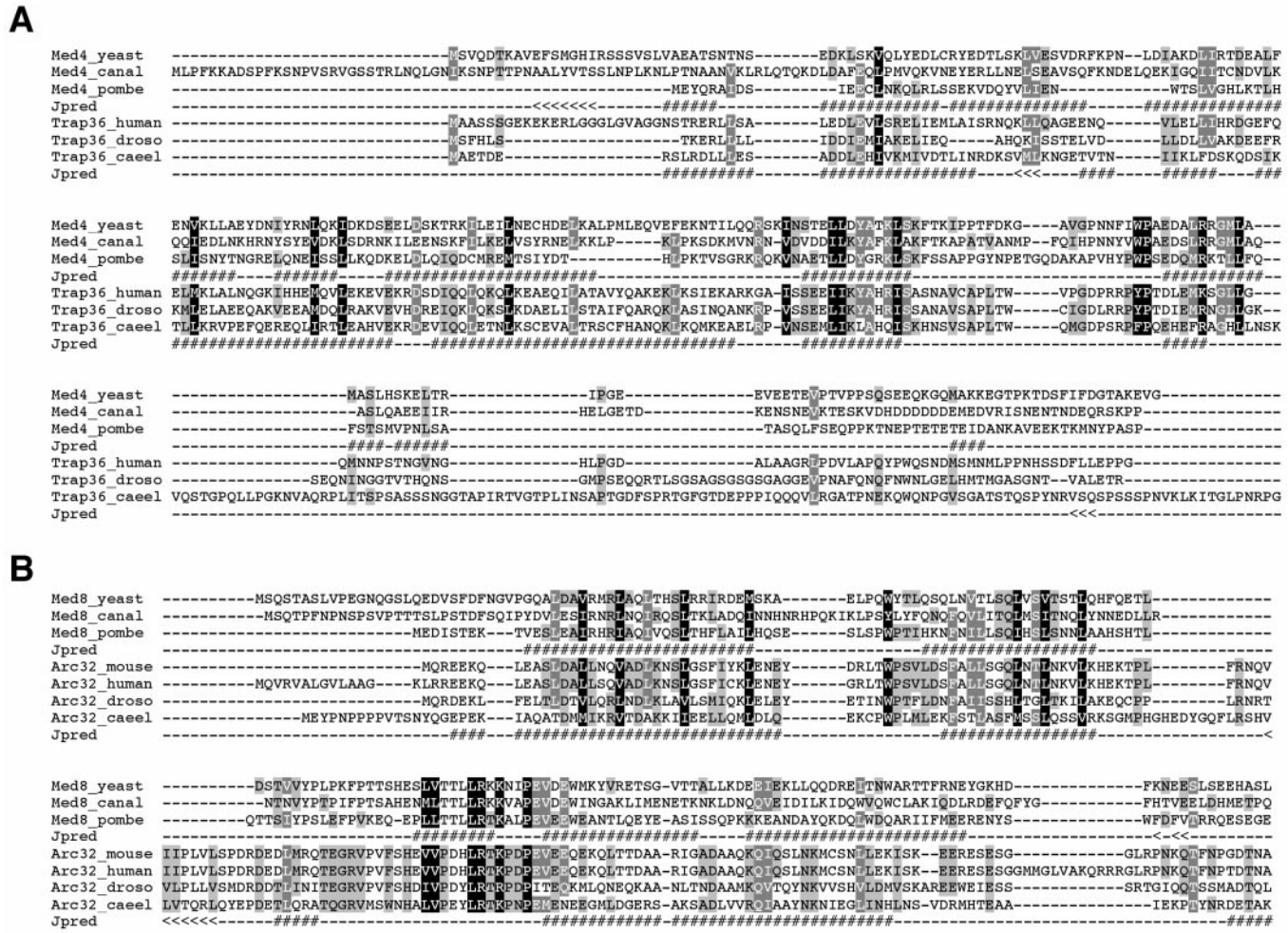

Fig. 3. Alignment of yeast mediator proteins and putative metazoan homologs. Multiple alignments of yeast and metazoan sequences, respectively, were used as a basis for secondary structure prediction by using the Jpred consensus method (34). Helical structures are indicated by "#" and beta structures by "<" symbols. (*A*) Alignments of yeast Med4 proteins and metazoan Trap36 homologs. The database accession codes for the proteins at the National Center for Biotechnology Information (NCBI) are NP_014817 (*S. cerevisiae*), T39283 (*S. pombe*), AAF29090 (*Homo*), AAF50591 (*Drosophila*) and AAA68747 (*C. elegans*). (*B*) Alignments of yeast Med8 proteins and metazoan Arc32 homologs. The accession nos. are NP 009752 (*S. cerevisiae*), T39886 (*S. pombe*), NP064384 (mouse), AAF57476 (*Drosophila*). and T31475 (*C. elegans*). The *Homo sapiens* sequence is the protein hCP40597 in the Celera protein database. The Med4 and Med8 sequences from *C. albicans* were obtained by an analysis of unfinished sequence data from the *C. albicans* genome project (http://www-sequence.stanford.edu/group/candida/index.html).

cerevisiae seem essential for viability, whereas the *S. pombe*-specific subunits Pmc2 and Pmc3 are nonessential.

spMed4 and spMed8 Have Metazoan Homologs. We reasoned that the 10 subunits shared between *S. pombe* and *S. cerevisiae* could represent an essential Mediator core. However, for four of these conserved genes (*med4*$^+$, *med8*$^+$, *rox3*$^+$, and *srb6*$^+$), no homologs have been identified in metazoan Mediator-like complexes. Based on our analysis of *S. pombe* Mediator, we decided to reinvestigate the existence of homologs to these proteins in metazoan cells. Profile-based methods to search databases, such as position-specific iterated (PSI)-BLAST, are often effective tools to reveal distant protein relationships. After the second iteration, a PSI-BLAST search of the National Center for Biotechnology Information protein database with the scMed4 sequence indicated that the N-terminal region (≈210 amino acids) of the enzyme is homologous not only to spMed4 ($E = 1 \times 10^{-4}$) but also to the *C. elegans* Mediator subunit TRAP36 ($E = 1 \times 10^{-2}$). A third iteration also identified TRAP36 homologs in human, mouse, and *Drosophila melanogaster*. A multiple alignment of Med4 from *S. pombe*, *S. cerevisiae*, and *Candida albicans*, and Trap36 homologs from *C. elegans*, *D. melanogaster*, and human cells is shown in Fig. 3*A*. A secondary structure prediction also seems to give support for a relationship between the two protein families (Fig. 3*A*). We would therefore be inclined to regard the Trap36 protein as a Med4 homolog.

A blastp search of the Celera human protein database identified hCP40597 as a human homolog to spMed8 ($E = 6 \times 10^{-04}$), with 28% identity and 48% similarity in a region spanning amino acids 19 and 135 in spMed8. hCP40597 is identical to the Arc32 protein, which has been identified as a component of the human Mediator (12). A multiple alignment of Med8 and Arc32 homologs is shown in Fig. 3*B* together with secondary structure predictions. The results of this analysis indicate that Arc32 is the metazoan homolog of spMed8. Sequence similarity searches with the *S. pombe* and *S. cerevisiae* homologs of Rox3 and Srb6 failed to identify any conserved counterparts in metazoan cells. We conclude that Med4 and Med8 have homologs in higher cells, and that 8 of 10 gene products conserved between *S. cerevisiae* and *S. pombe* also are present in metazoan Mediator.

Activator–Mediator Interaction. In *S. cerevisiae*, three different transcription factors (Gal4, Gcn4, and VP16) belonging to the

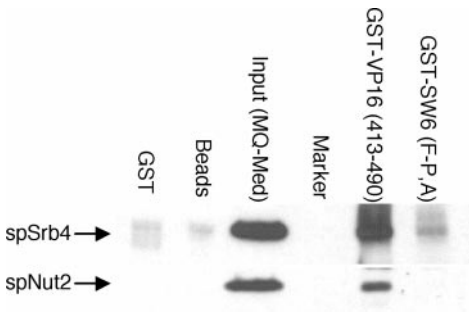

Fig. 4. GST pull-down assay of *S. pombe* Mediator by VP16 transactivation domain. RNA polymerase II holoenzyme was incubated with GST beads containing the GST activator fusion proteins indicated at the top of each lane. GST alone, beads alone, and the GST-SW6 mutant version of VP16 were used as negative controls. The samples were analyzed on SDS/PAGE, and subsequent immunoblotting with Mediator Abs is indicated (*Left*).

acidic-rich class interact with the mediator in a manner requiring the Gal11 module (Med2, Pgd1/Hrs1, Gal11, Sin4; ref. 23). These proteins are notably absent from the *S. pombe* Mediator. To test for direct physical interactions between VP16 and the *S. pombe* mediator complex, we performed a GST pull-down assay (Fig. 4). Two GST fusion proteins containing either VP16 (413–490) or SW6–VP16 (mutant) were incubated with the *S. pombe* RNA polymerase II holoenzyme. The SW6-VP16 fragment is mutated at four phenylalanine residues, which has been shown to inactivate VP16 transcriptional activity and to abolish interaction with the mammalian Mediator complex (12, 24). The pull-down assay was monitored with polyclonal Abs against spSrb4 and spNut2. We observed interactions of VP16 with the mediator complex but not with its SW6 mutant. We conclude that *S. pombe* Mediator can interact specifically with VP16 even in the absence of homologs to the *S. cerevisiae* Gal11 module. Presumably, other subunits in *S. pombe* Mediator can substitute for the subunits of the Gal11 module and mediate a direct interaction with the VP16 activation domain.

Discussion

We have reported on the purification and partial subunit characterization of an *S. pombe* RNA polymerase II holoenzyme (19). In this article, we finalize the subunit characterization of this complex and identify eight new Mediator components. Two of the new Mediator subunits, Pmc3 and Pmc6, are encoded by previously uncharacterized ORFs. Similar to the *pmc2*$^+$ gene product, these proteins seem to lack homologs in Mediator complexes isolated from other species. Gene disruption experiments demonstrated that *pmc2*$^+$ and *pmc3*$^+$ are not essential for *S. pombe* viability. The *S. cerevisiae* Mediator also contains a group of subunits not identified in other species, e.g., Med2 and Sin4. These nonconserved gene products are not essential for viability in *S. cerevisiae* and they are needed only for the regulated expression of specific genes (2). It therefore seems likely that Pmc2, Pmc3, and possibly Pmc6 belong to a category of nonconserved nonessential gene products that could be involved in the regulated expression of specific genes. BLAST and PSI-BLAST searches were also performed in an attempt to identify homologs to nonessential *S. cerevisiae* Mediator subunits in the *S. pombe* genome, because the absence of these could be the result of dissociation during purification. We failed to identify homologs to 9 of the 10 nonessential *S. cerevisiae* proteins in the *S. pombe* genome database. The only exception is a putative Gal11 homolog (http://bio.lundberg.gu.se/medb). However, in *S. cerevisiae* the Gal11 protein is also a member of the Paf1–Cdc73 complex, which is needed for the regulated

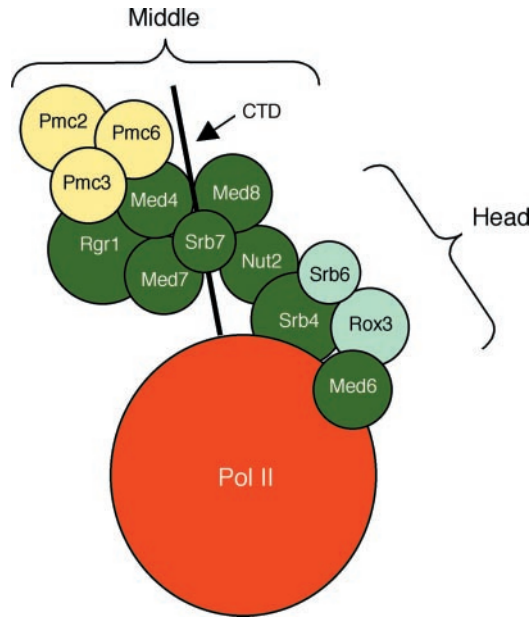

Fig. 5. Tentative organization of the *S. pombe* Mediator complex. Subunits unique to *S. pombe* are shown in yellow. The green subunits define essential Mediator components identified in both *S. cerevisiae* and *S. pombe*. The dark green components also have homologs in metazoan Mediator and may represent a conserved Mediator core. The exact placement of individual subunits is based on previous studies of *S. cerevisiae* Mediator (17).

expression of certain promoters (25). It is therefore possible that Gal11 has a role separate from Mediator in *S. pombe*.

Six of the new Mediator subunits are *S. pombe* homologs to Mediator proteins Med4, Med6, Med8, Rox3, Srb6, and Srb7 previously characterized in *S. cerevisiae*. With the identification of these new Mediator components, 10 of 20 *S. cerevisiae* Mediator subunits have a homolog in *S. pombe* (Table 1). It is noteworthy that the conserved subunits correspond to the subset of Mediator subunits, which are encoded for by essential genes in *S. cerevisiae*. Thus far, spSrb4, spMed4, spMed7 (this article), and spMed8 (26) have been demonstrated to be essential subunits in *S. pombe*. One could speculate that the set of essential subunits conserved between *S. pombe* and *S. cerevisiae* represents a Mediator core responsible for contacts formed with RNA polymerase II and TFIIH. This view is also supported by sequence analysis, which reveals homologs to Med4 and Med8 in metazoan Mediator-like complexes. The TRAP36 subunit of the SRB/MED-containing cofactor complex (SMCC) demonstrates significant sequence homologies with the spMed4 protein, and the Arc32 subunit of the activator-recruited cofactor (ARC) is homologous to spMed8. Others have recently reported homology between spSrb4 and the TRAP80 protein (15). Therefore, 8 of the 10 essential gene products conserved between *S. cerevisiae* and *S. pombe* also have a homolog in human cells, indicating the existence of a conserved Mediator core present in all eukaryotic cells (Fig. 5).

Perhaps the absence of homologs for the nonessential subunits can be explained from an evolutionary perspective. Genetic analysis indicates that the nonessential Mediator is needed for the regulated expression of subsets of genes. Because the regulation of specific genes probably have arisen relatively late during the course of evolution, the nonconserved Mediator proteins could represent a dynamic interface between the world of rapidly evolving gene-specific regulator proteins and the basal transcription machinery. According to this view, the subunit composition will vary to a large extent among different eukaryotic cell types, but the Mediator mechanism is highly conserved.

Genetic studies seem to lend support to this interpretation. Mutations in the conserved Srb4 protein in *S. cerevisiae* basically eliminate all RNA polymerase II-dependent transcription, whereas deletion of the nonessential *SRB5* and *MED2* genes affect only a subset of genes, expressed under certain conditions (1, 27). A notable exception is the Med6 protein. A temperature-sensitive mutation in this conserved Mediator subunit only effects the expression of about 10% of all genes (28). In mouse, disruption of the conserved *Srb7* gene in embryonic stem cells revealed that it was essential for cell viability and early embryonic development (29). In contrast, primary embryonic fibroblasts lacking TRAP220 function, a gene specifically found in higher eukaryotes, show a prominent decrease of thyroid hormone receptor function but they display no defect in activation by Gal4-RARalpha/RXRalpha, p53, or VP16 (30). Thus the mouse Srb7 protein seems to play a more general role for transcription in higher eukaryotes, whereas the nonconserved TRAP220 is needed for the function of specific activators.

Structural studies also seem to indicate the existence of a core Mediator conserved between yeast and metazoan cells. Single-particle analysis by electron microscopy and image processing has demonstrated striking structural similarities between Mediator isolated from yeast, mouse, and human cells (16, 17). The structures indicate that Mediator consists of three distinct submodules: head, middle, and tail. The similarities between yeast and metazoan Mediators are especially clear in the head region, which is the region of most pronounced contacts between Mediator and polymerase.

The *S. pombe* Mediator is considerably smaller than its counterparts in *S. cerevisiae* and higher cells (Table 1). It contains only 13 individual subunits and has a molecular mass of about 490 kDa. In comparison, *S. cerevisiae* and human Mediators contain 20 and 25 subunits, respectively, and have molecular masses of about 1.0 MDa and 1.5 MDa, respectively. In *S. cerevisiae*, a subcomplex of Mediator was identified recently and termed Medc (31). This complex lacks subunits of the Gal11 module (Hrs1/Pgd1, Med2, Gal11) as well as Nut1, Rgr1, and Rox3. In human, Mediator-like PC2 complex lacks many of the subunits present in SRB/MED-containing cofactor complex (SMCC) and is considerably smaller in size (32). It is possible that our *S. pombe* Mediator complex also represents a subcomplex of Mediator similar to Medc and PC2. Further genetic and biochemical studies are required to determine the existence of other forms of the *S. pombe* Mediator.

We thank G. Thon and O. Nielsen for strains and helpful discussions. This research was supported by grants from the Danish Research Councils and the Novo-Nordisk Foundation (to S.H.), and by grants from the Swedish Cancer Society (project 3947), the Human Frontiers Science Program, and the Swedish Foundation for Strategic Research (to C.M.G.). This work was also supported by the VIB (J.E.R., D.H., and I.E.). C.O.S. was supported by a grant from the Faculty of Natural Sciences, University of Copenhagen. Part of the work was carried out at the Protein Analysis Unit, Center for Structural Biochemistry.

1. Holstege, F. C., Jennings, E. G., Wyrick, J. J., Lee, T. I., Hengartner, C. J., Green, M. R., Golub, T. R., Lander, E. S. & Young, R. A. (1998) *Cell* **95,** 717–728.
2. Myers, L. C. & Kornberg, R. D. (2000) *Annu. Rev. Biochem.* **69,** 729–749.
3. Kim, Y. J., Bjorklund, S., Li, Y., Sayre, M. H. & Kornberg, R. D. (1994) *Cell* **77,** 599–608.
4. Myers, L. C., Gustafsson, C. M., Bushnell, D. A., Lui, M., Erdjument-Bromage, H., Tempst, P. & Kornberg, R. D. (1998) *Genes Dev.* **12,** 45–54.
5. Lorch, Y., Beve, J., Gustafsson, C. M., Myers, L. C. & Kornberg, R. D. (2000) *Mol. Cell.* **6,** 197–201.
6. Jiang, Y. W., Veschambre, P., Erdjument-Bromage, H., Tempst, P., Conaway, J. W., Conaway, R. C. & Kornberg, R. D. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 8538–8543.
7. Sun, X., Zhang, Y., Cho, H., Rickert, P., Lees, E., Lane, W. & Reinberg, D. (1998) *Mol. Cell.* **2,** 213–222.
8. Gu, W., Malik, S., Ito, M., Yuan, C. X., Fondell, J. D., Zhang, X., Martinez, E., Qin, J. & Roeder, R. G. (1999) *Mol. Cell.* **3,** 97–108.
9. Ryu, S. & Tjian, R. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 7137–7142.
10. Boyer, T. G., Martin, M. E., Lees, E., Ricciardi, R. P. & Berk, A. J. (1999) *Nature (London)* **399,** 276–279.
11. Rachez, C., Lemon, B. D., Suldan, Z., Bromleigh, V., Gamble, M., Naar, A. M., Erdjument-Bromage, H., Tempst, P. & Freedman, L. P. (1999) *Nature (London)* **398,** 824–828.
12. Naar, A. M., Beaurang, P. A., Zhou, S., Abraham, S., Solomon, W. & Tjian, R. (1999) *Nature (London)* **398,** 828–832.
13. Kwon, J. Y., Park, J. M., Gim, B. S., Han, S. J., Lee, J. & Kim, Y. J. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 14990–14995.
14. Malik, S. & Roeder, R. G. (2000) *Trends Biochem. Sci.* **25,** 277–283.
15. Boube, M., Faucher, C., Joulia, L., Cribbs, D. L. & Bourbon, H. M. (2000) *Genes Dev.* **14,** 2906–2917.
16. Asturias, F. J., Jiang, Y. W., Myers, L. C., Gustafsson, C. M. & Kornberg, R. D. (1999) *Science* **283,** 985–987.
17. Dotson, M. R., Yuan, C. X., Roeder, R. G., Myers, L. C., Gustafsson, C. M., Jiang, Y. W., Li, Y., Kornberg, R. D. & Asturias, F. J. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 14307–14310. (First Published December 12, 2000; 10.1073/pnas.260489497)
18. Forsburg, S. L. (1999) *Trends Genet.* **15,** 340–344.
19. Spahr, H., Beve, J., Larsson, T., Bergstrom, J., Karlsson, K. A. & Gustafsson, C. M. (2000) *J. Biol. Chem.* **275,** 1351–1356.
20. Pandey, A., Andersen, J. S. & Mann, M. (June 20, 2000) *Science's STKE*, http://stke.sciencemag.org/cgi/content/full/OC_sigtrans;2000/37/pl1.
21. Krawchuk, M. D. & Wahls, W. P. (1999) *Yeast* **15,** 1419–1427.
22. Egel, R., Willer, M., Kjaerulff, S., Davey, J. & Nielsen, O. (1994) *Yeast* **10,** 1347–1354.
23. Park, J. M., Kim, H. S., Han, S. J., Hwang, M. S., Lee, Y. C. & Kim, Y. J. (2000) *Mol. Cell. Biol.* **20,** 8709–8719.
24. Regier, J. L., Shen, F. & Triezenberg, S. J. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 883–887.
25. Shi, X., Chang, M., Wolf, A. J., Chang, C. H., Frazer-Abel, A. A., Wade, P. A., Burton, Z. F. & Jaehning, J. A. (1997) *Mol. Cell. Biol.* **17,** 1160–1169.
26. Zilahi, E., Miklos, I. & Sipiczki, M. (2000) *Curr. Genet.* **38,** 227–232.
27. Myers, L. C., Gustafsson, C. M., Hayashibara, K. C., Brown, P. O. & Kornberg, R. D. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 67–72.
28. Lee, Y. C., Min, S., Gim, B. S. & Kim, Y. J. (1997) *Mol. Cell. Biol.* **17,** 4622–4632.
29. Tudor, M., Murray, P. J., Onufryk, C., Jaenisch, R. & Young, R. A. (1999) *Genes Dev.* **13,** 2365–2368.
30. Ito, M., Yuan, C. X., Okano, H. J., Darnell, R. B. & Roeder, R. G. (2000) *Mol. Cell.* **5,** 683–693.
31. Liu, Y., Ranish, J. A., Aebersold, R. & Hahn, S. (2001) *J. Biol. Chem.* **276,** 7169–7175.
32. Malik, S., Gu, W., Wu, W., Qin, J. & Roeder, R. G. (2000) *Mol. Cell.* **5,** 753–760.
33. Gevaert, K., Demol, H., Sklyarova, T., Vandekerckhove, J. & Houthaeve, T. (1998) *Electrophoresis* **19,** 909–917.
34. Cuff, J. A., Clamp, M. E., Siddiqui, A. S., Finlay, M. & Barton, G. J. (1998) *Bioinformatics (Oxford)* **14,** 892–893.