The multiprotein transcriptional Mediator complex provides a key link between RNA polymerase II and upstream transcriptional activator proteins. Previous work has established that the multidrug resistance transcription factors Pdr1 and Pdr3 interact with the Mediator component Med15/Gal11 to drive normal levels of expression of the ATP-binding cassette transporter-encoding gene PDR5 in *Saccharomyces cerevisiae*. PDR5 transcription is induced upon loss of the mitochondrial genome (ρ0 cells) and here we provide evidence that this ρ0 induction is Med15 independent. A search through other known Mediator components determined that Med12/Srb8, a member of the CDK8 Mediator submodule, is required for ρ0 activation of PDR5 transcription. The CDK8 submodule contains the cyclin C homologue (CycC/Srb11), cyclin-dependent kinase Cdk8/Srb10, and the large Med13/Srb9 protein. Loss of these other proteins did not lead to the same block in PDR5 induction. Chromatin immunoprecipitation analyses demonstrated that Med15 is associated with the PDR5 promoter in both ρ+ and ρ0, whereas Med12 recruitment to this target promoter is highly responsive to loss of the mitochondrial genome. Coimmunoprecipitation experiments revealed that association of Pdr3 with Med12 can only be detected in ρ0 cells. These experiments uncover the unique importance of Med12 in activated transcription of PDR5 seen in ρ0 cells.

**INTRODUCTION**

The transcriptional Mediator complex is a group of more than 20 polypeptide chains that serves as a link between upstream activator proteins and the RNA polymerase II machinery (reviewed in Casamassimi and Napoli, 2007). The Mediator complex can be isolated in at least two different forms. The core Mediator (C-Mediator) lacks a four-protein module that is present in the larger L-Mediator complex. L-Mediator consists of C-Mediator and the so-called CDK module, which consists of the large proteins Med12/Srb8 and Med13/Srb9, the cyclin-dependent kinase Cdk8/Srb10, and the cyclin C (CycC)/Srb11 (Borggrefe et al., 2002; Samuelsen et al., 2003). Genetic and biochemical analyses of C- and L-Mediator argued that the C-Mediator acted as a positive regulator of transcription, whereas the L-Mediator served a repressive activity (Holstege et al., 1998; Elmlund et al., 2006).

More recent work suggests that this binary view of the function of C- and L-Mediator is insufficient to explain the roles of these complexes. For example, transcription of the *Drosophila Distal-less* gene requires Med12 but not Cdk8 or CycC (Carrera et al., 2008). Additionally, CDK8 is required for β-catenin–driven expression of several target genes important in colon cancer (Firestein et al., 2008) and certain p53-regulated genes (Donner et al., 2007). Coupled with earlier observations that loss of CDK module components in yeast led to induction of a large number of genes (Holstege et al., 1998), both negative and positive roles for the L-Mediator seem likely.

We have previously described the role of a Mediator component called Med15/Gal11 (Suzuki et al., 1998) in wild-type expression of a gene from *Saccharomyces cerevisiae* involved in multiple or pleiotropic drug resistance called PDR5. Expression of the ABC transporter protein-encoding PDR5 gene requires the presence of at least one of the Zn2+ Cys6 zinc cluster–containing transcription factors Pdr1 and Pdr3 (Delaveau et al., 1994; Katzmann et al., 1994). Analysis of the interaction between Pdr1 and Med15 was driven by the observation that these two proteins are in direct contact (Thakur et al., 2008). Hyperactive mutant forms of both Pdr1 and Pdr3 required Med15 to drive elevated levels of transcription as measured by use of a PDR5-*lacZ* reporter gene. However, closer examination of these data indicated that, although med15Δ strains exhibited lowered levels of PDR5-*lacZ* expression, introduction of hyperactive alleles of either PDR1 or PDR3 still led to a nearly 10-fold increase in β-galactosidase activity. We interpret these data to argue that both Pdr1 and Pdr3 still activate gene expression even in the absence of Med15, suggesting the presence of additional Mediator component targets. Here, we confirm this possibility for Pdr3-dependent activation of gene expression.

Pdr3 control of PDR5 transcription occurs via the same Pdr1/Pdr3 response elements (PDREs) present in the PDR5 promoter (Katzmann et al., 1996), but Pdr3 is differentially regulated from Pdr1. Loss of the mitochondrial genome (ρ0 cells) strongly induces PDR5 transcription in a Pdr3-depen-
dent but a Pdr1-independent manner (Hallstrom and Moye-Rowley, 2000). Additionally, changes in the levels of an enzyme involved in mitochondrial biosynthesis of phosphatidylethanolamine also acts via Pdr3 to induce PDR5 transcription (Gulshan et al., 2008). Because loss of Med15 reduced but did not eliminate the mitochondria-to-nucleus or retrograde induction of PDR5, we examined another nonessential Mediator components for their participation in this Pdr3 regulatory pathway. Loss of the Mediator component Med12/Srb8 prevented the retrograde activation of PDR5 seen in ρ0 cells. Med12 only plays an important role in PDR5 expression in ρ0 cells and is consistent with the view that this Mediator component may be intimately associated with retrograde regulation of multidrug resistance in this yeast.

MATERIALS AND METHODS

Yeast Media

Yeast cells were grown in YPD (2% yeast extract, 1% peptone, 2% glucose) under nonselective conditions or appropriate synthetic complete (SC) media under selective conditions (Sherman et al., 1979) at 30°C with shaking. Drug resistance was measured by the spot test assay on plates with or without drug as indicated. Midlog phase cells were serially diluted in water (1:10), and dilutions were spotted on plates that were then incubated at 30°C for 2–3 d and photographed. Briefly, gradient plates were produced by pouring 25 ml of medium containing the final desired drug concentration into a square 100-mm Petri dish held at a constant angle (roughly 20° degrees from the horizontal; Katzmann et al., 1995). The medium was allowed to solidify and additional 25 ml of medium was overlaid. Once the second layer of medium had solidified, the plates were used within 24 h. Cycloheximide, 0.2 mg/ml, was used for all assays except for Figure 6 in which 0.25 mg/ml cycloheximide was present in the medium. Transformation was performed using the LiOAc technique (Ito et al., 1983). Assays for β-galactosidase activity were carried out on permeabilized cells using o-nitrophenyl-β-D-galactopyranoside as substrate as described (Guarente, 1983).

Strain construction

Yeast strains used in this study were derived from SEY6210 or BY4742, and their genotypes are listed in Table 1. Deletion strains of open reading frames (ORFs) of MEDI5/GAL11, MEDI3/PGD1, MEDI3/SOH1, MEDI2/SRB2, MEDI12/SRB8, MEDI3/SSR89, and Cyc/SNR11 containing a KanMX cassette were obtained from Open Biosystems (Huntsville, AL) in the BY4742 background. PCR primers specific for nucleotide sequences 200 base pairs upstream and 200 base pairs downstream of the kanMX gene replacement were used to PCR-amplify the specific locus (primer list is available on request). Disruption cassettes were then transformed into SEY6210 ρ0 and ρ0 genetic backgrounds to get the corresponding disruptions. Deletion in the CDK8/SRB10 gene was made using plasmid pFA6-TRP1 by PCR-based disruption as described (Longtine et al., 1998). All disruption alleles were confirmed by PCR. Strains containing tandem affinity purification (TAP) tag fusions of MEDI5/GAL11, MEDI3/SRB8, and MEDI3/PGD1 in SEY6210 ρ0 and ρ0 were constructed by transforming these strains with a TAP-kanMX6 cassette amplified from the Open Biosystems TAP tag strain collection (Ghaemmaghami et al., 2003). The enhanced green fluorescent protein (eGFP) cassette flanked by targeting sequences was amplified from plasmid pYM29 and pYM30 (Janke et al., 2004) with S2-primer, the reverse complement of 45–55 bases downstream of the STOP-codon including STOP, followed by 5-ATCGATGAATTCGAGCTCG-3 and S3-primer, 45–55 bases before the 5′ end of the ORF. The PCR product was then transformed into yeast cells, resulting in eGFP tagging of MEDI2 in the SEY6210 ρ0 and ρ0 genetic backgrounds.

Plasmids

Acet-1-PDR3, PDR5-, SNQ2-, YOR1-α2, ·pRS15-PDR3/PDR3-11 plasmids have been described previously (Katzmann et al., 1994; Decottignies et al., 1995; Katzmann et al., 1995; Hallstrom and Moye-Rowley, 2000; Zhang et al., 2000).
The Myc-Pdr1-expressing plasmid (pPS1) was constructed by transferring a Myc-tagged wild-type PDR1 (Mamnum et al., 2002) allele into pRS315.

Quantitative Reverse Transcriptase-PCR mRNA Measurements

Cells were grown to midlog phase in the absence of any drugs. Total RNA was prepared, subjected to reverse transcription, and analyzed as described previously (Shahi et al., 2007).

Fluorescence Microscopy

The strains carrying different GFP-tagged versions of MED12 were grown to saturation. These cultures were then reincubated at a starting optical density of 600 nm (OD_{600}) of 0.1. Cells were allowed to grow for 2 h at which 1% formaldehyde was added. The cultures were further grown for 2 h to an approximate OD_{600} of 0.5 (TAP-tagged Mediator subunits) or 2 OD_{600} (untagged Mediator subunits) with 2 mM EDTA, dissolved in 100 μl SDS, 0.1 mM EDTA, 0.1 g/l bromophenol blue, supplemented with 1% proteinase K, and proteinase K was added. After incubation at 60°C, the samples were washed and immunoprecipitated proteins were recovered by adding 3X Laemmli dye (0.125 M Tris, pH 6.8, 4% SDS, 20% glycerol, 10% β-mercaptoethanol, 2% bromophenol blue dye). Immunoprecipitated proteins along with input proteins were then loaded on a 6% polyacrylamide gel and analyzed by Western blotting with anti-Myc or anti-TAP antibodies.

RESULTS

Med15/Gal11 Is Not the Sole Target of Pdr3-regulated Transcriptional Activation

Gain-of-function alleles of PDR3 (PDR3-11) are capable of inducing expression of PDR5-lacZ by 10-fold when compared with wild-type Pdr3 (Kean et al., 1997; Nourani et al., 1997). As described earlier (Thakur et al., 2008), although loss of Med15 reduced PDR5 expression to 10% of that seen in Med15 background, introduction of the PDR3-11 allele into med15Δ cells still induced this lower basal expression level by a factor of 10. To confirm that this effect on reporter gene expression was seen on the natural PDR3 locus, we introduced a low-copy-number plasmid containing or lacking PDR3-11 into isogenic wild-type and med15Δ cells. These transformants were then grown to midlog phase and placed on media containing cycloheximide, a drug known to be detoxified by the action of Pdr5 (Leppert et al., 1990). Plates were incubated at 30° and then photographed (Figure 1A).

Even in the absence of Med15, cells containing PDR3-11 were much more resistant to cycloheximide than cells with the wild-type PDR3 gene. This behavior supports the view that the increased transcriptional activation of Pdr3-11 compared with Pdr3 is retained even in the absence of Med15. To ensure that the increased drug resistance seen here was due to increased expression of the authentic PDR3 locus, expression of the Pdr5 protein was assessed using a polyclonal antibody directed against this ABC transporter protein (Egner et al., 1995). The transformants described above were grown to midlog phase, whole cell protein extracts prepared and analyzed by Western blotting with anti-Pdr5 antibody.

Coimmunoprecipitation Assay

To avoid this complication, we used a modified PDR3 promoter to drive expression of both the wild-type and hyperactive forms of Pdr3. This promoter was described previously (Zhang et al., 2005) and, in brief, contains copper response elements (CuRE; Pena et al., 1998) in place of the reporter gene expression was seen on the natural PDR3 locus, we introduced a low-copy-number plasmid containing or lacking PDR3-11 into isogenic wild-type and med15Δ cells. These transformants were then grown to midlog phase and placed on media containing cycloheximide, a drug known to be detoxified by the action of Pdr5 (Leppert et al., 1990). Plates were incubated at 30° and then photographed (Figure 1A).

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Consistent with the high-level drug resistance seen in the presence of Pdr3-11, the highest levels of Pdr5 expression were present in Med15 cells expressing this hyperactive regulatory protein compared with the same strain bearing the empty vector plasmid (Figure 1B). Importantly, loss of Med15 lowered the expression in the presence of either plasmid but the presence of the PDR3-11 allele still induced Pdr5 expression. These findings suggest that the transcriptional stimulation of PDR5 elicited by the hyperactive Pdr3-11 protein was still present even in the absence of Med15.

On the basis of the previous study of interaction of hyperactive Pdr1 protein with Med15 (Thakur et al., 2008), we anticipated that Pdr3-11 will interact more effectively with Med15 than its wild-type counterpart. This increased interaction would recruit more Mediator complex to the PDR5 promoter and lead to the enhanced transcriptional activation and Pdr3-dependent phenotypes demonstrated above. To test this expectation, a coimmunoprecipitation assay was carried out to directly compare the association of wild-type and hyperactive Pdr3 with Med15. One complication of this comparison comes from the autoregulatory nature of PDR3 gene expression (Delahodde et al., 1995). Because levels of Pdr3 are controlled by the activity of Pdr3 itself, Pdr3-11 expression to increased expression of the authentic PDR3 locus, expression of the Pdr5 protein was assessed using a polyclonal antibody directed against this ABC transporter protein (Egner et al., 1995). The transformants described above were grown to midlog phase, whole cell protein extracts prepared and analyzed by Western blotting with anti-Pdr5 antibody.

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native PDREs normally found in the PDR3 promoter. These CuREs are bound by the copper-inducible Ace1 transcription factor and eliminate autoregulation of PDR3, allowing both the wild-type and hyperactive forms of Pdr3 to accumulate to the same levels. These modified PDR3 genes carried on low-copy-number plasmids are referred to as Ace1-PDR3 and Ace1-PDR3-11, respectively. These plasmids were introduced into a strain containing a TAP-tagged form of Med15. Transformants were grown to early log phase and induced with copper to produce the two different forms of Pdr3, and whole cell protein lysates were prepared. Med15-containing protein complexes were recovered by anti-TAP immunoprecipitation and then analyzed by Western blotting with anti-HA (detects Pdr3) and anti-TAP antibodies (Figure 1C).

This coimmunoprecipitation analysis demonstrated that Pdr3-11 was more effective at association with Med15 compared with the wild-type Pdr3. Together, these data allow two important conclusions to be reached. First, Pdr3-11 exhibits increased interaction with Med15, stimulating elevated Mediator recruitment that, in turn, is likely to explain the increased expression of PDR5 in this background. Second, although Med15 is an important target of Pdr3-mediated transcriptional activation of PDR5, other routes exist for Pdr3 to activate expression of this key target gene. These experiments all rely on a genetically hyperactive allele of PDR3 to induce PDR5 expression. Our previous work has demonstrated that loss of the mitochondrial genome (ρ0 cells) strongly induced the activity of wild-type Pdr3, which in turn stimulated PDR5 transcription (Hallstrom and Moye-Rowley, 2000; Zhang and Moye-Rowley, 2001). To determine if ρ0 signaling exhibited a Med15 dependence similar to that seen for genetically activated Pdr3, the effect of removing Med15 from ρ0 cells was evaluated directly.

**Med15 Lowers But Does Not Eliminate ρ0 Induction of PDR5**

An isogenic series of ρ+ and ρ0 cells containing or lacking **MED15** was constructed by standard techniques. These strains were transformed with the PDR5-lacZ reporter plasmid and transformants assayed for two features of PDR5 expression (Figure 2A). First, appropriate transformants were analyzed for Pdr5-dependent cycloheximide resistance by placing transformants on medium containing an inhibitory concentration of this drug. Second, levels of PDR5-dependent β-galactosidase activity were determined from these same transformants.

Strains containing a normal mitochondrial genome dosage but lacking Med15 were extremely sensitive to cycloheximide as shown earlier (Thakur et al., 2008). Strikingly, cycloheximide resistance was still induced in ρ0 derivatives. Although ρ0 still enhances the cycloheximide resistance of a med15Δ strain, Med15 was clearly required for normal drug resistance in both ρ+ and ρ0 genetic backgrounds.

Expression of the PDR5-lacZ reporter gene correlated well with the observed effects on cycloheximide resistance. PDR5-dependent β-galactosidase activity increased by sevenfold upon loss of the mitochondrial genome, irrespective of the presence of Med15 (Figure 2B). Although this fold induction was maintained, loss of Med15 reduced PDR5-lacZ expression under both conditions by ~2.5-fold. These data indicate that Med15 is required for normal PDR5 expression in ρ+ and ρ0 cells but is not necessary for ρ0-dependent induction of expression. A SNQ2-lacZ gene was also assayed in these backgrounds as this gene is known to be responsive to Pdr3 regulation but not induced by mitochondrial signals (Zhang and Moye-Rowley, 2001). Expres-
sion of SNQ2 serves as a control for the specificity of ρ<sup>0</sup> signaling to Pdr-regulated genes.

**Med12 Component of Mediator Complex Is a Key Contributor to Induced Pdr3 Transcriptional Control**

The Mediator complex consists of more than 20 proteins and can be subdivided into multiple domains: head, middle, tail, and the CDK8 module (see Casamassimi and Napoli, 2007; for a review; Figure 3A). Biochemical isolation of Mediator from yeast extracts demonstrated that Mediator could be obtained as core Mediator (C-Mediator: head, middle, and tail) as well as L-Mediator (core Mediator/CDK8 module; Borggrefe et al., 2002; Samuelsen et al., 2003). Early experiments argued that C-Mediator was involved in positive regulation of transcription, whereas L-Mediator was thought to be a negative transcriptional regulator (reviewed in Bjorklund and Gustafsson, 2005). More recent studies (Larschan and Winston, 2005; Donner et al., 2007; Firestein et al., 2008) have called into question this clear-cut division of action and evidence is accumulating that L-Mediator is also involved in positive control of gene expression.

To probe the role of other Mediator components in Pdr3-mediated control of *PDR5* transcription, disruption mutations in various nonessential Mediator subunits were generated in isogenic wild-type and med15Δ cells containing (ρ<sup>+</sup>) or lacking (ρ<sup>0</sup>) their mitochondrial genomes were tested for cycloheximide resistance by spotting 10-fold serial dilution of midlog phase cells on rich medium (YPD) or YPD medium containing cycloheximide (Cyh). (B) Strains from above were transformed with low-copy-number plasmids containing gene fusions between *PDR5* or SNQ2- and *Escherichia coli* lacZ. Transformants were assayed for β-galactosidase activity as described (Guarente, 1983).

![](image)

**Figure 2.** Mitochondrial signals still induce *PDR5* in med15Δ cells. (A) Isogenic wild-type and med15Δ cells containing (ρ<sup>+</sup>) or lacking (ρ<sup>0</sup>) their mitochondrial genomes were tested for cycloheximide resistance by spotting 10-fold serial dilution of midlog phase cells on rich medium (YPD) or YPD medium containing cycloheximide (Cyh). (B) Strains from above were transformed with low-copy-number plasmids containing gene fusions between *PDR5* or SNQ2- and *Escherichia coli* lacZ. Transformants were assayed for β-galactosidase activity as described (Guarente, 1983).

![Graph A and B](image)
tion of PDR5, we wanted to confirm that the phenotypic changes described above were correlated with changes in PDR5 expression. Because any Mediator subunit is likely to affect transcription of hundreds of genes (for example, see

Figure 3. Mediator components influence resistance phenotypes in nonidentical ways. (A) The various subdomains of the Mediator complex are illustrated at the top of the figure and discussed in the text. This model is adapted from an earlier publication (Casamassimi and Napoli, 2007). (B) Isogenic $\rho^+$ and $\rho^0$ cells containing the disruption mutations indicated at the top of the figure were grown to midlog phase, and 10-fold serial dilutions were placed on media indicated. Strains containing all Mediator components are denoted as wild-type (wt) in both $\rho^+$ and $\rho^0$ backgrounds. The two media lacking all drugs are YPD and YPGE. Cycloheximide (Cyh) plates are based on YPD medium. (C) Isogenic $\rho^+$ and $\rho^0$ cells containing the indicated Mediator disruption mutations listed at the right of the figure were transformed with plasmids containing the PDR5- or SNQ2-lacZ reporter genes and grown to midlog phase. The levels of $\beta$-galactosidase activity were measured as described above.
van de Peppel et al., 2005), these alterations in drug resistance could be a composite phenotype resulting from alterations in global gene expression. To directly examine the effect of these Mediator subunit mutants on PDR5 expression, the mutant strains used above were transformed with a low-copy-number plasmid containing a PDR5-lacZ reporter gene. Transformants were assayed for PDR5-dependent β-galactosidase activity as described (Guarente, 1983). Additionally, SNQ2-lacZ also served both as a Pdr specificity control as well as a control for any effects on expression of the lacZ reporter gene as was reported for mutants lacking Med5 or Med10 (Tabtiang and Herskowitz, 1998).

The PDR5-lacZ reporter gene was induced by eightfold in \( p^0 \) cells compared with isogenic \( p^+ \) cells as seen before (Hallstrom and Moye-Rowley, 2000; Figure 3C). Loss of Med31 dramatically elevated PDR5-lacZ expression in \( p^+ \) cells, but this reflects the likelihood that the med31A strain, although originally constructed in a \( p^+ \) background, has converted to \( p^0 \) in the absence of this Mediator subunit. This suggestion is supported by the failure of the med31A mutant constructed in the initially \( p^+ \) strain to grow on YPGE medium (Figure 3B). Although loss of Med3 prevented PDR5 induction in \( p^0 \) cells, the usual \( p^0 \)-mediated increase in cycloheximide resistance was still observed in this background. Loss of Med15 reduced expression of both reporter genes in \( p^+ \) and \( p^0 \) cells. Strikingly, loss of Med12 had no effect on PDR5-lacZ expression in \( p^+ \) cells but strongly reduced the \( p^0 \)-dependent induction of this reporter gene. The med12A strains did not significantly alter SNQ2-lacZ expression. The other Mediator disruption mutants failed to exhibit PDR5 expression that correlated with their cycloheximide resistance profile and were not studied further.

Three important conclusions can be drawn from this analysis. First, loss of Med12 caused a selective defect in \( p^0 \)-mediated gene expression of PDR5. Second, although med15A lowered PDR5 expression in \( p^+ \) cells as reported previously (Thakur et al., 2008), PDR5 was still induced in a med15A \( p^0 \) cell by about eightfold, equivalent to the fold change seen in wild-type cells. Finally, interpretation of the results of a Mediator mutant in terms of a consistent effect on both drug resistance and PDR5 expression is complicated by the multiple roles of these proteins in transcription of many genes. Only in the case of Med12 and Med15 was a clear link seen between the drug resistance phenotype and PDR5-lacZ expression. Together, these data suggest that Med15 is required for Pdr3-mediated activation of PDR5 expression under all conditions, whereas Med12 is only required for \( p^0 \)-induced expression.

**Nonidentical Roles of CDK8 Mediator Subunits in PDR5 Regulation**

Med12 is a large subunit (167 kDa) of the CDK8 Mediator subcomplex that also contains the proteins Med13, Cdk8, and CycC (reviewed in Carlson, 1997). Previous studies have argued that these four proteins define a common central function (Balcunas and Ronne, 1995; Kuchin et al., 1995; Liao et al., 1995), but recently this view has been challenged (Carrera et al., 2008). To determine if the other three CDK8 subcomplex components played roles in PDR5 expression similar to that of Med12, disruption mutant strains were constructed lacking each of these other genes in isogenic \( p^+ \) and \( p^0 \) cells. These mutants were tested for their ability to tolerate cycloheximide and activate expression of a PDR5-lacZ reporter gene as described above.

Loss of Med12 or CycC prevented the large increase in cycloheximide resistance seen in \( p^0 \) cells (Figure 4A). Disruption mutants lacking CDK8 or MED13 did not affect the increases in cycloheximide resistance normally seen in a \( p^0 \) background. Further differences between CDK8 subunit members were seen when disruptions were generated in an initially \( p^+ \) strain. Mutants lacking Med13 or CycC were petite, whereas both cdk8A and med112A strains retained the ability to grow on nonfermentable carbon sources. The med13A mutant also exhibited elevated cycloheximide resistance when compared with all other strains. Loss of Med13 is likely to cause cells to become \( p^0 \) (see below).

These same strains were then transformed with low-copy-number plasmids containing gene fusions between PDR5 or SNQ2 to lacZ and β-galactosidase activities determined for all transformants (Figure 4B). Loss of Med13 in an initially \( p^+ \) background caused an increase in PDR5-lacZ expression equivalent to that seen in a wild-type \( p^0 \) cell. This result coupled with the fact that med13A cells fail to grow on YPGE medium supports the view that Med13 is required to prevent cells from becoming \( p^0 \). Interestingly, loss of CycC modestly elevated PDR5-lacZ, although not to the extent seen for med13A mutants. Strains lacking CycC were unable to grow on YPGE, which indicates that these cells are petite. When these same disruption mutations are generated in a \( p^0 \) background, PDR5-lacZ expression was found to be unaltered from wild-type except for med12A and cycCΔ \( p^0 \) strains. Loss of Med12 prevented \( p^0 \) induction of PDR5, whereas loss of CycC reduced PDR5 induction less than half that of wild-type cells. We believe the expression of PDR5 in \( p^+ \) and \( p^0 \) is equivalent because loss of CycC from the wild-type cell caused these cells to convert to \( p^0 \). As seen before, SNQ2 expression was not significantly affected in these backgrounds (Zhang and Moye-Rowley, 2001).

These data are consistent with the view that Med12 is required for wild-type induction of the PDR5 promoter in \( p^0 \) cells. Previous work has demonstrated that \( p^0 \) induction of PDR5 proceeds through activation of the Pdr3 transcription factor (Hallstrom and Moye-Rowley, 2000). Pdr3 expression is induced in \( p^0 \) cells via engagement of an autoregulatory circuit involving Pdr3 activation of its own transcription (Delahodde et al., 1995). To determine if the effect of Med12 in \( p^0 \) cells is restricted to the PDR5 promoter, expression of Pdr3 was evaluated. A low-copy-number plasmid containing an epitope-tagged version of PDR3 was introduced into an isogenic series of \( p^+ \) and \( p^0 \) cells containing or lacking MED12 or MED15. Transformants were analyzed for their level of Pdr3 expression by Western analysis (Figure 5A).

Expression of Pdr3 was induced in response to loss of the mitochondrial genome as seen before (Hallstrom and Moye-Rowley, 2000). However, loss of either Med12 or Med15 elicited distinct responses in Pdr3 expression. A med15A \( p^0 \) strain produced Pdr3 levels that were well below those of either wild-type or med12A cells. The absence of Med15 still supported \( p^0 \) induction of Pdr3 expression, although the induced level achieved was less than in a MED15 \( p^0 \) cell. Strikingly, no difference was seen in Pdr3 expression when comparing \( p^+ \) and \( p^0 \) med12A cells. These data indicate that Med15 is required for Pdr3-mediated activation of gene expression under all conditions, whereas Med12 is selectively important in \( p^0 \) activation. Additionally, the effects of Med12 and Med15 are exerted at both the PDR3 and PDR5 promoters.

To ensure that the differences observed by Western blotting were caused by changes in transcription, we analyzed the levels of PDR3 and PDR5 mRNA in isogenic \( p^+ \) and \( p^0 \) cells containing or lacking the MED12 gene. Transcript levels of ACT1 were also assessed as control for a gene insensitive to these genetic manipulations. Total RNA samples
were reverse-transcribed and analyzed by real-time PCR measurements (Shahi et al., 2007; Figure 5B). The changes in PDR3 and PDR5 mRNA levels correlated very well with the previously determined levels of epitope-tagged Pdr3. PDR3 mRNA levels were nearly 10-fold elevated in \( \rho^0 \) cells, whereas no detectable difference could be seen between the relative transcript levels produced in \( \text{med}12\Delta \) and \( \text{med}12\Delta \ \rho^0 \) cells. This same behavior was observed for PDR5 mRNA production. The simplest interpretation of these data are that Med12 is required to permit

**Figure 4.** Nonidentical roles of Cdk module subunits of Mediator in PDR5 activation. (A) Isogenic \( \rho^+ \) and \( \rho^0 \) cells containing the indicated disruption mutations in the four subunits of the Cdk module were grown to midlog phase, serially diluted as above and tested for their ability to grow on rich medium (YPD), YPD containing cycloheximide (Cyh), or rich medium with glycerol/ethanol as the carbon source (YPGE). (B) The strains described above were transformed with the indicated reporter plasmids and \( \beta \)-galactosidase activities determined.
Pdr3 to positively regulate transcription at both the PDR3 and PDR5 promoters in response to $\rho^0$ signaling.

**Retrograde Signaling Contribution of Med12**

Retrograde signaling refers to control of nuclear gene expression in response to mitochondrial dysfunction (reviewed in Butow and Avadhani, 2004) and was initially described for the $\rho^0$ induction of the citrate synthase-encoding gene CIT2 by the Rtg1/Rtg3 transcription factors (Parikh et al., 1987). The data above provided evidence that loss of Med12 blocked, whereas loss of Med15 only reduced $\rho^0$ induction of PDR5 expression. Other work has demonstrated that elevated expression of the mitochondrially localized phosphatidylserine decarboxylase enzyme (Psd1) also leads to PDR5 induction, even in $\rho^+$ cells (Gulshan et al., 2008). To determine if these Mediator components were also involved in Psd1 signaling, a strain that overproduced this enzyme was constructed and analyzed for the degree of cycloheximide resistance produced in the presence or absence of Med12 or Med15 (Figure 6A).

Increased expression of Psd1 led to an elevation in cycloheximide resistance as we have documented previously (Gulshan et al., 2008). Loss of Med15 prevented any Psd1-dependent increase in cycloheximide tolerance, whereas removal of Med12 had no effect on this regulatory circuit. Psd1 signaling, unlike $\rho^0$ activation of PDR5 expression, is Med12 independent.

To support the conclusion that the action of Med15 in Psd1-induced cycloheximide resistance is likely to come about through the direct action of this Mediator component on the Pdr pathway, ChIP experiments were carried out. A strain containing a MEDI15-TAP fusion protein was transformed with a high-copy-number plasmid overproducing Psd1 or the empty vector alone. Chromatin was prepared and subjected to ChIP as described (Gulshan et al., 2005). The presence of Med15-TAP at the PDR3 and PDR5 promoters was evaluated by qPCR with primers capable of detecting these two transcriptional control regions (Figure 6B).

Increased levels of Med15 were detected at both the PDR3 and PDR5 promoters when Psd1 was overproduced. These data support and extend previous work demonstrating that Pdr3 requires Med15 to normally positively regulate gene expression (Thakur et al., 2008). Taken in whole, our findings implicate Med15 as a key contributor to Psd1 control of Pdr3-mediated gene regulation in $\rho^+$ cells, whereas Med12 is dispensable under these same conditions.

Previous studies on retrograde regulation defined a suite of genes that were induced in $\rho^0$ cells but did not require Pdr3 for this induction (Zhang et al., 2005). The finding that Med12 was critical in Pdr3-mediated retrograde regulation prompted us to determine if Med12 influenced $\rho^0$ induction of other retrograde regulated genes. The $\delta$-lactate dehydrogenase– and aconitase-encoding genes DLD1 and ACO1 were selected as both of these transcripts showed robust $\rho^0$ induction in $\rho^0$ cells and were insensitive to the presence of Pdr3 (Zhang et al., 2005). RNA prepared from isogenic $\rho^+$ and $\rho^0$ cells containing or lacking Med12 was used to make total cDNA. Levels of DLD1, ACO1, and ACT1 transcripts were measured by qPCR using appropriate primers (Figure 6C).

Loss of Med12 reduced transcription of DLD1 and ACO1 in both $\rho^+$ and $\rho^0$ cells. This Med12 dependence is different from that seen for PDR5 since expression of this ABC transporter-encoding gene was only reduced by loss of Med12 from a $\rho^0$ strain, not $\rho^+$ cells. This analysis indicates that Med12 controls expression of DLD1 and ACO1 irrespective...
Med15 contribute to \( p^0 \) activation of PDR5 transcription. To explore the mechanism through which these Mediator components contribute to retrograde regulation, isogenic \( p^+ \) and \( p^0 \) cells were constructed containing or lacking MED12, MED15 or both of these genes. These strains were tested for their ability to tolerate cycloheximide as above (Figure 7A).

Loss of Med15 caused a loss of cycloheximide resistance in \( p^+ \) cells. Interestingly, although \( med12 \Delta \) cells had no detectable cycloheximide sensitivity in \( p^+ \) cells, removal of Med12 from \( med15 \Delta \) mutants suppressed the cycloheximide phenotype seen in single \( med15 \Delta \) strains. A \( p^0 \) \( med12 \Delta \) mutant failed to grow normally at the concentration of cycloheximide used, whereas a \( p^0 \) \( med15 \Delta \) grew slower than isogenic \( p^0 \) cells but better than the \( p^0 \) \( med12 \Delta \) mutant. A \( p^0 \) \( med11 \Delta \) \( med15 \Delta \) mutant was indistinguishable from a \( p^0 \) \( med12 \Delta \) mutant strain. These data indicated that although Med15 was important under both \( p^+ \) and \( p^0 \) conditions, Med12 was indispensable in a \( p^0 \) background.

To determine if these phenotypic effects could be explained by their effects on PDR5 expression, these same strains were transformed with the PDR5- and SNQ2-lacZ reporter plasmids described above. Transformants were grown to midlog phase, and levels of \( \beta \)-galactosidase activity were measured (Figure 7B).

Comparison of PDR5-directed \( \beta \)-galactosidase activity present in isogenic \( p^+ \) and \( p^0 \) \( med15 \Delta \) cells indicated that normal fold induction of PDR5-lacZ was retained, although the absolute level of PDR5 expression was lower than in the presence of Med15. These results indicated that Med15 exerted roughly equivalent effects in both \( p^+ \) and \( p^0 \) cells. The presence of a \( med12 \Delta \) allele, either alone or in combination with \( med15 \Delta \), had no influence on PDR5 expression in \( p^+ \) cells. Conversely, loss of Med12 prevented significant induction of PDR5-lacZ in \( p^0 \) cells. SNQ2-lacZ levels were not significantly influenced by these changes in genetic background, arguing for the specific influence of these Mediator components on activation of PDR5 transcription.

The data above suggested that Med12 played a uniquely important role in mediating Pdr3-dependent induction of PDR5 expression seen in \( p^0 \) cells, whereas Med15 was involved in expression irrespective of mitochondrial genome status. Because Mediator subunits affect the transcription of many genes (Holstege et al., 1998), the association of both Med12 and Med15 with promoters of different genes was determined. Isogenic \( p^+ \) and \( p^0 \) cells that contained either a MED12-TAP or MED15-TAP fusion gene were grown to midlog phase, total chromatin prepared and analyzed by ChIP using anti-TAP antibodies. Samples of total chromatin were reserved as input controls. Both total and immunoprecipitated DNA were analyzed by PCR using primers specific for the PDR5 promoter (left) or the PDR3 promoter (right).

Relative transcript level analysis by reverse transcriptase qPCR analysis of \( DLD1 \) and \( ACO1 \) mRNA levels in \( p^+ \) and \( p^0 \) cells containing or lacking \( MED12 \) was carried out as described above. Quantitation of qPCR was carried out as described above.
the PDR3 gene described above. This construct was introduced into an isogenic series of /H9267 and/or /H9267 0 cells. Additionally, we constructed a /H9267 0 med15 /H9004 strain to evaluate any requirement for the presence of this Mediator component in recruitment of Med12. Finally, all these strains were engineered to contain the MED12-TAP fusion gene. Appropriate transfectants were grown with or without copper in the medium (to induce Pdr3 production), lysates were prepared, and immunoprecipitation was carried out using anti-TAP antibody. These immunoprecipitates were analyzed by Western blotting with anti-TAP (to ensure equal recovery of Med12-TAP) and anti-HA (to detect epitope-tagged Pdr3). Pdr3 did not associate with Med12 in /H9267 0 cells but was easily detectable in a /H9267 0 background (Figure 8B). Loss of Med15 had no significant effect on Pdr3–Med12 association, arguing that Pdr3–Med12 association in /H9267 0 cells occurs independent of Med15. Taken as a whole, these data are consistent with the view that Med12 is an essential cofactor in /H9267 0 stimulation of PDR5 transcription by Pdr3.

A possible explanation for the dramatic difference seen in Med12/Pdr3 interaction in comparison of /H9267 and /H9267 0 cells could be provided by exclusion of Pdr3 from the nucleus in /H9267 cells as Pdr3 has been shown to be nuclear in these cells (Mamnun et al., 2002). A MED12-eGFP fusion gene was constructed in /H9267 and/or /H9267 0 cells and subcellular distribution evaluated by fluorescence microscopy (Supplemental Figure 1). Med12-eGFP was found in the nucleus irrespective of mitochondrial genome status. We interpret these data to indicate that posttranslational modification(s) of Pdr3 and/or Med12 are responsible for the observed difference in association of these two proteins.

Figure 7. Comparison of Med12 and Med15 roles in control of PDR5 expression. (A) Isogenic /p+ and /p0 strains containing disruption mutations of the either MED15 and/or MED12 were grown to midlog phase, and serial dilutions were tested for their ability to grow on YPD or YPD-containing cycloheximide (Cyh) media. (B) The strains described above were transformed with the indicated reporter genes and assayed for the levels of /β-galactosidase produced in appropriate transfectants.

Pdr1 encodes a protein that is 33% identical to Pdr3 (Delaueau et al., 1994; Katzmann et al., 1994). These proteins exhibit extensive functional overlap and have previously been demonstrate to interact with Med15 in /p+ cells (Thakur et al., 2008). We wondered if Med12 could associate with Pdr1 as this Mediator subunit does with Pdr3. To address this question, isogenic /p+ and /p0 pdr1 pdr3 strains expressing either a Med12-TAP or Med15-TAP fusion protein were transformed with a plasmid producing a Myc-tagged form of Pdr1. Protein extracts were made and processed for immunoprecipitation using an anti-TAP antibody. Samples of the input and anti-TAP precipitated proteins were analyzed by Western blotting using anti-Myc and anti-TAP antibodies (Figure 8C). Med12 did not associate with Pdr1 in either /p+ or /p0 cells. The Myc-Pdr1 protein was able to associate with Med15 as expected, confirming that this epitope-tagged protein functions normally. These data support the view that the specific association of Pdr3 with Med12 in /p0 cells is a determinative feature allowing this factor to induce a unique program of target gene expression in response to loss of the mitochondrial genome.

DISCUSSION

The Mediator complex is a critical link between the action of transcription factors and RNA polymerase II–dependent gene transcription. Biochemical experiments have provided evidence that two different forms of Mediator can be isolated from cells: C-Mediator, containing ~20 proteins, and C-Mediator containing an additional the four protein CDK8
subcomplex (L-Mediator; Casamassimi and Napoli, 2007). Early studies with these two different forms of Mediator suggested that the core complex was involved in positive transcriptional control, whereas addition of the CDK8 subcomplex was associated with repression of target gene expression (Björklund and Gustafsson, 2005). More recent experiments, including the present work, indicate that this simple view of the regulatory roles of the different forms of Mediator is inadequate to explain the activities of this transcriptional regulatory complex.

Med12 was first identified as a negative regulator of invertase gene expression (Carlson et al., 1984) and was later found to interact with C-terminal mutant forms of RNA polymerase II (Hengartner et al., 1995). Global microarray analyses of various Mediator components demonstrated that loss of the CDK8 constituents typically led to increased transcript levels (van de Peppel et al., 2005). Biochemical experiments also provide evidence that the isolated CDK8 module is capable of inhibiting Mediator-dependent transcription when added to an in vitro system (Knuesel et al., 2009). However, these observations are balanced by demonstrations in yeast (Larschan and Winston, 2005), Drosophila (Loncke et al., 2007), and mammalian cells (Donner et al., 2007) that normal levels of gene expression require the presence of functional CDK8 subcomplex. Together, these findings are most consistent with the view that the CDK8 subcomplex influences transcriptional regulation in a context-dependent manner and is capable of inhibiting or stimulating gene expression.

Med15 has already been shown to be an important participant in the positive transcriptional regulation of PDR5 by both Pdr1 and Pdr3 (Thakur et al., 2008). The gain-of-function form of Pdr3 exhibits enhanced association with Med15, consistent with increased recruitment of this Mediator com-

Figure 8. Med12 recruitment to the PDR5 promoter and its association with Pdr3 is highly inducible in ρ⁰ cells. (A) Isogenic ρ⁺ and ρ⁰ cells expressing the indicated Mediator subunit-TAP fusion protein were processed for ChIP as described above. Immunoprecipitated (IP) DNA was analyzed by qPCR using primers designed to amplify the PDR5 promoter. Antibody independent DNA recovery was estimated by performing identical immunoprecipitations but without the addition of any primary antibody (No IgG). The ratio of IP to input signals was calculated for the signal from the PDR5 promoter primer pairs divided by the corresponding value for the control (No IgG). The mean and SEs of the resulting normalized IP/Input ratios from replicate cultures, calculated with three PCR measurements, are plotted. (B) Strains with the indicated relevant genotypes (top of panel) expressing both Med12-TAP and epitope-tagged Pdr3 (under copper control: Ace1-Pdr3) were processed for communoprecipitation using anti-TAP antibody as described in Figure 1. Immunoprecipitated samples were analyzed by Western blotting with anti-TAP (detects Med12-TAP) and anti-HA (detects epitope-tagged Pdr3). (C) Isogenic ρ⁺ and ρ⁰ pdr1Δ pdr3Δ strains expressing the indicated Mediator subunit-TAP fusion protein were transformed with a low-copy-plasmid expressing Myc-Pdr1 and processed for communoprecipitation using anti-TAP antibody. Input and anti-TAP immunoprecipitated (IP) samples were electrophoresed on SDS-PAGE and subjected to Western blotting using anti-TAP (detects mediator subunit) and anti-Myc (detects epitope-tagged Pdr1) antibodies.
ponent during transcriptional activation by this hyperactive transcription factor. This interpretation is supported by previous data demonstrating that PDR5 activation by the Pdr3-11 factor is compromised in the absence of Med15 (Thakur et al., 2008). In opposition to the dependence of Pdr3-11 on Med15, our preliminary evidence suggests that loss of Med12 has no significant influence on Pdr3-11-mediated gene activation, at least when this measurement is done in p+ cells (data not shown). We interpret these findings as further support for the strictly inducible nature of Pdr3 interaction with Med12 and that the gain-of-function form of Pdr3 enhances interaction with Med15 rather than Med12.

Comparison of the phenotypes of different CDK8 subcomplex members indicates that disruption mutant alleles of these genes cause very different phenotypic effects, inconsistent with a common role for all these subcomplex proteins. Earlier work using global transcriptional profiling as a measure of the in vivo roles of the CDK8 subcomplex members demonstrated that loss of individual components led to common transcriptional defects (van de Peppel et al., 2005). Our experiments reveal additional complexity in the activities of the CDK8 subcomplex through focus on PDR5 transcription. Med12 has a unique importance in PDR5 expression in p+ cells but is dispensable in a p- background. This finding illustrates the differential requirements for Mediator components when different transcriptional demands are placed on a gene. Med12 is critical to maintain high-level PDR5 transcription in p+ cells but unimportant in when less PDR5 expression is required, such as in p+ cells. Evaluation of the importance of a given Mediator subunit in expression of any gene should be conducted under conditions of varying transcription rates to avoid missing important contributions.

Another striking feature of the behavior of CDK8 disruption mutants was the generation of a petite phenotype in CYCC and MED13 mutants but not in either CDK8 or MED12 disruptants. We believe that the cycda and med13Δ mutants are p0 for two reasons. First, ethidium bromide staining indicated that mitochondrial nucleoids are absent from both mutants. Second, both mutants exhibit elevated expression of PDR5, a diagnostic indicative of p0 status (Hallstrom and Moye-Rowley, 2000). An interesting feature of the elevated PDR5-lacZ levels in the cyca strain is the reduced expression compared with that seen in the med13Δ background. We suggest that CycC is required for full induction of PDR5 in response to loss of the mitochondrial genome. This is provocative in light of the lack of an effect on PDR5-lacZ expression seen in the corresponding p0 cdkaΔ strain. Because Cdk8/CycC form a kinase-cyclin pair (Liao et al., 1995), these data support the view that CycC may have Cdk8 independent roles.

Finally, the singular importance of Med12 in p0 activation of PDR5 transcription provides an alternative possibility to the induction of Pdr3 activity seen in the absence of the mitochondrial genome. We speculate that changes in the ability of Pdr3 to associate with Med12 could explain both the autoregulation of PDR3 and activation of PDR5 in p- cells. Because we have demonstrated that Pdr3–Med12 association is greatly enhanced in p+ cells, it is possible that Pdr3 may not be directly modified in this genetic background but rather that Med12 is the key target. Previously, others have demonstrated that protein kinase A influences gene expression through control of Med13 phosphorylation (Chang et al., 2004). Given the global rewiring of gene expression seen when comparing transcriptional profiles of p+ and p- cells (Epstein et al., 2001; Traven et al., 2001; Devaux et al., 2002), targeting a global transcriptional regulator like Med12 would provide a parsimonious means of both specifically inducing Pdr3-dependent transcriptional events as well as to trigger the range of responses necessary to ensure viability in response to loss of the mitochondrial genome.

Induced Pdr3–Med12 interaction would also explain one of the poorly understood features of p0 induction of PDR5 in wild-type cells. A variety of experiments including lacZ fusion genes and Western blot measurements (Hallstrom and Moye-Rowley, 2000; Mamnun et al., 2002) have indicated that in p+ cells, levels of Pdr3 only approach 1% of the levels of Pdr1. Because both Pdr1 and Pdr3 bind to the same DNA elements (Katzmann et al., 1996), the ability to induce Pdr3 in the presence of an excess of Pdr1 has been difficult to explain. If Med12 is specifically recruited to Pdr3 to enhance the transactivation capability of this factor, then this would allow Pdr3 activity to be elevated even in the presence of a large excess of Pdr1. Once the autoregulatory induction of PDR5 is complete, levels of Pdr3 rise by a factor of 10 (Delahodde et al., 1995), increasing the levels to a dose more comparable to that of Pdr1.

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REFERENCES

Balciunas, D., and Ronne, H. (1995). Three subunits of the RNA polymerase II mediator complex are involved in glucose repression. Nucleic Acids Res. 23, 4421–4425.

Bjorklund, S., and Gustafsson, C. M. (2005). The yeast Mediator complex and its regulation. Trends Biochem. Sci. 30, 240–244.

Borggrefe, T., Davis, R., Erdjument-Bromage, H., Tempst, P., and Kornberg, R. D. (2002). A complex of the Srb8, -9, -10, and -11 transcriptional regulatory proteins from yeast. J. Biol. Chem. 277, 44202–44207.

Boutow, R. A., and Avadhani, N. G. (2004). Mitochondrial signaling: the retrograde response. Mol. Cell. 14, 1–15.

Carlson, M. (1997). Genetics of transcriptional regulation in yeast: connections to the RNA polymerase II CTD. Annu. Rev. Cell Dev. Biol. 13, 1–23.

Carlson, M., Osmond, B. C., Neigeborn, L., and Botstein, D. (1984). A suppressor of Snf1 mutations causes constitutive high-level invertase synthesis in yeast. Genetics 107, 19–32.

Carrera, I., Janody, F., Leeds, N., Duveau, F., and Treisman, J. E. (2008). Pygopus activates Wingless target gene transcription through the mediator complex subunits Med12 and Med13. Proc. Natl. Acad. Sci. USA 105, 6644–6649.

Casamassimi, A., and Napoli, C. (2007). Mediator complexes and eukaryotic transcription regulation: an overview. Biochimie 89, 1439–1446.

Chang, Y.-W., Howard, S. C., and Herman, P. K. (2004). The Ras/PKA signaling pathway directly targets the Srb9 protein, a component of the general RNA polymerase II transcription apparatus. Mol. Cell 15, 107–116.

Davis, J. A., Takagi, Y., Kornberg, R. D., and Asturias, F. A. (2002). Structure of the yeast RNA polymerase II holoenzyme: Mediator conformation and polymerase interaction. Mol. Cell 10, 409–415.

Decottignies, A., Lambert, L., Catty, P., Degand, H., Epping, A. E., Moye-Rowley, W. S., Balzi, E., and Goﬀeau, A. (1995). Identiﬁcation and characterization of SNF1 mutations causes constitutive high-level invertase synthesis in yeast. Genetics 143, 1–15.

Delahodde, A., Delaveau, T., and Jacq, C. (1995). Positive autoregulation of the yeast transcription factor Pdr3p, involved in the control of the drug resistance phenomenon. Mol. Cell. Biol. 15, 4043–4051.

Delaveau, T., Delahodde, A., Carvajal, E., Subik, J., and Jacq, C. (1994). PDR3, a new yeast regulatory gene, is homologous to PDR1 and controls the multidrug resistance phenomenon. Mol. Gen. Genet. 244, 501–511.

Devaux, F., Carvajal, E., Moye-Rowley, S., and Jacq, C. (2002). Genome-wide studies on the nuclear PDR3-controlled response to mitochondrial dysfunction in yeast. FEBS Lett. 515, 25–28.

Donner, A. J., Szostek, S., Hoover, J. M., and Espinosa, J. M. (2007). CDK8 is a stimulus-speciﬁc positive coregulator of p53 target genes. Mol. Cell 27, 121–133.

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Egner, R, Mahé, Y, Pandalait, R, and Kuchler, K (1995). Endocytosis and vacular degradation of the plasma membrane-localized Pdr5p ATP-binding cassette multidrug transporter in S. cerevisiae. Mol. Cell. Biol. 15, 5879–5887.

Elmlund, H, Baraznenok, V, Lindahl, M, Samuelsen, C O, Koec, P J, Holmberg, S, Hebert, H, and Gustafsson, C M (2006). The cyclin-dependent kinase 8 module sterically blocks Mediator interactions with RNA polymerase II. Proc. Natl. Acad. Sci. USA 103, 15788–15793.

Epstein, C B, Waddle, J A, Hale, W, 4th, Dave, V, Thomson, J, Macatee, T L, Garner, H R, and Butow, R A (2001). Genome-wide responses to mitochondrial dysfunction. Mol. Biol. Cell 12, 297–308.

Firestein, R et al. (2008). CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity. Nature 455, 547–551.

Ghannamgahi, S H, Huh, W K, Bower, K, Howson, R W, Belle, A, Deplouve, O, O’Shea, E K, and Weissman, J S (2003). Global analysis of protein expression in yeast. Nature 425, 737–741.

Guarante, L (1983). Yeast promoter and lacZ fusions designed to study expression of cloned genes in yeast. Methods Enzymol. 101, 181–191.

Gulsman, K, Rovinsky, S A, Coleman, S T, and Moye-Rowley, W S (2005). Oxidant-specific folding of Yap1p regulates both transcriptional activation and nuclear localization. J. Biol. Chem. 280, 40524–40533.

Gulsman, K, Schmidt, J, Shahi, P, and Moye-Rowley, W S (2008). Evidence for the bifunctional nature of mitochondrial phosphatidyserine decarboxylase: role in Pdr3-dependent retrograde regulation of PDR5 expression. Mol. Cell. Biol. 28, 5851–5864.

Hallstrom, T C, and Moye-Rowley, W S (2000). Multiple signals from dysfunctional mitochondria activate the pleiotropic drug resistance pathway in Saccharomyces cerevisiae. J. Biol. Chem. 275, 37347–37356.

Hengartner, C J, Thomas, C M, Zhang, J, Chao, D M, Liao, S M, Koleske, A J, Okamura, S, and Young, R A (1995). Association of an activator with an RNA polymerase II holoenzyme. Genes Dev. 9, 897–910.

Holstege, F C P, Jennings, E G, Wyrric, J J, Lee, T L, Hengartner, C J, Green, M R, Golub, T R, Lander, E S, and Young, R A (1998). Dissecting the regulatory circuitry of a eukaryotic genome. Cell 95, 717–728.

Ito, H, Fukuda, Y, Murata, K, and Kimura, A (1983). Transformation of intact yeast cells treated with alkali cations. J. Bacteriol. 153, 163–168.

Janke, C, Magiera, M M, Rathfelder, N, Taxis, C, Reber, S, Maekawa, H, Eulm, H, Baraznenok, V, Lindahl, M, Samuelsen, C O, Koeck, P J, Elmlund, H, and Holstege, F C P (2006). The cyclin-dependent kinase 8 module sterically blocks Mediator interactions with RNA polymerase II. Proc. Natl. Acad. Sci. USA 103, 15788–15793.

Katzmann, D J, Hallstrom, T C, Voet, M, Wysock, W, Golin, J, Volckaert, G, and Moye-Rowley, W S (1995). Expression of an ATP-binding cassette transporter encoding gene (YOR1) is required for oligomycin resistance in Saccharomyces cerevisiae. Mol. Cell. Biol. 15, 6875–6883.

Kean, L S, Grant, A M, Angelettii, C, Mahé, Y, Kuchler, K, Fuller, R S, and Nichols, J W (1997). Plasma membrane translocation of fluorescent-labeled phosphatidylethanolamine is controlled by transcription regulators, PDR5 and PDR3. J. Cell Biol. 138, 255–270.

Knuesel, M T, Meyer, K D, Bernecky, C, and Taatjes, D J (2009). The human CDK8 subcomplex is a molecular switch that controls Mediator coactivator function. Genes Dev. 23, 439–451.

Kuchin, S, Yeghiayan, P, and Carlson, M (1995). Cyclin-dependent protein kinase and cyclin homologs SNN3 and SNS8 contribute to transcriptional control in yeast. Proc. Natl. Acad. Sci. USA 92, 4006–4010.

Larschan, E, and Winston, F (2005). The Saccharomyces cerevisiae Srb8-Srb11 complex functions with the SAGA complex during Gal4-activated transcription. Mol. Cell. Biol. 25, 114–123.

Leppert, G, McDevitt, R, Falco, S C, Van Dyk, T K, Ficke, M B, and Golin, J (1990). Cloning by gene amplification of two loci conferring multiple drug resistance in Saccharomyces. Genetics 125, 13–20.

Liao, S M, Zhang, J, Jeffery, D A, Koleske, A J, Thompson, C M, Chao, D M, Vlijmen, M, van Vuuren, H J, and Young, R A (1995). A kinase-cyclin pair in the RNA polymerase II holoenzyme. Nature 374, 193–196.

Loncle, N, Boume, M, Ojoula, L, Boscioh, C, Werner, M, Cribbs, D L, and Bourbon, H M (2007). Distinct roles for Mediator Cdk8 module subunits in Drosophila development. EMBO J. 26, 1043–1054.

Longtime, M S, Wickem, A, Demarini, D J, Shah, N G, Wach, A, Bracht, A, Philippus, P, and Pringle, J R (1998). Additional modules for versatile and modular PCR-based gene deletion and modification in Saccharomyces cerevisiae. Yeast 14, 953–961.

Mammon, Y M, Pandalait, R, Mahé, Y, Delahodde, A, and Kuchler, K (2002). The yeast zinc finger regulators Pdr1p and Pdr3p control pleiotropic drug resistance (PDR) as homo- and heterodimers in vivo. Mol. Microbiol. 46, 1429–1440.

Nourani, A, Papajova, D, Delahodde, A, Jacq, C, and Subik, J (1997). Clustered amino acid substitutions in the yeast transcription regulator Pdr3p increase pleiotropic drug resistance and identify a new central regulatory domain. Mol. Gen. Genet. 256, 397–405.

Parikh, V S, Morgan, M M, Scott, R, Clements, L S, and Butow, R A (1987). The mitochondrial genome can influence nuclear gene expression in yeast. Science 235, 576–580.

Pena, M M, Koch, K A, and Thiele, D J (1998). Dynamic regulation of copper uptake and detoxification genes in Saccharomyces cerevisiae. Mol. Cell. Biol. 18, 2514–2523.

Samuelsen, C O, Baraznenok, V, Khorosjutina, O, Spahr, H, Kieselbach, T, Holmberg, S, and Gustafsson, C M (2003). TRAP230/ARC240 and TRAP240/TRAP230 Mediator subunits are functionally conserved through evolution. Proc. Natl. Acad. Sci. USA 100, 6422–6427.

Shahi, P, Gulsman, K, and Moye-Rowley, W S (2007). Negative transcriptional regulation of multidrug resistance gene expression by an Hsp70 protein and a transcription factor. J. Biol. Chem. 282, 26822–26831.

Sherman, F, Fink, G, and Hicks, J (1979). Methods in Yeast Genetics, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Suzuki, Y, Nogi, Y, Abe, A, and Fukasawa, T (1988). GAL11 protein, an auxiliary transcription activator for genes encoding galactose-metabolizing enzymes in Saccharomyces cerevisiae. Mol. Cell. Biol. 8, 4991–4999.

Tabtiang, R K, and Herskovitz, I (1998). Nuclear proteins Nut1p and Nut2p cooperate to negatively regulate a Swi4p-dependent lacZ reporter gene in Saccharomyces cerevisiae. Mol. Cell. Biol. 18, 4707–4718.

Thakur, J K, et al. (2008). A nuclear receptor-like pathway regulating multidrug resistance in fungi, Nature 452, 604–609.

Traven, A, Wang, J M, Xu, D, Supta, M, and Ingles, C J (2001). Interorganelar communication. Altered nuclear gene expression profiles in a yeast mitochondrial DNA mutant. J. Biol. Chem. 276, 4020–4027.

van de Peppel, J, Ketelarji, N, van Bake, H, Kockelkorn, T T, van Leenen, D, and Holstege, F C (2005). Mediator expression profiling epitasis reveals a signal transduction pathway with antagoistic submodules and highly specific downstream targets. Mol. Cell 19, 511–522.

Zhang, X, Kolaczkowska, A, Devaux, F, Panwar, S L, Hallstrom, T C, Jacq, C, and Moye-Rowley, W S (2005). Transcriptional regulation by Lge1p requires a function independent of its role in histone H2B ubiquitination. J. Biol. Chem. 280, 2759–2770.

Zhang, X, and Moye-Rowley, W S (2001). Saccharomyces cerevisiae multidrug resistance gene expression inversely correlates with the status of the Fo component of the mitochondrial ATPase. J. Biol. Chem. 276, 47844–47852.