TRANSCRIPTION
2022, VOL. 13, NOS. 1–3, 39–52
https://doi.org/10.1080/21541264.2022.2085502

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REVIEW

Function and dynamics of the Mediator complex: novel insights and new frontiers

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ABSTRACT

The Mediator complex was discovered in the early 1990s as a biochemically fractionated factor from yeast extracts that was necessary for activator-stimulated transcriptional activation to be observed in *in vitro* transcription assays. The structure of this large, multi-protein complex is now understood in great detail, and novel genetic approaches have provided rich insights into its dynamics during transcriptional activation and the mechanism by which it facilitates activated transcription. Here I review recent findings and unanswered questions regarding Mediator dynamics, the roles of individual subunits, and differences between its function in yeast and metazoan cells.

ARTICLE HISTORY
Received 22 April 2022
Revised 24 May 2022
Accepted 27 May 2022

KEYWORDS
Mediator; transcription; heat shock; *saccharomyces cerevisiae*; CRISPR; gnomAD; ChIP-seq

Mediator was discovered as a large, multi-subunit complex present in a fractionated extract from yeast that was needed for stimulation of Pol II transcription *in vitro* by activators bound to upstream sites in promoters [1–4]. Biochemical purification found Mediator associated with Pol II, and genetic experiments revealed Mediator to be generally required for Pol II transcription in yeast. This was first demonstrated by the effect of a temperature sensitive mutant on bulk mRNA transcript levels, and later by genome-wide assays of transcript levels, nascent transcription, and Pol II recruitment, all of which were reduced to 5–15% of wild type levels by depletion of Mediator [5–11]. Subunits of Mediator were identified, the complex was found to be conserved across eukaryotes, and high-resolution structures of Mediator by itself and in complex with the Pol II preinitiation complex (PIC) have been determined [12–15].

On the basis of structural, biochemical, and genetic data, Mediator is generally viewed as having a modular structure comprising head, middle, and tail modules, and a Cdk8 kinase module, variously referred to as the kinase module or CKM, that associates with the remainder of the complex in dynamic fashion. Given the intimate connections among various Mediator subunits revealed in X-ray and cryo-EM structures, and the ambiguous assignment of some subunits to specific modules (not to mention a drastic revision of subunit placement into the larger structure [16,17]), this modular structure is perhaps best viewed as representing a useful heuristic device rather than a stringent segregation of parts [18,19]. Along these lines, it has been proposed to define the core Mediator as comprising middle and head modules together with the scaffold subunit Med14, with core Mediator being distinct from the tail module and dynamically associated kinase module, both of which are dispensable for stimulation of transcription by activators *in vitro* [8,20].

Recruitment and dynamics of Mediator in yeast

Two early microarray studies reported contradictory results regarding genome-wide localization of Mediator, with one finding high levels of Mediator association with genes induced by heat shock or growth in galactose, but a lack of association with intergenic regions in yeast grown in rich medium [21], while the other reported Mediator occupancy upstream of both active and inactive genes, as well

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as at coding regions of some highly active genes in yeast grown under anaerobic conditions [22]. The apparent absence of Mediator association in the first study may have reflected sensitivity constraints, as later ChIP-seq and ChEC-seq studies indicated variable association with UASs [6,23–26], while the reported association with coding regions was likely due to artifactual ChIP signal at highly transcribed regions, as documented several years later [23,25,27,28].

The variable association of Mediator with UAS regions in ChIP-seq and ChEC-seq studies was puzzling on two levels. First, Mediator occupancy at UASs correlated only weakly with transcription levels and Pol II occupancy at the associated genes; most conspicuously, Mediator peaks were weak or absent upstream of the highly transcribed ribosomal protein (RP) genes, corroborating one of the earlier microarray studies [21]. Second, in spite of abundant evidence for interactions of Mediator subunits from the head and middle modules with PIC components, including TBP and Pol II (reviewed in [29]), Mediator ChIP-seq peaks were seen only at UAS regions, and not at proximal promoters of transcriptionally active genes. A resolution to this conundrum was provided by experiments conducted in the Struhl and Robert labs that showed that inhibiting Pol II escape from the PIC resulted in conspicuous Mediator ChIP-seq peaks at proximal promoter regions (Figure 1) [25,26]. Inhibition of Pol II promoter escape was achieved by inactivating or depleting the TFIIH-associated kinase, Kin28, or by mutating its target, the Ser5 residues in the carboxy terminal domain of Rpb1. These results suggested that Mediator occupancy at promoters was normally transient, being coupled to Pol II recruitment, and was lost upon Pol II movement away from the promoter, thus accounting for the difficulty in detecting it at promoters in ChIP experiments. Two subsequent studies from the same labs provided strong evidence, including sequential ChIP experiments demonstrating a single Mediator complex contacted both UAS and proximal promoter regions, for initial recruitment of Mediator to UAS regions followed by its transit to the proximal promoter and rapid eviction upon Pol II escape [18,30]. Simultaneous depletion of TBP and Kin28 was found to result in a shift of Mediator ChIP-seq peaks from promoters to UAS regions, indicating that TBP was required to enable Mediator association with the proximal promoter (Figure 1) [31].

The ability to control Mediator dynamics by depletion or inactivation of Kin28 has also provided novel insight into Mediator recruitment. The Mediator tail module was identified in early biochemical and genetic experiments as a direct target of activators, and hence likely important for recruitment of Mediator [32,33]. Specifically, subunits in the tail module triad of Med2, Med3, and Med15, together with Med16, which connects the triad with core Mediator, were found in these and subsequent investigations to be important for transcription of and/or Pol II recruitment to genes activated by Gal4, Gcn4, and heat shock, among others [34–39]. The large Med15 subunit (previously known as Gal11, 1081 amino acids in length against 431 aa for Med2 and 397 aa for Med3) has been most intensively studied with regard to its physical interaction with activators, and unstructured activation domains from a variety of activators have been found to contact Med15 via "fuzzy" interactions (i.e., not involving shape complementarity) between clusters of hydrophobic amino acids with multiple Med15 surface regions [40–42].

Tail module subunit deletions affecting transcription of inducible genes had less effect on constitutive gene expression, and the tail module was not needed for "basal" transcription, defined as depending only on the general transcription machinery without the aid of activators, *in vitro* [32,33]. Moreover, yeast lacking all three of the tail module triad subunits are viable, as are *med16Δ* yeast, in which the connection between the tail module triad and core Mediator is disrupted [31,39,43,44]. Mediator association is severely reduced at UASs in *med3Δ med15Δ* yeast as well as in the triad knockout [18,30,31,34]. Nonetheless, Mediator association is retained at proximal promoter regions upon inactivation or depletion of Kin28 in tail module-deficient yeast, albeit at reduced levels, with corresponding reduced association of Pol II. Mediator association with proximal promoters appears to depend on cooperative interactions with Pol II, as depletion of Rpb1 together with Kin28 results in greatly reduced association of Mediator, consistent with

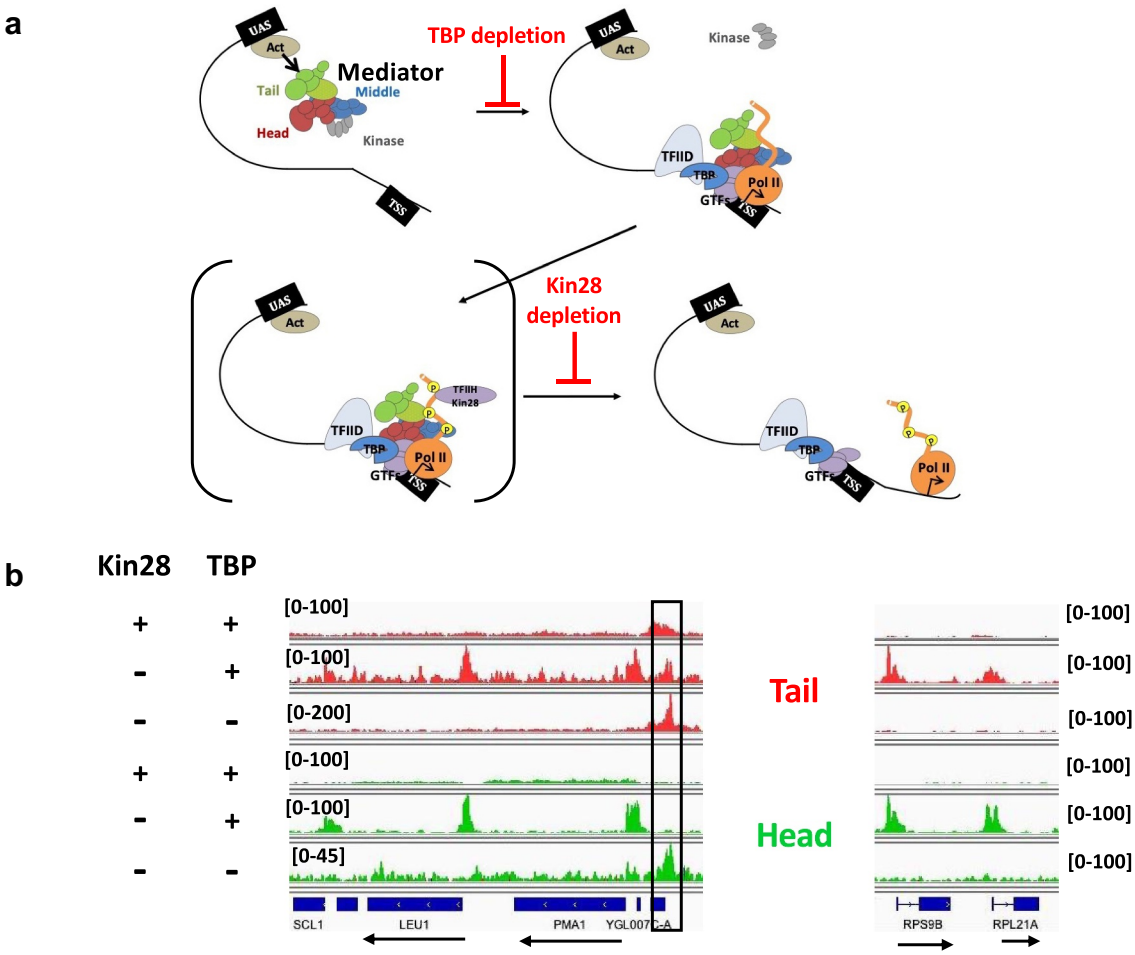


Figure 1. Mediator dynamics during activated transcription in yeast. (a) Mediator is recruited to UASs by DNA sequence-specific activator proteins via tail module subunits. Mediator then engages PIC components and transits to the proximal promoter region, with accompanying loss of the kinase module. Although the precise mechanism of Mediator transit from UAS to proximal promoter and engagement with the PIC remains unknown, TBP is required for this step. Mediator remains at the proximal promoter only transiently, with eviction accompanying promoter escape by Pol II, but can be captured at this stage by preventing Pol II promoter escape. (b) Browser scans showing occupancy of Mediator tail (Med15) or head (Med18) subunits at the *LEU1* and *PMA1* promoters (left) and *RPS9B* and *RPL21A* promoters (right) with or without depletion of Kin28 or Kin28 and TBP. The box indicates the *PMA1* UAS; the peak to the left at the proximal promoter is seen only when Kin28 is depleted and TBP is not. Relative scales normalized to the total number of mapped reads are indicated. All browser scans were produced using the integrative genome viewer [85]. Data is from [31,71].

the transient association seen in wild type yeast due to the rapid promoter escape by Pol II [31]. In accord with this idea, depletion of TBP, Taf1, or Rpb1 in KIN28+ yeast resulted in increased Mediator association with UAS regions, indicating that Mediator transit from UASs to proximal promoter regions is inhibited when PIC assembly is impaired [31,45]. Depletion of Taf1, a component of TFIID, together with Kin28 results in greatly reduced Mediator occupancy at proximal promoters of TFIID-dominated genes, which are enriched for constitutively expressed, housekeeping genes, while the ~15% of genes that are SAGA-dominated and enriched for stress response and other inducible genes exhibit a less pronounced effect [31,46]. (For reconsideration and revision of TFIID-dominated and SAGA-dominated gene categories, see [47,48]). Conversely, loss of a functional tail module triad affects the expression and the association of Mediator with the proximal promoter of SAGA-dominated genes more strongly than at TFIID-dominated genes [6,31,34,49] (but see [18] for an opposing argument). Together, these results point to two possible avenues for Mediator recruitment, one in which Mediator is recruited to UASs in a tail-

dependent fashion, and a second in which Mediator is recruited to proximal promoter regions independently of the tail module through the interactions of the middle and head modules with the general transcription factors and Pol II in the PIC.

Does direct recruitment of Mediator to proximal promoters, independently of UASs or the tail module, occur in unperturbed, wild type yeast? In a recent study, the Pugh lab has concluded that a large number of yeast genes (2474 genes, with an estimated "false negative" rate – meaning a bound TF was missed – of <10%) were bound only by a PIC, and lack promoter-bound activator proteins [50]. This assertion was based on ChIP-seq experiments targeting ~400 DNA-associated (not necessarily directly binding) proteins, including 78 sequence specific transcription factors, and is supported by comparison with TF binding determined in a large microarray study [51], as only 1459 binding events (defined as having $p < 0.001$) were observed over 2374 "UNB" genes for 150 TFs. These UNB genes, which are mostly transcribed at low levels and mainly comprise TFIID-dominated genes, would be candidates for direct recruitment of Mediator to proximal promoter regions. Analysis of ChIP-seq data in which association of the head module subunit Med17 was monitored after depletion of Kin28, reveals Mediator association at UNB proximal promoters that is only moderately weaker than observed at the STM genes, which are characterized by association with sequence specific TFs and cofactor assemblages SAGA, TUP, and Mediator and/or the SWI/SNF complex and are enriched for SAGA-dominated genes (Figure 2(a)). Intriguingly, the Med17 peak differs in character at these two cohorts, with upstream association apparent at STM promoters but absent from UNB gene promoters. In spite of this indication of possible association of Mediator with UASs at STM genes but not at UNB genes, a similar reduction in normalized peak intensity in the tail triad deletion mutant is seen at both cohorts (Figure 2 (a)). Differential behavior is also seen between STM and UNB genes upon simultaneous depletion of Kin28 and TBP, which results in an upstream shift in association of the tail module subunit Med15 at STM genes with no significant reduction in intensity, while Med15 association is lost at UNB genes (Figure (2b)). These observations suggest that if activators are bound upstream of UNB genes, their interaction with Mediator must be very weak. Alternatively, recruitment of the PIC at these genes may occur simply as a consequence of a nucleosome free region at the proximal promoter, as suggested by Pugh and colleagues, with Mediator recruitment occurring via its interaction

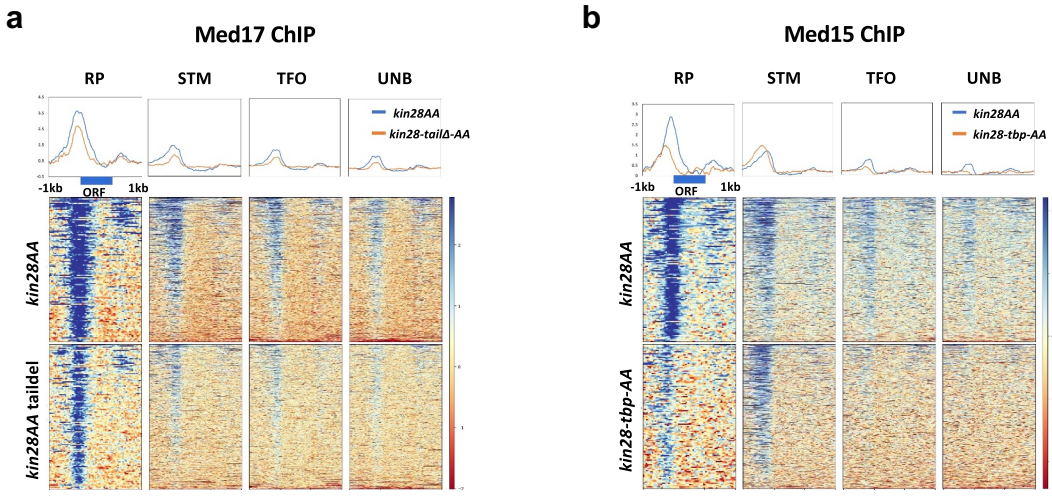


Figure 2. Mediator occupancy after Kin28 depletion at 125 ribosomal protein (RP) genes, 845 STM genes (see text), 1469 TFO genes (associated with sequence specific TFs but not with Tup1, SAGA, or mediator/SWI-SNF), and 2008 UNB genes (see text) as defined by [50] and with divergent promoters removed [86]. Heat maps and line graphs showing normalized occupancy of (a) Med17 (head) in *kin28AA* and *kin28AA med2Δ med3Δ med15Δ* yeast treated with rapamycin and (b) Med15 (tail) in *kin28AA* and *kin28-tbp-AA* yeast treated with rapamycin. Heat maps and line graphs produced using the galaxy server [87]. Data is from [31].

with Pol II and GTFs. If recruitment of Mediator does occur directly to, or as part of, the PIC, the contribution of the tail module to this recruitment remains to be explained.

Distinct modes of Mediator recruitment at Ty1 elements

The retrotransposon Ty1 is present in about 30 copies in laboratory strains of *Saccharomyces cerevisiae*. Ty1 expresses a single 5.7 kb transcript that encodes necessary components for the retrotransposon life cycle, including reverse transcriptase, integrase, Gag structural proteins that form cytoplasmic viral-like particles in which Ty1 protein processing and reverse transcription of the Ty1 transcript take place, and protease required for processing [52]. In addition, Ty1 has evolved an internally initiated, inhibitory transcript termed Ty1i [53]. This transcript initiates about 700 bp downstream of the transcription start site of the full-length Ty1 transcript and encodes a truncated Gag protein that inhibits Ty1 processing [54]. Ty1i is produced at a higher ratio relative to Ty1 when Ty1 copy number is greater; thus, Ty1i has apparently evolved as part of a molecular compromise to limit Ty1 propagation.

Numerous host factors impact rates of Ty1 retrotransposition, which normally occurs about once per 0.1–10 million cells per Ty1 element per generation. In an effort to understand Ty1 regulation, we investigated the effect of deletion of all non-essential Mediator subunits on Ty1 retromobility [55]. We found that deletion of head and middle subunits increased retromobility by >100 fold, while deletion of tail module triad subunits reduced retromobility to undetectable levels. Deletion of kinase module subunits had no effect on retromobility. Further characterization of the tail and middle/head module deletions revealed their impact on retromobility to be due to differential effects on transcription of Ty1 and Ty1i stemming from opposing effects on Mediator recruitment at the two promoters. Under conditions of Kin28 depletion, Mediator association is apparent at both Ty1 and Ty1i promoters; deletion of the tail module triad nearly abolishes Mediator association at Ty1 while leaving residual ChIP-seq signal at Ty1i, consistent with loss of retrotransposition in tail module triad subunit deletion mutants (Figure 3(a)) [55]. In contrast, deletion of *MED18* or *MED20*, encoding head module subunits, decreased Mediator association with Ty1i relative to Ty1, consistent with increased retrotransposition. These differential effects were intriguing in light of Ty1 possessing characteristics of SAGA-dominated promoters, including a well-defined TATA element and dependence on SAGA and the SWI/SNF chromatin remodeling complex; while Ty1i, in contrast, lacks a consensus TATA element and is not decreased in its transcription by loss of the SAGA component Spt3 that essentially abolishes Ty1 transcription [56,57]. The strong dependence of Ty1 transcription on tail module triad subunits was consistent with previous results indicating enrichment for SAGA-dominated genes among those down-regulated by deletion of tail module triad subunits [6,34]. Further supporting differential regulation of Ty1 and Ty1i promoters, TFIID subunits Taf1 and Taf4 show greater association with Ty1i than with Ty1, and depletion of Taf1 affects Mediator association with Ty1i more than with Ty1 (Figure 3(b)).

The differential effects of Mediator subunit deletions at Ty1 and Ty1i suggest that Mediator recruitment may occur by different mechanisms at SAGA-dominated and TFIID-dominated genes. Effects of subunit deletion at some specific loci provides some support for this, as for example deletion of the tail module triad has a larger effect on Mediator association at the SAGA-dominated *PMA1* promoter than at the TFIID-dominated *LEU1* promoter, while deletion of *MED18* or *MED20* has opposite effect (Figure 3(c)). These effects are variable, however, and either deletion results in a large decrease in Mediator association at many genes (e.g. *HSP150* and *CIS3*; Figure 3(c)). Ty1 transcriptional regulation is governed by several TFs, most of which have binding sites downstream of the Ty1 TSS but upstream of that for Ty1i (Figure (3d)). (Note also that Mediator occupancy shifts to this region upon simultaneous depletion of Kin28 and TBP; Figure 3(b)). Which of these TFs contribute to Ty1i transcription, and the roles of these various TFs in the different Mediator subunit dependence of Ty1 and Ty1i, is currently unknown. Thus, although it is clear that the subunit dependence of Mediator recruitment

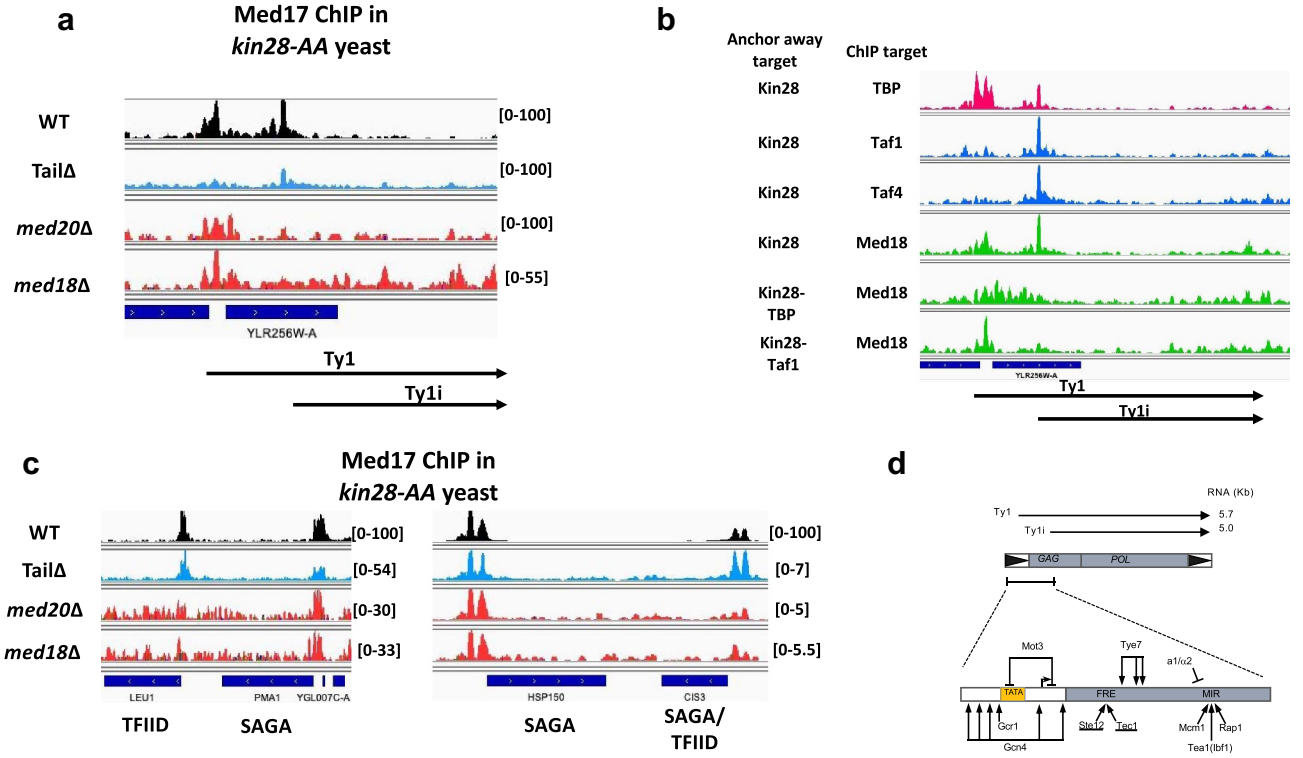


Figure 3. Opposing effects of deletion of mediator tail and head module subunits on mediator association with Ty1 and Ty1i promoters. (a) Med17 (head) ChIP-seq reads mapped to YLRWTy1-3 in wild type, *med2Δ med3Δ med15Δ, med18Δ*, and *med20Δ* yeast. Transcripts corresponding to Ty1 and Ty1i are indicated below. Note that reads cannot be unambiguously assigned to individual Ty1 elements; hence, YLRWTy1-3 serves as a proxy for a "representative" Ty1 element. Relative scales per total number of mapped reads are indicated. Data is from [31,55]. (b) ChIP-seq reads for TBP, Taf1 and Taf4 (TFIID subunits), and Med18 (head) mapped to YLRWTy1-3 with depletion of Kin28, Kin28 and TBP, or Kin28 and Taf1, as indicated. Data is from [31,88]. (c) Browser scans showing Med17 (head) occupancy at *LEU1, PMA1, HSP150*, and *CIS3* in wild type, *med2Δ med3Δ med15Δ, med18Δ*, and *med20Δ* yeast after Kin28 depletion. Designation of genes as SAGA-dominated or TFIID-dominated is from [46]. Relative scales per total number of mapped reads are indicated. Data is from [31,55]. (d) Schematic diagram of Ty1. Ty1 and Ty1i transcripts are indicated at the top; expanded view at bottom indicates binding sites for the indicated TFs [52].

to the PIC varies in a promoter-specific manner, the underlying mechanism for this variability remains to be determined.

Escape from upstream: dynamics of kinase module association

The ability to trap Mediator at proximal promoters by Kin28 depletion or inactivation has also allowed insight into another aspect of Mediator dynamics. Low resolution cryo-EM studies, confirmed more recently at higher resolution, showed that Pol II and the kinase module bind overlapping regions of the core Mediator, and biochemical experiments revealed their binding to be mutually incompatible [58–60]. In agreement with these findings, the kinase module was observed by ChIP-seq to associate with UASs but was not present at proximal promoters with the core Mediator when Kin28 was depleted or inactivated [18,30]. Thus, engagement of the PIC by Mediator is accompanied by loss of the kinase module. Loss of the kinase module upon transit of Mediator to the proximal promoter does not appear, however, to be rate limiting, as there was little effect on Mediator occupancy at core promoters in yeast lacking Med13, which is needed for the kinase module to associate with the remainder of the Mediator complex. Unexpectedly, Mediator association with UASs increased substantially in *med13Δ* yeast, as well as in a kinase-defective mutant; thus, the kinase module apparently inhibits Mediator recruitment to UASs, but not its transit to proximal promoter regions. The inhibition by the kinase module of Mediator recruitment to UASs is consistent with

earlier work demonstrating that the kinase module negatively regulates a subset of genes by antagonizing the tail module [61]. This antagonism may occur at least in part by indirect means, as phosphorylation by Cdk8 results in degradation of several primary activators, including Gcn4, Msn2, Ste12, and Phd1, and may in some cases autoregulate the stability of Mediator itself [62–65].

With regard to the function of the kinase module, it is interesting to note that all four of its subunits were identified as *srb* mutants in early genetic screens in which nine Mediator subunits were eventually identified [66,67]. *Srb* mutants were identified through their suppression of temperature and cold sensitivity (and also inositol auxotrophy) of yeast in which the carboxy terminal domain (CTD) of Rpb1, the largest subunit of Pol II, is truncated from the normal 26 repeats and near repeats of the consensus YSPTSPS to 10–12 repeats [68]; the *SRB* designation stands for *S*uppressor of *R*NA Polymerase *B*, where Polymerase B was the European designation for Pol II. The Pol II CTD is intrinsically disordered, and consequently mostly unresolved in high resolution structures of Mediator and PIC [15]. However, modeling and cross-linking experiments indicate its interaction with middle and head subunits of Mediator [69]; *srb* mutants include subunits from the middle and head, but not the tail module of Mediator. At the time that the kinase module subunits were identified as *srb* mutants, it was suggested that the *SRB10*-encoded kinase, now known as Cdk8, could target the CTD, but given the mutually exclusive association of Pol II and the kinase module with the head-middle-tail Mediator complex, this no longer seems tenable. How then could mutants in kinase module subunits suppress a truncated Pol II CTD? Interactions between Mediator and Pol II appear to depend strongly on the CTD [69]; perhaps this interaction is disfavored by a truncated CTD, and *srb* mutants in kinase module subunits alter the competing interaction of that module with core Mediator, thereby compensating for the weaker CTD-Mediator interaction.

Mediator under stress: holding back

Recent work provides evidence for altered Mediator dynamics under conditions of stress. In a study examining a possible role for Cdk8 kinase activity in regulating interaction between Mediator and Pol II, induction of genes activated by heat shock was virtually abolished by inhibition of Cdk8 kinase activity [59]. In a related study, cyclin C, another kinase module subunit, was shown to be required for induction of genes in response to oxidative stress in mammalian cells [70]. Inhibition of Cdk8 kinase activity had no effect on gene expression in yeast growing under steady-state conditions. Several targets of Cdk8 phosphorylation were identified in Mediator at sites on the kinase module binding surface, and phosphorylation of these sites weakened kinase module interaction with core Mediator. Based on these findings, a model was proposed in which association of the kinase module prevents Mediator at UASs from associating with Pol II to facilitate PIC assembly or recruitment; stress-induced activation of the kinase activity is proposed to destabilize this association and allow induction of stress-induced genes. This model would predict Mediator to be associated with UASs of stress-induced genes when Cdk8 kinase activity is inhibited, but this has not yet been tested.

Altered Mediator dynamics following stress induction have also been observed in ChIP-seq experiments [71]. First, conspicuous ChIP-seq peaks were observed at UASs of genes activated by Hsf1 and Msn2/4 (activators of heat shock and stress-induced genes, respectively) after heat shock, consistent with earlier work reporting strong Mediator ChIP signal at genes induced by heat shock or galactose [21]. This association showed little change (primarily a slight shift downstream, toward the proximal promoter) when Kin28 was depleted, in contrast to many active genes that show increased Mediator peaks upon Kin28 depletion in yeast grown under steady-state conditions (Figure 1(b)). This result does not so much speak to general Mediator dynamics as to underscore that Mediator occupancy is apparently high at the UAS of some active genes (whether under steady-state or stress-induced growth conditions) but not at others (e.g. RP genes under steady-state growth conditions); at strongly induced genes, the former situation may predominate.

More surprisingly, persistent Mediator occupancy was observed upstream of RP genes in spite of their nearly complete repression by heat shock (Figure (4a)). Mediator occupancy was previously reported at UAS regions of two repressed genes, *ROX1* and *ZRT2*, in yeast grown under anaerobic conditions, and at genes repressed by treatment with sulfometuron methyl, which mimics starvation conditions [22,24]. Persistent association at RP genes repressed by cadmium chloride administration was also observed [71]. The large majority (129 out of 137) of RP genes depend on the general regulatory factor Rap1 for their activation, and Mediator occupancy peaks coincided with Rap1 binding sites for RP genes repressed by heat shock. This was true even under conditions of Kin28 depletion, consistent with the absence of a PIC at the repressed RP genes [71,72]. Further investigation revealed persistent Mediator association at UAS regions not only at RP genes, but also at many other genes repressed by heat shock. In accord with the observed association of Mediator at UASs rather than at proximal promoters of repressed genes, ChIP-seq signals were more prominent for the tail module subunit Med15 than for head module subunit Med18.

Examination of the RP genes (prompted by an insightful referee) shed some light on the mechanism underlying the strong association of Mediator with genes repressed by heat shock. The 129 RP genes that are bound by Rap1 are nearly evenly divided into two groups, one of which is strongly bound by the HMG-related protein Hmo1 while the other group is only weakly associated [73–76]. Remarkably, Mediator association was much

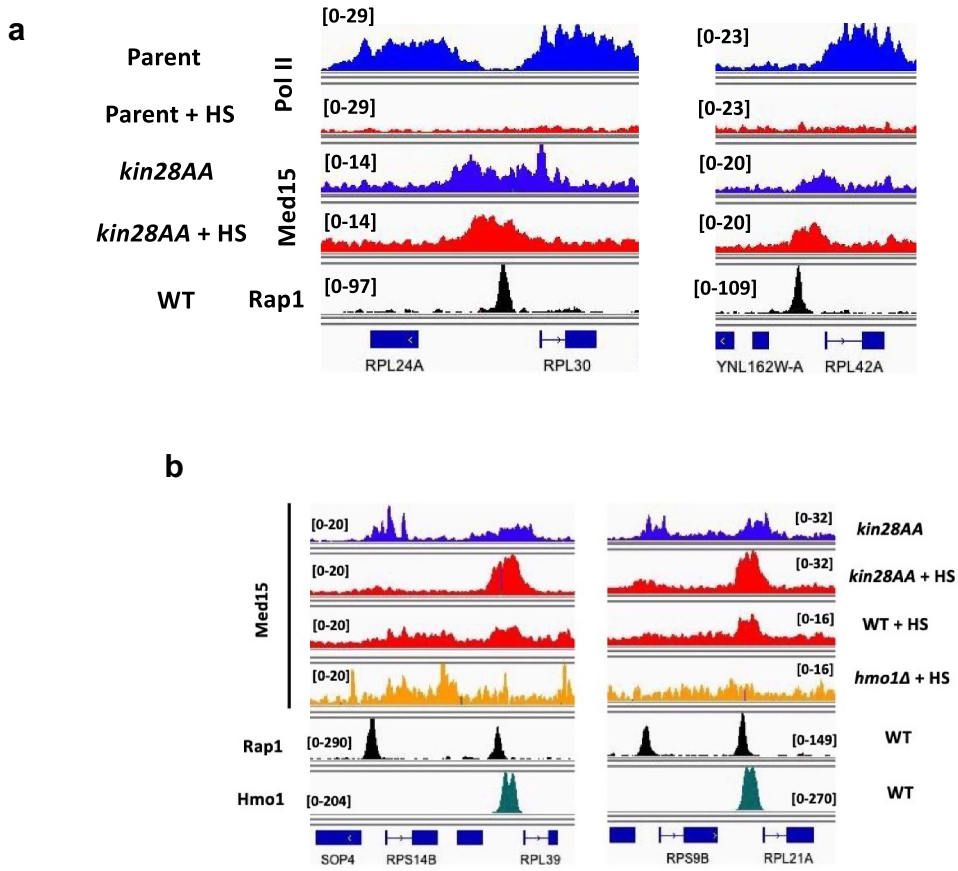


Figure 4. Persistent Mediator association with RP genes repressed by heat shock. (a) Browser scans showing occupancy of Pol II in the parent anchor away strain (YFR1321) treated with rapamycin, with or without 15 min heat shock; Med15 (tail) in *kin28AA* yeast treated with rapamycin with or without 15 min heat shock; and Rap1 in wild type yeast (strain BY4741). (b) Browser scans showing occupancy of Med15 (tail) for *kin28AA* yeast treated with rapamycin with and without 15 min heat shock, and for wild type (BY4741) and *hmo1Δ* yeast heat shocked for 15 min; Rap1 and Hmo1 occupancy is shown in wild type yeast (BY4741 and W303, respectively). Scale, in reads per million mapped reads, is indicated for each scan. Data is from [71,75].

greater at repressed genes having high Hmo1 occupancy than at those with weak Hmo1 occupancy, and this association was substantially reduced in *hmo1Δ* yeast (Figure 4(b)). Similar findings were made following analysis of repressed non-RP genes, although the number of these associated with high levels of Hmo1 is much smaller. Although these results point to a contribution from Hmo1 in facilitating persistent Mediator association with genes repressed by heat shock, several outstanding questions remain, including the mechanism by which Hmo1 facilitates Mediator association, what accounts for weak but continued Mediator association with repressed genes in *hmo1Δ* yeast, and what benefit strong and persistent Mediator association with a subset of RP genes might confer.

What prevents Mediator associated with UASs of repressed genes – particularly RP genes – from recruiting Pol II and facilitating assembly of a productive PIC? If Mediator engagement with Pol II following heat shock requires its release from the kinase module by Cdk8-mediated phosphorylation, as hypothesized by Osman et al., perhaps this step is inhibited at repressed genes by Hmo1 or other accessory factors. The mechanism behind differential retention of the kinase module under steady-state as compared to stress-induced growth conditions, if this is indeed the case, is currently obscure, but likely to involve signaling pathways that impinge on Mediator and related targets [5,59,77].

Mediator in metazoans: the same as yeast, but different

Much of what is known regarding Mediator dynamics and subunit function derives from studies with yeast, due to its genetic tractability. Cryo-EM studies reveal close correspondence between structures of yeast and metazoan Mediator both in isolation and in complex with the PIC [15], while recent analyses and novel genetic methods have begun to reveal differences in functional aspects. Based on ChIP-seq studies in murine embryonic stem cells (mESCs), Mediator was reported to be associated with both enhancers and promoters [78]. Following the paradigm established in yeast, this suggested that Mediator was recruited to enhancer sites by DNA sequence-specific activators, followed by PIC engagement and association with the promoter. Because the enhancers that correspond to activator binding sites are typically distant from promoters, communication between the two requires DNA looping. Although it would seem that this looping would likely involve Mediator, a recent study reported little effect on promoter-enhancer looping upon auxin-induced degradation of MED4 in mESCs, despite decreased recruitment of Pol II [79]. Moreover, a meta-analysis of ChIP-seq experiments concluded that Mediator is stably associated with promoters, and not with activator binding sites, in metazoan cells [80]. This study points out that most of the ChIP signal observed for the Mediator subunit Med1 arises from a small proportion of the total enhancer cohort (~10,000 out of ~150,000 enhancers), and the authors suggest that Mediator occupancy at these sites, which on average peaks ~90 bp distance from E2F and DP1 activator binding sites, reflects binding to sites that act as promoters for transcription of enhancer RNAs rather than to activator binding sites. This controversial assertion needs further testing; however, it is indirectly supported by findings that Mediator association with promoters and enhancers in mESCs was decreased by TAF12 degradation [79], analogous to the strong decrease in Mediator occupancy at promoters seen with simultaneous depletion of Kin28 and Taf1 in yeast, whereas depletion of Taf1 or TBP alone does not result in substantial loss of Mediator association [31,45].

Effects of deletion or depletion of Mediator subunits in metazoan cells is only just beginning to be seriously explored. While degron-induced depletion of the scaffold subunit Med14, which is required for the structural integrity of Mediator, produced a global decrease in mRNA production in murine B cells similar to that seen upon Mediator inactivation in yeast [81], a more rapid depletion of core Mediator subunits in "near-haploid" human KBM7 cells affected only a subset of genes, suggesting that the universal requirement for Mediator in transcription seen in yeast might not apply to metazoan cells [82]. The need for more work to establish Mediator requirements in metazoan gene transcription, and the

mechanism of possible Mediator-independent transcriptional activation, is evident.

Research on Mediator in metazoans may also benefit from resources stemming from high-throughput investigations. One such resource is the BioGRID Open Repository of CRISPR Screens (ORCS; https://orcs.thebiogrid.org/), which as of this writing includes data from 1514 CRISPR screens conducted in four major model organisms, 736 cell lines and 125 cell types. As an example, a cursory search of this resource indicates that Med14 is essential in numerous cell lines, as might be expected, while the requirement for Med12 from the kinase module appears variable in different murine cell lines. Data from individual publications can provide more focused information (Figure 5(a)). As with any such high-throughput data, there are sure to be false positives and negatives, and reported phenotypes require verification. Nonetheless, the scope of the data and variety of phenotypes examined, including cell proliferation, viability, effects on signaling pathways, and resistance to specific drugs, represent a rich resource for data mining.

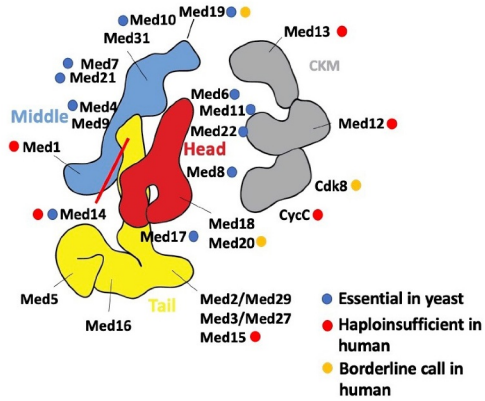
Another resource, the genome aggregation database, or gnomAD, provides information on the frequency of missense and loss of function (LoF) mutations in a compilation, as of this writing, of ~125,000 exome sequences and ~15,700 complete genome sequences derived from various disease-specific and population genetic studies [83]. Analysis of the frequency of LoF mutations compared to expected frequencies in the absence of detrimental effects allows insight into whether specific genes are haploinsufficient at some point during development or whether a single functional allele is sufficient for survival to adulthood. Differences between essentiality in yeast and haploinsufficiency in humans are notable (Figure 5(b)). For example, the kinase module, which has not thus far been found to be essential in any growth condition in yeast, appears to be haploinsufficient in humans: no LoF mutations have been detected in MED12 or

a

Viswanatha et al. eLife 2018;7:e36333. DOI: https://doi.org/10.7554/eLife.36333
Drosophila
"Hits" (essential genes) designated for Z< -3.99

Symbol	Rep1	Rep2
MED1	-0.011316	-1.0956
MED10	-8.5114	-9.4722
MED11	-0.92213	-1.3922
MED14	0.32039	-1.0002
MED15	-4.6189	-4.8058
MED16	6.1636	-2.9911
MED17	0.063768	-4.0549
MED18	-7.821	-11.199
MED19	-2.2401	1.235
MED20	-1.2508	-4.1924
MED21	-14.704	-14.858
MED22	-0.055763	-2.2087
MED23	-0.46527	-0.43566
MED24	3.9258	-1.4611
MED25	-5.3406	-1.747
MED26	-2.945	-1.2459
MED27	-0.087543	0.53481
MED28	-6.7903	-4.7892
MED30	-4.0522	-3.3136
MED31	-1.1136	1.4568
MED4	2.2759	-1.158
MED6	-1.4157	0.19507
MED7	0.2666	0.050454
MED8	-8.9115	-8.727
MED9	-3.5445	-4.3057

b



c

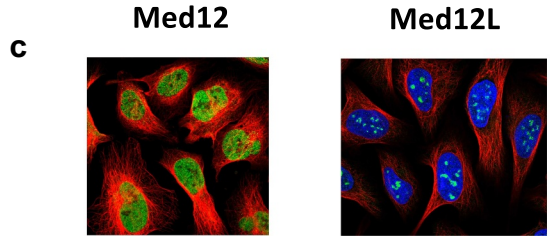


Figure 5. Mediator in metazoans. (a) Essentiality of Mediator subunits in *Drosophila* derived from a CRISPR screen; highlighted subunits were deemed likely to be essential [89]. (b) Mediator subunits labeled according to whether they are essential in yeast or likely to be haploinsufficient in humans, based on data from gnomAD [83]. (c) The paralogs Med12 and Med12L from the kinase module may have distinct nuclear localization. Left, Med12 stained green and microtubules stained red shows Med12 in the nucleoplasm; right, Med12L stained green, nucleus stained blue and microtubules stained red indicate that Med12L is localized to nucleoli (images from the Human Protein Atlas (https://www.proteinatlas.org/search/med12) [90]), which may suggest a special role for Med12L in regulating Pol II in the nucleolus [91].

CycC, while 98 and 20, respectively, would be expected in the cohort examined if loss was completely tolerated. In addition to the rather blunt phenotype represented by the presence or absence of LoF alleles, gnomAD and similar resources may also prove useful in allowing disease-causing mutations to be mapped onto structural data (e.g [84].).

Conclusions

The last few years have seen major advances in our understanding of Mediator dynamics during transcriptional activation based on studies in yeast. A clear picture has emerged for activated transcription, in which activators recruit Mediator to their binding sites via Mediator tail module triad subunits, after which Mediator associates with the proximal promoter in a TBP-dependent fashion, accompanied by loss of the kinase module. At this stage, Mediator engages the PIC and facilitates its assembly, which rapidly leads to productive transcriptional elongation and loss of Mediator association with the promoter. Numerous questions remain: Precisely how does Mediator engage PIC components during PIC assembly? What is the role of Mediator at promoters that function without sequence-specific activators, if such really exist, and what are its dynamics at these promoters? How are Mediator dynamics altered under conditions of environmental stress? And to what extent do the principles elucidated in yeast translate to metazoan cells, and what surprises may emerge from the flood of data coming from high-throughput studies (Figure 5(c))? We may with some confidence expect new insights into the basic biology of Mediator and into its role in health and disease in the coming few years.

Acknowledgments

I thank Joe Wade for helpful discussions, and Lis Knoll, Alicia Salinero, Debasish Sarkar and other members of the Morse lab, along with Joan Curcio, Iris Zhu, and David Landsman for their contributions to work discussed here. I thank Lis Knoll for her rendition of Mediator structure in (Figure 5(b)) (after [16]). This work was supported by the National Science Foundation under grant MCB1516839.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by the National Science Foundation [MCB1516839].

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