Distinct role of Mediator tail module in regulation of SAGA-dependent, TATA-containing genes in yeast

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The evolutionarily conserved Mediator complex is required for transcription of nearly all RNA Pol II-dependent promoters, with the tail module serving to recruit Mediator to active promoters in current models. However, transcriptional dependence on tail module subunits varies in a gene-specific manner, and the generality of the tail module requirement for transcriptional activation has not been explored. Here, we show that tail module subunits function redundantly to recruit Mediator to promoters in yeast, and transcriptome analysis shows stronger effects on genome-wide expression in a double-tail subunit deletion mutant than in single-subunit deletion mutants. Unexpectedly, TATA-containing and SAGA-dependent genes were much more affected by impairment of tail module function than were TFIID-dependent genes. Consistent with this finding, Mediator and preinitiation complex association with SAGA-dependent promoters is substantially reduced in *gal11/med15Δ med3Δ* yeast, whereas association of TBP, Pol II, and other Mediator modules with TFIID-dependent genes is largely independent of the tail module. Thus, we have identified a connection between the Mediator tail module and the division of promoter dependence between TFIID and SAGA.

The EMBO Journal (2012) 31, 44–57. doi:10.1038/emboj.2011.362; Published online 4 October 2011

Subject Categories: chromatin & transcription

Keywords: Mediator; *Saccharomyces cerevisiae*; SAGA; TFIID; transcription

Introduction

Transcription initiation by eukaryotic RNA Polymerase II (Pol II) is a multistep process that involves gene-specific activators, co-activators, chromatin remodellers, and general transcription factors. In general, activators bound to upstream elements recruit the general transcription machinery, includ-

ing Pol II, with the help of co-activators. One such co-activator is the Mediator complex, which is proposed to act as a bridge between transcription activators bound far upstream and the general transcription machinery formed close to the transcription start site (Kornberg, 2005; Malik and Roeder, 2010). Mediator is highly conserved structurally and functionally from yeast to humans (Bourbon *et al.*, 2004; Bourbon, 2008). In yeast (*Saccharomyces cerevisiae*), the core complex comprises 21 subunits that can be assigned to three distinct modules, termed as head, middle, and tail (Myers and Kornberg, 2000; Guglielmi *et al.*, 2004). A fourth module, termed the Cyclin-Cdk or Srb8-11 module, contains four additional proteins, interacts differentially with different promoters, and is mainly repressive in nature (Holstege *et al.*, 1998; Borggreve *et al.*, 2002; Elmlund *et al.*, 2006).

Early studies using yeast harbouring a temperature-sensitive mutant of the Mediator head subunit Med17/Srb4 showed that loss of Mediator function causes about 93% of Pol II transcribed genes to be downregulated by more than two-fold in yeast (Thompson and Young, 1995; Holstege *et al.*, 1998). This suggested that Mediator acts as a general regulator of Pol II transcription at least in yeast, and more recent studies have supported this view (Andrau *et al.*, 2006; Takagi and Kornberg, 2006; Ansari *et al.*, 2009). However, genome-wide expression analysis of single-deletion mutant yeast strains of all non-essential Mediator subunits indicated that deletions of different individual subunits affect the expression of different subsets of genes, with some subunits showing overlaps (van de Peppel *et al.*, 2005). These results, together with numerous studies of individual genes, suggest that although Mediator is a general regulatory factor, it nonetheless functions differently at specific genes, consistent with the view of Mediator as a conduit for both repressive and activating signals (Myers and Kornberg, 2000).

Genetic and structural studies indicate that the tail module is composed of Med3, Med2, Gal11/Med15, and Sin4/Med16 subunits whereas Rgr1/Med14 bridges the tail module with the middle module (Dotson *et al.*, 2000; Kang *et al.*, 2001; Guglielmi *et al.*, 2004). The subunits of the tail module, particularly the triad comprising Gal11/Med15, Med3, and Med2, have been shown to interact with different activators including Gal4, Gcn4, Pdr1, Oaf1, and others (Lee *et al.*, 1999; Myers *et al.*, 1999; Natarajan *et al.*, 1999; Park *et al.*, 2000; Thakur *et al.*, 2008, 2009). This also supports the idea that the tail module of Mediator is required for activated transcription. However, gene-specific studies have shown varying effects of deletion of individual tail subunits on gene expression, with expression oftentimes being reduced but not abrogated (Swanson *et al.*, 2003; Zhang *et al.*, 2004; Leroy *et al.*, 2006), leaving open the possibility that tail module subunits might function redundantly. Furthermore, recruitment of remaining tail subunits to activated promoters in single-tail subunit deletion mutants has seldom been exam-

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Received: 6 April 2011; accepted: 7 September 2011; published online: 4 October 2011

ined (Zhang *et al*, 2004). Thus, the role of the tail module of Mediator in activated transcription, as opposed to the function of the individual subunits, remains to be addressed.

Here, we investigate the role of the tail module of Mediator in transcription by using yeast mutants in which the function of the Gal11/Med2/Med3 triad is lost or severely impaired. We found that the tail module preferentially supports the expression of genes belonging to specific functional categories such as the transport of drug/toxins, metabolic processes such as carbohydrate and amino-acid metabolism, and stress response. Unexpectedly, we also found that the genes downregulated by the loss of function of tail module are mainly SAGA dependent, whereas most TFIID-dependent genes, which comprise the large majority of genes in yeast (Huisinga and Pugh, 2004), are relatively unaffected. Based on the results presented here, we propose that the Gal11/Med2/Med3 submodule of Mediator complex is selectively required for the expression of genes which are tightly regulated and require chromatin remodellers such as SAGA and SWI/SNF for their expression, whereas this module is dispensable for less tightly regulated, TFIID-dependent housekeeping genes.

Results

Mediator tail module subunits contribute redundantly to induced *CHA1* expression

Previous work from our laboratory showed that induced expression of the *CHA1* gene is strongly reduced in *srb4/med17 ts* mutant yeast, which harbour a *ts* mutation in an essential head module subunit of Mediator, at the non-permissive temperature (He *et al*, 2008). Correspondingly, recruitment of Srb5/Med18 (head module) and Rgr1/Med14 (middle module), as well as TBP, TFIID, and Pol II, were substantially reduced. Surprisingly, Gal11/Med15 (tail module) was present at wild-type levels. A subsequent study also found continued tail module recruitment in *srb4/med17 ts* yeast at several different promoters at the non-permissive temperature, in spite of loss of association of subunits from the head and middle modules (Ansari *et al*, 2009). These results suggest that in the absence of the head/middle modules, the tail module remains associated with actively transcribed promoters. Furthermore, the loss of middle and head modules along with the general transcription machinery, together with retention of the tail module, suggests that tail module subunits are targets for Mediator recruitment at these various promoters.

To address explicitly the role of the tail module of Mediator, we examined *CHA1* expression in yeast strains harbouring deletions of each of the four tail subunits, Gal11/Med15, Med3, Med2, and Sin4/Med16. Quantitative analysis of serine-induced *CHA1* expression showed no change in strains lacking individual tail module subunits compared with wild-type yeast (Figure 1A). In contrast, in the absence of Med9 from the middle module or Srb2/Med20 or Srb5/Med18 from the head module, *CHA1* expression is reduced nearly two-fold (He, 2008). The lack of effect of single-subunit tail module deletions could indicate that the tail module is not important for *CHA1* activation; alternatively, the tail module could remain otherwise intact when lacking individual subunits, and the different tail subunits may function redundantly. To determine whether

the tail module retained function in the absence of individual subunits, we tested whether deletion of single subunits from the tail module affected recruitment of other tail subunits. For this purpose, we tagged the Gal11/Med15 subunit with a 13-myc tag in *med3Δ*, *med2Δ*, and *sin4/med16Δ* strains. Chromatin immunoprecipitation (ChIP) analysis was performed to assess recruitment of Gal11/Med15-myc to the uninduced and induced *CHA1* promoter in wild-type and Mediator tail deletion mutant strains (Figure 1B). The results show that, in *med2Δ* and *sin4/med16Δ* strains, Gal11/Med15 recruitment to the induced *CHA1* promoter is similar to that seen in wild-type yeast. Interestingly, however, in a *med3Δ* strain, increased recruitment of myc-tagged Gal11/Med15 is no longer seen upon induction. At the same time, expression of *CHA1* in these Gal11/Med15-myc-tagged strains was severely reduced in the *med3Δ* background, while little effect was seen for the *med2Δ* and *sin4/med16Δ* counterparts (Figure 1C). Since we did not observe any change in expression of *CHA1* in the *med3Δ* strain expressing untagged Gal11/Med15, we conclude that the 13-myc tag leads to at least partial loss of function of Gal11/Med15 at the *CHA1* promoter, revealing functional redundancy for *CHA1* expression between Gal11/Med15 and Med3. A previous study also suggested that 13 myc-tagged Gal11/Med15 did not associate with Mediator complex in a *med3Δ* strain (Zhang *et al*, 2004).

To confirm the redundancy between Gal11/Med15 and Med3, we analysed *CHA1* expression in a *gal11/med15Δ med3Δ* strain. This double-mutant strain, which displays a strong reduced growth phenotype not seen in the *gal11/med15-myc med3Δ* strain, showed severe reduction in induced *CHA1* expression (Figure 1D). Furthermore, ChIP experiments revealed that another tail subunit, Med2, is recruited to the induced *CHA1* promoter in wild-type but not in *gal11/med15Δ med3Δ* yeast (Figure 1E). Taken together, these results indicate that the simultaneous loss of Gal11/Med15 and Med3 subunits leads to the loss of recruitment of the Gal11/Med3/Med2 triad to the induced *CHA1* promoter and a consequent severe reduction in *CHA1* expression.

Effect of the tail module of Mediator complex on global gene expression

Previous genome-wide studies of the Mediator tail module have examined deletions of individual tail subunit genes. Since Mediator tail integrity is maintained in single-subunit deletions, and since Mediator tail subunits sometimes function redundantly (Figure 1), these genome-wide experiments do not test the function of the Mediator tail module as a unit, nor of the Gal11/Med3/Med2 triad. To address this issue, we carried out microarray expression analysis on wild-type, *gal11/med15-myc med3Δ*, and *gal11/med15Δ med3Δ* strains grown in rich medium (YPD). Although *gal11/med15Δ med3Δ* yeast grows much more slowly than *gal11/med15-myc med3Δ* yeast, we observed a strong correlation between expression changes in *gal11/med15-myc med3Δ* and *gal11/med15Δ med3Δ* strains versus wild type ($r^2 = 0.82$ for 1052 genes showing $>1.5 \times$ altered expression in either mutant strain grown in YPD medium; Figure 2A and B). Comparison of the magnitude in change in expression for genes affected in *gal11/med15Δ med3Δ* or *gal11/med15-myc med3Δ* yeast indicated a stronger effect in the *gal11/med15Δ* mutant, as expected (Supplementary Figure S1A). These data demonstrate

Figure 1 Mediator tail subunits contribute redundantly to *CHA1* expression. (A) The expression of the serine-induced *CHA1* gene from WT, *med2Δ*, *med3Δ*, *sin4Δ*, and *gal11Δ* strains was analysed by qRT-PCR and the values normalized to *SNR6*; the *CHA1*/*SNR6* ratio was then set to 1 for wild type. (B) ChIP was performed to analyse Gal11-myc recruitment to the *CHA1* core promoter in WT, *med3Δ*, *med2Δ*, and *sin4Δ* strains by using anti-myc antibody. The ChIP'd DNA was analysed by quantitative real-time PCR. The bars represent log₂ of the IP/Input ratios, normalized to a non-transcribed region of ChrV (Keogh and Buratowski, 2004). (C) *CHA1* expression was analysed by qRT-PCR in *med3Δ*, *med2Δ*, and *sin4Δ* strains bearing a 13-myc tag on the Gal11/Med15 subunit and expression was compared with WT. Values were normalized to *SNR6* and the uninduced *CHA1*/*SNR6* ratio was then set to 1 in wild type. (D) Expression of the serine-induced *CHA1* gene was analysed by qRT-PCR in the *gal11Δ med3Δ* double-mutant strain and compared with WT and *gal11-myc med3Δ* strains. Values were normalized to *SNR6* and the *CHA1*/*SNR6* ratio was then set to 1 for wild type. (E) ChIP was used to measure binding of a TAP-tagged Med2 subunit of the tail module in WT and *gal11Δ med3Δ* strains to the serine-induced *CHA1* core promoter. ChIP'd DNA was analysed as described in (B). Each experiment was performed 3–4 times and error bars represent standard deviations.

that the myc-tagged Gal11/Med15 subunit is functionally impaired in *med3Δ* yeast.

Gene ontology analysis showed enrichment of downregulated genes in C-compound and carbohydrate metabolism, drug-toxin transport, detoxification, cellular import, and stress/heat-shock response, whereas upregulated genes were enriched for metabolism, methionine biosynthesis, sporulation, and sulphur metabolic processes (Figure 2B; Supplementary Table S1). We also observed enrichment among affected genes for those encoding proteins that localize to plasma membrane, vacuole, cell wall and cell periphery, and for transport families such as Major facilitator superfamily proteins and ABC transporter (Supplementary Table S1). This suggests that the severe impairment of tail module function in the double mutants specifically affects

genes involved in the transport of nutrients/metabolites across the cell membrane, which in turn affects other genes involved in metabolic processes such as C-compound and carbohydrate metabolism, sulphur metabolism, and amino-acid metabolism.

As the tail module is generally considered to be primarily involved in gene activation, we were somewhat surprised to find a substantial number of genes showing increased expression in the double mutants. To verify these findings, expression changes observed by microarray for several genes downregulated (*CHA1*) or upregulated (*MET2*, *MET32*, *SPS19*, and *SPO20*) in the Mediator tail mutants were confirmed by northern hybridization and qRT-PCR (Figure 2C and D). Expression changes for these genes were also examined in *srb8/cdk8Δ* and *srb10/cycCΔ* strains to

Figure 2 Genome-wide expression analysis shows that loss of function of the Mediator tail module affects specific gene classes. Microarray expression profiling was done in WT, *gal11-myc med3 Δ* , and *gal11 Δ med3 Δ* strains by isolating total RNA from cells grown in YPD media. (A) Scatterplot of expression changes in *gal11-myc med3 Δ* and *gal11 Δ med3 Δ* strains as compared with WT strain for those genes with altered expression in at least one mutant by at least 1.5-fold. (B) A heat map was generated by carrying out clustering of expression changes ≥ 1.5 -fold in *gal11-myc med3 Δ* and *gal11 Δ med3 Δ* strains by utilizing a k-means algorithm. (C) Northern analysis was performed for the expression of *CHA1*, *MET2*, *MET32*, and *SPS19* genes in WT, *gal11/med15 Δ med3 Δ* , *gal11-myc med3 Δ* , *srb8/med12 Δ* , and *srb10/cdk8 Δ* strains using gene-specific probes. The lower band seen in the *MET32* panel corresponds to the transcript that is seen upon induction. Ethidium bromide stained 25S rRNA is shown as loading control. (D) Expression of *MET2*, *MET32*, *SPS19*, and *SPO20* was analysed by qRT-PCR. The fold differences between WT and mutant strains are shown by setting the values in the WT strain as 1 after normalizing to *SNR6* expression.

determine whether upregulation in tail mutants might be indirectly due to the loss of the Cyclin-Cdk (Srb8-11) module in the double-tail mutants. Little change was observed in the expression of *SPS19* or *SPO20* relative to 25S rRNA or *SNR6* in either the *srb8/cdk8 Δ* or *srb10/cycCA* strain (Figure 2C and D), while induced *CHA1* expression showed a modest decrease in the Cyclin-Cdk subunit mutants. On the other hand, *MET2* and *MET32* genes showed significantly increased expression in *srb8/cdk8 Δ* and *srb10/cycCA* yeast, comparable to or higher than that seen in tail deletion mutants. We conclude that impaired function of the Mediator tail module results in both increased and decreased expression for substantial numbers of genes in yeast grown in rich medium.

Comparison of the effect of *gal11/med15 med3* mutants with single-subunit deletions from the tail or Cyclin-Cdk module on global gene expression

We next compared genome-wide expression changes in *gal11/med15 Δ med3 Δ* and *gal11/med15-myc med3 Δ* strains to expression changes in yeast harbouring single-tail subunit deletions.

Because we observed derepression of a number of genes in *gal11/med15 Δ med3 Δ* and *gal11/med15-myc med3 Δ* strains, we also included comparison of expression changes in *srb8/med12 Δ* and *srb10/cdk8 Δ* strains, as the Cyclin-Cdk module of Mediator, which includes Srb8/Med12 and Srb10/Cdk8, is reported to exert a largely repressive function (Holstege *et al*, 1998; Kuchin and Carlson, 1998; Lee *et al*, 2000; Zaman *et al*, 2001; van de Peppel *et al*, 2005). For this analysis, all strains were grown in complete synthetic medium.

K-means clustering of the expression data of all genes showing altered transcription by at least 1.5-fold in at least one mutant identified five principal groups (Figure 3A). Cluster 2 includes genes showing the strongest downregulation in the tail double-mutant strains. Many genes in this group are similarly downregulated in *gal11/med15 Δ* , *med3 Δ* , and *med2 Δ* mutants (60–70% are down by at least 1.5-fold for each of the single-subunit deletion mutants), but the effects are generally smaller in magnitude, and some genes that are affected in the double mutant show little effect in the single mutants (Figure 3B; Supplementary Figure S1B). The

Figure 3 Genome-wide analysis of expression in Mediator tail and Cyclin-Cdk module mutants. (A) Heat map comparing genome-wide expression changes between double-tail subunit mutants and single-tail subunit deletion strains. The genome-wide expression changes upon the deletion of tail module subunits Med3, Med2, Gal11/Med15, Sin4/Med16, and Srb8-11 module subunits Srb8/Med12 and Srb10/Cdk8 were compared with double-tail mutant strains *gal11-myc med3Δ* and *gal11Δmed3Δ*. Total RNA was prepared from cells grown in CSM media, and data are shown for all genes showing altered expression of at least 1.5-fold for at least one mutant. Data were then subjected to k-means clustering using $k = 5$. See Materials and methods for additional details. (B) Scatterplots for all genes shown in (A) of Log2 expression values for *gal11/med15Δ med3Δ* yeast against the single-tail module deletion mutants indicated.

majority of the genes in Cluster 2 show upregulation in *srb8/med12Δ* and *srb10/cdk8Δ* mutants, suggesting that the tail module and Cyclin-Cdk module exert opposing effects on a subset of Cluster 2 targets. This is consistent with previous genome-wide epistasis analysis showing that the Cyc-Cdk module antagonizes Med2 at many targets (van de Peppel *et al*, 2005). However, some genes (Cluster 4) are repressed by the Cyclin-Cdk module via a mechanism that does not involve the tail module subunits.

Previous work indicated that incorporation of Gal11/Med15 and Med3 into Mediator requires Med2, and genome-wide expression data were interpreted as being consistent with this interpretation (Lee *et al*, 1999; Myers *et al*, 1999; Park *et al*, 2000; van de Peppel *et al*, 2005). However, comparison of double-tail mutants with *med2Δ*, *med3Δ*, and *gal11/med15Δ* yeast indicates that although some genes are similarly affected in all of these mutants, the majority shows stronger effects in the double-tail mutants than in single-tail subunit deletions (Clusters 2, 3, and 5; Figure 3B; Supplementary Figure S1B). Furthermore, recruitment of myc-tagged Gal11/Med15 is not impaired in *med2Δ* yeast (Figure 1B). We conclude that Med2, Med3, and Gal11/Med15 sometimes function redundantly, and that loss of any one of these subunits does not result in loss of the other two. Other published data are also consistent with double-tail module subunit deletions exhibiting stronger

phenotypes than single-tail subunit deletions (Zhang *et al*, 2004). As these latter authors suggest, the apparent discrepancy between a requirement for individual tail subunits for tail module integrity in purified Mediator and the *in vivo* effects of single-subunit deletion mutants may reflect less stable interactions that are disrupted upon purification.

Interestingly, *sin4/med16Δ* affects expression of some genes similarly to *gal11/med2/med3* deletions (Cluster 3 and subset of Cluster 2), and others more similarly to Cyclin-Cdk module deletions (Cluster 4 and subset of Cluster 2). This is consistent with reports showing both activating and repressive roles for Sin4/Med16 (Jiang *et al*, 1995; Li *et al*, 1995). A possible explanation for these observations is that Sin4/Med16 has a modest effect on the *in vivo* association of both the Gal11/Med3/Med2 triad and the Cyclin-Cdk module, so that loss of Sin4/Med16 results in a corresponding effect on genes whose regulation is controlled by those complexes. Biochemical evidence and ChIP data are consistent with stabilization of the Gal11/Med2/Med3 triad by Sin4/Med16 (Li *et al*, 1995; Myers *et al*, 1999; Dotson *et al*, 2000; Zhang *et al*, 2004); however, we are not aware of data implicating Sin4/Med16 in stabilizing association of the Cyclin-Cdk module with the Mediator complex.

In addition to genes showing decreased expression in Mediator tail subunit deletion mutants, many showed

increased expression (Clusters 3 and 5). As noted above, most of these genes showed stronger effects in double mutants than in single mutants (Figure 3B; Supplementary Figure S1B). We also observed one cluster (Cluster 1) containing genes that show a modest downregulation in *gal11Δ med15Δ* and *med2Δ* strains, but no major effect in any other deletion mutants. At present, we do not understand the nature of these effects.

Overall, our data indicate that the pattern of expression changes in *gal11Δ med15Δ med3Δ* and *gal11Δ med15Δ myc med3Δ* strains is broadly similar to that seen in yeast lacking Gal11/Med15, Med2, or Med3, although these double mutants show stronger effects than seen upon loss of any single subunit from the Gal11/Med15 submodule. These findings are consistent with the redundancy between Gal11/Med15 and Med3 that we observed for *CHA1* expression (Figure 1).

Effect of *gal11Δ med3Δ* on recruitment of PIC components

Loss or severe impairment of function of the Mediator tail module in *gal11Δ med3Δ* yeast results in altered expression of 5–10% of the yeast genome. In contrast, loss of function of the Med17/Srb4 subunit from the head module in *srb4 ts* yeast causes loss of expression of >90% of Pol II transcribed genes (Thompson and Young, 1995; Holstege *et al*, 1998). Binding of Mediator middle and head modules and of Pol II is rapidly lost from the induced *CHA1* promoter and from the

RPS11B and *RPL12A* promoters, among others, upon shift to the restrictive temperature in *srb4 ts* yeast (Ansari *et al*, 2009). TBP association is also substantially reduced in *srb4 ts* yeast at several promoters that have been examined, including the induced *CHA1* promoter (Kuras and Struhl, 1999; Li *et al*, 1999; Qiu *et al*, 2004; He *et al*, 2008). We, therefore, hypothesized that at genes having reduced expression in *gal11Δ med3Δ* yeast, binding of Mediator, Pol II, and TBP would be decreased, while unaffected genes would retain Mediator middle and head modules, Pol II, and TBP at normal levels.

To test this idea, ChIP was performed for the Srb5/Med18 subunit from the head module of Mediator, the Pol II subunit Rpb3, and TBP. Association of Srb5/Med18, Rpb3, and TBP was reduced in *gal11Δ med3Δ* yeast at the promoters for *CHA1* and *PMA1*, both of which are downregulated in the tail deletion mutants (Figure 4A–C). In contrast, at the promoters for *RPS11B* and *RPS12A*, whose transcription is not affected in *gal11Δ med3Δ* yeast, Srb5 association is unaffected and Pol II shows a very modest decrease (Figure 4A and B). TBP shows a slightly larger decrease, to about 70% of wild-type levels, but this is a much smaller reduction than seen at *CHA1* or *PMA1* (Figure 4C). We conclude that the tail module is differentially required for Mediator recruitment; it is essential at *CHA1* and important at *PMA1*, while Mediator is recruited to other genes, such as *RPS11B* and *RPL12A*, by a mechanism that does not require

Figure 4 Effect of *gal11Δ med3Δ* mutation on recruitment of PIC components. (A–C) Association of Srb5/Med18 from the head module of Mediator, TBP, and Pol II subunit Rpb3 was analysed at the core promoter regions of indicated genes by ChIP. WT and *gal11Δ med3Δ* strains expressing myc-tagged Srb5/Med18 were grown in YPD media at 30°C and immunoprecipitations were carried out using anti-myc antibody and direct antibodies against TBP and Rpb3. ChIP'd DNA was analysed by real-time PCR. The bars represent IP/Input ratios for the indicated genes normalized to a non-transcribed region of ChrV (Keogh and Buratowski, 2004). Error bars represent standard deviation. Each experiment was performed 3–4 times. (D) The binding of activator Met4 and PIC components Srb5/Med18, TBP, and Pol II was analysed on the core promoter region of *MET2* gene in uninduced and induced (0.05 mM CdCl₂) condition by ChIP as described above.

the Gal11/Med2/Med3 module. Recruitment of TBP and Pol II directly reflects the effect of *gal11Δ med3Δ* on the recruitment of Mediator itself.

We also observed a number of genes to be upregulated in *gal11Δ med3Δ* yeast (Figures 2B and 3). This could be due to a direct repressive effect of the Mediator tail module at these genes, in which case we would expect to see association of Mediator at the repressed gene promoters in wild-type yeast. However, ChIP of Srb5/Med18 at *GAP1*, *MAL33*, *MET2*, *MET32*, *POX1*, and *SPS19*, all of which are upregulated in tail delete strains, did not reveal significant binding in either WT or *gal11Δ med3Δ* strains and no evidence was seen for altered Srb5/Med18 binding in *gal11Δ med3Δ* yeast (Figure 4A). To address the possibility that the tail module might repress these genes independently of the head and middle modules of Mediator, we assessed binding of Med2, another tail module subunit (Supplementary Figure S2A). This subunit shows significant association with the *CHA1* and *PMA1* promoters that is substantially reduced in *gal11Δ med3Δ* yeast, consistent with the results for Srb5/Med18. However, little if any binding was observed at the *MET2* or *SPS19* promoters in wild-type or mutant yeast (Supplementary Figure S2A). Consistent with these findings, Rgr1/Med14 and Gal11/Med15 were found to be associated with the *PMA1* promoter but not with *POX1*, *SPS19*, *MET32*, or *MIG3* (Supplementary Figure S2B). These results do not support a direct role for Mediator, or for the tail module acting independently of the rest of Mediator, in repression of genes that are upregulated in *gal11Δ med3Δ* yeast. However, we cannot rule out that direct effects may occur that are below the sensitivity of our ChIP assays, or that do not depend directly on binding of those Mediator subunits that we have examined.

We also examined Rpb3 association with promoters of this same set of repressed genes. We observed moderate levels of Pol II binding (2–5-fold above background) for some (*GAP1*, *MAL33*, *MET2*, and *MET32*), while others (*POX1*, *SPS19*, and *SPO20*) showed no significant binding above background in wild-type yeast (Figure 4B). A modest increase in Pol II association was seen in *gal11Δ med3Δ* yeast at the *SPS19*, *SPO20*, *POX1*, and *MAL33* promoters (Figure 4B), while TBP binding was very low in all of these promoters except for *MET2*, and showed no significant change between wild type and mutant (Figure 4C). In contrast, association of Srb5/Med18, Pol II, and TBP was substantially increased upon induction of the *MET2* promoter in the presence of CdCl₂, as expected (Figure 4D; Cormier *et al*, 2010). Association of Srb5/Med18, Pol II, and TBP with the induced *MET2* promoter was decreased in *gal11Δ med3Δ* yeast, although the primary activator Met4 showed no change in its association (Figure 4D), consistent with previous observations using single-tail subunit deletions (Leroy *et al*, 2006).

Taken together, these findings indicate that the downregulation in expression of genes in *gal11Δ med3Δ* yeast is caused by reduced binding of the head module of Mediator and subsequent decreased recruitment of TBP and Pol II, while upregulation may not be due to a direct effect of the loss of tail module from the promoters of affected genes.

Distinct role for the Mediator tail module at SAGA-dependent and TFIID-dependent genes

Our microarray results show that loss or severe impairment of a functional tail module from Mediator strongly affects

transcription of a select cohort of genes comprising 5–10% of the yeast genome. To further investigate interactions between the Mediator tail module and components involved in transcriptional activation and chromatin remodelling pathways, we modified an approach used by Steinfeld *et al* (2007). Using a compendium of gene expression profiles for 170 yeast strains harbouring mutations in a variety of ‘chromatin modifiers’, we calculated the average effect on gene expression of each mutation for the cohort of genes showing greatest downregulation or upregulation in our *gal11Δ med3Δ* yeast strain and compared it with the average effect of the same mutation on all genes.

Figure 5A and B shows the fraction of mutants in the compendium plotted against ΔExpression where ΔExpression = (average change in expression of the indicated cohort (genes showing decreased or increased expression in *gal11Δ med3Δ* yeast)) – (average change in expression of all genes). Supplementary Table S2 lists the mutations showing most significant effects on genes that are strongly affected in *gal11Δ med3Δ* yeast, which correspond to mutants falling in the regions outside the vertical lines in Figure 5A and B. Strikingly, these mutations include several that are functionally related to TFIID or SAGA. Genes downregulated in *gal11Δ med3Δ* yeast were also strongly downregulated in *spt3* and *spt8* mutants, as well as in several *tbp* mutants, while the same cohort was oppositely affected in *mot1*, *bur6*, *taf1*, and *taf2* mutant yeast (Supplementary Table S2; Figure 5A, B, D, and E). Spt3 and Spt8 are subunits of the SAGA complex and have been implicated in regulating recognition of the TATA element by TBP (Madison and Winston, 1997; Bhaumik and Green, 2002), and the TBP mutants identified are likely to affect the stability of the TBP/DNA complex (Chitikila *et al*, 2002). Mot1 and Bur6 play negative regulatory roles at TATA-containing genes, while Taf1 and Taf2 are components of TFIID, which functions primarily at genes lacking functional TATA elements and not using the SAGA complex (Basehoar *et al*, 2004; Huisinga and Pugh, 2004). Thus, the distinct effects of these mutants on the cohort of genes downregulated in *gal11Δ med3Δ* yeast suggests a preferential role for the Mediator tail in activating genes that contain a functional TATA element and are controlled by the SAGA complex, as opposed to those using TFIID (Basehoar *et al*, 2004; Huisinga and Pugh, 2004). Indeed, genes downregulated in *gal11Δ med3Δ* yeast are highly overrepresented among SAGA-dominated genes and underrepresented among TFIID-dominated genes (Figure 5C; $P < 10^{-47}$, Fisher’s exact test). In accord with this idea, genes containing functional TATA elements and using SAGA are more highly regulated than those depending on TFIID (Huisinga and Pugh, 2004), consistent with a role for the tail in contacting specific activator proteins (Lee *et al*, 1999; Zhang *et al*, 2004; Thakur *et al*, 2008, 2009; Herbig *et al*, 2010).

Genes downregulated in *gal11Δ med3Δ* yeast were also strongly downregulated in *swi/snf* mutants (Figure 5A; Supplementary Table S2). This suggests that the Swi/Snf and SAGA complexes impinge on a set of target genes that depend strongly on the Mediator tail module for their activation. Previous studies have also pointed to an interrelationship between Mediator and the Swi/Snf complex (Roberts and Winston, 1997; Sharma *et al*, 2003; Lemieux and Gaudreau, 2004), and in some cases, Mediator has been found to contribute to Swi/Snf recruitment (Sharma *et al*, 2003; Lemieux and Gaudreau, 2004; Biddick *et al*, 2008a).

Figure 5 Distinct effects of mutations in SAGA and TFIID components on gene cohorts affected by Mediator tail deletions. (A, B) The fraction of all members in a compendium of chromatin modifier mutants (Steinfeld *et al*, 2007) having a specific Δ Expression value is plotted as a function of Δ Expression, where the latter is equal to the difference between the average change in expression of the indicated cohort (genes showing decreased or increased expression in *gal11Δ med3Δ* yeast) and the average change in expression of all genes for each mutant in the compendium. The vertical lines indicate one standard deviation above and below the mean Δ Expression for each plot. (C) Venn diagram showing interrelationships between genes showing decreased expression in *gal11Δ med3Δ* yeast, and TFIID-dominated and SAGA-dominated genes (Huisinga and Pugh, 2004). (D–F) Plots showing relative frequency of genes having indicated changes in expression in *spt3*, *taf1*, and H3 K5, 9, 14, 18, 23, 27Q mutants for all genes (solid lines) and for the cohort of genes showing decreased (D, E) or increased (F) expression (dashed lines) in *gal11Δ med3Δ* yeast. Calculation of the Kolmogorov–Smirnov (K–S) statistic, which is useful for assessing disparities between non-normal distributions, for the distributions in (D–F) yielded *P*-values $< 10^{-15}$ in all three cases.

Genes upregulated in *gal11Δ med3Δ* yeast were also upregulated in several H3 and H4 tail mutants as well as in mutants affecting modifications of the histone tails (*rad6*, *tup1*, *ssn6*, and *yng2*) (Figure 5B and F; Supplementary Table S2). This may indicate that Mediator collaborates (directly or indirectly) with repressors that act on the H3 and H4 amino termini to repress this cohort of genes. Genes upregulated in *gal11Δ med3Δ* yeast were significantly downregulated, or showed less upregulation than the average for all genes, in several mutants belonging to histone deacetylase complexes (*rpd3*, *sap30*, *hda1*, *sin3*, *sds3*, and *sir4*). A likely explanation is that gene repression via the complexes associated with these genes operates on a distinct set of genes from that which is repressed by the Mediator tail module. Thus, although these mutants cause derepression of many genes, they have less effect on those that are upregulated in

gal11Δ med3Δ yeast. This also implies that those genes at which Mediator and the histone H3 and H4 amino termini apparently collaborate in repressing are repressed by mechanism(s) not involving these particular histone deacetylase complexes.

Disruption of tail module function preferentially affects PIC assembly on SAGA-dependent promoters

The results from the preceding section suggest that the Gal11/Med2/Med3 triad is generally important for Mediator recruitment at SAGA-dependent genes, but is much less so at TFIID-dependent genes. Alternatively, it could be that recruitment is equally affected but some other aspect of Mediator function that depends on the Gal11/Med2/Med3 triad is differentially required between these two gene categories. To address this issue, we used ChIP to examine recruitment of the Mediator

subunit Srb5/Med18 (head module), TBP, and Pol II at several SAGA-dependent and TFIID-dependent promoters in wild-type and *gal11Δ med3Δ* yeast (Figure 6). In addition to *PMA1* and the induced *CHA1* and *MET2* promoters (also shown in Figure 4), we examined eight additional SAGA-dependent genes (Huisinga and Pugh, 2004). *RPS11B*, *RPL12A* (also shown in Figure 4), and nine additional promoters served as examples of TFIID-dependent genes (Huisinga and Pugh, 2004).

We observed significant binding of Srb5/Med18, Pol II, and TBP at all promoters in WT cells, consistent with active transcription. Binding of TBP and Srb5/Med18 was visibly reduced for most SAGA-dependent promoters, whereas decreases seen at TFIID-dependent promoters were modest (Figure 6A, B, D, and E). Binding of TBP decreased an average of 2.7-fold for 11 SAGA-dependent genes examined in *gal11/med15Δ med3Δ* yeast, and 1.4-fold in 11 TFIID-dependent genes, a significant difference ($P=0.0002$). Srb5/Med18 binding

Figure 6 Differential dependence on the Mediator tail module of recruitment of PIC components to SAGA-dependent and TFIID-dependent genes. Association of Srb5/Med18 from the head module of Mediator, TBP, and Pol II subunit Rpb3 was analysed at the core promoter regions of indicated SAGA-dependent (A–C) and TFIID-dependent (D–F) genes by ChIP. Underlined genes showed significantly reduced expression in *gal11/med15Δ med3Δ* yeast. *CHA1*, *MET2*, and *MET6* were analysed under inducing conditions (*CHA1* is induced in YPD medium, which contains serine, and ChIP for *MET2* and *MET6* was performed using yeast grown in YPD plus 0.05 mM CdCl₂). WT and *gal11/med15Δ med3Δ* strains expressing myc-tagged Srb5/Med18 were grown in YPD media at 30°C and immunoprecipitations were carried out using anti-myc antibody and primary antibodies against TBP and Rpb3. ChIP'd DNA was analysed by real-time PCR. The bars represent IP/Input ratios for the indicated genes normalized to a non-transcribed region of ChrV (Keogh and Buratowski, 2004). Error bars represent standard deviation. Each experiment was performed 3–4 times.

decreased 2.0-fold for SAGA-dependent genes and only 1.1-fold for TFIID-dependent genes ($P < 10^{-4}$). Binding of Pol II exhibited a similar trend between the two gene classes; being reduced an average of 3.6-fold for SAGA-dependent genes and 1.8-fold for TFIID-dependent genes ($P = 0.008$). We previously showed that binding of Srb5 and Pol II is substantially reduced at *RPS11B* and *RPL12A* in *srb4 ts* yeast (Ansari *et al*, 2009), and also found that binding of Srb5, TBP, and Pol II was reduced at *RPS28A*, *PIK1*, and *RPL3* in *srb4 ts* yeast at the non-permissive temperature (Supplementary Figure 3A–C), indicating that Mediator is associated with these genes at levels above background and contributes to recruitment of TBP and Pol II. Thus, the Gal11/Med2/Med3 module appears to contribute preferentially to recruitment of Mediator, TBP, and Pol II at SAGA-dependent genes compared with those depending on TFIID. However, this preference is not absolute, as is also reflected in some TFIID-dependent genes showing decreased expression in *gal11/med15 med3* mutant yeast.

We also considered the possibility that the loss of Gal11 and Med3 subunits might still allow Med2, the remaining tail module subunit (Figure 3), to function as a target for Mediator recruitment to TFIID-dependent genes. To test this, we used ChIP to monitor association of Med2 with several TFIID-dependent genes in WT and *gal11Δmed3Δ* strains. The results show significantly reduced binding of Med2 in *gal11Δmed3Δ* yeast as compared with WT at all of the TFIID-dependent genes tested (Supplementary Figure S4). Thus, recruitment of Mediator head module, TBP, and Pol II shows little change in the absence of Gal11 and Med3, and in spite of substantially reduced Med2 association, at TFIID-dependent genes. The minor but significant decreases in binding of Pol II and TBP to the TFIID-dependent genes examined here suggest that the tail module might have a modest stabilizing effect on association of the PIC components distinct from that of the head module. Taken together, these results show a preferred role for the Mediator tail module and distinct mechanism for recruitment of the Mediator head and middle modules at SAGA-dependent and TFIID-dependent gene promoters.

Discussion

Since the discovery of the Mediator complex nearly 20 years ago, a large body of work has established its broad role in transcription in eukaryotes and elucidated some of the mechanisms by which it activates or represses transcription (Kornberg, 2005; Malik and Roeder, 2010; Conaway and Conaway, 2011). One central idea that has emerged is that the tail module functions mainly as a target for activators to recruit Mediator to active genes, while the middle and head modules aid in preinitiation complex formation through contacts with Pol II. However, while loss of function of Srb4/Med17 from the head module leads to rapid loss of transcription of most transcribed genes (Thompson and Young, 1995; Holstege *et al*, 1998), loss of individual tail module subunits results in variable effects on transcription from a relatively small fraction of the genome (van de Peppel *et al*, 2005).

In the work reported here, investigation of a *gal11Δ med3Δ* mutant lacking two of the three subunits from the Gal11/Med2/Med3 triad has revealed that Gal11 and Med3 can

Figure 7 Two modes of Mediator participation in transcriptional activation. (A) At TATA-containing, SAGA-dependent genes, Mediator recruitment by activators requires tail subunits. SAGA may be recruited directly by activators or indirectly, depending on the activator and promoter. (B) At TATA-less, TFIID-dependent genes, the Mediator tail subunits are dispensable for Mediator recruitment. At this latter class of genes, recruitment of the PIC may occur via activator interactions with TAF subunits of TFIID, among other possible mechanisms; Mediator recruitment evidently occurs via contacts with the head and/or middle modules, which may involve TFIID or other components of the PIC.

function redundantly at some targets. The *CHA1* promoter provides an extreme example of this redundancy, as individual tail subunit deletions have essentially no effect on *CHA1* induction or Mediator recruitment, while both are abrogated in the double mutant. Based on the observed redundancy between tail subunits, we have re-examined the role of Mediator tail in genome-wide transcription by using a mutant in which tail function is strongly impaired. Our analysis of genome-wide expression in *gal11Δ med3Δ* yeast revealed an unexpected division of labour for Mediator modules: the Gal11/Med2/Med3 module is largely dedicated to activation of SAGA-dependent, TATA-containing genes (Figure 7). In addition, we found that the tail module has an important role in responses to environmental perturbations, supporting transcription of genes involved in stress response, drug toxin transport, and cellular import.

The tail module regulates gene expression through multiple pathways

Our transcriptome analysis revealed that expression of a substantial number of genes is affected in Mediator tail mutants. In agreement with the redundancy of individual tail subunits seen for *CHA1* expression (Figure 1), stronger effects on expression were generally observed for the *gal11/med15 med3* mutants than for the corresponding single-tail subunit deletion mutants or for *med2Δ* yeast (Figure 3). Consistent with an earlier study, we observed substantial numbers of both upregulated and downregulated genes in Mediator tail mutants (Figure 3; van de Peppel *et al*, 2005). Many of the genes affected in Gal11/Med2/Med3 deletion mutants are oppositely affected by deletion of subunits from the Cyclin-Cdk module, which is in accord with a previous analysis showing that the latter module exerted an inhibitory effect on the tail module (van de Peppel *et al*, 2005). However, we also observed many genes affected in tail module mutants that were unaffected by loss of Cyclin-Cdk subunits. For example, of 225 genes downregulated by at least two-fold in *gal11Δ med3Δ* yeast, 84 are upregulated at least 1.5-fold in *cdk8/srb10Δ* yeast while 74 are not upregulated in the latter mutant. Similarly, genes that are upregulated in tail module mutants include genes that are

downregulated in Cyclin-Cdk module mutants and genes that are unaffected (Figures 2C and 3). Thus, the tail module functions in gene regulation through at least two distinct mechanisms.

As noted above, many genes showed increased expression in tail mutants. However, we did not find evidence for this repression being direct, as significant effects on Mediator recruitment at these targets in the double mutant were not observed. In contrast, we found decreased Mediator association at genes downregulated in tail mutants, indicating a direct effect. Another study also did not find Mediator association with repressed genes that were upregulated in an *rgr1/med14* mutant (Biddick *et al*, 2008a,b; Young *et al*, 2009). The C-terminal region of Rgr1 connects the tail domain to the rest of Mediator (Dotson *et al*, 2000; Guglielmi *et al*, 2004), so it might be expected that an *rgr1/med14* mutant would behave similarly to *gal11Δ med3Δ* yeast to the extent that both mutations cause loss of tail module function. Because expression data for the *rgr1/med14* mutant were collated from multiple platforms (Young *et al*, 2009), we were not able to rigorously compare those results with ours, but we did find both substantial overlap and notable differences upon limited comparison of data sets. For example, 15 of 19 targets of the activator Adr1 that were listed as being upregulated in *rgr1/med14* yeast are also upregulated in *gal11/med15Δ med3Δ* yeast, but 20 of 30 listed genes that are downregulated in *rgr1/med14* yeast were not significantly affected in *gal11Δ med3Δ* yeast. Thus, although Rgr1 behaves similarly to tail module subunits at some genes, at others it appears that Mediator functions differently between *rgr1* and *gal11Δ med3Δ* mutants. This is also consistent with another study that reported increased basal transcription of genes induced by heat shock in yeast in various Mediator mutants including *rgr1*, whereas basal transcription was unaffected in mutants lacking Gal11/Med15 or Srb10/Cdk8 (from the Cyclin-Cdk module; Singh *et al*, 2006).

A role for the Mediator tail module in regulating environmental response

Gene ontology analysis indicated that the loss of tail module function in *gal11Δ med3Δ* yeast preferentially affects expression of genes involved in the transport and metabolism of diverse nutrients/metabolites such as drug/toxin molecules, carbohydrates, amino acids, and metal ions; stress response genes are also strongly affected (Supplementary Table S1). These results suggest that the tail module of Mediator complex participates in responding to nutrient/metabolite status. This idea is consistent with several previous reports that suggest a role for Mediator tail subunits in the regulation of specific metabolic genes from yeast to human. For example, the Med15 subunit of the tail module has been shown to regulate the expression of genes involved in fatty acid metabolism by directly interacting with activators, SREBP α , SBP-1, and Oaf1 in human, *C. elegans*, and *S. cerevisiae*, respectively, suggesting functional conservation of this subunit (Taubert *et al*, 2006; Yang *et al*, 2006; Thakur *et al*, 2009). The requirement for Med15 in the metabolism of xenobiotics and in lipid metabolism has also been reported in yeast and *C. elegans* (Thakur *et al*, 2008; Taubert *et al*, 2008), and another tail subunit, Rgr1/Med14, has been implicated in regulating the expression of PPAR γ -dependent genes and adipogenesis in the mouse through its direct interaction with nuclear receptor PPAR γ (Grontved *et al*, 2010).

These studies lead us to speculate that the subunits of the tail module might specifically regulate the expression of genes involved in diverse metabolic pathways by either directly or indirectly interacting with their respective transcriptional activators. This role for the tail is likely to be connected with its preferential function in activation of the highly regulated TATA-containing genes in yeast, as these genes also are characterized by varied expression in response to environmental perturbations (Tirosh and Barkai, 2008). As this function of tail module subunits seems to be conserved among eukaryotes from yeast to human, it will be of interest to examine in future studies whether metazoan genes that depend strongly on Mediator tail subunits for activation retain other properties that distinguish this class of targets in yeast.

Evidence for distinct modes of Mediator recruitment

To determine whether the Mediator tail module acts in concert with specific chromatin- or transcription-related complexes or pathways, we compared the effect of 170 mutants reported in the literature on the cohorts of genes upregulated or downregulated in *gal11Δ med3Δ* yeast with the effect on all genes (Figure 5; Supplementary Table S2; Steinfeld *et al*, 2007). Mutants that affected these cohorts differently from their effect on the entire genome were particularly enriched in TFIID and SAGA components, indicating that the Mediator tail is largely dedicated to regulation of TATA-containing, SAGA-regulated genes. Since these genes are particularly enriched for transcriptionally plastic genes—that is, genes that respond differently to different environmental perturbations—this is consistent with the enrichment also observed for genes involved in stress response and nutrient and metabolite sensing, as noted above (Tirosh and Barkai, 2008).

In accord with the stronger effect on expression of TATA-containing, SAGA-regulated genes of Mediator tail mutations, we also found that recruitment of Mediator, Pol II, and TBP was much more affected at this class than at TFIID-regulated genes by loss of tail module function (Figure 6). Other studies have also found dependence on subunits from the Gal11/Med2/Med3 submodule for recruitment of Mediator, Pol II, and TBP at targets of the activators Gcn4 and Met4; these targets are also SAGA dependent (Zhang *et al*, 2004; Leroy *et al*, 2006). However, Mediator is also important for expression of TFIID-dependent genes, and loss of Mediator function results in rapid loss of Pol II association with genes in this class (Thompson and Young, 1995; Holstege *et al*, 1998; Ansari *et al*, 2009). This raises the question of how Mediator is recruited to these genes. The essential head module subunit Srb4/Med17 is important for Mediator association with promoters in this class, and Rap1 is important for its association with promoters of ribosomal protein genes that depend on Rap1 for activation (Ansari *et al*, 2009). These observations imply that subunits from the head or middle module may be targets for activators that are responsible for expression of TFIID-dependent genes; alternatively, Mediator may be recruited indirectly through interactions with TFIID, for example. The establishment of distinct mechanisms for recruitment of Mediator to SAGA-regulated and TFIID-regulated genes calls for new studies to determine how Mediator is recruited to the latter category of gene promoters.

Materials and methods

Yeast strains and growth conditions

The *S. cerevisiae* strains used in this study are listed in Supplementary Table S3 except for single-subunit deletion strains used in Figure 3, which are all derived from BY4742 (Benschop *et al*, 2010). The strains RMY410, RMY412, and RMY414 were derived from strain RMY511 (He *et al*, 2008) by replacing the chromosomal copy of *MED3*, *MED2*, and *MED16* ORFs, respectively, with a *URA3* marker. The strains LS01 and LS10 were kindly provided by Alan G Hinnebusch (Zhang *et al*, 2004). RMY415 was generated by inserting coding sequences for *SRB5* as a *myc13-HIS3* cassette in the strain LS10 (Longtine *et al*, 1998). RMY416 was generated by inserting a TAPtag-*HIS3* cassette to the 3' end of *MED2* ORF in LS10 (TAP-tag collection).

Transformations were done using a standard lithium acetate protocol (Hill *et al*, 1991). Yeast cells were grown at 30°C on complete synthetic dropout media lacking appropriate amino acids (6.7 g/l YNB w/o AA, 2% glucose, CSM (DropOut) powder) or YPD (20 g/l bactopectone, 10 g/l yeast extract, 2% glucose, 0.15 g/l L-tryptophan) media.

Chromatin immunoprecipitation

ChIP was performed essentially as described previously (He *et al*, 2008; Ansari *et al*, 2009) with the following modifications: the samples were sonicated using a Diagenode Bioruptor at high power setting, 30 s 'on'/30 s 'off' for a total of 10 min to obtain sheared DNA 200–600 bp in length. For induction of the *CHA1* gene, 1 mg/ml serine was added to the medium 30 min before crosslinking. To induce methionine biosynthesis genes, 0.05 mM CdCl₂ was added to the medium for 90 min (Cormier *et al*, 2010) before crosslinking. For ChIP of myc-tagged proteins, whole cell extract was incubated with ~2 µg of anti-myc antibody (Roche Applied Science). For ChIP of TAP-tagged proteins, antibody to protein A (Sigma) was used. For Pol II ChIP, antibody against Rpb3 subunit of Pol II (Neoclone) was used, whereas anti-TBP antibody was a generous gift of PA Weil (Vanderbilt University).

Quantitations were done by real-time PCR; log₂ ratios of IP/Input are depicted in figures after subtracting log₂ ratios obtained for a non-transcribed region of ChrV (Keogh and Buratowski, 2004). The sequences of the primers used in this study are available upon request.

Microarray expression analysis

For transcriptome analysis of *gal11Δ med3Δ* and *gal11-myc med3Δ* yeast, RNA was prepared from exponentially growing yeast cells (A₆₀₀ = 0.8–1.0) grown in YPD or CSM using the Masterpure Yeast RNA Purification Kit (Epicenter Technology, Madison, WI); three biological replicates were performed for each strain and condition. RNA was further purified using the RNeasy purification kit (Qiagen). Processing and hybridization using Affymetrix Yeast Genome 2.0 Arrays (Affymetrix, Santa Clara, CA) were performed as per the manufacturer's instructions. Changes in gene expression were calculated by averaging log₂ expression changes and using false discovery rates using Gene Spring software, after normalizing within each experiment (i.e., wild-type and tail subunit deletion mutant strains analysed in parallel). Profiling of single-subunit deletion mutants was performed as described (Benschop *et al*, 2010). Comparative analyses were done using Excel (Microsoft). Functional categorization of significantly affected genes was carried out using a web-based cluster interpreter program (Robinson *et al*, 2002) and *P*-values were corrected for multiple category testing. K-S statistics (Figure 5D–F) were calculated in R (Stephens, 1970).

Clustering was performed using Cluster 3.0 (<http://bonsai.ims.u-tokyo.ac.jp/~mdphoon/software/cluster/>), program and visualized

using Java Treeview (<http://jtreeview.sourceforge.net/>). For K-means clustering, the default options were used. Both the *gal11Δ med3Δ* and *gal11-myc med3Δ* mutant expression profiles were compared with the BY4741 strain for consistency with auxotrophic markers; since these strains are opposite in mating type, mating type genes (*HMLALPHA1*/// *MATALPHA1*, *MFA2*, *MF(ALPHA)1*, *HMRA1*, *MF(ALPHA)2*, *AGA2*, *MFA1*, *STE2*, and *STE3*) were removed prior to analysis. In addition, we discovered a large number of genes on Chromosome V whose expression was abnormally high in strain LS10 (*gal11Δ med3Δ*) during growth in YPD, suggesting a duplication of Chromosome V had occurred in this experiment (Figure 2B) (also indicated by analysis using T-profiler) (Boorsma *et al*, 2005). We, therefore, excluded all genes on Chromosome V upregulated by more than two-fold from our analysis. Similarly, we found an apparent aneuploidy in Chromosome II in our wild-type control (BY4741) during growth in CSM, and therefore discarded Chromosome II genes before clustering (Figure 3). For clustering, we selected genes changing by at least 1.5-fold in expression in at least one of the mutants shown, totalling 1505 genes in all.

Microarray data for double-tail mutants and corresponding wild type have been deposited at the Gene Expression Omnibus and are available under accession number GSE31774; data for single-subunit mutants and wild type can be found at ArrayExpress (<http://www.ebi.ac.uk/arrayexpress>) with accession numbers E-TABM-654 (mutants) and E-TABM-773 (wild type). Processed data can be downloaded from <http://www.wadsworth.org/resnres/bios/morse>.

RNA analysis

Northern analysis was performed as described previously (Yarragudi *et al*, 2004). Probes for *CHA1*, *MET2*, *MET32*, and *SPS19* genes were prepared by PCR. For quantitation of gene expression by real-time PCR, purified RNA was reverse transcribed using first-strand cDNA synthesis kit (USB) and the resulting cDNA was amplified by quantitative real-time PCR using an ABI 7500 Fast real-time PCR system. The values for all of the genes analysed were normalized to *SNR6*, which is transcribed by Pol III. Values depicted in Figure 2D represent fold changes in the mutants compared with wild-type values, which were set as 1.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

Acknowledgements

We thank Alan Hinnebusch for providing yeast strains, Martin Kupiec for providing the data in the Chromatin Modifier Compendium, and Tony Weil (Vanderbilt University) for a generous gift of antibody to TBP. We also acknowledge Mike Palumbo in the Wadsworth Center Bioinformatics Core Facility for computational help, and the Wadsworth Center's Applied Genomic Technologies Core Facility for microarray services. This work was supported by grants from the NSF to RHM (MCB0517825 and MCB0949722).

Author contributions: SAA, JTW, and RHM planned the experiments; SAA performed the experiments, with the exception of the microarray expression analysis depicted in Figure 3, which was planned and conducted by JJB and FCPH; SAA, MG, and RHM analysed the data; and SAA, MG, JTW, and RHM wrote the paper.

Conflict of interest

The authors declare that they have no conflict of interest.

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