The Structural and Functional Role of Med5 in the Yeast Mediator Tail Module

Jenny Béve1, Guo-Zhen Hu1, Lawrence C. Myers5, Darius Balciunas1,1, Olivia Werngren6, Kjell Hultenby**, Rolf Wibom1, Hans Ronne1,1, and Claes M. Gustafsson1,2

From the 1Division of Metabolic Diseases and 2**Clinical Research Center, Karolinska Institutet, Novum, Karolinska University Hospital, SE-141 86 Stockholm, Sweden, the 3Department of Medical Biochemistry and Microbiology, Uppsala University, Box 582, SE-751 23 Uppsala, Sweden, the 4Department of Plant Biology and Forest Genetics, Uppsala Genetic Center, Swedish University of Agricultural Sciences, Box 7080, SE-750 07 Uppsala, Sweden, and the 5Department of Biochemistry, Dartmouth Medical School, Hanover, New Hampshire 03755-3844

Med5 (Nut1) is identified here as a component of the Mediator tail region. Med5 is positioned peripherally to Med16 (Sin4) together with the three members of the putative Gal11 module, Med15 (Gal11), Med2, and Med3 (Pgd1). The biochemical analysis together with the three members of the putative Gal11 module, Med5 (Nut1), a tail region. Med5 is positioned peripherally to Med16 (Sin4) and enhancement of phosphorylation of the C-terminal domain of pol II by the transcription factor IIH kinase (2, 3). Mediator also contains a histone acetyltransferase (HAT) activity, which is not found in other eukaryotic Mediator complexes (4). The HAT activity was localized to Med5 (Nut1), a S. cerevisiae-specific protein, which lacks homologues in higher eukaryotes (4, 5). The MED5 gene was originally isolated in a screen for mutants that would suppress the Swi4/Swi6 dependence of a synthetic reporter gene containing part of the HO promoter (6). Several other genes encoding Mediator proteins were identified in the same screen, including MED10 (NUT2), MED16 (Sin4), MED19 (ROX3), MED12 (SRB8), MED13 (SRB9), CDK8 (SRB10), and CYCC (SRB11). The MED5 gene is nonessential in yeast. A deletion of MED5 relieves repression at the LRR2 element in the HO promoter but only in combination with a mutant allele of either MED10 or CCR4 (6). These effects on the HO promoter were seen with a lacZ reporter gene but not at the endogenous HO gene locus. The in vivo role of Med5 in Mediator-dependent gene expression therefore remains an open question.

In the presence of RNA pol II, Mediator adopts an extended conformation that embraces the globular pol II core complex (7). The extended structure reveals three distinct submodules of Mediator. Direct contacts are formed between pol II and the head and middle region (7, 8). The largest part of Mediator is made up of an elongated tail region, which does not appear to contact pol II. Structural analysis of mutant Mediator complex has demonstrated that the tail region contains the Med2, Med3, Med15 (Gal11), and Med16, proteins, which are involved in interactions with a number of different activators, including Gal4 and Gcn4 (9). The Med2, Med3, and Med5 proteins form a module (the Gal11 module), which in part function as a separate entity. In cells lacking Med16, the Gal11 module is detached from Mediator and recruited as a separate entity to the ARGI promoter by the yeast activator Gcn4 (10). Interestingly, this free Med2-Med3-Med15-containing complex recruits TBP and pol II to the promoter, and cells that lack Med16 show no defects in the induced expression levels of either of the two Gcn4-dependent ARG1 and SNZ1 genes, even though the middle or head module Mediator subunits cannot be detected at these promoters. It is possible that alternative activator-coactivator complex formations are functional at the ARGI and SNZ1 genes and that the isolated tail module might interact with, for example, the SAGA or SWI/SNF complexes in a way that is sufficient for the assembly of a functional preinitiation complex.

We have characterized the Med5 protein here and demonstrate that it is a component of the Mediator tail domain. We find that the in vivo role of Med5 is related to the function of other components in the tail module. Med5 influences the regulated expression of nuclear encoded components of the oxidative phosphorylation (OXPHOS) machinery, and deletion of the MED5 gene affects both carbon source usage and oxygen consumption.

MATERIALS AND METHODS

Antibodies and Immunoblot Analyses—Synthetic polypeptides corresponding to amino acids 1–15 of Med5 were used to immunize rabbits. The antisera used in this study were taken 10 days after the second booster injection (Innovagen AB, Lund, Sweden). Immunoblot analysis for Mediator proteins was as described (3, 5). Other antibodies used in this study were kindly provided by Drs. R. A. Young (Med18 (Sr5) and Med20 (Sr22)), Y.-J. Kim (Med14 (Rgr1) and Med16), and A. Aguilera (Med3).
Recombinant Proteins—DNA fragments encoding S. cerevisiae Med2, Med3, Med5, Med15, and Med16 were amplified from genomic DNA. PCR products were cloned into the vectors pBacPAK8 or pBacPAK9 (Clontech). Autographa californica nuclear polyhedrosis viruses recombinant for the individual proteins were constructed as described in the BacPAK manual (Clontech). Spodoptera frugiperda (S9) cells were maintained and propagated in suspension in SFM 900 medium (Invitrogen) containing 5% fetal calf serum at 27 °C. For protein expression, S9 cells were grown in suspension and harvested 60–72 h postinfection. Infected cells were frozen in liquid nitrogen and thawed at 4 °C in lysis buffer containing 25 mM Tris-HCl, pH 8.0, 20 mM β-mercaptoethanol, and 1× protease inhibitors (for all purifications a 100× stock of protease inhibitors contained 100 mM phenylmethylsulfonyl fluoride, 200 mM pepstatin A, 60 mM leupeptin, and 200 mM benzamidine in 100% ethanol). The cells were incubated on ice for 20 min, transferred to a Dounce homogenizer, and disrupted using 20 strokes of a tight-fitting pestle. NaCl was added to a final concentration of 1.5 M, and the homogenate was swirled gently for 40 min at 4 °C. The extract was cleared by centrifugation at 45,000 rpm for 45 min at 4 °C using a Beckman TLA 100.3 rotor.

Protein Purification—Purification of the RNA pol II holoenzyme and mutant versions thereof was described previously (9). For purification of the Med2-Med3 dimer, S9 cells were co-infected with recombinant baculoviruses expressing Med2 and Pgd1 at a multiplicity of infection of 10 for each virus. After lysis and centrifugation, the supernatant was dialyzed against buffer Q-0.1 (20 mM Tris acetate, pH 7.8, 10% glycerol, 1 mM dithiothreitol, and 1× protease inhibitors; the number after the hyphen (e.g. Q-0.1) indicates the potassium acetate concentration in molar units) overnight. The dialyzed material was loaded on a Mono S 5/5 FPLC column (Amersham Biosciences) that had been equilibrated in buffer Q-0.1. The MED2-PGD1 dimer eluted at about 700 mM KAC. The eluate was dialyzed against buffer A-0.08 (25 mM Hepes-KOH, pH 7.6, 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol, and 1× protease inhibitors; the number after the hyphen (e.g. A-0.08) indicates the potassium acetate concentration in molar units) overnight. The dialyzed material was applied to a Mono S 5/5 column (Amersham Biosciences) that had been equilibrated in buffer A-0.1. The column was washed with 5 ml of buffer A-0.1 and eluted with a linear gradient (15 ml) of buffer Q-0.1–1.0. The MED2-PGD1 dimer eluted at about 700 mM KAC. The eluate was dialyzed against buffer A-0.08 (25 mM Hepes-KOH, pH 7.6, 10% glycerol, 1 mM EDTA, 1 mM dithiothreitol, and 1× protease inhibitors; the number after the hyphen (e.g. A-0.08) indicates the potassium acetate concentration in molar units) overnight. The dialyzed material was applied to a Mono S 5/5 column (Amersham Biosciences) that had been equilibrated in buffer A-0.1. The column was washed with 5 ml of buffer A-0.1, and proteins were eluted with a linear gradient (15 ml) of buffer A-0.1–1.0. A peak of the MED2-MED3 dimer eluted around 45 mM KAC. The peak fractions were dialyzed against buffer H-0.07 (20 mM KPO4, pH 7.6, 10% glycerol, 1 mM dithiothreitol, and 0.2 mM EDTA, pH 8.0; the number after the hyphen (e.g. H-0.07) indicates the potassium acetate concentration in molar units) for 4 h and loaded on a 1-ml HiTrap heparin column (Amersham Biosciences) that had been equilibrated with buffer H containing 100 mM KAC. Essentially pure MED2-MED3 eluted around 0.4 M KAC. The peak fractions were frozen in liquid nitrogen and finally stored at −80 °C. To determine the apparent molecular weight of the MED2-MED3 dimer and the MED2-MED3-MED5-MED15 tetramer, a portion (0.5 ml) of the Mono S (MED2-MED3) or the heparin peak (MED2-MED3-MED5-MED15) was applied to a Superose 12 column (Amersham Biosciences) equilibrated in Q-0.2.

Affymetrix GeneChip Probe Array Analyses and Real-time PCR—Total yeast RNA was isolated using a hot acid phenol extraction protocol (11). Poly(A)+ RNA was prepared from total RNA using the Oligotex Midi Kit (Qiagen). The cDNA synthesis, cRNA synthesis, and labeling, as well as array hybridization to Affymetrix yeast S98 arrays, were performed at the Karolinska Institute Affymetrix core facility as described in the Affymetrix users’ manual (30). Washing and staining of arrays were performed using the GeneChip Fluidics Station 400 and by scanning with the Affymetrix GeneArray scanner. Acquisition and quantification of array images as well as primary data analysis were performed using Affymetrix GeneChip operating software (GCOs) 1.2. The transcription analysis was performed with RNA prepared from two wild type and two medΔ cell cultures. Each of the two wild type RNA preparations was compared with both of the medΔ RNA preparations, generating a total of four comparisons. Genes absent according to the Affymetrix detection algorithm on both arrays of a comparison were excluded from the presented data. Genes were included if the Affymetrix change algorithm gave significant change calls (p < 0.0025) in all four comparisons. To verify changes in gene transcription, real-time quantitative PCR was carried out on ABI Prism 7700 sequence detection system with Absolute QPCR SYBR Green reagents (Applied Biosystem) and primers at a concentration of 0.5 μM. PCR conditions were standardized to 40 cycles: 95 °C for 15 s followed by 60 s for 1 min. The -fold change reported in TABLE THree is the average of at least three separate experiments. Primer sequences are available upon request.

Yeast Strains and Genetic Manipulations—Yeast strains used in this study are listed in TABLES ONE and TWO. For disruption of MED5, the KanMX4 cassette was amplified from Y04518 (med5:KanMX4) with primers binding to sequences 500 bp upstream and downstream of the cassette. The KanMX4-containing DNA fragment was used to construct the JB001 and JB002 strains using standard genetics methods (12, 13). The Y04393, Y01742, Y01976, Y04518, and Y13701 strains were from the Euroscarf collection. A MED5-encoding DNA fragment was amplified from the yeast strain W303-1A including 500 bp upstream and downstream of the open reading frame by PCR (forward primer: 5′-CCGGGGATCCCTTCTCATACTCTCTCATAATGGT-3′; reverse primer: 5′-CCGCGCCGCGGCG-CCAGCGTACGACTGTTATA-3′). The MED5-encoding fragment was digested with BamHI and Smal and subsequently cloned into the pHR81 vector (20).

### Table One

| Strain   | Relevant genotype |
|----------|-------------------|
| H966     | MATα med15–1::HIS3 |
| DY1699   | MATα med16::LEU2  |
| JB001 (H1425) | MATα med5::KanMX4 |
| JS004    | MATα med5::URA3   |
| JB002 (H1426) | MATα med5::KanMX4 med15–1::HIS3 |
| H1310    | MATα med21–101 ts |
| H1285    | MATα med21–101 ts |
| H845     | MATα cyC–1::LEU2  |
| H707     | MATα med1–2::HIS3 |
| JS005 (JSY130) | MATα el3::LEU2 |
| JS006 (JSY141) | MATα gen5::HIS3 |

**Med5 Is a Component of the Mediator Tail Domain**

| Yeast strains congenic to W303-1A |
|-----------------------------------|
| These strains carry the SUC2 ade2-1 can 1-100 his3-11,15 leu2-3,112 trp1-1 ural3-1 markers. |
Med5 Is a Component of the Mediator Tail Domain

Growth Curves and Measurements of Respiration Rates—Yeast strains grown in YPD (1% yeast extract, 2% peptone, 2% dextrose) were used as inocula. Exponentially growing cells were collected by centrifugation, and the pellet was washed once with water and once with YPG (1% yeast extract, 2% peptone, 3% glycerol). The cultures were diluted with YPG to an initial OD of 0.1–0.2, and the growth was monitored by OD measurements for ~60 h.

For measurements of respiration rates, cells were grown exponentially for 12–24 h in synthetic complete (SC) medium (yeast nitrogen base supplemented with ammonium sulfate (Qbiogene catalog no. 4027-512), 2% glucose, and amino acids). The cultures were then diluted to OD 0.1–0.15, and respiration was measured after 2–3 generations. Oxygen consumption in whole cells was measured using a Clarke electrode connected to a CBI D3–02 control box (Hansatech, Kings Lynn, United Kingdom). Exponentially growing cells were harvested by centrifugation at 1800 rpm in a clinical centrifuge (Jouan CR422) and the pellet was washed once with water and once with 40 mM NaPO4, pH 7.4. We estimated the cell count using a Bürkert counting chamber.

The respiration rate was measured at 30 °C by adding 2.5 × 107 cells to a water-jacked chamber containing 1 ml of 40 mM NaPO4, pH 7.4, with 1% glucose added as substrate. Cyanide-sensitive respiration was calculated after the addition of 1 mM KCN when the cyanide-insensitive respiration was subtracted from the total respiration. Measurements were repeated at least three times for each individual strain.

Measurement of Citrate Synthase Activity and Mitochondrial Volume Density—Exponentially growing cells (OD 0.5–0.8) in SC-glucose medium were harvested by centrifugation at 1800 rpm and +4 °C in a clinical centrifuge (Jouan CR422) and washed once with water followed by two washes with buffer J (0.138 M NaCl, 2.7 mM KCl, 0.01 M NaPO4, pH 7.4, 1 M sorbitol). Approximately 10^8 cells were resuspended in 0.6 ml of buffer J and incubated with 400 units of lytase at 30 °C for 50–60 min to digest the cell wall. Spheroplasts were collected by centrifugation, washed twice with buffer J, and frozen at −20 °C. Citrate synthase activity was determined in triplicate for each strain as described previously (14, 15). Volume density measurements of mitochondria on electron micrographs were performed as described previously (16). Twenty-five randomly selected cells from each strain were selected for analysis.

RESULTS

Med5 Is a Component of the Mediator Tail Module—We and others have reported previously that the Med5 protein is present in highly purified preparations of the S. cerevisiae Mediator complex (5, 17). To further verify that Med5 is a stable component of Mediator, we raised polyclonal antibodies against a synthetic peptide corresponding to the N-terminal 15 amino acids of Med5. The Med5 protein co-purified with Mediator on Bio-Rex70, DEAE-Sepharose FF, hydroxyapatite, Mono Q, and Superose 6. Immunoblot analysis showed co-elution from Superose 6 of Med5 with Med2 and Med20 (Fig. 1A).

Several nonessential Mediator subunits (Med2, Med3, Med15, and Med16) have previously been localized to the Mediator tail module, and we therefore investigated the presence of Med5 in Mediator purified from med2Δ, med3Δ, med15Δ, and med16Δ mutant strains. We found Med5 present in Mediator from the med2Δ, med3Δ, and med15Δ strains, demonstrating that Med5 does not depend on any of the encoded proteins for stable interaction with the rest of the Mediator (Fig. 1B). Analysis of Mediator from the med16Δ strain did, however, not only reveal a loss of the known Gal11 module proteins, as reported previously (9), but also a loss of Med5 (Fig. 1C). We therefore conclude that Med5 is a new component of the Mediator tail module and that it is located peripherally to Med16, together with subunits of the Gal11 submodule. Our attempts to purify Mediator from a med5Δ strain and analyze its subunit composition proved difficult, because the mutant Mediator complex precipitated during later stages of protein purification. We could however use immunoblot analysis to demonstrate that the med5Δ mutant Mediator purified as a multiprotein complex, which still contained the other tail module components Med2 and Med16 (Fig. 1D).

Med5 Interacts with the Gal11 Module—We constructed recombinant baculoviruses for expression of Med2, Med3, Med5, Med15, and Med16 and investigated the association of these subunits by co-expression in insect cells. When Med2 and Med3 are expressed together, they form a soluble complex that can be purified to near homogeneity. Gel
filtration analysis demonstrated that the Med2-Med3 complex had an apparent molecular weight of about 90 kDa, suggesting that the two proteins form a stable dimer that can be purified to homogeneity. Peak fractions from a Superose 12 column (Amersham Biosciences) were analyzed by 12% SDS-PAGE. Proteins were stained with Coomassie Brilliant Blue. B, Med2, Med3, Med15, and Med5 that had been co-expressed in insect cells from a complex that co-migrates on an S-Sepharose column. Fractions were analyzed by 10% SDS-PAGE and immunoblotted with antibodies directed against the different proteins, as indicated in the figure. C, the peak from the S-Sepharose column was further fractionated by gel filtration on a Superose 12 column. Fractions were analyzed as described in B. D, proposed organization of the S. cerevisiae Mediator tail module.

Genetic Interactions of MED5 with MED15 and MED21—We next monitored genetic interactions between MED5 and genes encoding other Mediator subunits. We found a synthetic lethality between the med5/H9004 knock-out and med21-ts (Fig. 3A). The latter allele, which is temperature-sensitive (med21-ts); Wt, wild type. Note that predicted double mutants are all dead. B, MED5 interacts genetically with MED15. The med5/H9004/med15/H9004 double mutant is temperature-sensitive on YPD at 36 °C. The intact MED5 gene restores growth at 36 °C when present on a plasmid (pMED5). Wt, wild type. C and D, deletion of MED5, MED16, or CYCC causes increased growth, whereas deletion of ELP3, GCN5, or MED15 causes decreased growth on rich glycerol medium (YP-Glycerol). After an initial lag period, a med1/H9004 strain also displayed increased growth. Growth was monitored by OD measurements for ~60 h.
Med5 Is a Component of the Mediator Tail Domain

![Figure 4](image-url)

**FIGURE 4. Mediator tail module mutants display increased respiration and mitochondrial activity.** 
A, oxygen consumption was measured at 30 °C as described under “Materials and Methods.” The bars indicate mean ± S.D. B, citrate synthase (CS) activity was measured as described under “Material and Methods.” The bars indicate mean ± S.D. The activities are given as fold change compared with the wild type strain. C, mitochondrial volume density (the mitochondrial volume fraction in the cytoplasm) in wild type, med5Δ, and med16Δ strains. The bars indicate mean ± S.E.

Med5 has a few unique properties. First, Med5 is temperature-sensitive at 38 °C, encodes an N-terminally truncated Med21 protein that lacks amino acid residues 2–8.4 We also found a synthetic interaction between the med5Δ and med15Δ knock-outs. The med5Δ med15Δ double knock-out strain is temperature-sensitive at 35 °C, as compared with 38 °C for the med15Δ strain and no temperature sensitivity for the med5Δ strain (Fig. 3B). No synthetic interaction was observed between the med5Δ and med16Δ knock-outs (data not shown), which is consistent with the finding that loss of Med16 also leads to loss of Med5 (Fig. 1C) and supports a localization of Med5 peripherally to Med16 (Fig. 2D).

**med5Δ Strain Displays Increased Respiration**—The med2Δ, med3Δ, med15Δ, and med16Δ strains all exhibit gal− phenotypes, whereas the med5Δ strain is gal+ (data not shown). This is consistent with our observation that Med5 is not required for association of the Gal11 module with Mediator (Fig. 1D). Interestingly, the med5Δ strain grows significantly better than the wild type strain on the nonfermentable carbon source glycerol (Fig. 3C and data not shown). Enhanced growth on glycerol was also seen for some other Mediator mutant strains, including med16Δ, med1Δ and cycCΔ. In contrast, deletion of MED15, GCN5 (encoding a component of the SAGA complex), or ELP3 (encoding a component of the Elongator complex) produce a slow growth phenotype (Fig. 3C and D). We also compared oxygen consumption in wild type and mutant yeast strains using a Clarke electrode. The med5Δ deletion caused a near 2-fold increase in oxygen consumption compared with a wild type strain (Fig. 4A).

**Mitochondrial Density**

We next used DNA chip technology to identify genes that are dependent on Med5 for their expression. We compared med5Δ and wild type strains grown in YPD and observed no significant changes in global gene transcription (data now shown). When the cells were grown in synthetic complete glucose medium (SC-glucose), we saw dramatic changes in global gene transcription, and distinct functional patterns emerged. The three most affected genes were ANB1, DAN1, and TIR1, which displayed an almost 10-fold decrease in transcription (TABLE THREE). These genes belong to the group of anaerobic genes, which are induced in anaerobic cells and repressed in aerobic cells. The DAN/TIR genes encode homologous mannoproteins, which contribute to the anaerobic remodeling of the cell wall (18, 19), whereas ANB1 encodes an anaerobic isofrom of translation initiation factor elf-5A (21). We also noted an up-regulation of MUC1 (FLO11), a cell surface glycoprotein required for diploid pseudohyphal formation and haploid invasive growth (20, 21). In agreement with these changes in MUC1 gene transcription, scanning electron microscopy revealed a weak chain-forming phenotype for both the med5Δ and med16Δ strains (data not shown).

Finally, we observed changed expression for a large number of genes encoding components involved in oxidative phosphorylation. No less than 32 nuclear encoded OXPHOS genes were significantly up-regulated in the med5Δ strain, and only one single nuclear OXPHOS gene was down-regulated. The down-regulated gene was COX5b, which encodes an anaerobically expressed isofrom of the cytochrome-c oxidase subunit V. The COX5a gene, which encodes the aerobically expressed isofrom of the same cytochrome-c oxidase subunit, was up-regulated in the med5Δ strain. The observed expression pattern is therefore consistent with the increased respiration observed in the med5Δ strain. In contrast to the nuclear genes, which were up-regulated, transcription of the mitochondrial OXPHOS genes AAT1, COX2, COX3, ATP6, and COB was down-regulated. Expression of nuclear and mitochondrial OXPHOS genes are coordinated in wild type cells, and the opposite effects observed in the med5Δ strain were therefore unexpected.

**DISCUSSION**

We here identify Med5 as a structural component of the Mediator tail domain. Deletion of the MED16 gene leads to a loss of Med5 from the purified Mediator complex along with the Gal11 module components Med2, Med3, and Med15. Consistent with the idea that Med5 resides on the periphery of Med16 together with the Gal11 module, the four proteins form a soluble complex when co-expressed in insect cells. The localization of Med5 to the tail domain is also supported by the in vivo interactions between Med5 and Med16 observed in a recent yeast two-hybrid screen (22).

On the basis of our biochemical characterization, we postulate a model for the Mediator tail domain subunit architecture (Fig. 2D). To support the model, we investigate the functional importance of Med5.

---

4 M. Hallberg, G.-Z. Hu, D. Balcıunus, Z. Shaikhlibrahim, S. Björklund, and H. Ronne, manuscript submitted for publication.
Med5 Is a Component of the Mediator Tail Domain

TABLE THREE
Genes affected in a med5Δ strain

Shown are genes in which expression is most affected in a med5Δ strain as compared with the wild type. The -fold change is given as an average of all four comparisons. For expression analysis, we used the Affymetrix yeast S98 arrays as described under “Materials and Methods.” Some of the changes in gene transcription were verified with real-time quantitative PCR, and real-time fold changes (RT/FC) from these experiments are indicated in the table. GPI, glycosylphosphatidylinositol.

| Genes                                      | Systematic name | Common name | -Fold change | RT/FC |
|--------------------------------------------|-----------------|-------------|--------------|-------|
| Nuclear encoded OXPHOS-related genes       |                 |             |              |       |
| Ubiquinol-cytochrome-c oxidoreductase complex | YHR001W-A       | QCR10       | 3.7          | 4.4   |
| Protein that inhibits ATP hydrolysis by the F1F0-ATP synthase | YDL181W       | INH1        | 3.3          | 3.9   |
| Cytochrome cβ                                | YMR256C         | CYT1        | 2.6          |       |
| Ubiquinol-cytochrome-c reductase            | YEL024W         | RIP1        | 2.5          | 3.9   |
| Subunit IV of cytochrome-c oxidase          | YGL187C         | COX4        | 2.4          | 2.6   |
| Iron-sulfur protein subunit of succinate dehydrogenase | YLL041C     | SDH2        | 2.4          | 1.3   |
| Subunit VII of cytochrome-c oxidase         | YMR256C         | COX7        | 2.3          | 2.6   |
| Subunit Va of cytochrome-c oxidase          | YNL052W         | COX5A       |             |       |
| Subunit 2 of the ubiquinol cytochrome-c reductase complex | YPR191W       | QCR2        | 2.1          |       |
| Subunit 7 of the ubiquinol cytochrome-c reductase complex | YDR529C       | QCR7        | 2.1          |       |
| Subunit VI of cytochrome-c oxidase          | YHR051W         | COX6        | 1.9          |       |
| Subunit d of the F1F0-ATP synthase          | YKL016C         | ATP7        | 1.8          |       |
| Subunit 9 of the ubiquinol cytochrome-c reductase complex | YGR183C       | QCR9        | 1.8          |       |
| Subunit g of the mitochondrial F1F0-ATP synthase | YPR020W       | ATP20       | 1.8          |       |
| NADHubiquinone oxidoreductase               | YML120C         | ND11        | 1.8          |       |
| ε subunit of the F1 sector of mitochondrial F1F0-ATP synthase | YPL271W       | ATP15       | 1.8          |       |
| Flavo protein subunit of succinate dehydrogenase | YKL148C      | SDH1        | 1.7          |       |
| Subunit 6 of the ubiquinol-cytochrome-c reductase complex | YFR033C       | QCR6        | 1.7          |       |
| Subunit 8 of ubiquinol-cytochrome-c reductase complex | YJL166W       | QCR8        | 1.7          |       |
| β subunit of the F1 sector of mitochondrial F1F0-ATP synthase | YJR121W       | ATP2        | 1.7          |       |
| Subunit 5 of the stator stalk of mitochondrial F1F0-ATP synthase | YDR298C       | ATP5        | 1.7          |       |
| Subunit h of the F1 sector of mitochondrial F1F0-ATP synthase | YLR295C       | ATP14       | 1.6          |       |
| δ subunit of the central stalk of mitochondrial F1F0-ATP synthase | YDL004W       | ATP16       | 1.6          |       |
| Subunit b of the mitochondrial F1F0-ATP synthase | YPL078C       | ATP4        | 1.6          |       |
| Cytochrome b subunit of succinate dehydrogenase | YKL141W       | SDH3        | 1.6          |       |
| γ subunit of the F1 sector of mitochondrial F1F0-ATP synthase | YBR039W       | ATP3        | 1.5          |       |
| Subunit f of the F1 sector of mitochondrial F1F0-ATP synthase | YDR377W       | ATP17       | 1.5          |       |
| Subunit j of the mitochondrial F1F0-ATP synthase | YML081C-A     | ATP18       | 1.5          |       |
| Subunit k of the mitochondrial F1F0-ATP synthase | YOL077W-A     | ATP19       | 1.5          |       |
| Subunit e of mitochondrial F1F0-ATPase       | YDR322C-A       | TIM11       | 1.4          |       |
| Membrane anchor subunit of succinate dehydrogenase | YDR178W       | SDH4        | 1.4          |       |
| Subunit VIIa of cytochrome-c oxidase         | YDL067C         | COX9        | 1.3          |       |
| Subunit VIIb of cytochrome-c oxidase         | YIL111W         | COX5B       | −2.4         |       |
| Mitochondrially encoded OXPHOS-related genes |                 |             |              |       |
| Subunit 8 of the F1F0-ATP synthase          | Q0080           | AAP1        | −2.3         |       |
| Subunit 6 of the F1F0-ATP synthase          | Q0085           | ATP6        | −2.6         |       |
| Subunit III of cytochrome-c oxidase          | Q0275           | COX3        | −3.4         |       |
| Subunit II of cytochrome-c oxidase           | Q0250           | COX2        | −3.6         |       |
| Cytochrome b                                 | Q0105           | COB         | −11.9        |       |
| Other processes                             |                 |             |              |       |
| GPI-anchored cell surface glycoprotein       | YIR019C         | MUC1        | 12.4         | 25.8  |
| Plasma membrane-localized protein           | YFL014W         | HSP12       | 5.3          |       |
| Putative plasma membrane protein            | YBR054W         | YRO2        | 4.4          |       |
| Citrate synthase                            | YNR001C         | CIT1        | 1.5          | 2.04  |
| Protein involved in vitamin B₆ biosynthesis | YMR096W         | SN21        | −3.2         |       |
| High affinity sulfate permease              | YBR294W         | SUL1        | −4.7         |       |
| Cell wall mannoprotein of the Srp1p/Tip1p family | YER011W       | TIR1        | −4.8         |       |
| Translation initiation factor eIF-5A         | YJR047C         | ANB1        | −8.4         |       |
| Cell wall mannoprotein with similarity to Tir1p | YJR150C       | DAN1        | −8.1         | −4    |

and its genetic interactions with other tail module subunits. We found that med5Δ and med15Δ interact genetically (Fig. 3B) but failed to observe genetic interactions between med5Δ and med16Δ (data not shown). The former observation supports a physical interaction between Med5 and Med16, whereas the latter is consistent with the biochemical finding that Med5 is lost in the med16Δ Mediator. We
Med5 Is a Component of the Mediator Tail Domain

Further found that a temperature-sensitive MED21 allele (med21-ts) is lethal in the med5Δ background (Fig. 3A). This observation is in nice agreement with a previous report of synthetic lethality between the temperature-sensitive MED10 allele med2-ts70 and a med5Δ knock-out (6). Med10 and Med21 are both part of the middle domain of Mediator (26) and interact both genetically and physically with each other.4 The reason why Med5, which is normally dispensable, becomes an essential protein in the med21-ts and med2-ts70 mutants remains to be determined. Possibly, Med5 participates in protein-protein interactions that help to stabilize the middle domain in these mutants.

Med5 interacts with the Gal11 module but is not absolutely required for the structural integrity of this module in vivo. Mediator purified from a med5Δ strain still retains the Gal11 module (Fig. 1D), and whereas deletion of MED2, MED3, or MED15 generates a gal− phenotype, the med5Δ and med16Δ strains remain gal+ (data not shown). Furthermore, it appears that Med5 is structurally and functionally more related to Med11 than to the other subunits of the tail module. First, Med5 is lost from Mediator purified from a med16Δ strain (Fig. 1B). Second, the med5Δ and med16Δ mutants have similar phenotypic characteristics with increased growth on the nonfermentable carbon source glycerol, increased oxygen consumption, and increased citrate synthase activity. We further found that loss of the entire Gal11 module (med5Δ) generates a dramatic chain-forming phenotype with grape-like clusters, whereas the med5Δ and med16Δ strains showed only a mild chain-forming phenotype. Med14 is thought to link the Mediator tail and middle domains, and a similar chain-forming phenotype has been reported for med14 mutant strains (23). Finally, MED16 functions as a negative regulator of HO gene expression, and both the MED5 and MED16 genes were isolated in a screen for mutants that would suppress the Swi4/Swi6 dependence of a synthetic reporter gene containing part of the HO promoter (6). In this context, it should be noted that deletion of the med5Δ gene influences chromatin structure (24). The mechanisms remain obscure, but because the Med5 protein is an acetyltransferase (5), it may be involved. Unfortunately, mutations in the Med5 HAT motif destabilize the protein,5 and we have therefore been unable to characterize the specific contribution of the HAT activity for Med5 function.

Our data suggest that deletion of MEd5 causes an increased expression of nuclear encoded mitochondrial proteins that are involved in oxidative phosphorylation and a simultaneous down-regulation of genes that are encoded by the mitochondrial genome. The exact mechanisms for this uncoupling of nuclear and mitochondrial gene regulation remain elusive. Most nuclear encoded aerobic genes are subjected to glucose repression, and it is therefore possible that a deletion of MED5 leads to release from glucose repression similar to what has been reported for med14 (rgr1) and med16 mutations (25). Arguing against this possibility, we could not observe any increase in transcription of classical glucose repressed genes, e.g. SUC2, GAL1 or CYB2. Another possibility is that Med5 is involved in the retrograde response, a signaling pathway that communicates information from the mitochondria to the nucleus and affects many cellular processes including stress response, metabolic pathways, and mitochondrial biogenesis (26). Interestingly, the PGD1 gene, encoding the tail module protein Med3, was originally identified as a multicopy suppressor of a mutant mitochondrial RNA polymerase (27).

Finally, our finding that the med5Δ knock-out has only limited effects on gene transcription in YPD, but dramatic effects in synthetic complete medium, suggests that the growth conditions may influence the activity of or the requirements for Med5. It should be noted that the Ca2+ concentration in synthetic complete medium (900 μM) is significantly higher than in YPD (140 μM) (28). This difference in Ca2+ levels may be significant, because mitochondria-to-nucleus stress signaling occurs through elevated levels of cytosolic Ca2+ in mammalian cells (29). In future studies we will investigate the HAT activity of the Med5 protein and how this activity is influenced by mitochondrial stress.

Acknowledgments—We thank Dr. David Stillman for the gift of DY1699 (to H. R.) and Dr. Jesper Svejstrup for the gift of JS004, JS005, and JS006 (to C. M. G.).

REFERENCES

1. Myers, L. C., and Kornberg, R. D. (2000) Annu. Rev. Biochem. 69, 729–749
2. Kim, Y. J., Bjerklund, S., Li, Y., Sayre, M. H., and Kornberg, R. D. (1994) Cell 77, 599–608
3. Myers, L. C., Gustafsson, C. M., Bushnell, D. A., Liu, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R. D. (1998) Genes Dev. 12, 45–54
4. Lorch, Y., Beve, J., Gustafsson, C. M., Myers, L. C., and Kornberg, R. D. (2000) Mol Cell 6, 197–201
5. Gustafsson, C. M., Myers, L. C., Beve, J., Spahr, H., Liu, M., Erdjument-Bromage, H., Tempst, P., and Kornberg, R. D. (1998) J. Biol. Chem. 273, 30851–30854
6. Tabtiang, R. K., and Hershkoavitz, I. (1998) Mol. Cell. Biol. 18, 4707–4718
7. Asturias, F. J., Jiang, Y. W., Myers, L. C., Gustafsson, C. M., and Kornberg, R. D. (1999) Science 283, 985–987
8. Dotson, M. R., Yuan, C. X., Roeder, R. G., Myers, L. C., Gustafsson, C. M., Jiang, Y. W., Li, Y., Kornberg, R. D., and Asturias, F. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 14307–14310
9. Myers, L. C., Gustafsson, C. M., Hayashibara, K. C., Brown, P. O., and Kornberg, R. D. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 67–72
10. Zhang, F., Sunmiety, L., Hinnebusch, A. G., and Swanson, M. J. (2004) Mol. Cell. Biol. 24, 6871–6886
11. Schmitt, M. E., Brown, T. A., and Trumpower, B. L. (1990) Nucleic Acids Res. 18, 3091–3092
12. Rothstein, R. (1991) Methods Enzymol. 194, 281–301
13. Sherman, F. (1991) Methods Enzymol. 194, 3–21
14. Wibom, R., Hagenfeldt, L., and von Dobeln, U. (2002) Anal. Biochem. 311, 139–151
15. Wredenberg, A., Wibom, R., Wilhelmsen, H., Graff, C., Wiener, H. H., Burden, S. J., Oldfors, A., Westerbland, H., and Larsson, N. G. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 15066–15071
16. Ekstrand, M. I., Falkenberg, M., Rantanan, A., Park, C. B., Gaspari, M., Hultken, H., Rustin, P., Gustafsson, C. M., and Larsson, N. G. (2004) Hum. Mol. Genet. 13, 935–944
17. Liu, Y., Ranish, J. A., Aebbersold, R., and Hahn, S. (2001) J. Biol. Chem. 276, 7169–7175
18. Sertkil, O., Cohen, B. D., Davies, K. J., and Lowry, C. V. (1997) Gene (Ann Arbor) 192, 199–205
19. Abramova, N. E., Cohen, B. D., Sertkil, O., Kapoor, R., Davies, K. J., and Lowry, C. V. (2001) Genetics 157, 1169–1177
20. Lo, W. S., and Dranginis, A. M. (1998) Mol. Biol. Cell 9, 161–171
21. Lo, W. S., and Dranginis, A. M. (1996) J. Bacteriol. 178, 714–7151
22. Guglielmi, B., van Berkum, N. L., Klapholz, B., Bijma, T., Boubé, M., Boschiero, C., Bourbon, H. M., Holytege, F. C., and Werner, M. (2004) Nucleic Acids Res. 32, 5379–5391
23. Sakai, A., Shimizu, Y., Kondou, S., Chibazakura, T., and Hishinuma, F. (1999) Mol. Cell. Biol. 19, 4130–4138
24. Jiang, Y. W., and Stillman, D. J. (1992) Mol. Cell. Biol. 12, 4503–4514
25. Wang, X., and Michels, C. A. (2004) Proc. Natl. Acad. Sci. U. S. A. 101, 1527–1537
26. Beve, J., unpublished observation.

5 J. Beve, unpublished observation.