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# IDENTIFICATION OF *SCHIZOSACCHAROMYCES POMBE* GENES THAT ENCODE PUTATIVE HOMOLOGUES OF *SACCHAROMYCES CEREVISIAE* MEDIATOR COMPLEX SUBUNITS*

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The mediator complexes transduce regulatory information from upstream regulatory elements to the transcription machinery in organisms ranging from yeasts to humans. By a genome-wide search we identified 14 ORFs and genes in the genome of the fission yeast *Schizosaccharomyces pombe* that encode putative homologues of *Saccharomyces cerevisiae* mediator subunits. The *Sch. pombe* proteins are smaller and appear to form a mediator of lower complexity, which is consistent with the hypothesized ancient origin of fission yeasts.

**Keywords**: fission yeast, mediator, transcription, BLAST, phylogenesis

## Introduction

All eukaryotes share a highly conserved mechanism of transcription regulation. The transcription initiation of protein-encoding genes requires interactions between cis-acting promoter elements and trans-acting factors [for a review, see 1]. The promoter consists of core elements, which include the TATA box and other DNA sequences that define transcription start sites and upstream regulatory elements, which either enhance or repress transcription in a gene-specific manner. The cis-acting factors include RNA polymerase II, general transcription factors, transcription activators and coactivators.

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The general transcription factors assemble at the core promoter and include the TATA-binding protein (TBP), TFIIB, TFIIE, TFIIF and TFIIH. Transcription activators – which bind sequence-specific regulatory elements located upstream of the core promoter – stimulate the rate of transcription in response to physiological or developmental stimuli. A third class of transcription factors, known as coactivators, facilitates the interaction between the sequence-specific activators and the general RNA polymerase II machinery. They are not gene-specific and usually mediate activation by a broad spectrum of activators. Coactivators include the TAF (TBP-associated factor) component of the general transcription factor TFIID, the mediator complex(es) (e.g. SRB/MED) that associate(s) with RNA polymerase II, TFIIA, the histon acetyltransferase complexes (e.g. SAGA), and the chromatin remodelling complexes (e.g. SWI/SNF) [for recent reviews, see 1, 2, 3].

The mediator complex (SRB/MED) discovered in *Saccharomyces cerevisiae* interacts with RNA polymerase II, forming a "holoenzyme" complex. It binds tightly to the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II and can physically interact with at least some activators. The *S. cerevisiae* mediator complex includes the Srb proteins, the Med proteins and the proteins Cse2, Gal11, Nut2, Rgr1, Rox3 and Sin4 [4, 5, 6, 7]. The components of the complex assemble into functional modules that regulate the transcription of distinct groups of genes [8, 9].

Mediator complexes have also been identified in various metazoans [reviewed in 3], indicating that the function of mediator as a coactivator for gene-specific transcription regulation is evolutionary conserved. These complexes contain homologues of several, but not all, components of the mediator of *S. cerevisiae* and also contain proteins that do not have counterparts in the budding yeast. Very recently, ten mediator proteins were also identified in the fission yeast *Schizosaccharomyces pombe* [10, 11]. Since these yeasts are almost as different from each other as from animals [12], a detailed comparison of their mediators might shed more light on the evolution of transcription regulation in eukaryotes. Here we report on the identification of homologues of the *S. cerevisiae* mediator genes in the Sanger Center database that contains the recently completed genomic *Sch. pombe* sequence (http://www.sanger.ac.uk/Projects/S_pombe). We also present an initial analysis of the data, describing some of the insight that can be obtained from the sequences.

## Methods

The *S. cerevisiae* mediator sequences were used as query sequences to conduct basic BLAST searches, using the blastp (basic local alignment search technique for protein sequences) search algorithm [13], to look for proteins (translated sequences)

that are similar in *Sch. pombe* in the non-redundant database at the Sanger Center, Cambridge. Peptide sequences were compared with entries in other nonredundant proteins and nucleotide databases with the use of the National Center for Biotechnology Information Advanced BLASTP programme [14]. For pairwise comparison of sequences we used the National Center for Biotechnology Information "Blast 2 sequences" programme [15]. The phylogenetic relationships among the proteins were investigated by the PROTPARS (Protein Parsimony) programme of the PHYLIP (Phylogeny Inference Package) package, version 3.573c [16].

## Results and Discussion

The *S. cerevisiae* mediator complex is composed of the Srb family of proteins (Srb2, Srb4, Srb5, Srb6 and Srb7), the Med proteins (Med1, Med2, Med4, Med6, Med8, Med9, Med10 and Med11), Cse2, Gal11, Rgr1, Rox3 and Sin4 (Table I) [4, 5, 6, 7]. Recently, two independent lines of research demonstrated that a similar complex exists in *Sch. pombe*. One of the cell separation genes, *sep15*$^{+}$, turned out to encode a protein with high degree of sequence similarity to Med8 [11]. Independently, the purification and partial characterisation of RNA polymerase II holoenzyme from *Sch. pombe* revealed a multiprotein complex that contains homologues to Rgr1, Srb4, Med4, Med7 and Nut2 proteins, representing each of the three subgroups of mediator proteins of *S. cerevisiae* [10]. It also contained four proteins denoted Pmc2, Pmc3, Pmc5 and Pmc6 with no discernible similarity to *S. cerevisiae* subunits. Its total mass was about 400 kDa, significantly less than 1000 kDa, the mass of the *S. cerevisiae* mediator. Interestingly, no homologues were found for 17 *S. cerevisiae* mediator components. Since the composition of the isolated complex is critically dependent on the methods applied to purification, the described proteins may represent only a subset of the subunits of the *Sch. pombe* mediator complex. Different purification strategies may lead to the identification of additional components.

An alternative approach to the identification of more components can be the search of the *Sch. pombe* sequence database for genes that encode amino acid sequences similar to those of the *S. cerevisiae* subunits. With the completion of the *Sch. pombe* genome project, the whole genome has become available for homology search. In order to identify putative homologues we performed a genome-wide Blastp search in the *Sch. pombe* genome database based at the Sanger Center (http//www.sanger.ac.uk). As shown in Table II, matching sequences were found for all but two *S. cerevisiae* proteins. For most of them more than one *Sch. pombe* sequence producing high-scoring segment pairs was found, but for further analysis we selected

only the most similar sequence from each group. We identified all genes previously described to encode homologues of *S. cerevisiae* mediator proteins such as Med7, Med8, Nut2, Pmc1 and Srb4. We also found fourteen uncharacterized ORFs whose putative products showed various degrees of similarity to additional *S. cerevisiae* mediator proteins. In principle, these might be the genes that encode the *Sch. pombe* counterparts. We were unable to identify sequences homologous to Med1 and Med2.

**Table I**

Saccharomyces cerevisiae *Mediator subunits*[1]

| Subunit | Size (amino acid) | Gene |
|---|---|---|
| Cse2 | 149 | *CSE2, MED9* |
| Gal11 | 1081 | *GAL11, SPT13, SDS4, RAR3* |
| Med1 | 566 | *MED1* |
| Med2 | 431 | *MED2* |
| Med3 | 431 | *PGD1, HRS1* |
| Med4 | 284 | *MED4* |
| Med6 | 295 | *MED6, MTR32* |
| Med7 | 222 | *MED7* |
| Med8 | 223 | *MED8* |
| Med11 | 131 | *MED11* |
| Nut2 | 157 | *NUT2, MED10* |
| Rgr1 | 1082 | *RGR1* |
| Rox3 | 220 | *ROX3, SSN7* |
| Sin4 | 974 | *SIN4, SSN4, TSF3* |
| Srb2 | 210 | *SRB2* |
| Srb4 | 687 | *SRB4* |
| Srb5 | 307 | *SRB5* |
| Srb6 | 121 | *SRB6* |
| Srb7 | 140 | *SRB7* |
| Srb8 | 1427 | *SRB8, SSN5, ARE2* |
| Srb9 | 1420 | *SRB9, SSN2, UME2* |
| Srb10 | 555 | *SRB10, SSN3, UME5, ARE1* |
| Srb11 | 323 | *SRB11, SSN8, UME3* |

[1]see references [2, 5, 6]

To verify the homologies revealed, each pair of sequences was subjected to a Blast 2 sequence comparison. This analysis did not confirm the homology between Cse2, Med3, Med11, Sin4, Srb2, Srb5, Srb9 of *S. cerevisiae* and the *Sch. pombe* sequences identified in the database search. Most probably, these proteins do not have counterparts in *Sch. pombe*. The rest of the proteins shared regions of homology with 24 to 53 % sequence identity. In certain proteins the similarity of amino acid sequences was confined to rather short regions. For example, Gal11 (1018 aa) and its putative

*Sch. pombe* homologue (653 aa) share only a very short (93 aa) homologous stretch (Fig. 1A). On the other hand, proteins like Med6, Med8 and Srb7 show similarity over the entire sequence (Fig. 1B, D and E). Altogether, 14 out of the 23 known *S. cerevisiae* mediator proteins seem to have homologues in *Sch. pombe*.

**Table II**

Schizosaccharomyces pombe *Mediator subunits*[1]

| *Sch. Pombe* | | | Homology with |
|---|---|---|---|
| Subunit | Size | Gene or ORF | *S. cerevisiae* subunits |
| Pmc4 | 239 | SPBC1105.06 | Med4 |
| SpMed7 | 376 | *med7* | Med7 |
| | 200 | *sep15* | Med8 |
| SpNut2 | 138 | SPBC31F10 | Nut2 |
| Pmc1 | 879 | *pmc1* (SPBC1A4.10C) | Rgr1 |
| SpSrb4 | 545 | *srb4* | Srb4 |
| Pmc2 | | *pmc2* (SPAC2F7.04) | |
| Pmc3 | | | |
| Pmc5 | | | |
| Pmc6 | | | |

[1]see references [10, 11]

Remarkably, 12 out of the 14 *Sch. pombe* proteins are smaller than their *S. cerevisiae* counterparts, which fits in fairly well with the earlier finding that the *Sch. pombe* mediator is smaller [10]. The smaller size of the *Sch. pombe* proteins might be attributed to the hypothesized ancient origin of the fission yeasts [12]. One can speculate that these proteins contain little more than the domains necessary for the basic activity, whereas the *S. cerevisiae* proteins have acquired longer sequence extensions required for specific tuning of the activity. With the exception of Med6, all *S. cerevisiae* proteins have these non-homologous extensions at their ends (examples are shown in Fig. 1). In Med6 the region of homology is split up in four parts by a shorter and two longer unique stretches that are absent in the *Sch. pombe* sequence (Fig. 1B). Med7 represents an exception to the rule that the *S. cerevisiae* proteins are longer. Its counterpart in *Sch. pombe* has a unique internal block and a unique terminal stretch that make the whole amino acid sequence longer than that of the *S. cerevisiae* Med7. It seems likely that the homologous regions separated by the internal block in *Sch. pombe* correspond to two specific protein domains.

**TableIII**

*Mediator homologs identified in the Sanger database of the* Sch. pombe *genome project*

| *Saccharomyces cerevisiae* | | *Schizosaccharomyces pombe* homologs in Sanger database[1] | | | Sequence homology *Sch. pombe /S. cerevisiae* | |
|---|---|---|---|---|---|---|
| Subunit | Size (amino acid) | Gene or ORF | BLASTP value[2] | Size (amino acid) | identity | pozitives |
| Cse2 | 149 | SPAC10F6.09C | 0.66 | 1234 | no significant similarity | |
| Gal11 | 1081 | SPBP35G2.15 | 9.8e-06 | 653 | 28/93 (30%) | 46/93 (49%) |
| Med1 | 566 | no hit | | | | |
| Med2 | 431 | no hit | | | | |
| Med3 | 431 | SPBC800.06 | 0.9992 | 305 | no significant similarity | |
| Med4 | 284 | SPBC1105.06 | 1.3e-06 | 239 | 34/125 (27%) | 64/125 (51%) |
| Med6 | 295 | SPAC1002.15c | 1.7e-09 | 216 | 63/214 (29%) | 99/214 (45%) |
| Med7 | 222 | SPBC14F5.08 | 1.2e-18 | 376 | 82/314 (26%) | 127/314 (40%) |
| Med8 | 223 | *sep15* | 2.1e-11 | 200 | 58/191 (30%) | 100/191 (51%) |
| Med11 | 131 | SPAC6G9.06C | 0.0075 | 1208 | no significant similarity | |
| Nut2 | 157 | SPBC31F10 | 2.7e-13 | 138 | 40/150 (26%) | 76/150 (50%) |
| Rgr1 | 1082 | SPBP23A10.01C | 1,2e-13 | 879 | 96/357 (26%) | 158/357 (43%) |
| Rox3 | 220 | SPCC1450.05C | 0.0042 | 138 | 24/60 (40%) | 37/60 (61%) |
| Sin4 | 974 | SPBC1347.01C | 0.9992 | 935 | no significant similarity | |
| Srb2 | 210 | *Lys1* | 0.98 | 1415 | no significant similarity | |
| Srb4 | 687 | SPBC31F10.04C | 1.4e-08 | 545 | 47/137 (34%) | 78/137 (56%) |
| Srb5 | 307 | SPAC1327.01C | 0.11 | 977 | No significant similarity | |
| Srb6 | 121 | SPAC29A4.07 | 0.061 | 136 | 25/100 (25%) | 44/100 (44%) |
| Srb7 | 140 | SPBC1604.10 | 0.019 | 138 | 41/129 (31%) | 67/129 (51%) |
| Srb8 | 1427 | SPAC688.08 | 6.8e-09 | 1233 | 52/214 (24%) | 91/214 (42%) |
| Srb9 | 1420 | SPBC30D10.15 | 0.83 | 516 | No significant similarity | |
| Srb10 | 555 | SPAC23H4.17C | 2.6e-92 | 352 | 160/299 (53%) | 215/299 (71%) |
| Srb11 | 323 | SPBC12D12.06 | 4.4e-17 | 228 | 63/163 (38%) | 99/163 (60%) |

[1]http:www.sanger.ac.uk
[2]smallest sum probability

*Fig. 1.* Sequence comparison of *S. cerevisiae* mediator subunits and their putative *S. pombe* homologues. Comparison of Gal11Sp with Gal11Sc (A), Med6Sp with Med6Sc (B), Med7Sp with Med7Sc (C), Med8Sp with Med8Sc (D), Srb7Sp with Srb7Sc (E), Srb10Sp with Srb10Sc (F), and Srb11Sp with Srb11Sc (G). Symbols of amino acids are A: alanine, C: cysteine, D: aspartic acid, E: glutamic acid, F: phenylalanine, G: glycine, H: histidine, I: Isoleucine, L: leucine, K: lysine, M: methionine, N: asparagine, P: proline, Q: glutamine, R: arginine, S: serine, T: threonine, W: tryptophane, Y: tyrosine, V: valine

*Fig. 1.* (continued)

*Fig. 1* (continued)

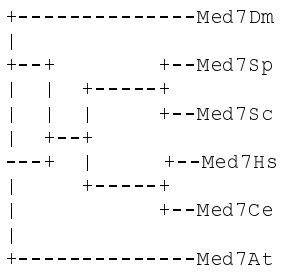


*Fig. 2*. Maximum parsimony tree for the *S. cerevisiae* Med7 protein (Med7Sc) and its homologues in *Arabidopsis thaliana* (Med7At), *Cenorhabditis elegans* (Med7Ce), *Drosophila melanogaster* (Med7Dm), *Homo sapiens* (Med7Hs) and *Sch. pombe* (Med7Sp)

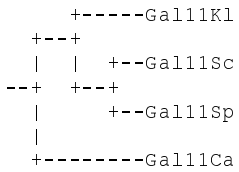


*Fig. 3*. A maximum parsimony tree inferred from the sequences of the *S. cerevisiae* Gal11 protein (Gal11Sc) and its homologues in *Candida albicans* (Gal11Ca), *Kuyveromyces lactis* (Gal11Kl) and *Sch. pombe* (Gal11Sp)

Recent studies indicate that mediator complexes are organized in a modular fashion [reviewed in 3]. In *S. cerevisiae* the core mediator subunits form two submodules, Cse2-Med1-Med2-Med4-Med7-Med8-Nut2-Rgr1-Srb7 and Med6-Rox3-Srb2-Srb4-Srb5-Srb6 [17], that interact with the modules composed of Gal11-Med3-Sin4 and Srb8-Srb9-Srb10-Srb11 [18]. Combined biochemical and genetic studies demonstrated that specific functions can be attributed to each subcomplex [19].For example, *SRB10* and *SRB11* encode a kinase-cyclin pair involved in the phosphorylation of CTD in a cell cycle-dependent manner [20, 21]. We found putative *Sch. pombe* homologues of components of all these modules, indicating that the *Sch. pombe* mediator performs functions similar to those of the *S. cerevisiae* mediator. However, none of the modules are completely represented in *Sch. pombe*, which suggests that the two yeasts may also differ in the organisation and activity of their mediators. The Pmc proteins (Pmc2, Pmc3, Pmc5 and Pmc6), for which no homology was found in *S. cerevisiae* [10], might be substitutes for the missing homologues in these modules.

Numerous mammalian mediator-like complexes have been identified recently, including SMCC/TRAP, NAT, DRIP, ARC, murine Mediator and CRSP [for a review, see 3]. Their molecular composition has not been fully defined but they all appear to contain homologues of Med6, Med7, Rgr1, Srb10 and Srb11. *Cenorhabditis elegans* also has genes that code for homologues of Med6, Med7, Rgr1, Srb7 [22]. We found the counterparts for these genes is *Sch. pombe*, too, which indicates that these proteins may constitute the key mediator components that are evolutionarily conserved from yeasts to mammals. Remarkably, none of the mammalian and *C. elegans* complexes described to date contain readily apparent homologues of yeast Srb2, Srb4, Srb5 or Srb6. These proteins constitute a distinct submodul in *S. cerevisiae* and are critical for activator interactions [23]. *Sch. pombe* appears to have two members of this group, Srb2 and Srb5, demonstrating that it may possess a similar submodul. This finding further indicates that the *S. cerevisiae* mediator shares more features in common with the *Sch. pombe* mediator complex than with the corresponding metazoan complexes. We hypothesized that a closer relationship should also be reflected in a higher degree of sequence similarity between mediator proteins of these yeasts. To test this idea, we tried to find homologues for them in as many species as possible by searching various sequence data bases. Srb10 showed high degree of homology to a wide spectrum of cyclin dependent protein kinases of a great number of species. Many proteins with highly similar sequences were also found for Srb11, which were mostly cyclins. However, the limited information available about the exact functions of many of these kinases and cyclins did not allow the identification of the real homologues. Therefore we did not use them for multiple sequence comparison. Instead, we choose Med7, for

which single highly similar homologues were found in three Metazoans and one plant species. The most parsimonious tree shown in Fig. 2. indicates a close relationship between the yeast proteins.

A search of the National Center for Biotechnology Information data base revealed Gal11 homologues only in yeasts. In addition to *S. cerevisiae* and *Sch. pombe,* genes encoding Gal11-like proteins were also identified in *Candida albicans* and *Kluyveromyces lactis*. Previously, it was suggested that Gal11 is absent in *Sch. pombe* because the RNA polymerase II holoenzyme does not contain a protein with molecular mass similar to that of *S. cerevisiae* Gal11 [10]. Its absence seemed to correlate with the inability of *Sch. pombe* to grow on galactose. In *S. cerevisiae*, the Gal11 protein is needed to support Gal4-dependent induction of genes involved in galactose metabolism [16]. Interestingly, we could find an ORF encoding an amino acid sequence that shares high degree of sequence similarity with Gal11 over a 93-amino acid region (Fig. 1A). This hypothetical protein is significantly shorter (653 aa) than Gal11 (1018 aa), which might account for the failure of its identification in SDS-PAGE analysis of the purified polymerase II holoenzyme [10]. In spite of the considerable size difference, it appears to be more closely related to Gal11 than their homologues in the other yeast species.

In this analysis we identified numerous *Sch. pombe* genes that code for putative homologues of *S. cerevisiae* mediator subunits. The conclusion one may draw from these results is that the components of the putative *Sch. pombe* mediator complex show a higher degree of similarity with those of *S. cerevisiae* mediator than with the components of the metazoan mediators. However, the revealed sequence homologies do not necessarily coincide with functional homologies, and certain similarities may be only accidental. Future experimental studies will test which of the identified genes code for *Sch. pombe* mediator subunits and how these proteins work together to regulate transcription.

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