Mediator binding to UASs is broadly uncoupled from transcription and cooperative with TFIID recruitment to promoters

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Abstract

Mediator is a conserved, essential transcriptional coactivator complex, but its *in vivo* functions have remained unclear due to conflicting data regarding its genome-wide binding pattern obtained by genome-wide ChIP. Here, we used ChEC-seq, a method orthogonal to ChIP, to generate a high-resolution map of Mediator binding to the yeast genome. We find that Mediator associates with upstream activating sequences (UASs) rather than the core promoter or gene body under all conditions tested. Mediator occupancy is surprisingly correlated with transcription levels at only a small fraction of genes. Using the same approach to map TFIID, we find that TFIID is associated with both TFIID- and SAGA-dependent genes and that TFIID and Mediator occupancy is cooperative. Our results clarify Mediator recruitment and binding to the genome, showing that Mediator binding to UASs is widespread, partially uncoupled from transcription, and mediated in part by TFIID.

Introduction

The Mediator complex is a conserved coactivator that is broadly required for eukaryotic transcription. Mediator integrates regulatory signals from DNA-bound transcriptional activators and *cis*-regulatory elements to modulate the basal RNA polymerase II (Pol II) transcription machinery. Mediator appears to exert its effects on transcription in part through interactions with other coactivator complexes such as SAGA and TFIID. Previous work has suggested that the Mediator tail module is preferentially required at SAGA-dependent promoters (Ansari *et al*, 2012). *In vitro* studies have also described cooperative DNA binding between TFIID and Mediator

(Baek *et al*, 2002; Johnson *et al*, 2002; Johnson & Carey, 2003; Takahashi *et al*, 2011), but it is unclear whether such a cooperative relationship exists *in vivo*. In metazoans, Mediator associates with distal enhancer elements and is critical for looping of enhancers to promoters (Kagey *et al*, 2010). Despite recent advances in understanding the structure and functions of Mediator (Allen & Taatjes, 2015), the mechanisms by which Mediator co-regulates global Pol II transcription remain poorly understood.

Key to understanding the *in vivo* function of Mediator is accurate determination of its genomic binding sites. However, genome-wide mapping of Mediator using various chromatin immunoprecipitation (ChIP)-based methods has yielded ambiguous results in budding yeast, complicating analysis of its global transcriptional role. Mediator has been reported variously to bind both upstream activating elements (UASs) (Jeronimo & Robert, 2014) and core promoters (Ansari *et al*, 2009, 2012). Recent findings also indicate that Mediator accumulates at yeast core promoters only upon inhibition of the TFIIH subunit Kin28 (Jeronimo & Robert, 2014; Wong *et al*, 2014). Multiple studies have also argued for (Andrau *et al*, 2006; Zhu *et al*, 2011; Wong *et al*, 2014; Paul *et al*, 2015) and against (Fan *et al*, 2006; Fan & Struhl, 2009; Jeronimo & Robert, 2014) gene body association of Mediator. Despite a decade of genome-wide Mediator mapping, ambiguity regarding its genome-wide binding persists: Two recently published Mediator ChIP-seq studies indicate predominant gene body binding of Mediator (Wong *et al*, 2014; Paul *et al*, 2015), while two other recent mapping studies show little binding of Mediator to gene bodies but robust association with upstream regions under normal growth conditions (Eyboulet *et al*, 2013; Jeronimo & Robert, 2014). Notably, ChIP-seq for Med17 using an antibody against Med17 (Paul *et al*, 2015) or HA-tagged Med17 (Eyboulet *et al*, 2013) gives substantially different results. Issues potentially leading to these conflicting ChIP results include low Mediator ChIP efficiency and corresponding low enrichment values, artifactual signals in sonicated input chromatin (Teytelman *et al*, 2009; Vega *et al*, 2009; Grokhovsky *et al*, 2011; Poptsova *et al*, 2014), and the reported hyper-ChIPability of highly expressed genes (Park *et al*, 2013; Teytelman *et al*, 2013). While genome-wide binding of Mediator has been studied mainly in budding yeast, the

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structure of Mediator and its mechanisms of action are conserved throughout the eukaryotic lineage (Cai *et al*, 2009; Allen & Taatjes, 2015). As such, the uncertainty surrounding the binding of Mediator to the budding yeast genome has implications for understanding Mediator function in all eukaryotes.

Here, we apply chromatin endogenous cleavage and high-throughput sequencing (ChEC-seq) (Zentner *et al*, 2015) to map Mediator binding to the yeast genome and ascertain its relationship to TFIID. ChEC-seq employs fusion of micrococcal nuclease (MNase) to chromatin-associated proteins, directing calcium-dependent cleavage to specific sites on chromatin *in vivo*. ChEC-seq is thus immunoprecipitation-independent and as such does not require cross-linking, chromatin solubilization, or antibodies, and is quantitative. ChEC-seq therefore provides a ChIP-independent means by which to establish high-confidence profiles of Mediator binding. Profiling two Mediator head module subunits (Med8 and Med17), we observe that Mediator globally associates with UASs, rather than core promoters or gene bodies, under all conditions tested. Unique patterns of Mediator enrichment at SAGA- and TFIID-dependent genes suggest distinct promoter architectures of their respective transcription initiation complexes on chromatin. A striking finding is that Mediator binding to UASs is widespread and at most genes only weakly correlated with expression levels, suggesting that Mediator occupancy is partly uncoupled from gene expression. However, loss of the Mediator tail subunit Gal11/Med15 strongly reduced Mediator recruitment to a subset of genes upregulated upon Gcn4 activation. Lastly, we find that Mediator is generally necessary for full recruitment of TFIID to TFIID- and SAGA-dependent genes and that TFIID is also required for full Mediator recruitment to chromatin. Our results clarify the genome-wide binding locations of Mediator and reveal a functional relationship between coactivators Mediator and TFIID in transcription initiation.

Results

ChEC-seq profiling of Mediator binding to the budding yeast genome

ChEC-seq uses strains containing a C-terminal fusion of the calcium-dependent endo/exonuclease MNase to a chromatin-binding protein. Addition of calcium to permeabilized cells activates MNase and cleaves DNA in proximity to the chromatin-bound factor. We previously showed that ChEC-seq provides high-resolution maps of binding of the general regulatory factors Abf1, Rap1, and Reb1 to the yeast genome with additional information regarding their orientation on DNA (Zentner *et al*, 2015). As MNase must be near DNA for cleavage to occur, structural consideration of the protein(s) under study is essential. While this is relatively straightforward for transcription factors with defined DNA binding domains, the yeast Mediator complex consists of 25 subunits, which are distributed between four modules (head, tail, middle, kinase), without any documented DNA binding ability. Available high-resolution structural information is limited to parts of the Mediator middle module (Larivière *et al*, 2013; Wang *et al*, 2014) and the Mediator head domain (Imasaki *et al*, 2011; Lariviere *et al*, 2012). Head subunits Med8, Med17, and Med20 were fused with 3×FLAG-MNase based on their exposed carboxyl-terminal ends (Appendix Fig S1A). When

assessed by agarose gel electrophoresis following ChEC, DNA from the Med8-MNase and Med17-MNase strains displayed a moderate amount of cleavage, while DNA from the Med20-MNase strain displayed little cleavage (Appendix Fig S1B). This was not due to lack of expression of Med20-MNase, as all three MNase-tagged subunits were appropriately expressed (Appendix Fig S1C).

We sequenced endogenously cleaved DNA fragments from the Med8-MNase and Med17-MNase strains and mapped fragment ends back to the yeast genome. For comparison, we also plotted published Med14 ChIP-seq data (Wong *et al*, 2014) and Gal11/Med15 ChIP-chip data which had been normalized to a control ChIP from a strain without tagged Gal11/Med15 (Jeronimo & Robert, 2014). At three exemplary highly transcribed SAGA-dependent genes (*CDC19*, *ILV5*, *PDC1*), ChEC-seq revealed robust enrichment of Med8 and Med17-MNase cleavages upstream of TSSs but not within gene bodies (Fig 1A). Enrichment of specific ChEC cleavages was specific to fusion of MNase to Mediator sub-units, as it was not observed in a strain expressing untethered MNase under the control of the *MED8* promoter (Fig 1A and B). Med14 ChIP-seq showed enrichment upstream and within the coding regions of all three genes, with gene body signal being particularly pronounced at *CDC19* (Fig 1A). Gal11/Med15 ChIP-chip effectively captured upstream enrichment at all three genes as well as modest gene body signal. We next examined Med8 and Med17 cleavages at three exemplary strongly transcribed TFIID-dependent genes (*EFB1*, *RPS5*, *YEF3*). As observed for SAGA-dependent genes, Med8 and Med17 cleavages were enriched upstream of TSSs but not within coding regions (Fig 1B). Med14 ChIP-seq displayed upstream of *EFB1*, across the upstream region and coding region of *RPS5*, and within the coding region of *YEF3* (Fig 1B). Gal11/Med15 ChIP-chip captured enrichment upstream of *EFB1* and *YEF3*, with some coding region signal at *YEF3*, and across the upstream region and coding region of *RPS5* (Fig 1B). In summary, ChEC shows Mediator interaction at many intergenic gene regulatory regions but not within coding sequences of mRNA genes. In addition, the contrasting patterns of genome-wide ChIP enrichment we observed for Med14 and Gal11/Med15 using recent datasets underscores the continuing lack of clarity regarding the genome-wide distribution of Mediator.

Distinct patterns of Mediator association with SAGA- and TFIID-dependent UASs

We next investigated the position of Mediator binding relative to TSSs. To this end, we assessed the average distance of the cleavage peak summit for each Mediator subunit and promoter class profiled, pooling data from ChEC time points (Fig 2A). For Med8, the distance from the peak summit to TSS at SAGA-dependent genes was 267 bp (332 bp for Med17) and 165 bp at TFIID-dependent promoters (127 bp for Med17). The distances of the Mediator cleavage maxima to TSSs support preferential binding to UASs, which are generally located 250–400 bp upstream of TSSs in yeast (Chambers *et al*, 1988; de Bruin *et al*, 2001; He *et al*, 2012; Yan *et al*, 2015), rather than core promoters, which typically span 75 bp upstream and 50 bp downstream of TSSs (Lubliner *et al*, 2013). We observed similar results when single ChEC time points were analyzed (Appendix Fig S2), indicating that pooling of ChEC time points does not distort average profiles. On the single gene level, our average
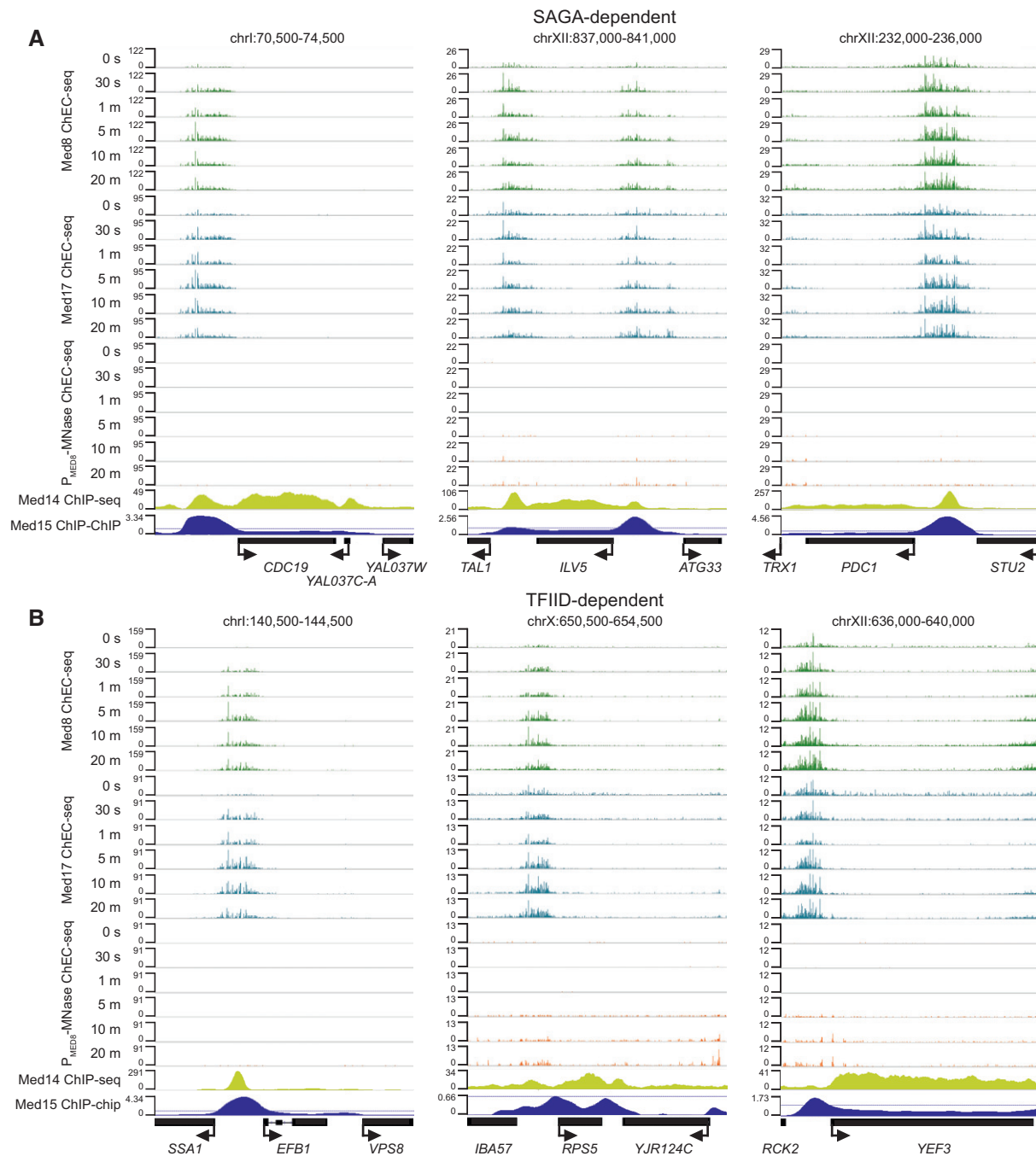
Figure 1. ChEC-seq mapping of Mediator at SAGA- and TFIID-dependent genes.

A, B   Signal tracks showing cleavages generated by Med8-MNase, Med17-MNase, and P_{MED8}-MNase in YPD at three highly expressed SAGA-dependent (A) and three highly expressed TFIID-dependent (B) loci. All time points for a given factor were scaled to the same data range, and P_{MED8}-MNase tracks were scaled to the lower Mediator-MNase fusion range. Med14 ChIP-seq and Med15 ChIP-chip data are shown for comparison. TSSs are indicated by arrows.

observations were confirmed by robust Med8 cleavage over the previously characterized UASs of the *CLB2* (SAGA-dependent) (Van Slyke & Grayhack, 2003) and *RPS5* (TFIID-dependent) (Li *et al*, 2002) genes (Fig 2B). We also explored the previously reported association of Mediator with gene bodies. Consistent with our single-locus results (Fig 1), we observed little Med8 or Med17-MNase

cleavage as far as 1 kb into gene bodies, indicating that the gene body enrichment of Mediator detected in many ChIP studies is not representative of Mediator's location. As Med8 and Med17 displayed very similar cleavage profiles at the UASs of SAGA- and TFIID-dependent genes in the preceding analyses, only Med8 was profiled in subsequent ChEC-seq experiments.
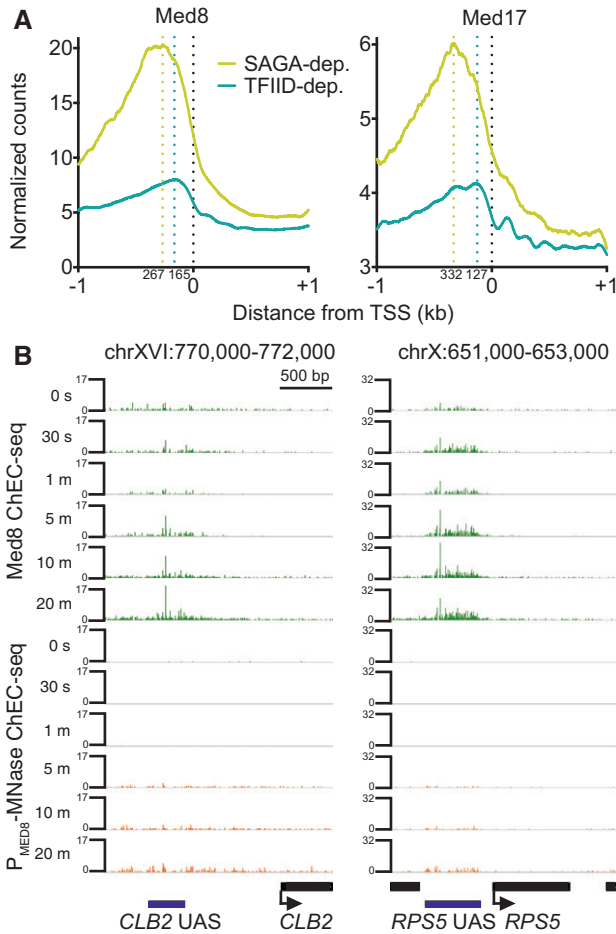
Figure 2. Mediator preferentially associates with the UASs of SAGA-dependent genes.

A   Average plots of Mediator cleavages around the TSSs of SAGA- and TFIID-dependent genes. The distance of cleavage maximum to TSS is indicated by the dotted lines and corresponding numbers.
B   Signal tracks of Med8-MNase and free MNase cleavages at the previously characterized UASs of the *CLB2* and *RPS5* genes. TSSs are indicated by arrows.

Figure 3. Lack of RNA Pol II CTD Ser5 phosphorylation does not lead to robust Mediator–UAS dissociation.

A   Average plots of Med8 cleavages around the TSSs of SAGA- and TFIID-dependent genes in Kin28AS cells treated with DMSO (-NA-PP1) or 6 μM NA-PP1 (+NA-PP1).
B   Signal tracks of Med8-MNase cleavages at the upstream regions of several genes previously shown to have increased core promoter association of Mediator by ChIP-qPCR following NA-PP1 treatment of a Kin28AS strain (Wong et al, 2014). All time points for a given treatment were concatenated to generate a combined track. TSSs are indicated by arrows.

Inactivation of Kin28 does not cause Mediator displacement from UASs

Two recent studies suggested that Ser5 phosphorylation of the Pol II C-terminal domain by the TFIIH-associated kinase Kin28 is important for Mediator release from the core promoter (Jeronimo & Robert, 2014; Wong *et al*, 2014). We thus wondered whether Mediator binding, as measured by ChEC-seq, would shift from UASs to core promoters in *kin28*-analog sensitive (Kin28AS) cells after treatment with the inhibitor NA-PP1. As shown earlier, 6 μM NA-PP1 was sufficient to fully inhibit cell growth of the Kin28AS strain (Appendix Fig S3). NA-PP1 treatment resulted in a moderate decrease in Mediator binding at the UASs of mainly SAGA-dependent, and, to a lesser extent, TFIID-dependent genes (Fig 3A, Appendix Fig S4). However, we did not detect Mediator cleavages at the core promoter (Fig 3A), indicating that a majority of Mediator remained bound to UASs. This observation was confirmed at the
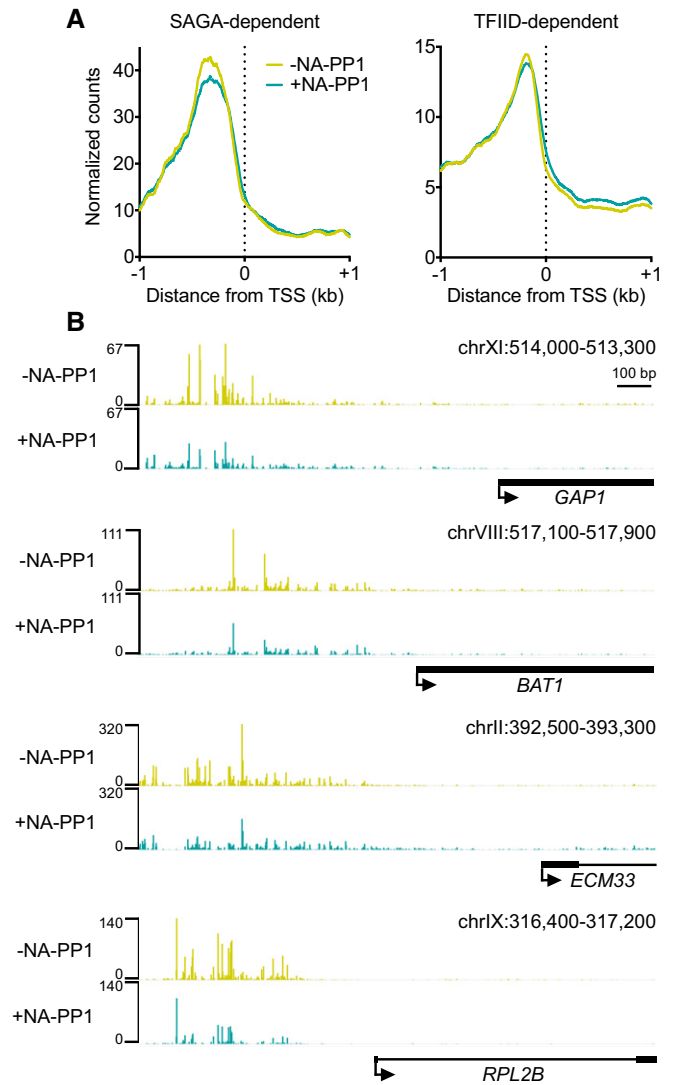
upstream regions of four genes (*GAP1*, *BAT1*, *ECM33*, *RPL2B*) previously shown to have increased Mediator association with core promoters following NA-PP1 treatment of a Kin28AS strain (Wong *et al*, 2014) (Fig 3B). These results contrast with those of two recent studies showing accumulation of Mediator at core promoters upon Kin28 inhibition (Jeronimo & Robert, 2014; Wong *et al*, 2014). We speculate that, when looped to core promoters from UASs, Mediator-tethered MNase may be too far from DNA for efficient cleavage or blocked from access to DNA by the PIC, and that Mediator may be detected at core promoters in ChIP experiments through cross-linking to the PIC or other promoter-associated factors.

Mediator binding is widespread and uncoupled from transcriptional activity at most genes

To further clarify the relationship of Mediator binding to the expression of SAGA- and TFIID-dependent genes, we stratified average Med8 cleavage levels at SAGA- and TFIID-dependent genes by expression level. As a measure for active transcription, previously published native elongating transcript sequencing (NET-seq) (Churchman & Weissman, 2011) signal within 200 bp downstream of the TSS was used. We found that Med8 occupancy was highest at the most highly transcribed quintile in both gene classes (Fig 4A), with significantly higher cleavage observed at the UASs of SAGA-dependent genes. To ensure that this was not due to the smaller number of genes in the SAGA-dependent quintiles (87 SAGA-dependent to 810 TFIID-dependent), we repeated the analysis using the 87 most highly transcribed TFIID-dependent genes. Again, we found that highly transcribed SAGA-dependent genes showed higher levels of Med8 cleavage at their UASs compared to the smaller TFIID-dependent gene set (Fig 4B), despite the fact that, on average, the top 87 most highly transcribed TFIID-dependent genes were expressed at significantly higher levels (Fig 4C, $P = 2.23 \times 10^{-13}$ by unpaired *t*-test). To more systematically assess the relationship between Mediator occupancy and transcription, we correlated average Med8 ChEC-seq signal in a 1-kb window upstream of the TSS with average NET-seq counts in a 200-bp window downstream of the TSS. In agreement with our above findings (Fig 4A), this revealed only modest correlations (SAGA-dependent Spearman's $\rho = 0.4126$, $P < 0.0001$; TFIID-dependent Spearman's $\rho = 0.3467$, $P < 0.0001$) (Fig 4D). We wondered whether the weak correlation of Med8 cleavage with transcriptional activity was driven by distinct subsets of TFIID- or SAGA-dependent genes. Hence, we determined correlations between Med8 cleavage and NET-seq for the SAGA- and TFIID-dependent NET-seq quintiles described in Fig 4A. While correlations were relatively poor across all quintiles, the best correlations were observed in the most highly transcribed quintile for both SAGA- and TFIID-dependent genes (Appendix Fig S5). Taken together, these observations indicate that Mediator occupancy and gene expression are largely but not completely uncoupled, and that Mediator binding to UASs is widespread.

The Mediator head module is recruited to activated genes and remains bound to downregulated genes

We next analyzed how a global perturbation of transcription affects Mediator association with the genome. We treated cells with sulfometuron methyl (SM), which mimics amino acid starvation (Jia *et al*, 2000). This treatment results in upregulation of the Gcn4 transcription factor, which in turn activates the transcription of amino acid biosynthetic genes (Hinnebusch, 2005) in part through interactions with the Med15/Gal11 subunit of the Mediator tail module (Herbig *et al*, 2010; Jedidi *et al*, 2010). We first assessed Mediator recruitment to Gcn4 binding sites previously determined by ChIP-chip (MacIsaac *et al*, 2006) and observed a robust increase in Med8 binding following SM treatment (Fig 5A). We also mapped Mediator binding via Med17 ChEC-seq in a strain lacking Med15 and observed a substantial reduction in signal at Gcn4 binding sites, confirming the tail module dependence of activator recruitment (Fig 5A). The majority of Gcn4 sites tested displayed an increase in
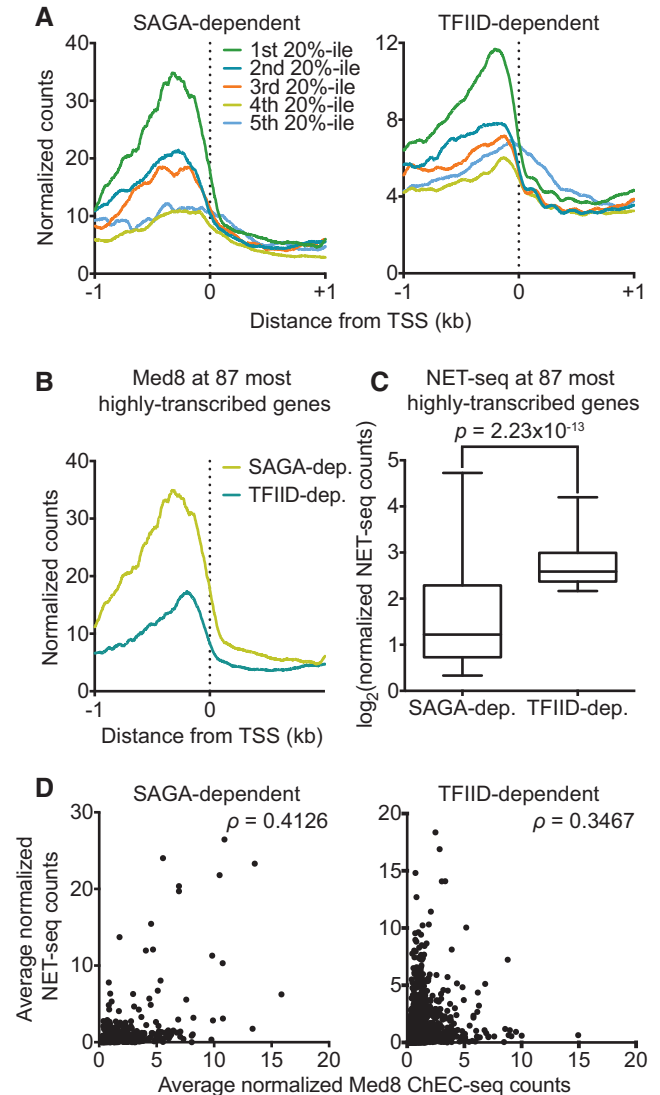

Figure 4. Mediator binding is widespread and partially uncoupled from transcription.

A Average plots of Med8 cleavages around SAGA- and TFIID-dependent gene TSSs stratified into quintiles by the level of NET-seq signal in a 200-bp window downstream of the TSS.

B Average plots of Med8 cleavage around the TSSs of the 87 most highly transcribed SAGA- and TFIID-dependent genes.

C Boxplots of the transcription levels of the 87 most highly transcribed SAGA- and TFIID-dependent genes as determined by NET-seq. Significance was assessed by *t*-test. Horizontal line = mean, box range = 10–90th percentile, error bars = min to max.

D Scatterplots of average NET-seq counts in a 200-bp window downstream of the TSS versus average Med8 cleavages in a 1-kb window upstream of the TSS for SAGA- and TFIID-dependent genes. Correspondence between the datasets was assessed by Spearman correlation.

Mediator cleavage upon SM induction, and these increases were strongly attenuated in *med15Δ* (Appendix Fig S6). We next analyzed Med8 occupancy around the TSSs of genes ≥ twofold up- or downregulated by SM treatment (Saint *et al*, 2014). We found that Mediator recruitment to UASs was increased at approximately 20% of upregulated genes (Fig 5B; Appendix Fig S6) and these
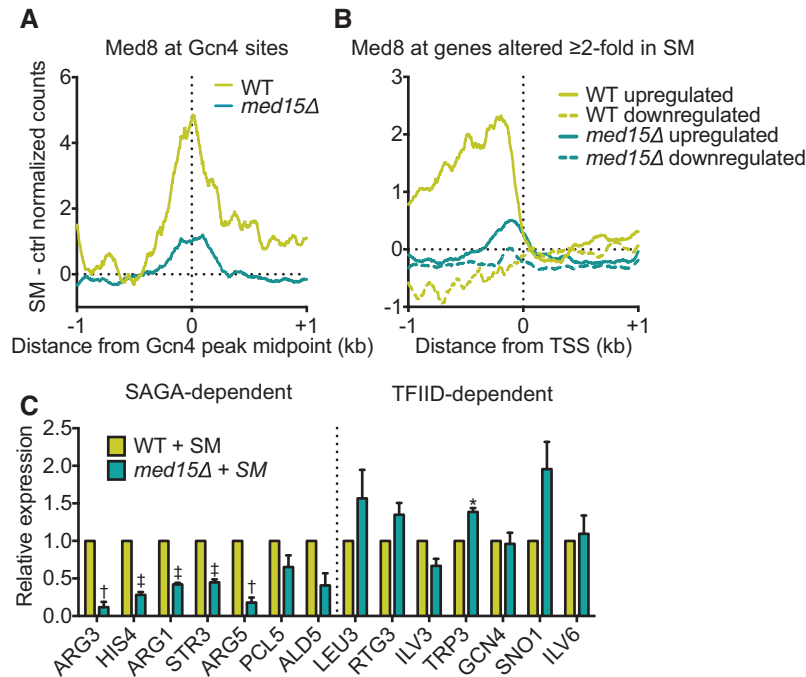
Figure 5. **The Mediator head module is recruited to Gcn4 sites and upregulated genes in SM and only moderately dissociates from SM-downregulated genes.**

A   Average plot of Med8 cleavages around 193 Gcn4 ChIP-chip peak midpoints (MacIsaac et al, 2006) in WT and med15Δ.

B   Average plots of Med8 cleavages around the TSSs of genes upregulated and downregulated ≥ twofold in SM (Saint et al, 2014) in WT and med15Δ. Control cleavages were subtracted from SM cleavages at each base position.

C   qRT–PCR analysis of SAGA- and TFIID-dependent genes in WT and med15Δ. Bars represent mean + SEM for two biological replicates performed in triplicate.
    *P < 0.05; †P < 0.01; ‡P < 0.005 by unpaired Student's t-test.

increases were diminished in med15Δ (Fig 5B, Appendix Fig S6). Unexpectedly, we observed only a modest decrease in Med8-dependent cleavage near SM-downregulated genes (Fig 5B). This decrease was observed at only a small minority of genes (Appendix Fig S6). Our findings suggest that the majority of Mediator remains bound to UASs even upon transcriptional downregulation, further supporting the hypothesis that Mediator occupancy and transcription are uncoupled at many genes.

The Mediator tail has previously been implicated in SAGA-dependent activated transcription (Ansari et al, 2012). We therefore used qRT–PCR to examine the effects of deleting Med15 on the expression of a set of representative Gcn4-activated genes consisting of seven SAGA-dependent and seven TFIID-dependent genes following SM induction. Deletion of the Mediator tail significantly downregulated 5/7 of the SAGA-dependent genes tested (ARG3, HIS4, ARG1, STR3, and ARG5), and the remaining SAGA-dependent genes (PCL5 and ALD5) showed a trend toward decreased expression. Of the TFIID-dependent genes tested, TRP3 expression was significantly increased, and three of the other tested TFIID-dependent genes (LEU3, RTG3, and SNO1) showed a trend toward increased expression (Fig 5C). These results confirm our genome-wide results that recruitment of Mediator through its tail module is important for activated transcription of predominantly SAGA-dependent genes.

TFIID depends on Mediator for maximal promoter interaction

To characterize the different recruitment pathways for Mediator at SAGA and TFIID-dependent genes, we analyzed the effect of Mediator loss on TFIID binding to chromatin, as previous studies have suggested Mediator-TFIID DNA binding cooperativity (Baek et al, 2002; Johnson et al, 2002; Johnson & Carey, 2003; Takahashi et al, 2011). To map TFIID binding genome-wide, we tagged the Taf1 subunit with MNase and performed ChEC-seq. Strikingly, we observed notable enrichment of Taf1 cleavages at both SAGA- and TFIID-dependent TSSs, though cleavages were slightly higher at annotated TFIID-dependent promoters (Fig 6A). As expected, the major site of Taf1 binding was within the nucleosome-depleted region (NDR) at core promoters (peak summit to TSS distance: 51 bp for SAGA-dependent, 68 bp for TFIID-dependent). We surprisingly observed a periodic enrichment of Taf1 cleavages into gene bodies, both upstream and downstream of TFIID-dependent TSSs in a pattern that is reminiscent of the nucleosomal arrays present in gene bodies. Comparison of MNase-seq (Henikoff et al, 2011) to Taf1 ChEC-seq data revealed a striking inverse relationship between Taf1 cleavages and nucleosome occupancy (Fig 6A), suggesting moderate cleavage of linker DNA between nucleosomes, perhaps due to interaction of the TFIID-associated and bromo-domain-containing subunit Bdf1 with acetylated nucleosomes in the promoter region (Matangkasombut et al, 2000; Durant & Pugh, 2007). This pattern was also observed downstream of SAGA-dependent TSSs, though to a lesser extent.

We next examined whether loss of Mediator would affect TFIID recruitment and subsequent PIC formation. We used anchor-away (Haruki et al, 2008) to rapidly deplete nuclear Med14, which functions as connector between all four Mediator modules (Tsai et al, 2014). Depletion of nuclear Med14 (Appendix Fig S7) resulted in a
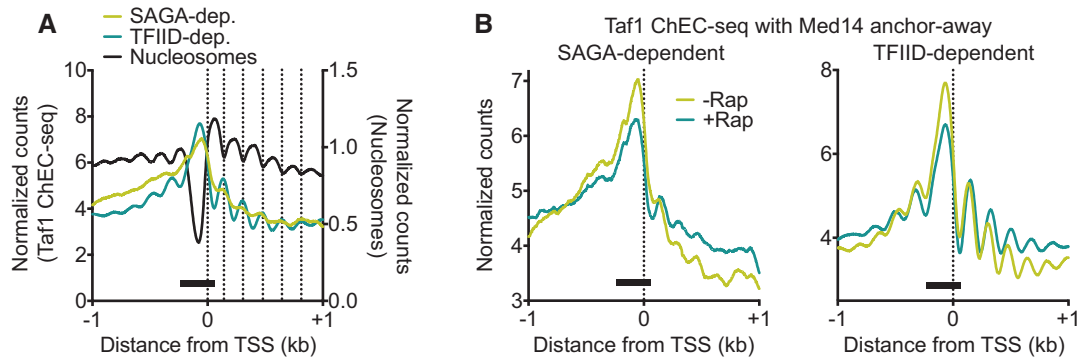
Figure 6. Mediator is required for full TFIID recruitment.

A   Average plot of Taf1 cleavages around the TSSs of SAGA- and TFIID-dependent genes. A nucleosome occupancy profile as determined by MNase-seq is also shown for comparison. Dotted lines represent Taf1 cleavage maxima in TFIID-dependent gene bodies. The NDR, taken to be the −1 to +1 nucleosome midpoint distance, is indicated by a black rectangle.

B   Average plots of Taf1 cleavages around the TSSs of SAGA- and TFIID-dependent genes ± rapamycin to deplete Med14-FRB.

reduction of TFIID occupancy at the core promoter, but a slight increase in and downstream shift of gene body cleavages regardless of coactivator dependence (Fig 6B). Approximately 70% of promoter NDRs displayed a modest decrease in Taf1 binding (Appendix Fig S8), indicating a moderate but widespread role for Mediator in TFIID recruitment.

Loss of TFIID impairs Mediator recruitment

Having found that disruption of Mediator impairs TFIID recruitment to promoters, we sought to determine whether the converse is true. We performed Med8 ChEC-seq following anchor-away depletion of Taf1 (Appendix Fig S7), an essential subunit of TFIID involved in promoter DNA binding (Louder *et al*, 2016). Med8 cleavages were, on average, strongly reduced at both SAGA- and TFIID-dependent promoters upon Taf1 depletion (Fig 7), implying an important role for TFIID in Mediator binding. Unexpectedly, Mediator recruitment to UASs of SAGA-dependent genes appeared to be more strongly affected by Taf1 depletion. Indeed, ~40% of SAGA-dependent upstream regions showed a noticeable decrease in average Med8 cleavages, while ~20% of TFIID-dependent upstream regions

showed decreased average Med8 cleavages (Appendix Fig S9). We also noted an increase in average Med8 cleavages upstream of ~20% of SAGA-dependent genes, but no such increase upstream of TFIID-dependent genes (Appendix Fig S9). Combined with our data indicating that TFIID recruitment to the majority of genes is partially dependent on Mediator (Fig 6B), these observations indicate mutual dependency between TFIID and Mediator for chromatin recruitment to many genes.

Discussion

The *in vivo* genomic distribution of the Mediator complex in budding yeast has presented a considerable obstacle to understanding its *in vivo* functions. We present a genome-wide map of Mediator binding in budding yeast generated using ChEC-seq, a method based on a completely different principle than ChIP, allowing us to identify Mediator-bound genomic loci with high resolution. We find that Mediator associates with the majority of UASs in the budding yeast genome. This pervasive binding is, to a large extent, uncoupled from the transcriptional activity and coactivator dependence of the genes associated with Mediator-bound UASs.

ChEC-seq refines the genome-wide map of Mediator binding

Our results led to several observations regarding the locations of Mediator association *in vivo*. First, we observed widespread Mediator-specific cleavage in the upstream region of the majority of genes in the budding yeast genome. We observed essentially no cleavage above background within gene bodies, indicating that Mediator enrichment within gene bodies is misleading, as has been previously speculated (Fan & Struhl, 2009; Teytelman *et al*, 2013; Jeronimo & Robert, 2014). Our data indicate that the predominant site of Mediator binding at TATA-containing genes is, on average, ~300 bp upstream of TSSs, consistent with UASs being the major site of Mediator association. At TFIID-dependent promoters, the Mediator cleavage maxima to TSS distance was ~150 bp, likely reflective of a shorter UAS-TSS distance. This is in agreement with previous
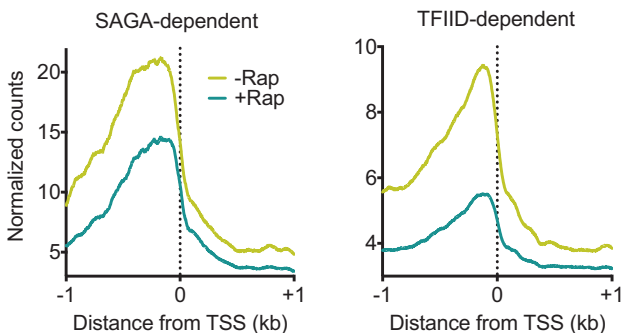

Figure 7. Loss of Taf1 impairs Mediator recruitment.
Average plots of Med8 cleavages around the TSSs of SAGA- and TFIID-dependent genes ± rapamycin to deplete Taf1-FRB.

studies suggesting that transcription factor binding sites, which tend to cluster in UASs, generally tend to be closer to the TSS at TATA-less promoters (Erb & van Nimwegen, 2011). Notably, the difference in the Mediator binding location to TSS between TATA-containing and TATA-less genes equals the approximate size necessary to accommodate a nucleosome, possibly due to distinct transcription initiation complex architectures for both promoter classes. We note that our results were obtained using MNase fused to subunits of the Mediator head module, and that ChEC-seq using MNase fused to subunits of the middle, tail, and kinase modules, if they are within reasonable proximity to DNA, could give slightly different results. However, since the head module directly interacts with Pol II and, along with the Mediator middle module, constitutes the core Mediator complex (Plaschka et al, 2015), we are confident that we have obtained functionally relevant maps of Mediator binding.

Two recent studies reported that abrogation of Kin28 kinase activity led to the appearance of Mediator ChIP-signal at core promoters. To explain these results, it was suggested that Mediator-PIC association is transient due to rapid transcription initiation upon PIC formation. Blocking the kinase was proposed to stall PICs at the promoter and allow detectable Mediator cross-linking to PIC components (Jeronimo & Robert, 2014; Wong et al, 2014). While we observed strong Mediator-MNase cleavages at the UASs under all conditions, we surprisingly did not observe a significant increase of Mediator-MNase cleavages at core promoters in Kin28AS cells when treated with the NA-PP1 analog. In higher eukaryotes, Mediator is important for the organization of chromatin into topological domains by forming gene loops, which enable coordinated transcription regulation (Plank & Dean, 2014). We suggest that, for activator-driven transcription, Mediator remains bound to UASs and transiently interacts with PICs via a DNA-looping interaction, bringing the core promoter close to the UAS. The long UAS to TSS distances we observe at SAGA-dependent genes may favor the formation of such loops. We speculate that, when looped to core promoters, MNase tethered to UAS-bound Mediator is either too far from DNA for efficient MNase cleavage or that DNA access is blocked by other factors in the PIC. However, ChIP may detect Mediator at some core promoters through cross-linking of Mediator to the PIC or other core promoter-bound transcriptional regulators.

Global mutual dependency in chromatin recruitment of Mediator and TFIID

We investigated in vivo the potential cooperativity between Mediator and TFIID suggested by several studies (Baek et al, 2002; Johnson et al, 2002; Johnson & Carey, 2003; Takahashi et al, 2011). Utilizing ChEC-seq to map TFIID occupancy in vivo, we find that in addition to being bound to TFIID-dependent promoters, TFIID is generally associated with TATA-containing SAGA-dependent core promoters. This association is partially Mediator-dependent, as anchor-away depletion of the Mediator middle module subunit Med14, expected to destabilize the entire Mediator complex, led to decreased Taf1 ChEC-seq signal at a majority of both SAGA- and TFIID-dependent core promoters. We also found that nuclear depletion of the Taf1 subunit of TFIID strongly reduced Mediator occupancy upstream of a sizable minority of SAGA- and TFIID-dependent genes. However, depletion of Taf1 also increased

Mediator binding to UASs of some SAGA-dependent genes, perhaps suggesting compensatory upregulation of these genes or an inhibitory role of TFIID in Mediator recruitment and transcription initiation at a particular subset of genes as described in higher eukaryotes (Tatarakis et al, 2008). Nevertheless, our results provide the first in vivo support for cooperative assembly of Mediator and TFIID on chromatin, previously described in vitro (Johnson et al, 2002). A direct cooperative relationship between TFIID and Mediator could be mediated by Taf1-Med16 interactions, which have been described in high-throughput studies (Gavin et al, 2002, 2006). Taken together, our findings suggest a general model for Mediator-facilitated TFIID recruitment to core promoters: Mediator-independent, likely non-specific binding of Taf1/TFIID to linker DNA between acetylated nucleosomes promoted by the loosely associated TFIID subunit Bdf1 could represent transient probing of chromatin by TFIID. In a cooperative process, Mediator guides TFIID to core promoters to facilitate transcription initiation. However, future studies are required to understand whether and how TFIID and Mediator directly interact with each other.

What are the functions of widespread Mediator binding?

The scale of the effect of Mediator on transcription (global versus selective) has been the subject of much debate (Fan & Struhl, 2009; Jeronimo & Robert, 2014). However, it has been clearly demonstrated that Mediator is globally required for transcription, as loss of the head module subunits Med17 or Med18 led to decreased synthesis of most mRNAs (Holstege et al, 1998; Plaschka et al, 2015). Consistent with this, we showed that the Mediator head module binds the majority of UASs in the budding yeast genome regardless of transcription level or coactivator dependence. We also found that Mediator binding is poorly correlated with transcriptional output in most cases, an exception being highly transcribed SAGA-dependent genes, which Mediator has been shown to activate through its tail module (Ansari & Morse, 2012). Together, these observations suggest that drawing inferences regarding the scale of Mediator activity in transcription based on simple correlations between Mediator occupancy and transcriptional output is problematic.

In addition to pervasive Mediator binding to UASs, we also observed binding of TFIID to most core promoters, regardless of their designation as SAGA- or TFIID-dependent (Basehoar et al, 2004). The presence of TFIID at essentially all promoters may suggest that the presence of Mediator and TFIID constitutes a transcriptional baseline that can be tuned by activators and repressors. Indeed, it has been suggested that TFIID is required at nearly all promoters in vivo, as loss of Taf11 leads to a general transcriptional shutdown even in the presence of excess TBP (Komarnitsky et al, 1999). In addition, it has been proposed that SAGA, too, is generally involved in Pol II transcription, not just the small fraction that has been classified SAGA-dependent (Bonnet et al, 2014). With SAGA important for and TFIID present at many Pol II genes, we feel that classifying genes based on their coactivator dependence might not be accurate. In contrast, we do see that recruitment of Mediator through its tail module is important for transcription activation of several TATA-containing genes in vivo.

There may also be a non-transcriptional role for widespread Mediator binding, related to genome architecture. In mammals,

where regulatory elements may be located great distances from their target promoters, Mediator has been implicated in the formation and stabilization of chromatin loops between such elements (Kagey *et al*, 2010). Yeast generally utilizes short-range gene loops in regulated transcription, and so such long-distance interactions are not a feature of the compact yeast regulatory landscape. However, the Med18 subunit of yeast Mediator has been implicated in the formation of loops between gene 5′ and 3′ ends (Mukundan & Ansari, 2013), and Mediator is proposed to regulate transcription activation distance in yeast (Reavey *et al*, 2015). Recent work in yeast has also shown that loss of Mediator results in global decompaction of chromatin (Hsieh *et al*, 2015). In agreement with this observation, we noticed a shift of Taf1 cleavages in the linker DNA when anchoring Med14, perhaps indicating a shift in nucleosome position upon nuclear depletion of Mediator. However, mapping of nucleosome positions following Med14 depletion is necessary to conclusively test this idea. We thus postulate that global Mediator binding, beyond its connection to transcription, has implications for genome architecture. Our study provides a high-confidence map of Mediator to the budding yeast genome that will help further our understanding of its multiple *in vivo* functions.

Materials and Methods

Yeast methods

Endogenous loci were tagged with 3×FLAG-MNase by lithium acetate transformation of gene-specific PCR products amplified from pGZ108 (kanMX6 marker) or pGZ109 (HIS3MX6 marker) (Zentner *et al*, 2015). The P_{MED8}-MNase fusion was constructed by Gibson assembly of 500 bp upstream of the *MED8* start codon and a PCR amplicon encoding 3×FLAG-MNase-SV40 NLS into the EcoRI/SpeI sites of pRS413. Yeast strains were grown in yeast–peptone–dextrose (YPD) medium (Figs 1, 2 and 4) or glucose complete (GC) medium lacking isoleucine and valine (Figs 3 and 5–7). For SM treatment, cells were grown in GC medium lacking isoleucine and valine and treated with 0.5 μg/ml SM for 90 min. For Kin28-AS inhibition, cells were treated with 6 μM NA-PP1 for 20 min (Liu *et al*, 2004). Med14 and Taf1 were tagged with FRB or FRB-GFP by transformation with PCR products amplified from pFA6a-FRB-kanMX6 or pFA6a-FRB-GFP-HIS3MX6 (Haruki *et al*, 2008). Nuclear depletion was induced by treatment with 1 μg/ml rapamycin for 1 h. For fluorescence microscopy, cells were fixed with 4% formaldehyde in PBS for 15 min at room temperature, washed with 500 μl 100 mM potassium phosphate/1.2 sorbitol, and resuspended in 50 μl of the same buffer. About 20 μl of fixed cells was placed on a concanavalin A-coated slide and let sit at room temperature for 5 min. Slides were then washed with 3 ml of 0.4% Photo-Flo and dried at room temperature for 5 min. Cells were imaged using a DeltaVision Elite (GE Healthcare). Plasmids and yeast strains used in this study are listed in Appendix Tables S1 and S2, respectively.

Quantification of gene expression

RNA extraction, cDNA synthesis, and qRT–PCR were performed as described (Knutson & Hahn, 2011). Two biological replicates were performed in triplicate for each condition.

ChIP-seq data analysis

Med14 ChIP-seq data (Wong *et al*, 2014) were converted from SRA to FASTQ using SRA Toolkit *fastq-dump*. Data were aligned to the sacCer3 genome build using Bowtie2. The resulting SAM file was converted to a tag directory and visualized as a reads per million (RPM)-normalized bedgraph with HOMER (http://homer.salk.edu) (Heinz *et al*, 2010).

ChEC-seq

ChEC, sequencing library preparation, alignment, and track visualization were performed as described (Zentner *et al*, 2015). Cleavage pattern analysis was performed as described (Zentner *et al*, 2015) with any modifications detailed below. All scripts used for data processing and analyses are available at https://github.com/zentnerlab/chec-seq.

Cleavage pattern analysis

TSS annotations from a previous study (Xu *et al*, 2009) were intersected with the lists of SAGA- and TFIID-dependent genes encoding verified ORFs, yielding 435 SAGA-dependent TSSs and 4,052 TFIID-dependent TSSs. Pairs files for all time points for a given factor or treatment were concatenated. Plots were smoothed using GraphPad Prism 6 using second-order smoothing and 100 neighbors. The distance from the Mediator binding site to the TSS was taken to be the distance from the base position of the smoothed cleavage maxima to the TSS. For SM experiments, smoothed non-SM-treated signal was subtracted from smoothed SM-treated signal at each base.

Comparison of ChEC-seq to NET-seq and microarray data

NET-seq data were aligned and normalized as described (Henikoff *et al*, 2011). The average NET-seq counts in a 200-bp window downstream of the TSS were then determined. Only NET-seq counts originating from the same strand as the analyzed TSS were considered for this analysis. All six genes used for visualization of ChEC-seq and ChIP-seq data in Fig 1 were in the top 100 most highly transcribed genes as determined by the above metric, and each had an average of ≥ 5.12 NET-seq counts in the 200 bp downstream of the TSS. For analysis of Mediator binding to genes with altered expression in SM, we only considered genes with a fold change ≥ twofold and an annotated TSS. This yielded 674 upregulated and 803 downregulated genes.

Cumulative frequency analysis

Pairs_single_end_sizes.pl was used to generate bedgraphs normalized to the number of fragment ends mapped as described (Zentner *et al*, 2015). BEDOPS *bedmap* (Neph *et al*, 2012) was used to determine the average normalized counts in a specified window upstream of the TSS of each SAGA- and TFIID-dependent gene.

Data availability

Primary data
Grünberg S, Henikoff S, Hahn S, Zentner GE (2016). Mediator binding to UASs is broadly uncoupled from transcription and

cooperative with TFIID recruitment to promoters. Gene Expression Omnibus GSE81289.

Referenced data

Churchman LS, Weissman JS (2011). Native elongating transcript sequencing (NET-seq) of wild type *Saccharomyces cerevisiae* and of DST1, RCO1, SET1, SET2, EAF3 deletion strains. Gene Expression Omnibus GSE25107 (NET-seq).

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MacIsaac KD, Wang T, Gordon DB, Gifford DK, Stormo GD, Fraenkel E (2006). An improved map of conserved regulatory sites for *Saccharomyces cerevisiae*. http://fraenkel.mit.edu/improved_map/ (Gcn4 ChIP-chip sites).

Natarajan K, Saint M (2014). Evolutionarily conserved C-terminal region of TAF9 is critical for SAGA and TFIID recruitment to promoters and transcriptional activation. Gene Expression Omnibus GSE44544 (Microarray expression data for control and SM treatment).

Wong KH, Jin Y, Struhl K (2014). Functional analysis of TFIIH (Kin28) in *Saccharomyces cerevisiae*. Sequence Read Archive SRX472130 (Med14 ChIP-seq).

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Author contributions

SG, SHenikoff, SHahn, and GEZ designed the study. SG and GEZ performed experiments. SG and GEZ analyzed data. SG and GEZ wrote the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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