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ORIGINAL PAPER

Genomic expression patterns in cell separation mutants of *Schizosaccharomyces pombe* defective in the genes *sep10*$^+$ and *sep15*$^+$ coding for the Mediator subunits Med31 and Med8

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Abstract Cell division is controlled by a complex network involving regulated transcription of genes and post-translational modification of proteins. The aim of this study is to demonstrate that the Mediator complex, a general regulator of transcription, is involved in the regulation of the second phase (cell separation) of cell division of the fission yeast *Schizosaccharomyces pombe*. In previous studies we have found that the fission yeast cell separation genes *sep10*$^+$ and *sep15*$^+$ code for proteins (Med31 and Med8) associated with the Mediator complex. Here, we show by genome-wide gene expression profiling of mutants defective in these genes that both Med8 and Med31 control large, partially overlapping sets of genes scattered over the entire genome and involved in diverse biological functions. Six cell separation genes controlled by the transcription factors Sep1 and Ace2 are among the target genes. Since neither *sep1*$^+$ nor *ace2*$^+$ is affected in the mutant cells, we propose that the Med8 and Med31 proteins act as coactivators of the Sep1-Ace2-dependent cell separation genes. The results also indicate that the subunits of Mediator may contribute to the coordination of cellular processes by fine-tuning of the expression of larger sets of genes.

Keywords Septum · Transcription · Regulation · Fission yeast

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Introduction

The eukaryotic cell cycle is the ordinary sequence of events that accomplish cell reproduction. The final event is cell division, in which a parent cell gives rise to two daughter cells that carry copies of the genetic information of the parent cell. Cell division is controlled by a complex network involving regulated transcription of genes and postranslational modification of proteins. The aim of this study is to demonstrate that the Mediator complex, a general regulator of transcription, is involved in the regulation of the second phase (cell separation) of cell division of the fission yeast *Schizosaccharomyces pombe*.

Division of the fission yeast cell is a seemingly straightforward process consisting of septum formation (septation, cytokinesis) and the concomitant fission of the septum (cell separation) (for reviews, see Johnson et al. 1982; Sipiczki 2007). The septum is a trilaminar structure composed of a central β-1,3-glucan layer (primary septum) and two lateral layers (secondary septa; composed of β-1,6-branched β-1,3-glucan and β-1,6-glucan) (Johnson et al. 1973; Humbel et al. 2001). It divides the cell symmetrically into a pair of daughter cells. To separate, the daughter cells degrade the mother cell wall at the junction with the septum by a process requiring the Agn1 endo-1,3-alpha-glucanase activity (Dekker et al. 2004). Once the septum-edging part of the cell wall has been eroded, the primary septum splits.

It is a rapid process driven by physical force generated by the swelling of the secondary septa (Sipiczki and Bozsik 2000) and supported by the enzymatic degradation of the primary septal material carried out by the Eng1 endo-β-1,3-glucanase (Martin-Cuadrado et al. 2003).

Numerous genes implicated in cell separation and its coordination with other cellular events have been described (for a review see Sipiczki 2007). The first gene to be identified was *sep1*$^+$ (Sipiczki et al. 1993), whose inactivation abolished cell separation and resulted in a switch from unicellular propagation to hyphal growth. The Sep1 protein is a fork-head transcription factor (Ribar et al. 1997) playing an important role in cell cycle-regulated transcription (Bähler 2005), including the coordination of cytokinesis initiation with mitosis initiation (Grallert et al. 1998). Sep1 regulates a large group of M-G1 phase genes, including *ace2*$^+$, which encodes another transcription factor that then activates genes functioning in cytokinesis and cell separation (Rustici et al. 2004). The Eng1 β-glucanase and the Agn1 α-glucanase are among the Ace2-regulated protein activities (Alonzo-Nunez et al. 2005).

In a screen for mutants being at least partially defective in cell separation, we identified 14 additional *sep* genes (Grallert et al. 1997, 1999). Besides the cell separation defect, most of the sep$^-$ mutants showed diverse and complex phenotypes involving reduced fertility, low spore viability, impaired stress response, increased resistance to cell wall lytic enzymes, formation of twin septa and anucleate minicells. Four of them were cloned, sequenced and found to encode *Sch. pombe* orthologs of conserved general transcription regulators. Sep9 is an ortholog of the Spt8 subunit of the *Saccharomyces cerevisiae* chromatin remodelling complex SAGA (Sipiczki et al. 1999). *sep10*$^+$, *sep11*$^+$ and *sep15*$^+$ encode proteins with high degree of amino acid similarity to Mediator complex subunits of *S. cerevisiae* (Zilahi et al. 2000; Szilagyi et al. 2002). The multi-subunit Mediator complex functions in eukaryotic cells as a general co-activator that conveys regulatory information from DNA-binding transcription factors to RNA polymerase II (for a recent review, see Blazek et al. 2005). Biochemical analysis of the *Sch. pombe* Mediator confirmed that the Sep10, Sep11 and Sep15 proteins are Mediator subunits (Spahr et al. 2001; Linder and Gustafsson 2004). *sep10*$^+$ encodes a conserved protein (Linder and Gustafsson 2004) referred to as Med31 in the proposed unified nomenclature for Mediator subunits (Bourbon et al. 2004). Its *S. cerevisiae* counterpart is associated with the middle part of the complex (Guglielmi et al. 2004). The Sep15 protein corresponds to Med8 located in the head part of the *S. cerevisiae* Mediator that establishes direct contacts with Polymerase II (Davis et al. 2002).

The *Sch. pombe sep10/ med31*$^+$ is a non-essential gene; its deletion confers subtle filamentous morphology, temperature sensitivity, longer cell generation time at permissive temperatures, almost complete sterility and low sporulation efficiency (Szilagyi et al. 2002). The deletion of *sep15/ med8*$^+$ is lethal (Zilahi et al. 2000), while the mutation *sep15/med8-598* is temperature sensitive (lethal at temperatures above 33°C). At permissive temperatures, it shows a partial cell separation defect (Grallert et al. 1999). Both *sep10/ med31*$^+$ and *sep15/med8*$^+$ (hereafter referred to as *med31*$^+$ and *med8*$^+$) were proposed to be involved in the regulation of larger sets of genes besides those involved in cell separation (Grallert et al. 1999).

Here, we report on the identification of genes by genome-wide gene expression profiling that are dependent on Med31 and/or Med8. The inactivation of the genes causes both down-regulation and up-regulation of larger groups of genes scattered over the entire genome. We show that, consistent with the complex mutant phenotypes, genes involved in diverse cellular functions are differentially regulated in the mutants. Based on the experimental results, we propose a mechanism for these proteins in the regulation of the Sep1-Ace2-dependent cell separation genes.

Materials and methods

Strains, strain construction and growth media

The *Sch. pombe* strains used in this study are listed in Table 1. Cultures were maintained in YEL (1% yeast extract, 2% glucose) or on YEA (YEL + 2% agar) media (Sipiczki and Ferenczy 1977). The synthetic agar minimal medium EMM was described in Mitchison (1970). For gene expression profiling, cultures were grown in EMML (EMM without agar) supplemented with nutrients according to the auxotrophy of the strain.

Strains 2-989 and 2-991 were isolated as meiotic recombinants from the hybrids 2-924 × B-13 and 2-506 × 0-38, respectively. Hybridisation was performed by protoplast fusion (Sipiczki and Ferenczy 1977). The hybrids were sporulated on SPAS (10 g glucose, 1 g KH_2PO_4, 1 ml EMML vitamin, 46 mg adenine, leucine, uracil, histidine, lysine, 15 g agar in 1,000 ml) plates (Moreno et al. 1991), and tetrads of spores were isolated from the asci by micromanipulation. Auxotrophic markers were detected by replica-plating on EMM plates supplemented with nutrients according to the expected marker combinations.

Visualisation of cytoskeletal structures

Actin was visualised by rhodamine-conjugated phalloidin, and tubulin was stained by immunofluorescence techniques as described by Alfa et al. (1993). Stained cells were viewed and photographed using an Olympus BH-2 microscope.

Table 1 *Sch. pombe* strains used in this study

Strain	Genotype	Source
0-1	L972 wild-type h^-	U. Leupold, Bern
0-3	L968 wild-type h^{90}	U. Leupold, Bern
0-103	SA21 h^+	L. Heim, Braunschweig
0-38	*leu1-32* h^-	U. Leupold, Bern
B-13	*ade6-M210 ura4-D18* h^-	U. Leupold, Bern
2-506	*med8/sep15-598 his3* h^{90}	Grallert et al. (1999)
2-689	*med8/sep15-598 ade3-58* h^{90}	Grallert et al. (1999)
2-870	*med8/sep15-598 his3* h^{90}	Grallert et al. (1999)
2-871	*med8/sep15-598 ura4D18* h^-	Zilahi et al. (2000)
2-921	*Med31/sep10::ura4*$^+$ *leu1-32 ade6-M26* h^{90}	Szilagyi et al. (2002)
2-923	*med31/sep10::ura4 ura4-D18 lys1-131* h^{90}	Szilagyi et al. (2002)
2-924	*med31/sep10::ura4 ura4-D18 lys1-131 leu1-32* h^{90}	This study
2-989	*med31/sep10::ura4 ura4-D18*	This study
2-991	*med8/sep15-598*	This study

Molecular methods

Recombinant DNA manipulations were performed by standard techniques (Sambrook et al. 1989; Moreno et al. 1991). The pREP3X ΔATG expression vector was described by Forsburg (1993). The primers used for PCR reactions are shown in Table 2. To identify the mutation site in *med8-598*, the mutant gene was amplified from genomic DNA using the primers EZ 359 and EZ 360. The *med8*$^+$ cDNA was amplified by PCR from a cDNA library (kind gift from Professor Jimenez, University of Malaga, Spain) with the primers, EZ 361 and EZ 362. The PCR fragments were sequenced on both strands, using the Geneart DNA sequencing service (http://www.geneart.de). The nucleotide sequences obtained were compared with the *med8*$^+$ gene sequence (Zilahi et al. 2000) using the ClustalW 1.7 algorithm (Thompson et al. 1994).

To overexpress the *med8*$^+$ gene, the PCR-amplified *med8*$^+$ cDNA was fused to the thiamine-repressible *nmt1*$^+$ promoter (*Bam*HI site) of the pREP3X expression vector. The resulting construct was transformed into 0–38 *leu1-32* h^- cells, and prototrophic transformants were selected on EMM plates supplemented with 5 μg/ml thiamine (thiamine represses the *nmt1*$^+$ promoter of the vector and inhibits the transcription of the gene fused to it).

For the determination of spliced and unspliced *med8* transcript levels, the 0-3 wild-type and 2-991 *med8-598* strains were propagated in EMML at 33°C until mid-log phase (OD_{600} = 0.5). The cells were harvested and used for the isolation of total RNA with the Sigma TRIZOL-reagent.

Table 2 List of primers

Gene	Primers
SPBC21.04 (*med8*)	EZ359 5′-cgggatccATGGAAGACATATCTACCGAAA (1–22) EZ360 5′-gcggaatccTTAACGTTTACCTGATTTCATG (719–740) EZ361 5′-CAGTGGAATCTTTGGAAGCAA (26–46) EZ362 5′-TTCGGAGTAGGGTGGTCAAT (408–427) ZS1 5′-AAGGGTATTTGCCTCCCATT (281–304) ZS2 5′-CTTGCAATTCTTCATCAAAGC (91–111) ZS3 5′-AAACTTCAACATTTTGCTGTCTCA (291–304)
SPAC14C4.09 (*agn1*)	5′-TGGTAAAGTGTTTGTTTCTACATTT (357–381) 5′-GGCTCTTTTACAGCAGAATCC (430–450)
SPAC17H9.11	5′-AGGCTCGTATGTTCACCATTTCG (11–33) 5′-AACTGATTTCTTCAACCGCAAACG (65–88)
SPBC18H10.04c (*sce3, tif48*)	5′-GTGACTGGGTTCGTCGTG (542–559) 5′-GGCGCTCAGAGGATTCAC (708–725)
SPBC1271.07c	5′-TGGAACAGCTCTAGGGTGTGTA (198–219) 5′-ACTCCTAATTTCAGACCTCGACTC (283–306)
SPBP4G3.03	5′-TACCCTCATAGGCCAAACCATTT(568–590) 5′-GACATCCGTGTAGCCCACTAC (681–701)

The isolated DNA was treated with DNAse to eliminate DNA contamination. One microgram RNA was reverse transcribed using the *med8*$^+$-specific reverse primer ZS1 and the Promega RT-system (Cat No. M314A). The *med8* cDNAs were amplified by PCR using the primers ZS1 and ZS2 and identified by Southern hybridisation and by digestion with a restriction endonuclease. For Southern hybridisation, the cDNA fragments were separated by electrophoresis and transferred to a nylon membrane. The membrane was hybridised with a DIG-labelled probe created by amplification of a fragment of the *med8*$^+$ ORF using wild-type (0-3) genomic DNA and the primers ZS1 and ZS3 (Fig. 4a). DIG labelling was carried out with the Roche-DIG DNA Labelling and Detection Kit (Cat. No. 11585614910) according to the manufacturer's recommendation. Signals were detected on X-ray films. Amplifications with the same RNA samples as template were also carried out to specifically detect DNA contamination that might affect the outcome of the experiment. These reactions did not give products. For the identification with restriction endonuclease, the amplified *med8* cDNA preparations were digested with *Stu*I. *med8*$^+$ has a *Stu*I site that overlaps with the end of the intron and the beginning of exon 2. Accordingly, the spliced cDNA does not have the *Stu*I site, whereas the wild-type *med8*$^+$ gene and the unspliced *med8*$^+$ cDNA have the *Stu*I site (lanes 5 to 8 in Fig. 4b). The disruption of the *med31*$^+$ gene was verified by PCR amplification and digestion of the amplified fragments with restriction endonucleases.

Stress response test

One hundred microlitre aliquots of suspensions of exponential-phase cultures ($OD_{600} = 0.5$) grown in YEL were dropped on YEA plates supplemented with various concentrations of sorbitol (0–2.6 M), $CaCl_2$ (0–0.9 M), $CdSO_4$ (0–1.2 mM), KCl (0–1.6 M), NaCl (0–1.6 M), $ZnSO_4$ (0–6 mM), formamide (0–4%) and caffeine (0–28 mM). The plates were incubated at 30°C. After 4 days, the intensity of growth was evaluated visually. The response to glucose and nitrogen starvation was tested by culturing cells in EMML free of nitrogen or glucose at 30°C. Samples were taken, diluted and plated on YEA at the beginning of the experiment and after 2 days. The number of colonies formed by the surviving cells was determined after 5 days of incubation at 25°C.

Gene expression profiling

The mutant strains 2-989 *med31::ura4*$^+$ and 2-*991 med8-598* and the wild type 0-1 were cultured until mid log phase ($OD_{600} = 0.5$) in EMML at 33°C. RNA was isolated using the protocol described at http://www.sanger.ac.uk/PostGenomics/S_pombe/ (Lyne et al. 2003). The RNA was purified with Qiagen RNeasy mini spin columns. Twenty micrograms of total RNA was labelled by directly incorporating Cy3- and Cy5-dCTP through reverse transcription. cDNA was synthesised with Promega M-MLV reverse transcriptase and an oligo-dT17 primer. The resulting cDNA was hybridised onto DNA microarrays containing all known and predicted *Sch. pombe* genes printed onto glass slides (Lyne et al. 2003). Microarrays were scanned using a GenePix4000B laser scanner, and the data were analysed with GenePix Pro software (Axon Instruments, Foster City, CA, USA). We normalised relative expression data as described by Lyne et al. (2003). To quantify global effects, we also normalised one repeat for each strain using spiked external control RNAs as previously described (Lee et al. 2005). We performed four independent biological repeats for each mutant relative to wild-type control strains, and one of these repeats for each mutant was normalised using external spikes. Modifications of the transcription levels, which were at least two times higher or lower than the control level in at least three repeats, were accepted as modified mRNA levels. The statistical significance for the relative overlap of gene lists was calculated using the hypergeometric distribution. All processed microarray data are available from http://www.sanger.ac.uk/PostGenomics/S_pombe/.

Results

Identification of genes dependent on Med31 and Med8

To identify genes whose transcription is altered in *med31* and *med8* mutants, mRNA was purified from exponentially growing wild-type (0-1), *med31::ura4*$^+$ (2-898) and *med8-598* (2-991) cultures and used for hybridisation to DNA microarrays containing all known and predicted *Sch. pombe* genes. The evaluation of the hybridisation signals identified 549 genes whose expression levels changed at least twofold (up or down) in at least one of the mutants (Table 3; Electronic Supplementary Material S1). 382 genes showed modulated transcription levels in *med31::ura4*$^+$, and 273 genes were modulated in *med8-598* cells. The mutants differed in the proportion of up-regulated and down-regulated genes. In *med31::ura4*$^+$ the majority (62%) and in *med8-598* the minority (40%) of genes were repressed. Normalisation using external spikes revealed that all genes in the *med31::ura4*$^+$ mutant (but not in the *med8-598* cells) were about 40% lower expressed on average, indicating that transcription is generally compromised in the *med31* mutant. Comparison of the gene sets most affected in the mutants revealed a considerable overlap: the transcription of 114 genes was affected in both mutants ($P \sim 6.1 \times 10^{-59}$; Fig. 1; Electronic Supplementary Material S1;

Table 3 Number of up- and down-regulated genes sorted by functional categories

Functional category[a]	In *med31::ura4*		In *med8-598*		In both		In *med31::ura4* up In *med8–598* down
	Up	Down	Up	Down	Up	Down	
Cell wall organisation and biogenesis (GO:0007047;)	5	1	5	2	1		
Transport and protein secretion (GO: 0006810, GO: 0009306)	8	47	7	21	1	13	
Carbohydrate metabolism (GO:0005975)	2	17	16	5		4	2
Tricarboxylic acid cycle, tricarboxylic acid cycle intermediate metabolism, ethanol metabolism, acetate metabolism, D-gluconate metabolism, hexitol catabolism, glycerol metabolism (GO: 0006099, GO:0006100, GO: 0006068, GO:006083, GO: 0019521, GO:0019407, GO: 0006071)		9	3	5		1	1
Heme metabolism (GO:0042168)	1	3		1		1	
Amino acid metabolism (GO: 0006520)	3	16	4	18		6	
Vitamin biosynthesis (GO0009110)	1	2	1				
Nucleobase, nucleoside and nucleotide metabolism (GO: 0009112, GO:0009116, GO:0009117)	4	6	1	3	1	2	
Cellular lipid metabolism (GO: 00044255)	3	11	3	2	2	2	
Mitotic cell cycle and cell separation during cytokinesis (GO:0000278 and GO: 0000920)	3	8	4	5	2	4	
Cytoskeleton, kinetochore organisation and biogenesis, centromeric DNA binding and cell morphogenesis (GO:0005856, GO:0051383, GO:0019237 and GO:0000902)	9	5	3	3	3	1	
DNA repair and DNA modification (GO:0006281 and GO:0006304)			1	1			
Conjugation with cellular fusion (GO: 0000747)	3	5	5	3	2	2	
Meiosis (GO:0007126)	1	7	5				
Regulation of transcription, DNA dependent (GO:0006355)	2	6	4	1	1		
Chromatin remodelling (GO:0006338)	1	3	1	3			
mRNA metabolism and mRNA export from nucleus (GO:0006379 and GO:0006406)	5	5	3		2		
Ribosome biogenesis and assembly and translation (GO:0042254 and GO:0043037)	18	3	1	6	1		
Protein modification (GO:0006464)	5	3	1	2		2	
Protein folding (GO0006457)		5	1	4		3	
Cell surface receptor linked signal transduction and intracellular signalling cascade (GO:0007165 and GO:0007242)	4	6	1				
Response to stress (GO:0006950)	2	4	5	1			1
Transposons and RNA-directed DNA polymerase activity (GO:0003964)		10					
Proton transport and electron transport (GO:0015992 and GO:0006118)	3		1	1			
Conserved proteins, conserved eukaryotic proteins, conserved fungal proteins and conserved yeast proteins with no similarity to other proteins	10	7	11	4	3	2	
Sch. pombe specific, with no similarity to other proteins	1		1				
Sequence orphans, with no similarity to other proteins	13	15	18	3	5	2	4
Hypothetical proteins		3	1	1		1	1
Pseudogenes	1	2	5	1	1		
Dubious	2		2		1		
Mitochondrial proteins			7				
Miscileneous	27	29	38	12	13	8	
misc_RNA	7		4	2	4		
	144	238	163	110	43	54	9

All genes that were induced greater than twofold or repressed greater than twofold

[a] Partially arbitrary because numerous genes can be assigned to two or more categories

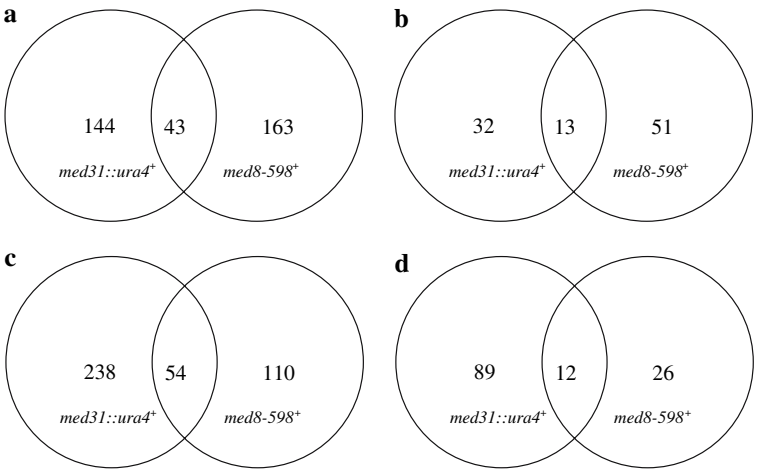

Fig. 1 Numbers of genes with modified expression levels in the *med31::ura4*$^+$ and *med8-598* strains. **a** Twofold up-regulation. **b** Threefold up-regulation. **c** Twofold down-regulation. **d** Threefold down-regulation

Table 3). In both the *med8* and *med31* mutants the repressed genes showed highly significant overlaps with genes repressed during cisplatin treatment ($P \sim 1 \times 10^{-28}$–1×10^{-47}; Gatti et al. 2004) and with genes repressed in cells compromised for the RNA polymerase II component Rpb4 ($P \sim 1 \times 10^{-24}$–1×10^{-56}) (Sharma et al. 2006).

Confirmation of array data by RT-PCR

To validate the array data, RT-PCR was performed with four selected genes. SPBC1271.07c and SPAC14C4.09 were chosen because they were down-regulated in both med$^-$ mutants. The former encodes a predicted *N*-acetyltransferase; the latter codes for the glucan endo-1,3-alpha-glucosidase Agn1. SPBP4G3.03 and SPAC17H9.11 were selected because they were up-regulated in both mutants. SPBP4G3.03 is a sequence orphan. The product of SPAC17H9.11 is a putative cofilin/tropomyosin family protein (inferred from homology). The RT-PCR results were in good agreement with the microarray data (Fig. 2).

Chromosomal location of the affected genes

Schizosaccharomyces pombe GeneDB lists 2,438, 1,913 and 963 ORFs on chromosomes I, II and III, respectively (http://www.genedb.org/genedb/pombe). We estimated that the transcription of 6.7% (chromosome I), 6.6% (chromosome II) and 7.3% (chromosome III) of these genes was strongly affected in the *med31::ura4*$^+$ cells. The corresponding values for the *med8-598* mutant were 5.3, 3.8 and 3.7%. Within the chromosomes, several groups of three and four adjacently located genes, simultaneously affected by one or both mutations, were detected. Examples of four-gene groups: SPAC750.05c to 08 (affected in *med8-598*), SPBP4H10.08 to 11c (affected in *med31::ura4*$^+$), SPBC660.13c to 16 (affected in *med31::ura4*$^+$), SPBC148.02 (affected in *med8-598*). Longer gene arrays interrupted with one or two

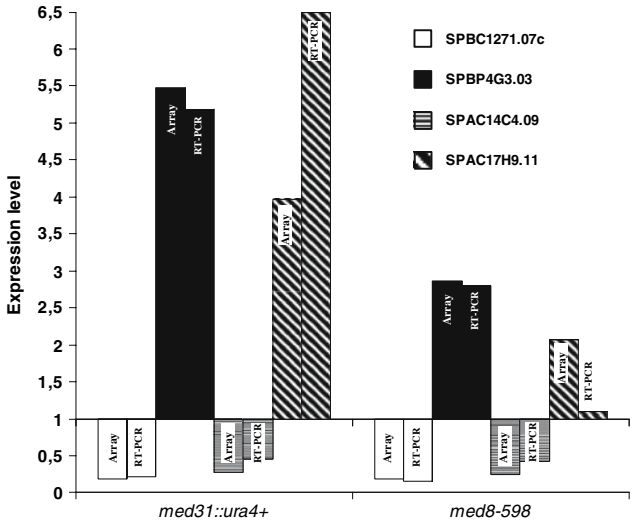

Fig. 2 Validation of microarray data by RT-PCR. *Columns* and *numbers* show the fold-changes relative to the wild-type control. Expression levels were normalised to *sce3*$^+$

non-affected genes were also found. For example in the regions SPBC1348.02 to 10 (6 of 8 genes were down-regulated in *med8-598*) and SPBC1683.02 to 11 (7 of 10 genes were affected in *med31::ura4*$^+$).

Functional categories of the affected genes

Table 3 also shows the major functional categories of the genes identified in the microarray analysis. Categorisation was based on Gene Ontology (GO) categories (updated 15.10.2006; Aslett and Wood 2006) (Electronic Supplementary Material S1). Classification of the genes into functional categories was complicated by the fact that many genes have multiple cellular functions. For example, the Ran-GTPase system (involving *ptr2*$^+$) affects various cellular processes including RNA metabolism, cell cycle progression, and protein transport (Azad et al. 1997).

The largest groups consisted of genes and ORFs encoding proteins involved in transport, carbohydrate metabolism and amino acid metabolism. Nevertheless, there were some remarkable differences between the functions of the two gene sets. In the *med8-598* profile, no transposons and no genes coding for mitochondrial proteins and only few genes involved in signal transduction or the formation of cytoskeletal structures were found. The two mutations had opposite effects on the transcription of carbohydrate metabolism and meiosis genes. In both functional categories, the *med8-598*-affected genes had elevated transcription levels, whereas the *med31::ura4*$^+$-affected genes were repressed. In the case of genes involved in ribosome biogenesis and translation, the *med31::ura4*$^+$ mutation enhanced and the *med8-598* mutation decreased expression levels. Interestingly, the inactivation of *med31*$^+$ facilitated the transcription of 13 ribosomal protein genes. None of them were affected by *sep8-598*. All hexose, gluconate, glycerophosphodiester, and acetate transporter genes were repressed in *med31::ura4*$^+$ but unaffected in *med8-598*. Fatty acid transport and mRNA export from nucleus were down-regulated in *sep31::ura4*$^+$ cells only. Six of the eight DUFF999 family genes had increased expression in *med8-598*, while only three of them were up-regulated in *med31::ura4*$^+$.

Ten out of 35 Ace2-dependent genes identified by Rustici et al. (2004) and Alonzo-Nunez et al. (2005) showed modified expression levels (mainly down-regulated) in at least one of the mutants. Remarkably, all Ace2-controlled cell separation genes were down-regulated (Table 4).

A group of four *Sch. pombe*-specific telomeric duplications (SPAC977.01, SPAC750.05c, SPBPB2B2.19c and SPBC1348.02) coding for putative proteins of 99–100% sequence identity were down-regulated in both mutants. Their function is unknown but their transmembrane domains (similar to those of the eukaryotic lung seven transmembrane receptors) suggest that these proteins are probably localised on cell surface.

Impaired stress response in mutant cells

A considerable number of the genes affected by the mutations *med8-598* and/or *med31::ura4*$^+$ code for proteins involved in stress response and MFS transporters (Table 3; Chen et al. 2003). Since their changed expression levels may affect the ability of the cells to cope with external stresses, we tested both mutants for sensitivity to various stress conditions. As shown in Fig. 3 and Table 5, both mutants were more sensitive than the wild type to formamid, caffeine and

Table 4 Effect of the *med8-598* and *med31::ura4*$^+$ mutations on the transcription of Ace2-regulated genes listed in *Sch. pombe* GeneDB (http://www.genedb.org/genedb/pombe/)

Gene	Gene product	Function	Transcription	
			In *med31::ura4*$^+$	In *med8-598*
adg1$^+$	Predicted GPI anchored protein	Cell separation[1]	Down	Down
adg2$^+$	Predicted GPI anchored protein	Cell separation[1]	Down	Not affected
adg3$^+$	Predicted beta-glucosidase, SUN4 family	Cell separation[1]	Down	Down
agn1$^+$	Glucan endo-1,3-alpha-glucosidase	Septum edging degradation[2]	Down	Down
cdc4$^+$	Myosin II light chain	Contractile ring formation[3]	Not affected	Not affected
cfh4$^+$*; chr1*$^+$	Predicted chitin synthase regulatory factor	Uncharacterised	Not affected	Down
cut2$^+$	Securing	Sister chromatid separation[4]	Not affected	Not affected
eng1$^+$	endo-1,3-beta-glucanase	Primary septum degradation[5]	Down	Down
kap111$^+$	Predicted karyopherin	Nucleocytoplasmic transport, uncharacterised	Not affected	Not affected
mid2$^+$	Anillin homologue	Septin ring assembly and cell separatioin[6]	Down	Not affected
par2$^+$*; pbp2*$^+$	Protein phosphatase regulatory subunit	Septation initiation[7]	Not affected	Not affected
pob1$^+$	Boi family protein	Cell growth and septation[8]	Not affected	Not affected
rgf3$^+$	Rho guanyl-nucleotide exchange factor	Septation/maintenance of septum[9]	Not affected	Not affected

[1] Alonzo-Nunez et al. (2005)
[2] Dekker et al. (2004)
[3] McCollum et al. (1995)
[4] Nagao et al. (2004)
[5] Martin-Cuadrado et al. (2003)
[6] Tasto et al. (2003)
[7] Jiang and Hallberg (2001)
[8] Toya et al. (1999)
[9] Mutoh et al. (2005)

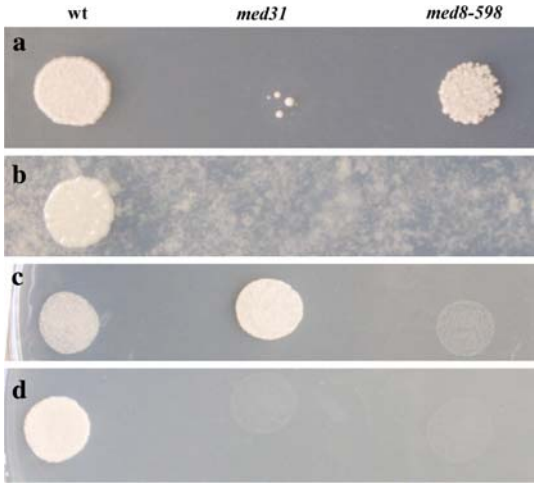

Fig. 3 Growth on media supplemented with salts. **a** 0.35 M NaCl. **b** 0.4 M Na_2SO_4. **c** 10 mM caffeine. **d** 3% formamide

Table 5 Effect of the *med8-598* and *med31::ura4*$^+$ mutations on stress response

Condition	Response		
	L972	*med31::ura4*$^+$	*med8-598*
Minimal inhibitory concentration (MIC)			
Sorbit	2.4 M	2.4 M	2.4 M
KCl	1.6 M	1.4 M	1.5 M
NaCl	0.6 M	0.5 M	0.5 M
$CaCl_2$	0.8 M	0.5 M	0.7 mM
$CdSO_4$	0.95 mM	0.65 mM	0.85 mM
Na_2SO_4	0.4 M	0.3 M	0.2 M
$ZnSO_4$	5.6 mM	5.0 mM	4.0 mM
Caffeine	12 mM	11 mM	8 mM
Formamid (on YEA)	3.0%	1.8%	1.8%
% survival			
Glucose starvation 6 days	85	33	84
Nitrogen starvation 6 days	94	53	24

Na_2SO_4, and to nitrogen starvation. $CaCl_2$ and $CdSO_4$ had stronger inhibitory effects on the *med31::ura4*$^+$ cells, whereas $ZnSO_4$ was more inhibitory to *med8-598*. The *med31::ura4*$^+$ cells were also sensitive to glucose starvation.

Cytoskeletal irregularities in mutant cells

In the *med8-598* and *med31::ura4*$^+$ mutant profiles, 10 genes with known or putative functions in the organisation of the actin-tubulin-cytoskeleton were affected (Table 3). Eight of them showed non-standard transcription in *med31::ura4*$^+$. This finding prompted us to compare the actin and tubulin structures in logarithmic phase cells of the wild-type (0-1) and the *med31::ura4*$^+$ (2-989) mutant cells. The mutant cells showed slightly abnormal actin and tubulin distribution, particularly at higher temperatures (Fig. 4). Actin dots appeared to be less organized, and the interphase tubulin cables were shorter than in the wild-type. About 20% of the cells showed random actin distribution and tubulin cables shifted to the cell side.

Identification of the *med8-598* mutation and its effect on splicing

Although *med8*$^+$ is an essential gene, the mutant *med8-598* is viable at 25°C and only shows a slight defect in cell separation, a non-essential cellular function (Grallert et al. 1999). To identify the *med8-598* mutation, we amplified and sequenced a nearly 0.8 kb fragment of the mutant allele from strain 2-689 *med8-598*. The comparison of its sequence with that of the wild-type *med8*$^+$ revealed a guanine-to-adenine single-nucleotide exchange at position 265. The replaced guanine is the last nucleotide of the predicted intron of the wild-type gene (129–265 nt). Since the location of the intron had not been confirmed experimentally before, we amplified and sequenced the corresponding cDNA from a wild-type cDNA library. Its nucleotide sequence did not contain the predicted intron, confirming that the detected mutation occurred at the intron/exon-2 boundary.

A mutation near an intron/exon boundary can affect the splicing of the RNA transcript of the gene. To examine the effect of the *med8-598* mutation on splicing, we isolated and reverse-transcribed total RNA from the mutant cells and from the wild-type cells grown at a semirestrictive temperature. The cDNAs of the *med8* transcripts were amplified, and the spliced and unspliced versions were identified by Southern hybridisation and *Stu*I digestion (Fig. 5). The level of the unspliced transcript was much higher in the mutant cells than in the wild-type cells, indicating that the *med8-598* mutation impaired the efficiency of splicing. When we translated the nucleotide sequence of the unspliced RNA into amino acid sequence, we encountered a stop codon at the fourth triplet in the intron. This early termination reduces the size of the protein to 22% of that of the wild-type Med8.

Over-expression of *med8*$^+$

To examine whether over-expression of the *med8*$^+$ gene had any effect on the cell, the coding region of the wild-type gene was PCR-amplified and cloned under the control of the inducible promoter *nmt1*$^+$ which is regulated by thiamine (Forsburg 1993). The resulting plasmid was introduced into a med$^+$ strain (0-38), and the transformants were grown on a medium supplemented with thiamine (*nmt1*$^+$ promoter repressed) and on medium containing no thiamine (*nmt1*$^+$ promoter induced). The two cultures did not show

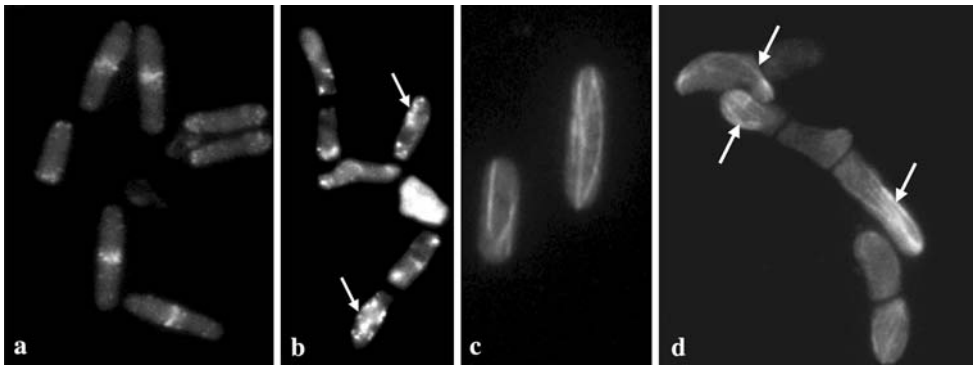

Fig. 4 Distribution of actin and tubulin in wild type and mutant cells. **a** Actin in 0–1 wild-type cells. **b** Actin in 2–989 *med31::ura4*$^+$ cells. **c** Tubulin in 0–1 wild-type cells. **d** Tubulin in 2–989 *med31::ura4*$^+$ cells. Arrows mark abnormal localisation of actin or tubulin

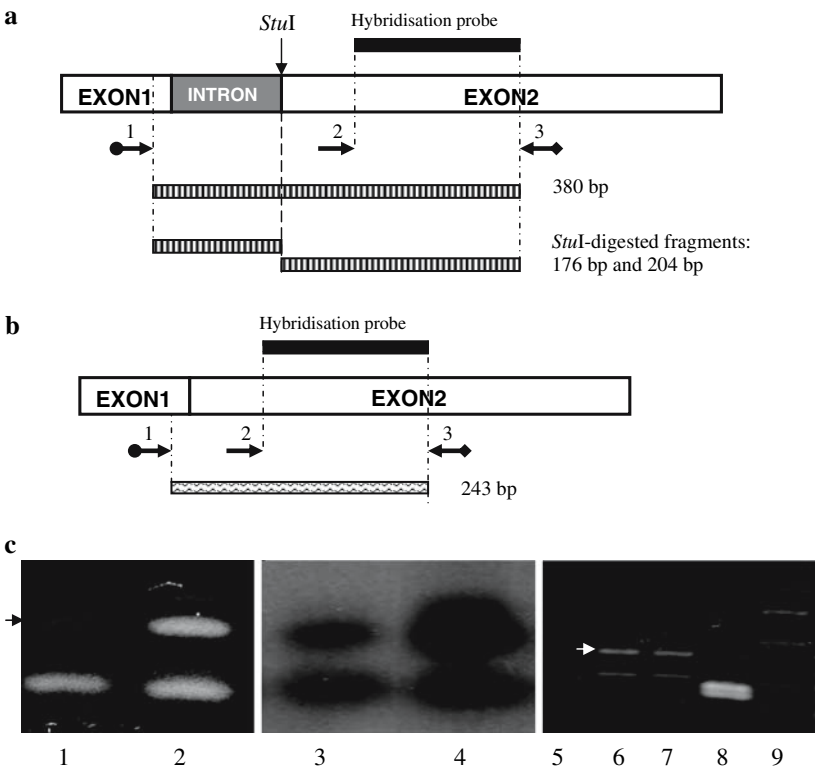

Fig. 5 Detection of unspliced *med8-598* RNA. **a** PCR fragments amplified from the *med8*$^+$ gene or the unspliced *med8*$^+$ cDNA. *1* primer ZS2; *2* primer ZS3; *3* primer ZS1. **b** PCR fragments amplified from the spliced *med8*$^+$ cDNA. *1* primer ZS2; *2* primer ZS3; *3* primer ZS1. **c** Detection of fragments. *Lane 1* *med8*$^+$ cDNA; *lane 2* *med8-598* cDNA; *lane 3* hybridisation of *med8*$^+$ cDNA with the probe; *4* hybridisation of *med8-598* cDNA with the probe; *5 Stu*I digestion of *med8*$^+$ cDNA (*Stu*I does not cut the spliced cDNA); *lanes 6* and *7 Stu*I digestion of *med8-598* cDNA (*Stu*I does not cut the unspliced cDNA either because the *med8-598* mutation eliminated the recognition site); *lane 8 Stu*I digestion of the *med8*$^+$ gene (*Stu*I cuts the wild-type DNA); *lane 9* 1-kb size marker. *Arrowheads* mark unspliced cDNA

any differences in growth or cell morphology, even after prolonged incubation. Thus, the over-expression of *med8*$^+$ has no detectable physiological effect.

Med8-598 is synthetically lethal with *med31::ura4*$^+$

As shown above, each med$^-$ mutation affected the expression of large sets of genes and caused drastic phenotypic changes. To examine their cumulative effect, we attempted to construct a strain that harboured both mutations. We hybridised 2-922 *med31::ura4*$^+$ with 2-870 *med8-598* by protoplast fusion and isolated tetrads of spores from the hybrid asci (Fig. 6a). The spore clones obtained were tested for the auxotrophic markers, for the presence of the *med31::ura4*$^+$ allele (Fig. 6b) and for cell morphology. Most tetrads were incomplete and no viable *med31::ura4*$^+$ *med8-598* spore was found in 20 tetrads. Among the viable spores, the wild-type:mutant proportion was 1:1 for each auxotrophic marker. These results indicate that the presence of both med$^-$ mutations in the genome is lethal to the cell.

Discussion

To obtain further insight into the functions of the cell separation genes *sep10*$^+$ (*med31*$^+$) and *sep15*$^+$ (*med8*$^+$), we performed genome-wide gene expression profiling in mutants defective in these genes. Since *med31*$^+$ is not essential (Szilagyi et al. 2002), we could use a mutant with a disrupted *med31*$^+$ allele. However, the complete inactivation (disruption) of *med8*$^+$ is lethal to the cell (Zilahi et al. 2000), and we therefore used the temperature sensitive

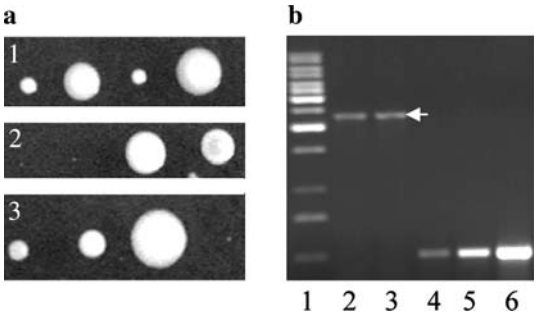

Fig. 6 Demonstration that the *med8-598 med31::ura4*$^{+}$ double mutant is not viable. **a** Three types of tetrads isolated from a *med8-598* × *med31::ura4*$^{+}$ hybrid . *1* All spores are viable; *2* two spores do not form colonies; *3* one spore does not form colony. **b** PCR-amplification of *med31* DNA from the spore clones of a tetrad containing three viable spores. *Lane 1* 1-kb size marker. *Lanes 2*, *4* and *5* spore clones. *Lane 3* positive control (*med31::ura4*$^{+}$ *ura4-D18 leu1-32* h^{90}). *Lane 6* negative control (0–1 wild-type). *Arrowhead marks med31::ura4*$^{+}$

med8-598 mutant. At a semi-permissive temperature the *med8-598* cells grow slowly and show partial cell separation defects (Grallert et al. 1999). Here, we show that the *med8-598* mutation is a single-nucleotide exchange at the intron/exon 2 boundary which impairs the efficiency of splicing. Consequently, a considerable proportion of the *med8-598* transcript remains unspliced. Since the unspliced transcript has a stop codon in the intron, the protein that can be produced from this transcript lacks almost 80% of the normal Med8 amino acid sequence. As mature mRNA is also produced, the mutant cells can synthesise wild-type Med8 proteins in an amount which is sufficient to keep the cells alive and ensure slow growth. This fact has to be taken into account when interpreting the microarray results. Due to the presence of wild-type Med8 proteins, the group of affected genes in *med8-589* may be smaller than the entire set of Med8-regulated genes. The temperature sensitivity of the *med8-589* cells might be due to the down-regulation of genes whose products are involved in the adaptation of the cell to higher temperatures or to unstable conformation of the truncated proteins produced from unspliced RNA molecules. If incorporated into Mediator, these undersized proteins may destabilise the structure of the complex, for example by undergoing conformational changes when the temperature is raised. The over-production of the wild-type Med8, on the other hand, does not affect Mediator function.

The genome-wide gene expression data raise the possibility that Med8 and Med31 are not absolutely required for the expression of all genes transcribed by RNA-polymerase II, but both are involved in the regulation of large subsets of genes. This finding is consistent with recent observations made in other organisms, where the lack of certain Mediator proteins also affected only subsets of genes (e.g. Holstege et al. 1998; Myers et al. 1999; Kim et al. 2004). Nevertheless, the analysis applied in this study did not allow the identification of all Med8 and Med31-modulated genes. The arbitrarily chosen threshold of twofold changes (up or down) as criterion for accepting genes as being regulated by Med8 or Med31 left all genes unidentified whose activity changed less than twofold in the mutant cells. Moreover, given that Med8 is essential, it is possible that transcription of many more or all genes is affected in the deletion mutant. It is also important to point out that the expression levels of all genes were ~40% lower on average in the *med31* mutant.

Since the mutations in the *med8*$^{+}$ and *med31*$^{+}$ genes cause both down-regulation and up-regulation, Med8 and Med31 appear to have both direct and indirect roles in the regulation of gene activity. Genes that are down-regulated in the mutants could be directly regulated by Med8 and/or Med31. At these genes (or at least some of them) the Med proteins could act as co-activators interacting directly with the gene-specific activators and the general transcriptional machinery. In numerous down-regulated groups, we found genes that code for other regulators of gene activity (e.g. *fhl1*, *rep2*, *rsv1*, SPAC1F5.11c, SPAC1399.05c, SPCC320.03, SPAC16E8.16). The indirect regulatory roles of the Med subunits could be attributed to these or some of these proteins. Many genes up-regulated in the mutants might be targets of repressors produced by the genes directly co-activated by the Med proteins. An alternative explanation for up-regulation in mutants can be that the Mediator subunits also have silencing functions. In *S. cerevisiae*, Med16 was found to have a repressive role at many promoters (Jiang and Stillman 1992). Nevertheless, it is impossible to elucidate the exact function of Med8 and Med31 purely based on microarray data. Notably, the mutation *med8-596* caused more up-regulation than down-regulation.

The med^{-} mutants differed in ~80% of the affected genes, which indicates that Med8 and Med31 also regulate distinct gene sets in the genome. What makes a gene a regulatory target of one or the other subunit? We examined two possibilities: the location of the genes in the genome and the biological function of the gene products. Since the genes of both sets are almost randomly scattered in all three chromosomes, neither of the Mediator subunits seems to specialise in any larger region of the genome. Nevertheless, small groups of adjacent genes were identified in every chromosome, which were simultaneously down- or up-regulated in one of the mutants. These islets suggest that the regulated genes are not entirely randomly distributed.

Med8 and Med31 do not seem to specialise in the regulation of genes on the basis of their biological functions either. The comparison of the functions of the affected genes revealed only few differences between the med^{-} mutants. A remarkable difference was found in the

regulation of transposons. In *Sch. pombe* two families of retrotransposons, Tf1 and Tf2, are known but only the Tf2 element is present in the form of full-length copies in the laboratory strain L 972 (Levin et al. 1990). We found that 10 of the 11 Tf2 transposons require the activity of *med31*$^+$ for transcription (although they are so similar at the DNA level that microarray probes may not be specific enough), whereas *med8*$^+$ does not seem necessary. Another difference was the proportion of genes involved in carbohydrate metabolism and energy production. It appears that *med31*$^+$ has an important role in energy household. For example all hexose transporter genes were down-regulated in *med31::ura4*$^+$ but were less affected in *med8-598*. *Sch. pombe* has six hexose transporter genes (*ght1* to *ght6*) (Heiland et al. 2000). A putative transcription factor gene, the ortholog of the *S. cerevisiae RTG1*, is a likely regulator of these transporter genes and was also down-regulated. *gti1*$^+$, *git3*$^+$ and *gpa2*$^+$ were also specifically repressed in *med31::ura4*$^+$. *gti1*$^+$ codes for a gluconate transporter inducer that is required for the onset of gluconate uptake triggered by glucose limitation (Caspari 1997). The products of *git3*$^+$ and *gpa2*$^+$ are members of the glucose-sensing mechanism performing negative regulation of transcription by glucose (Wang et al. 2005). The transcription of the hexokinase gene *hxk2*$^+$ and the invertase gene *inv1*$^+$ was also specifically reduced in *med31::ura4*$^+$. This finding points to a difference between *S. cerevisiae* and *Sch. pombe* in the functions of the Mediator subunits. The *S. cerevisiae* counterparts (*HXK2* and *SUC2*) of the latter two genes are regulated by Med8. The *S. cerevisiae* Med8 protein specifically binds to a downstream repressing sequence (*DRS*) of the *HXK2* gene and the upstream activating sequences of the *SUC2* gene (Chaves et al. 1999; Moreno-Herrero et al. 1999). The *S. cerevisiae* Hxk2 is a bifunctional protein with catalytic and regulatory functions. It is activated in the presence of glucose and inhibited when the levels of the sugar are low (Palomino et al. 2005). In our experiments, *hxk2* and *inv1* were not affected by the *med8-598* mutation, but both of them were down-regulated in *med31::ura4*$^+$. The *S. cerevisiae* Med8 binds directly to a heptameric motif (C/A G/A GAAAT) present in several glucose-regulated genes (Chaves et al. 1999). We could not find this or similar motifs in the promoters of the corresponding *Sch. pombe* genes. These results suggest that the *Sch. pombe* Med8 does not regulate the same group of genes as the *S. cerevisiae* Med8. The up-regulation of seven genes coding for mitochondrial proteins in *med8-598* represents still another difference between the med$^-$ mutants and indicate that Med8 may play a (probably indirect) role in the regulation of certain mitochondrial functions.

The cell separation defect characteristic of both mutants (Grallert et al. 1999) is probably due to the cumulative effect of the reduced transcription of genes involved directly or indirectly in septum dissolution and/or in cell wall degradation. The Sep1-controlled Ace2 transcription factor regulates many cell separation genes (for a review, see Sipiczki 2007). We found that six of those genes also required Med8 and/or Med31 for correct transcription. However, neither the *sep1*$^+$ nor the *ace2*$^+$ mRNA levels changed in the mutants. These results suggest that the Med8 and Med31 Mediator subunits do not act through the regulation of *sep1*$^+$ and/or *ace2*$^+$, but participate directly in the transcription of the Ace2-dependent cell separation genes, possibly as co-activators that interact with Ace2. They may also play indirect roles by co-activating additional genes such as *pmr1*$^+$ and *ubc4*$^+$. *pmr1*$^+$ codes for a Ca2$^+$-ATPase whose mutation has pleiotropic effects on septum formation and cytokinesis (Cortes et al. 2004). Ubc4 is a ubiquitin-conjugating enzyme involved in the degradation of the mitotic cyclin Cdc13 (Seino et al. 2003). Its inactivation causes abnormal mitosis with highly condensed chromosomes and arrests cells with unsplit septa. *pmr1*$^+$ was down-regulated in *med31::ura4*$^+$, whereas *ubc4*$^+$ was strongly up-regulated in both mutants.

In a recent study, we found a significant overlap between the transcription profiles of the med$^-$ mutants with those of *pmh1-26* and *mcs6*^{ts1} mutants (Lee et al. 2005). Pmh1 and Mcs6 are components of the Mcs6–Mcs2–Pmh1 complex (Lee et al. 2005), homologous to metazoan Cdk7-cyclin H-Mat1, which acts both as a CDK activating kinase and as a component of the general transcription factor IIH (reviewed by Harper and Elledge 1998; Fisher 2005). This suggests that gene groups are co-ordinately controlled by the Mcs6 complex and by the Mediator complex. Temperature-sensitive mutants of the genes *mcs6* and *pmh1* had previously been reported to arrest at restrictive temperatures as short chains of cells similar to the hyphae of sep$^-$ mutants (Saiz and Fisher 2002; Lee et al. 2005). Interestingly, nearly all *sep1*$^+$-regulated genes were also down-regulated in *mcs6*^{ts2} and *pmh1-26*, but *sep1*$^+$ itself was not affected. The overlap set included *ace2*$^+$, suggesting that the Mcs6 complex may have an indirect effect on cell separation, through the regulation of the transcription of *ace2*$^+$. This mechanism differs from that we suggest for *med8*$^+$ and *med31*$^+$ (see above), in which the Mediator subunits do not directly regulate *ace2*$^+$ but cooperate with the Ace2 transcription factor in the activation of cell separation genes.

The genes down-regulated in the *med31* and *med8* mutants showed also strong overlap with genes down-regulated after decreasing the expression levels of the *rpb4*$^+$ gene, which encodes the RNA polymerase II component Rpb4 (Sharma et al. 2006). Decreased *rpb4*$^+$ expression leads to a hyphal phenotype similar to the morphology of the *med31* and *med8* mutants, indicating that the general transcription machinery in combination with Mediator

components is particularly important for the transcription of genes required for cell separation (Sharma et al. 2006).

The conjugation defect and the low sporulation efficiency of the *med31::ura4*$^+$ cells (Szilagyi et al. 2002) could be attributed to the down-regulation of a group of 12 genes listed as conjugation genes and meiosis genes in Table 3 and numerous additional genes assigned to other functional categories (e.g. *isp5*$^+$, *plb1*$^+$ and *srk1*$^+$). *isp5*$^+$ encodes an amino acid permease homologue whose inactivation makes the cell sterile (Sato et al. 1994). The Plb1 protein is a phospholipase B homologue that acts as a mediator of the osmotic stress response and the nutrient-dependent repression of sexual differentiation (Yang et al. 2003). The Srk1 protein kinase is involved in the Sty1/Spc1-regulated process of sexual differentiation (Smith et al. 2002). Although *med8-598* does not cause sterility, it also reduces the transcription of some of the above genes.

We have found previously that mutations in *med8*$^+$and *med31*$^+$ make the cells hypersensitive to heat shock. The results of the present work reveal that both med$^-$ mutations change the expression of many genes that are known to act in stress response (e.g. Chen et al. 2003). The increased sensitivity of the mutant cells to heat shock might be the consequence of the down-regulation of the heat shock protein *ssa1*$^+$ and proteins associated with the heat-shock systems such as *srk1*$^+$ (Smith et al. 2002), *sti1*$^+$ (Yamashita et al. 1996), SPCC63.13 and SPCC63.03. The mutants were capable of mounting a nearly normal response to osmotic stress by sorbitol, NaCl and KCl but not to $CaCl_2$. The increased sensitivity of *med31::ura4*$^+$ cells to $CaCl_2$ may be due to the down-regulation of *asp1*$^+$, whose deletion is known to cause hypersensitivity to Ca^{++} ions (Feoktistova et al. 1999). The *med31::ura4*$^+$ cells died faster than the wild-type cells during glucose starvation, which may be the consequence of the low expression level of *rsv1*$^+$ that encodes a stationary phase protein required for cell viability in glucose-starved environments (Hao et al. 1997). We also found a significant overlap between the down-regulated genes in the med$^-$ mutants and the genes down-regulated during cisplatin treatment (Gatti et al. 2004). This overlap in gene expression signatures raises the possibility that cisplatin can interfere with Mediator function, but more work will be required to further explore this possible relationship.

Apart from its role in response to calcium ions, *asp1*$^+$ is also important for the function of the cortical actin cytoskeleton (Feoktistova et al. 1999). Three additional genes (*mid2*$^+$, SPAC17H9 and SPBC12C2.05c) with known or inferred functions in the organisation of actin had low transcription levels in the *med31::ura4*$^+$ mutant. Consistent with this and with the down-regulation of *klp5*$^+$ (kinesin-like protein), the *med31::ura4*$^+$ cells showed slight aberrations in the organisation of the actin and tubulin cytoskeleton.

Although numerous genes down-regulated in *med31::ura4*$^+$ are essential, the mutant cells are viable. Their viability indicates that the inactivation of the Med31 protein reduces rather than abolishes the activity of these genes. This further suggests that this Mediator subunit only modulates and fine-tunes the activity of the genes. Nevertheless, the lack of Med31 becomes lethal in combination with reduced Med8 activity.

In conclusion, the genome-wide gene expression profiling of mutants revealed that large, partially overlapping sets of genes of diverse biological functions are dependent on the activity of the Mediator subunits Med31 and Med8. Six Sep1-Ace2-dependent cell separation genes, not including *sep1*$^+$ or *ace2*$^+$, are among the target genes, suggesting that the Med8 and Med31 proteins may be direct coactivators of cell separation genes. Med31 also seems to be involved in the (direct or indirect) regulation of transposons, genes functioning in sexual differentiation, stress response and energy household. The large number of affected genes and the complexity of the mutant phenotypes also indicate that the subunits of Mediator may contribute to the coordination of cellular processes by fine-tuning of the expression of larger sets of genes.

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