Mediator dynamics during heat shock in budding yeast

Debasish Sarkar,1 Z. Iris Zhu,2 Elizabeth R. Knoll,1,3 Emily Paul,3,4 David Landsman,2 and Randall H. Morse1,3

1Wadsworth Center, New York State Department of Health, Albany, New York 12208, USA; 2Computational Biology Branch, National Center for Biotechnology Information, National Library of Medicine, NIH, Bethesda, Maryland 20814, USA; 3Department of Biomedical Sciences, University at Albany School of Public Health, Albany, New York 12208, USA

The Mediator complex is central to transcription by RNA polymerase II (Pol II) in eukaryotes. In budding yeast (Saccharomyces cerevisiae), Mediator is recruited by activators and associates with core promoter regions, where it facilitates preinitiation complex (PIC) assembly, only transiently before Pol II escape. Interruption of the transcription cycle by inactivation or depletion of Kin28 inhibits Pol II escape and stabilizes this association. However, Mediator occupancy and dynamics have not been examined on a genome-wide scale in yeast grown in nonstandard conditions. Here we investigate Mediator occupancy following heat shock or CdCl2 exposure, with and without depletion of Kin28. We find that Pol II occupancy shows similar dependence on Mediator under normal and heat shock conditions. However, although Mediator association increases at many genes upon Kin28 depletion under standard growth conditions, little or no increase is observed at most genes upon heat shock, indicating a more stable association of Mediator after heat shock. Unexpectedly, Mediator remains associated upstream of the core promoter at genes repressed by heat shock or CdCl2 exposure whether or not Kin28 is depleted, suggesting that Mediator is recruited by activators but is unable to engage PIC components at these repressed targets. This persistent association is strongest at promoters that bind the HMGB family member Hmo1, and is reduced but not eliminated in hmo1Δ yeast. Finally, we show a reduced dependence on PIC components for Mediator occupancy at promoters after heat shock, further supporting altered dynamics or stronger engagement with activators under these conditions.

[Supplemental material is available for this article.]

Transcription of protein-coding genes in eukaryotes is a complex process involving recruitment of coactivators, assembly of the preinitiation complex (PIC), including RNA polymerase II (Pol II), and transition from promoter melting and initiation to productive elongation (Schier and Taatjes 2020). The Mediator complex, a large, multisubunit complex present in plants, animals, and single-celled eukaryotes, is central to this process (Jeronimo and Robert 2017; Soutourina 2018). In yeast, Mediator has been divided into head, middle, tail, and cyclin-CDK modules on the basis of structural and genetic data (Plaschka et al. 2016). Functionally, yeast Mediator is first recruited to upstream activation sites (UASs) that are 200–400 bp upstream of the transcription start sites (TSSs) of most genes by sequence-specific DNA-binding activator proteins via the Mediator tail module (Jeronimo et al. 2016; Jeronimo and Robert 2017; Knoll et al. 2018; Soutourina 2018). Subunits in the middle and head module then engage PIC components, including Pol II, to facilitate PIC assembly (Plaschka et al. 2016; Robinson et al. 2016; Jeronimo and Robert 2017; Soutourina 2018). This process entails bridging between the UAS and the core promoter, where the PIC is assembled, by a single Mediator complex (Jeronimo et al. 2016; Petrenko et al. 2016). Association of Mediator with the core promoter is normally brief (estimated as being <1 sec for a single transcription initiation event) but is stabilized by inhibiting Pol II escape, for example, by depletion or inactivation of Kin28, which facilitates Pol II escape by phosphorylating the C-terminal domain of Rpb1 (Jeronimo and Robert 2014; Wong et al. 2014). Transit of Mediator from UASs to the core promoter depends on Spt15 (also known as TBP, as it will be referred to hereafter), and its association with the core promoter in the absence of Kin28 is destabilized by depletion of Taf1 or Pol II (Knoll et al. 2018).

Mediator occupancy measured by ChIP during the normal transcription cycle (i.e., with Kin28 active) is much higher at induced genes that are controlled by strong activators, such as those activated by heat shock or growth in galactose (Fan et al. 2006; Fan and Struhl 2009; Kim and Gross 2013). This could reflect stronger interactions between Mediator and activators, and hence less dependence on PIC components or other factors for stabilizing Mediator occupancy, at strongly induced genes compared with constitutively active genes during growth in rich medium (YPD). Increased Mediator occupancy has been observed at a few individual promoters induced by heat shock following Kin28 depletion (Petrenko et al. 2016); however, no genome-wide analysis has been reported examining whether promoter occupancy by Mediator is stabilized by Kin28 depletion at strongly induced genes or depends on PIC components as it does in yeast grown in rich medium (YPD). In this work, we examine genome-wide Mediator occupancy in Saccharomyces cerevisiae following two
perturbations, heat shock, and CdCl₂ administration, that induce large and rapid genome-wide transcriptional responses.

**Results**

**Pol II recruitment upon heat shock depends on Mediator**

To identify genes most active and most highly induced after heat shock, we performed ChIP-seq to determine Pol II occupancy in the commonly used laboratory strain BY4741 and in YFR1321, the parent strain (originally derived from W303) (Haruki et al. 2008) to that used for anchor-away experiments described in later sections, before and after 15 and 30 min of heat shock. Heat shock resulted in increased Pol II occupancy at targets of Hsf1, the primary transcription factor responding to heat shock, and the stress-responsive activators Msn2 and Msn4, whereas Pol II occupancy was nearly completely lost from RP genes, consistent with previous determinations of mRNA levels and nascent RNA production following heat shock (Supplemental Fig. S1A; Warner 1999; Gasch et al. 2000; Causton et al. 2001; Pincus et al. 2018). K-means clustering revealed two groups of genes strongly up-regulated by heat shock; these groups were both enriched for association with Hsf1 and Msn2 and Msn4 (Supplemental Fig. S1B). Transcription induced by Hsf1 has been reported to occur independently of Mediator (Lee and Lis 1998; McNeil et al. 1998), but we found a similar two- to threefold reduction in Pol II occupancy at Hsf1 and Msn2/4 targets after heat shock upon depletion of the essential Mediator head module subunit Med17, using the anchor-away method (see next section), as at other genes in the absence of heat shock, in agreement with recent results from the Struhl laboratory (Supplemental Fig. S1C; Petenko et al. 2017).

**Effect of Kin28 depletion on Mediator recruitment with and without heat shock**

To examine Mediator dynamics at genes induced upon heat shock, we performed ChIP-seq against Gal11 (also known as Med15) (Bourbon et al. 2004), from the Mediator tail module, in yeast engineered to allow depletion of Kin28 from the cell nucleus using the anchor-away method, and in the parent strain, YFR1321 (Jeronimo and Robert 2014; Wong et al. 2014; Knoll et al. 2018). The kin28ΔA yeast strain expresses Kin28 with a C-terminal FRB tag and the ribosomal subunit Rpl13A C-terminally tagged with the FKBP12 fragment; upon administration of rapamycin, the FRB and FKBP12 moieties are tightly coupled, and Kin28 is evicted from the nucleus following nuclear processing of Rpl13A. The parent strain is identical except for lacking the FRB tag, and both strains were both enriched for association with Hsf1 and Msn2 and Msn4 (Supplemental Fig. S2A). In the absence of Kin28, depletion of Kin28 resulted in an increase in Med15 ChIP signal at Hsf1 and Msn2/4 targets with or without Kin28 depletion (Fig. 1A). Quantitation of Med15 ChIP signal in non-heat-shocked cells at RP genes and at the approximately 300 non-RP gene promoters having highest Med15 occupancy after Kin28 depletion (also in non-heat-shocked cells) revealed an increase in signal in kin28ΔA compared with wild-type yeast, as expected (Fig. 1B; Supplemental Fig. S2B). In contrast, Med15 occupancy showed little or no increase upon Kin28 depletion in heat-shocked cells, either at Hsf1 targets or the 300 genes most highly occupied by Med15 in kin28ΔA yeast after heat shock (Fig. 1A,B; Supplemental Fig. S2A,B). Analysis of the 300 non-RP gene promoters with highest Pol II occupancy (in non-heat-shocked and heat-shocked YFR1321 yeast) yielded similar results (Supplemental Fig. S2C), as did comparison of genes having similar Pol II occupancy in the absence and presence of heat shock (Supplemental Fig. S2D).

Consistent with the trends evident from Figure 1, A and B, stronger induction of Med15 occupancy upon Kin28 depletion under non–heat shock than under heat shock conditions was observed upon inspection of browser scans at many genes (Fig. 1C; Supplemental Fig. S3, YDJ1). At the same time, some promoters behave counter to this trend, reflecting the range of altered Med15 occupancy observed upon Kin28 depletion (Fig. 1B; Supplemental Fig. S3, TPS1). Variable effects on the increase in Med15 occupancy upon Kin28 depletion were observed for Hsf1 and Msn2/4 targets as well as for genes induced by heat shock that are not known to be targets of either of these stress-responsive activators (Supplemental Fig. S3; Supplemental Table S1). The gene-specific increase in Mediator ChIP signal upon Kin28 depletion or inactivation is similarly evident in previous reports, but the cause of this remains unexplained (Jeronimo and Robert 2014; Knoll et al. 2018).

Under normal growth conditions in rich medium, many active gene promoters, most conspicuously those of many RP genes, show little or no association with Mediator as measured by ChIP (Jeronimo and Robert 2014; Wong et al. 2014; Paul et al. 2015). Some active genes, however, do show Mediator ChIP signal at UAS regions, but not at core promoter regions, under normal growth conditions; depletion or inactivation of Kin28 results in a shift in Mediator occupancy at these genes from UAS to core promoter, with the shift being greatest for head and middle module subunits that contact the PIC and least for tail module subunits (Jeronimo et al. 2016; Knoll et al. 2018). Correspondingly, we observed a shift toward the core promoter with little change in intensity of the Med15 ChIP signal upon Kin28 depletion in the absence of heat shock at a set of 498 genes, which we refer to as “UAS genes,” that show Mediator ChIP signal at UAS regions under normal growth conditions (Fig. 1D; Supplemental Table S2; Jeronimo et al. 2016). In contrast, RP genes show increased Mediator occupancy upon Kin28 depletion, along with a shift in the peak toward the TSS (Fig. 1D).

Taken together, these results indicate that Hsf1 targets that are active under non-heat-shocked conditions behave similarly to the large cohort of genes, exemplified by RP genes, that show little or no Mediator signal unless Kin28 is depleted, whereas under heat shock conditions, Hsf1 and Msn2/4 targets behave more like the “UAS genes” at which Mediator ChIP signal is seen under

Sarkar et al.

www.genome.org
normal growth conditions and shows modest increase upon deple-
tion of Kin28.

Mediator remains associated at genes repressed by heat shock

We were surprised to note a prominent Med15 peak upstream of
RP genes following heat shock (Fig. 1A), despite the near absence
of Pol II occupancy (Supplemental Fig. S1A). The position of this
peak was not affected by Kin28 depletion in heat-shocked cells
and was shifted upstream relative to the Med15 peak observed fol-
lowing Kin28 depletion in the absence of heat shock (Fig. 1A, RP
genes, and Fig. 2A). Thus, upon heat shock, Mediator association
with RP genes, under conditions of depleted Kin28, shifts from a
core promoter location close to the TSS to an upstream position
that closely coincides with that of the RP gene activator Rap1
(see below; Fig. 2A). ChIP-seq against the Mediator head module
subunit Srb5 (also known as Med18) (Bourbon et al. 2004) in
kin28AA yeast treated with rapamycin also showed persistent asso-
ciation with the repressed RP genes following heat shock, albeit
at reduced intensity relative to that seen with Med15, and the parent strain YFR1321, also treated with rapamycin, in the absence of heat shock, at “UAS genes” (see text and Supplemental Table S2) and RP genes.

Figure 1. Effect of heat shock on Mediator association. (A) Heat maps and line graphs depicting normalized occupancy of the Mediator tail module sub-
unit, Med15, in kin28AA yeast treated with rapamycin and the parent strain YFR1321, also treated with rapamycin, before and after 15 min of heat shock, at
42 Hsf1 targets and 213 Msn2-4 targets and 137 RP genes (see Methods; Supplemental Table S2). (B) Box and whisker plots showing the ratios of Med15
occupancy with and without Kin28 depletion for the approximately 300 genes showing the highest Med15 occupancy in Kin28-depleted cells without or
with heat shock; ratios are also shown for RP genes, Hsf1 targets, and Msn2-4 targets in heat-shocked cells. (C) Browser scans showing Med15 occupancy
upstream of BAP2 and UBI4 in kin28AA yeast and the parent strain YFR1321, both treated with rapamycin, with and without heat shock. UBI4 is a target of
Hsf1, whereas BAP2 is not a target of Hsf1 or Msn2-4. Scale, in reads per million mapped reads, is indicated for each scan. (D) Heat maps and line graphs
depicting occupancy of the Mediator tail module subunit, Med15, in BY4741 yeast, kin28AA yeast treated with rapamycin, and the parent strain YFR1321, also treated with rapamycin, in the absence of heat shock, at “UAS genes” (see text and Supplemental Table S2) and RP genes.

Mediator peaks upstream of repressed RP genes in heat-
shocked yeast were nearly coincident with the peak observed for
Hsf1, which binds upstream of the large majority of RP genes and
is essential for recruitment of the PIC to most RP genes (Fig.
2C; Lieb et al. 2001; Mencí’a et al. 2002; Ansari et al. 2009; Zeevi
et al. 2011; Knight et al. 2014; Reja et al. 2015). This observation
suggests that Mediator is recruited to these genes in heat-shocked
cells but does not transit to core promoters as it normally does in
non-heat-shocked cells. This notion is also consistent with the
lower signal observed at RP genes repressed by heat shock for
Med18 (from the head module) than for Med15 (from the tail
module), as Med15 is expected to have more direct contact with ac-
tivators bound to UASs and thus be more efficiently cross-linked
than Med18 (Jeronimo et al. 2016; Petrenko et al. 2016).
Many non-RP genes are also repressed by heat shock (Supplemental Fig. S1; Gasch et al. 2000; Causton et al. 2001). To assess whether Mediator occupies promoters of such genes after heat shock, we examined ChIP-seq signal at the UAS genes in BY4741. The signal observed at transcribed ORF regions (seen at Hsf1 targets under heat shock conditions, and at RP genes under non-heat-shocked conditions) is a ChIP artifact frequently observed at highly transcribed ORFs (Eyboulet et al. 2013; Park et al. 2013; Teytelman et al. 2013; Sarkar et al. 2018).

Upon heat shock, the two cohorts behaved differently with regard to Mediator association. At “UAS not down” genes, both Med15 and Med18 peaks increased in amplitude while not changing much in position, consistent with the overall increased expression of this cohort. In contrast, “UAS down” genes behaved similarly to the repressed RP genes, with both Med15 and Med18 showing peaks that were shifted upstream from their positions near the TSS, at the core promoter, with little change in amplitude (Fig. 3B, left panel, C).

Together, these results reveal persistent Mediator association with genes repressed by heat shock. This association was localized to UASs even under conditions of Kin28 depletion, suggesting that Mediator is recruited by activators at these repressed loci but is unable to facilitate PIC assembly and transcription. We next sought to gain insight into the mechanism underlying the persistent association of Mediator with genes repressed by heat shock.

Hmo1 contributes to persistent Mediator association at genes repressed by heat shock but is not the sole determinant

To test whether the nonproductive retention of Mediator at genes repressed by heat shock could be associated with specific activators or promoter configurations, we first examined the RP genes in more detail. Based on ChIP-seq experiments, RP genes have been divided into three categories. The large majority binds Rap1, which recruits Fhl1 and Ibf1, with Ibf1 being critical for transcriptional activation (Martin et al. 2004; Schawalder et al. 2004; Wade et al. 2004; Rudra et al. 2005). These 129 RP genes fall into two distinct classes, one of which binds the high mobility group family protein, Hmo1, downstream from Rap1 and is characterized by smaller Rap1-TSS and Rap1-Fhl1 separation than the other class, which lacks Hmo1 binding (Hall et al. 2006; Kasahara et al. 2006).
The remaining RP genes do not bind Rap1, Ifh1, or Fhl1 and are instead under control of the general regulatory factor Abf1.

All three classes of RP genes are repressed by heat shock (Supplemental Fig. S5A; Reja et al. 2015). However, these cohorts behave differently with respect to Mediator association: Mediator association persists at Hmo1-binding RP genes after heat shock in both kin28AA and parent yeast strains, whereas association is much lower in non-Hmo1-binding RP genes (Fig. 4A,B; Supplemental Fig. S5B). Abf1-bound RP genes show weak Med18 association only in non-HS kin28AA yeast and show no discernible Med15 peak. Med15 peaks upstream of Hsf1 targets, and non-Hmo1-binding RP genes showed a similar intensity in wild-type and hmo1Δ yeast, providing internal controls for the reduced intensity observed at Hmo1-binding RP genes repressed by heat shock in hmo1Δ yeast.

To determine whether Hmo1 itself or some other feature common to Hmo1-binding RP gene promoters determines persistent Mediator association at RP genes repressed by heat shock.

Mediator dynamics during heat shock in yeast

2007; Knight et al. 2014; Reja et al. 2015; Zencir et al. 2020). The remaining RP genes do not bind Rap1, Ifh1, or Fhl1 and are instead under control of the general regulatory factor Abf1.

All three classes of RP genes are repressed by heat shock (Supplemental Fig. S5A; Reja et al. 2015). However, these cohorts behave differently with respect to Mediator association: Mediator association persists at Hmo1-binding RP genes after heat shock in both kin28AA and parent yeast strains, whereas association is much lower in non-Hmo1-binding RP genes (Fig. 4A,B; Supplemental Fig. S5B). Abf1-bound RP genes show weak Med18 association only in non-HS kin28AA yeast and show no discernible Med15 peak. Med15 peaks upstream of Hsf1 targets, and non-Hmo1-binding RP genes showed a similar intensity in wild-type and hmo1Δ yeast, providing internal controls for the reduced intensity observed at Hmo1-binding RP genes repressed by heat shock in hmo1Δ yeast.

We also noted a decrease in intensity of the Med15 peak upstream of UAS genes in heat-shocked hmo1Δ yeast (Fig. 4C). Some RP genes are included among the set of UAS genes (Supplemental Table S2), but many non-RP genes also show Hmo1 peaks at upstream promoter regions, albeit of lower intensity (Supplemental Fig. S6A; Hall et al. 2006; Kasahara et al. 2007; Knight et al. 2014; Reja et al. 2015). To examine whether persistent Mediator binding in non-RP genes repressed by heat shock was influenced by Hmo1, we identified, among UAS genes repressed by heat shock, five genes having high-intensity Hmo1 ChIP-seq peaks and 105 genes having low-intensity Hmo1 peaks (Supplemental Fig. S6A; Supplemental Table S2). Med15 signal was observed at both sets of genes after heat shock both in kin28AA yeast treated with rapamycin and in the parent strain and, similarly to the RP genes, showed a greater increase upon heat shock at the small set growing or inviable (Dolinski and Heitman 1999; Kasahara et al. 2020). We therefore compared Mediator association following heat shock in hmo1Δ and wild-type BY4741 yeast. We found that Med15 association was still evident upstream of repressed, Hmo1-binding RP genes in hmo1Δ yeast but was reduced by approximately twofold relative to wild-type cells (Fig. 4C). Med15 peaks upstream of Hsf1 targets, and non-Hmo1-binding RP genes showed a similar intensity in wild-type and hmo1Δ yeast, providing internal controls for the reduced intensity observed at Hmo1-binding RP genes repressed by heat shock in hmo1Δ yeast.
of high Hmo1-binding than at those genes binding Hmo1 at lower levels (Supplemental Fig. S6B). In addition, Med15 association decreased about twofold at high Hmo1-binding UAS genes in hmo1Δ yeast while remaining nearly unchanged at low Hmo1-binding UAS genes repressed by heat shock (Supplemental Fig. S6C).

From these results, we conclude that Hmo1 evidently contributes to persistent Mediator association with both RP genes and UAS genes down-regulated by heat shock, although conclusions regarding the latter are limited by the small size of the high Hmo1-binding UAS genes repressed by heat shock (Supplemental Fig. S6C).

Figure 4. Persistent Mediator association preferentially occurs at Hmo1-binding RP genes repressed by heat shock. (A) Heat maps and line graphs showing normalized occupancy of Med15 and Med18 in kin28AA yeast treated with rapamycin, with and without heat shock, at RP genes divided into Hmo1-binding, non-Hmo1-binding, and Abf1-binding genes (Supplemental Table S2). (B) Browser scans showing occupancy of Med15 and Med18 in kin28AA yeast treated with rapamycin, with and without heat shock, and Rap1 and Hmo1 in non-heat-shocked yeast, at RPS24A and RPL21A (Hmo1-binding) and RPL40B and RPS9B (non-Hmo1-binding). Scale, in reads per million mapped reads, is indicated for each scan. (C) Heat maps and line graphs showing normalized occupancy of Med15 and Med18 at UAS genes, Hsf1 targets, and Rap1-binding RP genes that do or do not bind Hmo1, in wild-type (BY4741) and hmo1Δ yeast after 15 min of heat shock.

Sarkar et al.

116 Genome Research

www.genome.org
enrichment for binding, although 47 different TFs bind to these 39 gene promoters (Supplemental Table S3). We conclude that no single TF, including Hmo1, is required for persistent Mediator association at genes repressed by heat shock.

Role of PIC components in Mediator association following heat shock

We next addressed the role of PIC components TBP, TFIIID, and Pol II in stabilizing Mediator occupancy at gene promoters in heat-shocked cells. Previously, we reported on the effect of depletion of Taf1, TBP, or the Pol II subunit Rpb3 on association of Mediator with gene promoters in yeast growing in rich medium (Knoll et al. 2018). In KIN28+ yeast, depletion of PIC components had little effect with gene promoters in yeast growing in rich medium (Knoll et al. 2013; Teytelman et al. 2013; Jeronimo and Robert 2014; Paul et al. 2015; Grünberg et al. 2016). Finally, Mediator signal at RP genes after heat shock appeared mostly insensitive to depletion of Taf1, TBP, or Rpb3, with the exception being an increased Med18 signal upon depletion of Kin28 together with TBP (Fig. 5B). This insensitivity was not surprising, as inasmuch as all three of these PIC components are depleted from RP genes under heat shock conditions (Supplemental Fig. S1; Reja et al. 2015; Vinayachandran et al. 2018).

Effect of exposure to cadmium on Pol II and Mediator occupancy

The unexpected observation of persistent Mediator association with genes repressed by heat shock prompted us to examine the effect of another environmental stress on association of Mediator with gene promoters. To this end, we chose to examine the effect of exposure to CdCl2. Exposure of yeast to this toxic metal at submillimolar concentrations induces a rapid transcriptional response in which about 150–500 genes are induced and from 18 to around 400 genes repressed (Momose and Iwahashi 2001; Cormier et al. 2010; Hosiner et al. 2014; Huang et al. 2016). Induced genes are enriched for binding sites for Hsf1, Msn2, and Msn4, and genes involved in ribosomal biogenesis are repressed by CdCl2 exposure, in common with the response to heat shock and other environmental stresses (Gasch et al. 2000; Causton et al. 2001; Hosiner et al. 2014). However, CdCl2 exposure also induces genes involved in sulfur compound metabolism, such as the MET genes, among others, that are not involved in the heat shock response (Momose and Iwahashi 2001; Cormier et al. 2010; Hosiner et al. 2014; Huang et al. 2016), thus providing an environmental perturbation that results in a distinct but overlapping response to that elicited by heat shock.

We first tested the effect of CdCl2 exposure on Pol II association by ChIP-seq (Fig. 6A). Marked induction of Pol II association was observed with the 50 genes showing the largest increase in mRNA abundance upon CdCl2 exposure (Momose and Iwahashi 2001), whereas association of Pol II with RP genes decreased to near baseline levels, in agreement with previous studies showing a decrease in RP gene transcript levels after CdCl2 exposure (Momose and Iwahashi 2001; Hosiner et al. 2014; Huang et al. 2016). Promoters showing at least a threefold increase in normalized Pol II occupancy upon CdCl2 administration, and from the top 1000 Pol II-occupied genes after CdCl2 exposure, overlapped strongly with those identified in microarray studies (Supplemental Table S4; Momose and Iwahashi 2001). Gene Ontology analysis revealed enrichment for categories related to sulfur compound metabolism and amino acid biosynthesis, as well as with response to stress, as expected (Supplemental Table S4). Transcription factors enriched for binding to induced genes included Hsf1, Met4, Met3, Snf2, Msn2, Msn4, and Yap1, all identified previously in a microarray study (Hosiner et al. 2014), as well as Ahn2, Cbf1, Met31, and Skn7 (Supplemental Table S4).

We next examined the effect of CdCl2 exposure on Mediator association. ChIP-seq of Med15 and Med18 under conditions of Kin28 depletion revealed increased association at genes identified as showing increased expression upon CdCl2 administration and at those showing increased Pol II association (Fig. 6B). Similar to our observations of the effect of heat shock, RP genes, despite
showing greatly reduced Pol II association upon CdCl₂ exposure, showed persistent association of both Med15 and Med18 (Fig. 6B). Also, in accord with the effect of heat shock, Med18 showed relatively lower signal at RP genes after CdCl₂ exposure than did Med15. Unlike Mediator association after heat shock, little if any upstream shift in the peaks for Med15 and Med18 at RP genes was observed after CdCl₂ exposure (Fig. 6B,C; compare Figs. 1, 2). Also, in contrast to the effect of heat shock on Mediator association with repressed RP genes, persistent Mediator association was observed at both Hmo1-binding and non-Hmo1-binding RP genes.

Figure 5. Effect of depleting PIC components on Mediator association with gene promoters. (A) Heat maps and line graphs showing normalized occupancy of Med15 (tail) and Med18 (head) at TATA-containing, Taf1-depleted promoters from the 1000 genes with highest Pol II occupancy (228 genes); TATA-less, Taf1-enriched genes excluding RP genes from the 1000 genes with highest Pol II occupancy (330 genes); and RP genes after depletion of Kin28 alone or together with Taf1, TBP, or Rpb3, as indicated. (B) Heat maps and line graphs showing normalized occupancy of Med15 (tail) and Med18 (head) at the approximately 300 genes with highest Pol II occupancy after heat shock, Hsf1 targets, and RP genes after depletion of Kin28 alone or together with Taf1, TBP, or Rpb3, as indicated, without or with 15 min of heat shock, as indicated.
although it appeared slightly lower at the latter (Supplemental Figs. S11, S12). Finally, as with UAS genes following heat shock, Med15 and Med18 association persisted at UAS genes showing decreased Pol II occupancy, while increasing (Med15) or staying constant (Med18) at genes having unchanged or increased Pol II occupancy (Supplemental Fig. S13). However, unlike the case for heat shock and mirroring the results for RP genes, little or no shift of Mediator to more upstream sites was observed after CdCl$_2$ exposure. We conclude that the stresses of heat shock and CdCl$_2$ exposure both allow continued Mediator association with repressed genes while suppressing Pol II association, but they differ in the extent to which they allow Mediator to remain associated with core promoters rather than UAS regions under conditions of Kin28 depletion.

**Discussion**

Mediator association with strongly induced genes, as assayed by ChIP, appears stronger than at many constitutively active genes, even those expressed at high levels (Fan et al. 2006; Fan and Struhl 2009; Kim and Gross 2013). However, whether this reflects altered dynamics of Mediator has not been closely examined. In this work, we used ChIP-seq in combination with rapid depletion of Kin28 and PIC components to investigate Mediator association with activated and repressed genes following heat shock.

Consistent with previous work, we find increased association of Pol II and Mediator with genes induced by heat shock, with Mediator ChIP signal being evident even without Kin28 depletion (Fan et al. 2006; Kim and Gross 2013; Petrenko et al. 2016, 2017). Pol II occupancy was reduced two- to threefold upon depletion of the essential Med17 (head module) subunit of Mediator, under both heat shock and non–heat shock conditions, and the reduction in occupancy did not differ for targets of Hsf1 or Msn2/4 compared with other genes expressed under heat shock conditions (Supplemental Fig. S1C). Reduction in Pol II occupancy or nascent mRNA transcription of two- to eightfold has been reported upon acute depletion or inactivation of essential Mediator subunits in yeast under standard growth conditions.
Thus, genes expressed during heat shock show dependence on Mediator similar to genes expressed during normal growth. Dependence of gene expression on Mediator may show greater variability in mammalian cells: Although degron-induced depletion of the scaffold subunit Med14 in murine B cells resulted in a global, approximately seven-fold reduction in mRNA after 60 h, more acute depletion of core Mediator subunits in “near-haploid” human KMB7 cells strongly affected only a subset of genes, with many genes showing little effect on nascent transcription (El Khattabi et al. 2019; Jaeger et al. 2020).

Depletion of Kin28, which inhibits promoter escape by Pol II (Wong et al. 2014), increases Mediator ChIP signal at proximal promoters in the absence of heat shock (Fig. 1A). Under heat shock conditions, Med15 association is not increased at Hsf1 targets by Kin28 depletion, rather showing only a slight shift toward the promoter (Fig. 1A,B). These observations suggest that Mediator dynamics may be altered during heat shock, such that its residence time and consequent “ChIP-ability” increase relative to non-heat shock conditions. In a possibly related observation, a genetic screen uncovered mutations in several Mediator subunits that reduced the dynamic range in the transcriptional response to heat shock (Singh et al. 2006); whether these mutations might affect dynamics of Mediator is unknown.

An unexpected finding was the persistent association of Mediator with promoters of repressed genes having low or negligible Pol II association following heat shock or exposure to CdCl$_2$ (Figs. 1–3, 6). We first noticed this persistent association at repressed RP genes but additionally found it to occur at many repressed non-RP genes. Mediator association has also been observed by ChEC-seq upstream of genes down-regulated by sulfometuron methyl treatment, which mimics amino acid starvation (Grünberg et al. 2016). At genes repressed by heat shock, ChIP-seq peaks for Mediator were observed upstream of core promoter regions and, at RP genes, were approximately coincident with binding sites for Rap1, which is required for Mediator recruitment to most RP genes (Ansari et al. 2009). This was true whether or not Kin28 was depleted; in contrast, Mediator ChIP-seq peaks at active RP and non-RP genes are observed at the core promoter nearer the TSS when Kin28 is depleted. These results, together with the stronger signal for Med15 (from the tail module) than for Med18 (from the head module) upstream of repressed genes suggest that Mediator is still recruited by UAS-bound activators under repressed conditions but is unable to transit to the core promoter (Supplemental Fig. S14). This scenario is reminiscent of the effect of depleting TBP together with Kin28, which results in Mediator being stranded at UAS regions (Knoll et al. 2018), and is consistent with the absence of a PIC at RP genes repressed by heat shock (Vinayachandran et al. 2018). The lack of a PIC at repressed RP genes also fits well with depletion of TBP, Taf1, or RpB3 having little effect on association of Med15 and Med18 with RP genes following heat shock (Fig. 5).

Given the localization of Mediator ChIP-seq peaks to UAS regions of repressed genes, it seems likely that bound activators are still able to recruit Mediator under repressive conditions. Consistent with this idea, Mediator can be recruited to activator binding sites in the absence of PIC formation (Bohte et al. 2001; Knoll et al. 2018). What then prevents PIC formation at Mediator-bound, repressed genes? Two possibilities seem most likely: (1) a factor or factors actively block assembly of the PIC, with consequent failure of Mediator to transit from the UAS to the core promoter (Knoll et al. 2018), or (2) activators or coactivators that are required for PIC assembly at heat shock–repressed genes are prevented from functioning by heat shock, whereas other factors remain that allow continued association of Mediator. In either case, the mechanism must allow transcription of those genes induced or not repressed by heat shock to be active while preventing PIC assembly at a cohort of genes that includes, but is not limited to, RP genes. Considering only the Rap1-binding RP genes, repression by heat shock results in eviction of the critical activator Ifh1, whereas Rap1 and Fhl1 remain bound (Martin et al. 2004; Schwalger et al. 2004; Wade et al. 2004; Rudra et al. 2005). A plausible mechanism, therefore, is that Rap1, possibly together with Fhl1, is able to recruit Mediator, whereas Ifh1 is needed for PIC assembly. Further studies will be needed to test the mechanism by which Mediator is recruited nonproductively to both RP and non-RP genes repressed by heat shock or CdCl$_2$, and to establish whether some activators may recruit Mediator but nonetheless be insufficient to facilitate PIC assembly and activate transcription.

We also discovered a strong correlation between persistent Mediator association after heat shock and of Hmo1 binding at RP genes. This association was reduced, but not eliminated, in hmo1 Δ yeast. Hmo1 at RP genes is reduced upon heat shock to ~30% of non-heat-shocked levels (Knight et al. 2014; Reja et al. 2015); whether the residual Hmo1 directly facilitates Mediator retention and how it might do so remain topics for future research. Furthermore, the observed association of Mediator with genes repressed by heat shock in hmo1 Δ yeast indicates that some other feature also contributes to its retention. RP genes that bind or do not bind Hmo1 also differ in the distance between Rap1 and Fhl1 binding sites, Rap1-TSS distance, G/C content, and chromatin structure, with Hmo1-binding RP genes having a larger nucleosome-depleted region (Knight et al. 2014; Reja et al. 2015; Zencir et al. 2020). Moreover, Fhl1 shows binding over an extended region in Hmo1-binding RP gene promoters, in contrast to the narrow binding region seen in RP gene promoters that do not bind Hmo1, and this difference persists even after heat shock (Reja et al. 2015). The relationship among these distinguishing features of Hmo1-binding and nonbinding RP genes is not clear, and one or a combination of these features may contribute to persistent Mediator association after heat shock.

In contrast to our observations following heat shock, Mediator association is seen at both Hmo1-binding and non-Hmo1-binding RP genes following CdCl$_2$ exposure. In addition, under conditions of Kin28 depletion, Mediator ChIP-seq peaks show less of an upstream shift at repressed genes following CdCl$_2$ exposure than after heat shock, suggesting that Mediator still associates with core promoters of repressed genes to some extent after CdCl$_2$ exposure despite the near absence of Pol II. We currently have no explanation for this difference but note that considerable differences exist in the response of yeast to various stresses in both transcriptome changes and signaling pathways used (Gasch and Werner-Washburne 2002; Jin et al. 2008; Zencir et al. 2020).

The dynamics of Mediator recruitment and participation in PIC assembly have only recently begun to be appreciated, principally in the model organism S. cerevisiae (Jeronimo and Robert 2017). It is clear that in budding yeast, these dynamics vary in a gene-dependent fashion, as Mediator occupancy varies at UAS regions and does not correlate with transcriptional output (Jeronimo and Robert 2014; Paul et al. 2015; Grünberg et al. 2016). The work reported here shows that Mediator dynamics, including its association with repressed genes, also can vary in a condition-dependent
fashion, Mediator itself is affected in its post-translational modifications and its composition by alterations in environment such as osmotic shock and the transition to stationary phase (Holstege et al. 1998; Miller et al. 2012); whether and how Mediator dynamics are affected by such alterations are currently obscure. Finally, in metazoan organisms, Mediator associates with enhancers that are sometimes many kilobases removed from the sites of PIC assembly at which Mediator participates via loop formation (Kagey et al. 2010); the dynamics of Mediator association and its participation in loop formation and PIC assembly in metazoans are just beginning to be explored (Sun et al. 2021).

**Methods**

**Yeast strains and growth**

Additional details can be found in the Supplemental Material as Supplemental Methods. *S. cerevisiae* strains used in this study are listed in Supplemental Table S5. For simplicity, epitope-tagged strains are referred to by the parent strain names in the text and figures; for example, “BY4741” refers also to TBY100, which harbors the *med15-myc* allele. Cultures were grown in yeast peptone dextrose (YPD) medium (1% yeast extract, 2% dextrose, 2% glucose). Anchor-away experiments were performed as reported previously (Knoll et al. 2018). In brief, yeasts were grown to 0.6–0.8 OD_{600} at 30°C and then rapamycin (LC Laboratories) was added from a 1 mg/mL stock solution in ethanol to a final concentration of 1 µg/mL. One hour after rapamycin treatment, one-third volume of prewarmed media (13 mL at 57°C added to 39 mL culture for a final temperature of 37°C, or one-third volume of media at 30°C for non-heat-shocked cells, was added to each flask, and flasks were immediately placed at 37°C (or at 30°C for non-heat-shocked cells) in a shaking incubator for 15 min (30 min for indicated samples in Fig. 1) before crosslinking with formaldehyde. Both *kin28A* yeast and the parent strain YFR1321 were treated with rapamycin in all experiments unless indicated otherwise. For CdCl2-treated samples, after 1 h of rapamycin treatment, CdCl2 was added from a 1 M stock solution to a final concentration of 0.5 mM and incubated for another hour before crosslinking.

**ChIP-seq experiments and analyses**

ChIP-seq experiments, library construction, and analyses were performed as described previously (Knoll et al. 2018). Additional details can be found in the Supplemental Material as Supplemental Methods. The various gene sets used in analyses are listed in Supplemental Table S6. Correlation analysis (determined using the Galaxy server) of ChIP-seq replicate experiments examining Med13 occupancy is shown in Supplemental Figure S15.

**Data access**

ChIP-seq reads generated in this study have been submitted to the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA657372.

**Competing interest statement**

The authors declare no competing interests.

**Acknowledgments**

We thank Todd Benziger for strain construction, Francois Robert and Kevin Struhl for generously providing yeast strains, and Jason Lieb and Colin Lickwar for help in ChIP-seq of Rap1. We thank the Wadsworth Center Applied Genomics Technology and Tissue Culture and Media Cores for help. This work was supported by the National Science Foundation (MCB1516839 to R.H.M.) and in part by the National Institutes of Health Intramural Research Program at the National Library of Medicine (Z.I.Z. and D.L.).

**Author contributions**

D.S. conducted most experiments; E.R.K. and R.H.M. also conducted experiments, and E.P. conducted ChIP-seq experiments for Rap1 binding. Z.I.Z., D.L., D.S., and R.H.M. analyzed the data, and Z.I.Z. managed data deposition. D.L. and R.H.M. supervised the project; R.H.M. conceptualized the project and wrote the manuscript.

**References**

Ansari SA, He Q, Morse RH. 2009. Mediator complex association with constitutively transcribed genes in yeast. *Proc Natl Acad Sci USA* **106**:16734–16739. doi:10.1073/pnas.0905103106

Bhose LT, Yu Y, Stillman DJ. 2001. The Swi5 activator recruits the Mediator complex to the HO promoter without RNA polymerase II. *Genes Dev* **15**:2457–2469. doi:10.1101/gad.921601

Bourbon HM, Aguillera A, Ansari AZ, Asturias FJ, Berk AJ, Bjorklund S, Blackwell TK, Borggrefe T, Carey M, Carlson M, et al. 2004. A unified nomenclature for protein subunits of Mediator complexes linking transcriptional regulators to RNA polymerase II. *Mol Cell* **14**:553–557. doi:10.1016/j.molcel.2004.05.011

Bruzzone MJ, Grünberg S, Kubik S, Zentner GE, Shore D. 2018. Distinct patterns of histone acetyltransferase and Mediator deployment at yeast protein-coding genes. *Genes Dev* **32**:1252–1265. doi:10.1101/gad.132173.118

Causton HC, Ren B, Koh SS, Harbison CT, Kanin E, Jennings EG, Lee TI, True HL, Lander ES, Young RA. 2001. Remodeling of yeast genome expression programs in response to environmental changes. *Mol Biol Cell* **12**:323–337. doi:10.1091/mbc.12.2.323

Cormier L, Barber R, Kuras L. 2010. Transcriptional plasticity through differential assembly of a multiprotein activation complex. *Nucleic Acids Res* **38**:4998–5014. doi:10.1093/nar/gkq257

de Jonge WJ, O’Dubhthair E, Lijnzaad P, van Leenen D, Groot Koerkamp MJ, Kemmeren P, Holstege FC. 2017. Molecular mechanisms that distinguish THID housekeeping from regulatable SAGA promoters. *EMBO J* **36**:274–290. doi:10.15252/embj.201695621

Dolinski KJ, Heitman J. 1999. Hmo1p, a high mobility group 1/2 homolog, genetically and physically interacts with the yeast FBP12 prolyl isomerase. *Genetics* **151**:935–944. doi:10.1093/genetics/151.3.935

Donczew R, Warfield L, Pacheco D, Erijman A, Hahn S. 2020. Two roles for Mediator during heat shock in yeast. *Genes Dev* **34**:1673–1678. doi:10.1101/gad.312173.119

Ebyoulet F, Cibot C, Eychenne T, Neill H, Albright O, Werner M, Soutourina J. 2013. Mediator links transcription and DNA repair by facilitating Rad2/XPG recruitment. *Genes Dev* **27**:2549–2562. doi:10.1101/gad.225813.113

Fan X, Struhl K. 2009. Where does Mediator bind in vivo? *PLoS One* **4**:e5029. doi:10.1371/journal.pone.0005029

Fan X, Chou DM, Struhl K. 2006. Activator-specific recruitment of Mediator in vivo. *Nat Struct Mol Biol* **13**:117–120. doi:10.1038/nsmb1049

Gasch AP, Werner-Washburne M. 2002. The genomics of yeast responses to environmental stress and starvation. *Func Integ Genomics* **2**:181–192. doi:10.1007/s12251-002-0058-2

Gasch AP, Spellman PT, Kao CM, Carmel-Harel O, Eisen MB, Storz G, Botstein D, Brown PO. 2000. Genomic expression programs in the response of yeast cells to environmental changes. *Mol Biol Cell* **11**:4241–4257. doi:10.1091/mbc.11.12.4241

Gross DS, English KE, Collins KW, Lee SW. 1990. Genomic footprinting of the yeast HSP82 promoter reveals marked distortion of the DNA helix and constitutive occupancy of heat shock and TATA elements. *J Mol Biol* **216**:611–631. doi:10.1016/0022-2836(90)90387-2
Tirosh I, Barkai N. 2008. Two strategies for gene regulation by promoter nucleosomes. Genomes Res 18: 1084–1091. doi:10.1101/gr.076059.108
Vinayachandran V, Reja R, Rossi MJ, Park B, Rieber L, Mittal C, Mahony S, Pugh BF. 2018. Widespread and precise reprogramming of yeast protein-genome interactions in response to heat shock. Genomes Res 28: 357–366. doi:10.1101/gr.226761.117
Wade JT, Hall DB, Struhl K. 2004. The transcription factor Ifh1 is a key regulator of yeast ribosomal protein genes. Nature 432: 1054–1058. doi:10.1038/nature03175
Warfield L, Ramachandran S, Baptista T, Devys D, Tora L, Hahn S. 2017. Transcription of nearly all yeast RNA polymerase II-transcribed genes is dependent on transcription factor TFII D. Mol Cell 68: 118–129.e5. doi:10.1016/j.molcel.2017.08.014
Warner JR. 1999. The economics of ribosome biosynthesis in yeast. Trends Biochem Sci 24: 437–440. doi:10.1016/S0968-0004(99)01460-7
Wong KH, Jin Y, Struhl K. 2014. TFIIH phosphorylation of the Pol II CTD stimulates Mediator dissociation from the preinitiation complex and promoter escape. Mol Cell 54: 601–612. doi:10.1016/j.molcel.2014.03.024
Zeevi D, Sharon E, Lotan-Pompan M, Luhling Y, Shipony Z, Raveh-Sadka T, Keren L, Levo M, Weinberger A, Segal E. 2011. Compensation for differences in gene copy number among yeast ribosomal proteins is encoded within their promoters. Genomes Res 21: 2114–2128. doi:10.1101/gr.119669.110
Zencir S, Dilg D, Rueda MP, Shore D, Albert B. 2020. Mechanisms coordinating ribosomal protein gene transcription in response to stress. Nucleic Acids Res 48: 11408–11420. doi:10.1093/nar/gkaa852

Received May 7, 2021; accepted in revised form November 13, 2021.