The Mediator co-activator complex regulates Ty1 retromobility via alternative transcription start site selection

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Abstract

Retrotransposons are mobile genetic elements that replicate via an RNA intermediary and constitute a significant portion of most eukaryotic genomes. *Saccharomyces cerevisiae* has been invaluable to retrotransposon research due to the presence of an active retroelement known as Ty1. The retromobility of Ty1 is regulated both positively and negatively by numerous host factors, including several subunits of the Mediator transcriptional co-activator complex. Paradoxically, previous studies have implicated the nuclear Mediator complex in the regulation of post-translational steps in Ty1 retromobility. To attempt to resolve this apparent paradox, we have examined the effects of deleting non-essential Mediator subunits on the various stages of Ty1 retromobility. The Mediator core complex is organized into genetically and structurally defined head, middle, and tail modules, along with a transiently associated kinase module. We show that with the exception of the kinase module, deleting Mediator subunits has a major impact on Ty1 mobility. Disrupting the Mediator tail module decreases Ty1 activity to undetectable levels, while disrupting the head or middle module increases Ty1 retromobility substantially. These major changes in retromobility are accompanied by insignificant differences in Ty1 RNA or Gag protein levels in Mediator mutants relative to the wild-type strain. Decreased retromobility in tail module gene deletion mutants requires the Ty1 promoter in the 5′ LTR and is correlated with increased expression of an internal transcript known as Ty1i, which encodes a dominant negative inhibitor of Ty1 retromobility. We present evidence that Mediator preferentially associates with the Ty1 or Ty1i promoters in strains lacking specific Mediator head or tail subunits, respectively, indicating that Mediator controls Ty1 retromobility by governing transcription start site selection within Ty1 elements. This work elucidates a mechanism of host control of retrotransposon activity via promoter competition and provides mechanistic insight into transcriptional regulation of Ty1i RNA.

Author Summary

Retrotransposons are mobile genetic elements that copy their RNA genomes into DNA and insert the DNA copies into the host genome. These elements contribute to genome instability, control of host gene expression and adaptation to changing environments. Retrotransposons depend on numerous host factors for their own propagation and control. The retrovirus-like retrotransposon, Ty1, in the yeast *Saccharomyces cerevisiae* has been an invaluable model for retrotransposon research, and hundreds of host factors that regulate Ty1 retrotransposition have been identified. Non-essential subunits of the Mediator transcriptional co-activator complex have been identified as one set of host factors implicated in Ty1 regulation. Here, we report a systematic investigation of the effects of loss of these non-essential subunits of Mediator on Ty1 retrotransposition. Our findings reveal a heretofore unknown mechanism by which Mediator influences the choice of transcription start site (TSS) in Ty1 to modulate expression of an autoinhibitory transcript known as Ty1i RNA. Our results provide new insights into host control of retrotransposon activity via TSS selection and elucidate a novel mechanism by which different promoters compete for the Mediator co-activator.

Introduction

Retrotransposons have been extensively characterized as catalysts of evolutionary change and agents of genome instability [1–6]. In humans, retrotransposons have been shown to be upregulated in cancerous cells [7], and have been implicated in tumorigenesis [8]. Long terminal repeat (LTR) retrotransposons are of particular interest because they are the evolutionary progenitors of retroviruses, [9], and have been found to be influenced heavily by host factors that control retroviral propagation [10–17].

Research focused on the LTR-retrotransposons in *Saccharomyces cerevisiae* has been fundamental to our understanding of the mechanism by which LTR-retrotransposons replicate, and how they interact with the host genome [3,6,18]. Ty1 is the most abundant and active of these LTR-retrotransposons, with 31 copies in the haploid genome and a mobility rate of approximately $1x10^{-7}$ to $1x10^{-5}$ per Ty1 element in each cell generation [19]. Retrotransposition initiates with transcription of the element (Fig 1A & B). The U3 region of the 5′ LTR contains promoter sequences recognized by DNA binding transcriptional activators, as well as two TATA box sequences (Fig 1C). The 5.7kb Ty1 transcript extends from the R region of the 5′ LTR and terminates in the R region of the 3′ LTR (Fig 1B & C). This transcript contains two partially overlapping open reading frames, *GAG* and *POL*, the latter of which is translated only when a specific +1 frameshift event occurs in the overlapping region (Fig 1B). This frameshifting event enables production of two translational products, the p49-Gag protein and the p199-Gag-Pol polyprotein. Ty1 protease, a factor that is encoded in the *POL* ORF, processes p49-Gag to p45-Gag as an integral part of Ty1 protein maturation. The Gag-Pol polyprotein is processed to yield p45-Gag, protease, reverse transcriptase and integrase. In addition to its protein coding function, the Ty1 transcript serves as a template for reverse transcription of the element. This process occurs within a cytoplasmic capsid of Gag protein known as the virus-like particle (VLP) (Fig 1A & B) [18]. Following successful reverse transcription within the VLP, Ty1 cDNA is transported back to the nucleus and integrated into the host genome through the activity of integrase (Fig 1A) [20,21].

Ty1 relies extensively on autoregulatory factors and host factors to successfully complete its mobility cycle and limit its mobility so as not to destabilize the host genome [18,22]. One mechanism of auto-inhibition known as copy number control (CNC) is defined by a copy number-dependent decrease in Ty1 retrotransposition observed both in *S. cerevisiae* and its close relative, *S. paradoxus* [23–25]. The process has been studied extensively but until recently remained poorly understood. CNC is enforced by a dominant, *trans*-acting regulatory protein known as p22-Gag [25–28] that is translated from an internal

transcript, Ty1i, which begins 1 kb from the beginning of the Ty1 element within the *GAG* ORF (Fig 1B) [25,29]. Binding sites for several DNA binding activator proteins are located within the *GAG* ORF upstream of the Ty1i start site (Fig 1C). Translation of Ty1i RNA produces a 22 kDa protein that lacks the N-terminal region of Gag. This truncated Gag protein retains the ability to associate with p49- or p45-Gag. However, incorporation of p22-Gag into the VLP disrupts particle formation, thereby halting Ty1 protein maturation and production of Ty1 cDNA [25–27,30].

Host factors that regulate Ty1 mobility include subunits of the Mediator transcriptional co-activator complex [31–36]. In *Saccharomyces cerevisiae*, Mediator is a 1.4 MDa complex composed of 25 individual subunits organized into four modules (Fig 2A) [37–40]. The core Mediator complex contains the "head," "middle," and "tail" modules, while a fourth kinase module is transiently associated with the core complex in a context-specific manner [41–43]. The tail domain is generally responsible for Mediator's association with transcriptional activator proteins, while the head and middle are involved in association of RNA Polymerase II (Pol II) and pre-initiation complex (PIC) formation [37,42]. The Mediator complex plays a crucial role in the formation of the PIC at all Pol II transcribed genes by acting as a bridge between DNA binding transcriptional activator proteins and the RNA Pol II transcription machinery [42–49].

Several studies have implicated individual Mediator subunits as either activators or repressors of Ty1 retromobility. Efforts to identify retromobility phenotypes in the absence of these subunits indicate that they affect a step in retrotransposition between transcription and integration [18]. The tail subunit Med16 was identified as an activator of Ty1 mobility [32,34,36] that acts at a stage following mRNA export to the cytoplasm [34], but prior to cDNA integration [32]. Med2 was also identified as an activator, but was not further characterized [36]. Conversely, the tail subunit Med3 was found to negatively affect mobility without altering levels of cytoplasmic RNA foci wherein VLPs are assembled [34,36]. Mediator head subunits Med20 and Med18 and the middle subunits Med1, Med10, Med31 and

Med9 were identified as repressors of Ty1 mobility [31,33–35]. Absence of the head or middle subunits Med20, Med18, Med31, or Med9 had no significant effect on Ty1 mRNA expression [34], but increased the amount of unintegrated Ty1 cDNA [35]. Additionally, in the absence of the middle subunits Med1 or Med10 Ty1 cDNA increased concurrently with elevated levels of Ty1 mobility [31]. Med1 has also been found to influence Ty1 mobility with minimal changes to Ty1 mRNA levels [31] or localization [34]. Mediator kinase module subunits Med12 and Med13 were identified as activators of Ty1 mobility [32,36], with *med13*Δ exhibiting a decrease in cDNA production that aligns with Ty1 hypomobility [32]. Taken together, these studies suggest that individual Mediator subunits positively or negatively regulate a post-transcriptional step in Ty1 retrotransposition. This conclusion is strikingly incongruous with Mediator's canonical role as a transcriptional regulator. To date, no mechanistic characterization of Mediator's positive and negative influences on Ty1 mobility has been undertaken, and an explanation for its post-transcriptional function in Ty1 mobility has been elusive.

In this study, we systematically determine the effects of deleting non-essential subunits of the Mediator complex on various steps in Ty1 retrotransposition, from Ty1 and Ty1i RNA expression to completion of the retrotransposition event. We show that deletion of Mediator complex subunits results in substantial, module-specific effects on the level of Ty1 retrotransposition. Consistent with previous findings, we find that Mediator subunit deletions have minimal effects on the levels of Ty1 RNA and Gag protein, but do result in substantial changes in the level of unintegrated cDNA that correspond to changes in the level of retrotransposition. We report for the first time that deletion of individual subunits of the tail module triad, Med2-Med3-Med15, increases recruitment of Mediator and Pol II to a secondary promoter within the Ty1 *GAG* ORF. Use of this internal promoter results in expression of Ty1i RNA, whose translation product, p22-Gag, is a potent inhibitor of VLP formation and Ty1 cDNA synthesis. In contrast, loss of Mediator head module subunit Med18 or Med20 decreases Mediator association with the internal Ty1i promoter and results in increased Ty1 mobility. Thus, Mediator

153 subunits control a post-transcriptional step in Ty1 mobility by modulating transcription of Ty1i RNA.
154 Based on these observations, we propose a mechanism by which the Mediator tail anchors the pre-
155 initiation complex to the Ty1 LTR promoter, thereby dampening transcription from the internal Ty1i
156 promoter, while Mediator head subunits are strictly required for expression from the internal Ty1i
157 promoter.
158
159 **Results**
160
161 *The Core Mediator Complex Has a Profound Effect on Ty1 Mobility*
162 Previous genetic screens have indicated the existence of a relationship between the Mediator
163 complex and Ty1 retrotransposition [31,32,34,36,50]. These screens determined that individual
164 Mediator subunits influence Ty1 mobility through post-transcriptional mechanisms; however, they
165 employed different assays for retromobility, and differed in their identification of specific Mediator
166 subunits contributing to Ty1 mobility. To systematically investigate the role of all non-essential subunits
167 of Mediator in Ty1 mobility, a collection of strains, each containing a deletion of a non-essential
168 Mediator subunit, was generated from a BY4741 progenitor strain containing a chromosomal *his3AI*-
169 marked Ty1 element (S1 Table). These strains were then tested for Ty1*his3AI* mobility using an
170 established quantitative mobility assay in which cells that sustain a retromobility event are detected as
171 His+ prototrophs (Fig 2B) [19]. A mutant lacking the SAGA complex component Spt3 was chosen as a
172 negative control for Ty1 mobility due to the well-characterized requirement for Spt3 in Ty1 transcription
173 and mobility [51,52].
174 Results from this assay indicate that Mediator influences Ty1*his3AI* mobility in a profound,
175 module-specific manner. Deletion of genes encoding subunits in the head or middle module increased
176 Ty1*his3AI* mobility approximately 100-fold. Conversely, deleting any subunit from the Med2-Med3-

Med15 tail module triad resulted in a mobility level that was more than 100-fold decreased relative to the wild-type strain, and below detection limits. The Med2-Med3-Med15 subunits are direct targets of DNA-binding activator proteins [42,47,53,54], and deletions of these subunits exhibit similar phenotypes [42,53]. In contrast, two other tail module subunits that exhibit distinct phenotypes when deleted, Med5 and Med16, do not appear to affect Ty1 mobility. The transiently associated kinase module also does not substantially influence Ty1 mobility. Kinase module deletion strains were omitted from further analysis in this work. The disparate effects of deleting subunits in different modules is consistent with previous data indicating that the Mediator complex regulates the expression of some gene sets in a module-dependent manner [41].

Mediator Influences Ty1 cDNA without Altering Ty1 Transcript Levels

Given Mediator's role as a transcriptional co-activator, we first sought to determine whether the changes in Ty1 mobility observed in the Mediator subunit deletion strains were caused by altered Ty1 transcription. Changes in the abundance of Ty1 RNA observed by northern blotting in head, middle or tail module gene deletion strains relative to the wild-type strain were modest and not statistically significant ($p>0.1$, one-way ANOVA) (Fig 3A). These data indicate that Mediator head, middle and tail subunits do not regulate Ty1 retromobility by affecting production of full-length Ty1 transcripts.

A series of non-coding antisense transcripts are also expressed from Ty1 elements via an internal promoter (Fig 1B & C). These transcripts initiate from distinct positions within the first 700 bp of the Ty1 element, and their expression is enhanced in certain hypomobile strains, most notably in an *spt3*Δ mutant [24,29]. Levels of Ty1 antisense transcripts (Ty1AS RNA) were measured to determine whether Mediator was altering expression of these transcripts and thereby influencing Ty1 mobility (Fig 3A). A modest increase of approximately 2-fold in Ty1AS RNA was observed in the mediator tail subunit deletion strains relative to the wild-type strain (Fig 3A). When compared with increases of a similar

magnitude in the *med31*Δ hypermobile strain, and contrasted with the 10-fold increase in the hypomobile *spt3*Δ strain, it would appear that Ty1AS RNA expression is not likely to be a major contributing factor to tail-mediated repression of Ty1.

To independently confirm that the substantial changes in retromobility in Mediator subunit deletion strains were not a result of minor changes in Ty1 RNA levels, or in alterations to Ty1 polyadenylation that would result in translational defects, the levels of Gag, the major product of Ty1 RNA, were measured using western blot analysis (Fig 3B). As with Ty1 RNA and Ty1AS RNA, there were only moderate (<2 fold relative to the wild-type strain) changes in the levels of Gag protein. Together, these data strongly support the argument that Mediator regulates a post-transcriptional step in Ty1 retromobility, and does so without altering the steady state levels of the Gag protein (Fig 3A & B).

Assessing the level of unintegrated cDNA provides an indication of the efficiency with which Ty1 has undergone VLP formation and reverse transcription of the template mRNA. This assay involves the separation of *SphI*-digested genomic DNA, which is subsequently probed for Ty1sequences at the 3′ end of *POL* [15,31], allowing for the visualization of bands representing the junction between the 3′ end of each Ty1 element and flanking genomic DNA. Differences in the size of the bands are due to the different location of *SphI* sites in DNA flanking Ty1 at different locations. In this assay, the smallest band represents unintegrated Ty1 cDNA because of the absence of flanking genomic DNA. We performed this assay to compare the ratio of unintegrated cDNA to genomic Ty1 DNA in wild-type yeast to that in Mediator mutants. We observed increased cDNA levels for *med18*Δ, *med20*Δ, *med31*Δ, and *med1*Δ mutants, while loss of tail module triad subunits Med2, Med3, or Med15 reduced cDNA to nearly undetectable levels (Fig 3C). Thus, both hyper- and hypomobile mutants exhibited cDNA levels that strongly correlated with changes in Ty1*his3AI* mobility (Fig 2B). The magnitude of changes in Ty1 cDNA levels are not as great as those of Ty1 mobility, but this is expected because this assay measures steady-state levels of cDNA, whereas the mobility assay measures accumulated mobility events.

Together, these data indicate that Mediator core subunits regulate a post-transcriptional step in Ty1 mobility wherein deletion of Mediator complex genes alters the accumulation of Ty1 cDNA without substantially altering overall Ty1 transcript or Gag protein levels.

The Tail Module Triad Regulates Ty1 via Modulation of Ty1i Expression

The observation that Mediator regulates a post-transcriptional step in Ty1 retromobility, and that Mediator subunits regulate the level of Ty1 cDNA, led us to consider the possibility that Mediator influences the expression of the internal Ty1i transcript, which encodes p22-Gag, the dominant negative inhibitor of retromobility. The post-transcriptional effects on Ty1 mobility caused by increasing expression of p22-Gag [25] are very similar to those observed in Mediator tail triad deletion mutants. We therefore sought to determine whether expression of Ty1i RNA is altered by deletion of Mediator subunits.

Ty1i RNA is not easily detected in northern blots of total cellular mRNA (Fig 3A), as it is obscured by the highly abundant Ty1 transcript. Therefore polyA$^+$ mRNA of the wild-type strain and Mediator deletion strains was subject to northern analysis to achieve better separation of the Ty1 and Ty1i transcripts, as shown previously [25]. This analysis revealed an increase in Ty1i mRNA relative to levels of Ty1 mRNA in strains with tail module triad gene deletions, suggesting a likely mechanism for the decreased Ty1 mobility seen in these mutants (Fig 4A). Notably, Ty1i mRNA levels in the tail module subunit deletion *med5*Δ were similar to those in the wild-type strain, which is consistent with the wild-type mobility seen in this strain. In contrast to tail module triad gene deletion strains, the total amount of Ty1i mRNA and in the ratio of Ty1i RNA to Ty1 RNA in head and middle subunit gene deletion strains were not significantly altered relative to the wild-type strain. Given the low level of Ty1i mRNA in all these strains, we were unable to determine whether decreased Ty1i mRNA levels accompany the hypermobility phenotypes of head and middle subunit gene deletion strains.

Previous work from the Morse lab compared genome-wide occupancy of Pol II in wild type and *med3*Δ *med15*Δ yeast [55,56]. The effects of the *med3*Δ *med15*Δ mutation are similar to those of the single Mediator tail module triad subunit deletions [55], and so we used this data to compare Pol II occupancy on all Ty1 elements in wild type and *med3*Δ *med15*Δ yeast (Fig 4B). The results show that Pol II occupancy in *med3*Δ *med15*Δ yeast is reduced exclusively in the first 1 kb of the Ty1 element. Pol II occupancy in the *med3*Δ *med15*Δ double mutant and wild-type strain became equivalent near the Ty1i transcription start site (TSS) and remained so until the transcription end site (TES) of both Ty1 sense-strand transcripts (Fig 4B). This indicates that the Mediator tail preferentially directs Pol II to the primary Ty1 TSS. Absence of subunits from the tail module triad increases transcription from the internal Ty1i TSS, explaining the elevated levels of Ty1i RNA in tail module triad mutants (Fig 4A).

The elevated Ty1i RNA levels observed in Mediator tail module triad mutants would be predicted to give rise to increased levels of p22-Gag and its cleavage product, p18-Gag. To test this prediction, we performed western blotting using an anti-p18 polyclonal antibody. As expected, elevated levels of the internal Ty1i transcript were accompanied by substantial increases in p22-Gag in Mediator tail module triad gene deletion strains, whereas no p22-Gag was detected in the wild-type strain or Mediator head and middle module mutants (Fig 4C). Notably, levels of p18-Gag were not correlated with p22-Gag or Ty1 retromobility levels in the wild-type strain or any Mediator subunit deletion strain. This is in contrast to a previous report in *Saccharomyces paradoxus* [25], a close relative of *S. cerevisiae* that lacks endogenous Ty1 elements, where increasing the copy number of introduced Ty1 elements results in higher levels of both p22-Gag and p18-Gag. Nonetheless, the data presented demonstrate that diminished Ty1 retromobility is inversely correlated with elevated Ty1i RNA and p22-Gag levels in Mediator tail triad gene deletion strains, strongly suggesting that enhanced occupancy of Pol II at the internal Ty1i promoter is the underlying cause of undetectable Ty1 retromobility in tail mutants. Given

that p22-Gag blocks post-transcriptional steps in Ty1 mobility, the findings also reveal how Mediator functions in its canonical role as a transcriptional co-activator to block Ty1 retrotransposition at a post-transcriptional step.

While the molecular phenotypes of tail module triad mutants present a clear mechanistic paradigm, the results for head and middle mutants do not shed light on whether an opposing mechanism of Ty1i RNA regulation causes the hypermobility phenotype of head and middle mutants. Basal levels of Ty1i RNA and p22-Gag in the wild-type strain remain below detection in northern blots (Fig 4A) and western blots (Fig. 4C), respectively, thus making it impossible to detect a reduction in Ty1i RNA or p22-Gag in head and middle mutants.

The Mediator Tail Module Regulates Ty1 Mobility via an Interaction with the LTR Promoter

We next sought to gain broader mechanistic insight into tail module-regulated repression of Ty1i RNA. The Ty1 LTR contains two TATA boxes within its U3 region (Fig 1), and its transcription is Spt3-dependent (Fig 3 & 4), indicating that Ty1 belongs to the SAGA-dependent class of genes. This class is enriched in highly regulated genes such as stress-response genes, and is characterized by a promoter structure that is distinct from that of the largely constitutively-active, TATA-less, TFIID-dependent genes [57,58]. In contrast to the Ty1 promoter, the region upstream of the Ty1i TSS lacks any consensus TATA element, indicating that Ty1i belongs to the class of TFIID-dependent genes (Fig 4A). As SAGA-dependent genes show a preferential dependence on the tail module triad relative to TFIID-dependent genes [55], we theorized that the tail module triad might operate by favoring the association of Mediator with the SAGA-dependent U3 promoter that is upstream of Ty1 (Fig 1). As a result of this association, the Mediator head and middle modules would be tethered to the Ty1 promoter, and would in turn preferentially recruit Pol II to the Ty1 TSS rather than the internal Ty1i TSS. Disruption of this Mediator-U3 interaction via deletion of a tail module gene could allow for Mediator and the PIC to

297 instead associate with the less robust TFIID-dependent Ty1i promoter. To test the role of the U3
298 promoter in governing the effect of Mediator mutations on Ty1 mobility, the U3 region of the Ty1 LTR
299 was swapped for the transcriptionally robust, TFIID-dependent *TEF1* promoter sequence on a *CEN*-
300 plasmid-based Ty1*his3AI* element (Fig 5A). The *TEF1* promoter was chosen based on findings that
301 deletions of non-essential Mediator subunits do not alter *TEF1* expression substantially [55,59]. The
302 effects of Mediator subunit deletions on retromobility of the *TEF1* promoter-driven Ty1*his3AI* element
303 versus the LTR promoter-driven Ty1*his3AI* element expressed from the same vector were then
304 compared (Fig 5B).

305 To our surprise, the retromobility frequency of the Ty1*his3AI* element on a single-copy *CEN*-
306 plasmid (referred to as LTR-Ty1*his3AI*) was nearly identical to that of P_{TEF1}-Ty1*his3AI* on the same
307 vector in a wild-type background (1.13×10^{-5} versus 1.64×10^{-5} His^+ prototrophs per cell; Fig. 5B). The
308 only difference between these elements is the promoter, and unlike the *TEF1* promoter, the Ty1
309 promoter has been reported to be weak [60]. To determine whether similar levels of Ty1*his3AI* RNA are
310 expressed from the plasmid-borne LTR-Ty1*his3AI* and P_{TEF1}-Ty1*his3AI* elements, we performed
311 northern blotting of $polyA^+$ RNA using a *HIS3* probe to detect Ty1*his3AI* and Ty1i*his3AI* RNA. The
312 level of Ty1*his3AI* RNA was only about 3-fold higher when expressed from the *TEF1* promoter relative
313 to the LTR in the wild type strain (Fig. 5C). Together with the fact that retromobility of the *CEN*-based
314 LTR-Ty1*his3AI* element is >30-fold higher than the chromosomal Ty1*his3AI* element (compare Fig 5B
315 to Fig 2B), the findings suggest that overall Ty1 RNA levels, including Ty1*his3AI* RNA, are markedly
316 increased by LTR-Ty1*his3AI* or P_{TEF1}-Ty1*his3AI* expression. Moreover, the internal Ty1i*his3AI*
317 transcript was not detected in the wild-type strain expressing LTR-Ty1*his3AI* or P_{TEF1}-Ty1*his3AI*,
318 suggesting that the total Ty1i/Ty1 RNA ratio in the presence of these plasmids is substantially lower
319 than in their absence. Therefore, we propose that retromobility in the strain expressing LTR-Ty1*his3AI*
320 is unexpectedly high because of derepression of the *CEN*-based LTR promoter by an unknown

mechanism, high levels of Ty1 RNA bearing the *his3AI* reporter and a very low total Ty1i /Ty1 RNA ratio.

In the wild-type, LTR-Ty1*his3AI* plasmid-bearing strain with low Ty1i /Ty1 RNA ratios, deletion of *MED3* diminished retromobility more than 1000-fold (Fig. 5B). In contrast, deleting either tail module triad gene, *MED3* or *MED15*, had little effect on retromobility of the P_{TEF1}-Ty1*his3AI* element (Fig 5B). These findings provide strong evidence that the LTR promoter is required for diminished retromobility in tail triad module mutants. This lends further support to the idea that the tail module triad promotes the association of Mediator with the SAGA-dependent U3 promoter in the LTR, and that disruption of the tail module allows increased Mediator recruitment to the Ty1i promoter.

In contrast to the above results, deletion of genes encoding head (*MED20*) or middle module (*MED31* or *MED1*) subunits had modest and equivalent effects on retromobility of P_{TEF1}-Ty1*his3AI* and LTR-Ty1*his3AI*. These results suggest that head and middle modules do not depend on the SAGA-dependent U3 promoter for their repression of retromobility. We hypothesize that deletion of head and middle module subunits increases retromobility by favoring Mediator association with the Ty1 promoter and therefore decreasing the Ty1i/Ty1 RNA ratio. Deleting head and middle module genes may have only a modest effect on retromobility in a wild-type strain expressing LTR-Ty1*his3AI* or P_{TEF1}-Ty1*his3AI* because the Ty1i /Ty1 RNA ratios are very low to begin with.

Levels of Ty1*his3AI* RNA driven from the LTR or P_{TEF1} promoter were only modestly altered (≤4-fold up or down) by deletion of Mediator head and middle subunits, and not in a manner consistent with the observed retrotransposition frequencies. P_{TEF1}-driven Ty1*his3AI* RNA levels in the *med15*Δ mutant were about 4-fold higher than in the wild-type strain. It is possible that this elevated level of Ty1*his3AI* RNA contributes to overcoming the inhibitory effect of a high Ty1i/Ty1 RNA ratio in tail module triad mutants (Fig. 4A), thereby allowing Ty1*his3AI* to transpose at similar frequencies in wild-type, *med3*Δ and *med15*Δ strains with P_{TEF1}-Ty1*his3AI* (Fig. 5B). However, our data taken together

345 support the idea that a failure to induce Ty1i*his3AI* RNA expression when the LTR promoter is replaced
346 by P_{TEF1} is the major reason why retromobility is not repressed in tail module triad gene deletion strains
347 expressing P_{TEF1}-Ty1*his3AI*.

348 Because the internal Ty1i*his3AI* transcript was not detected, even in the *med3*Δ mutant harboring
349 the LTR-Ty1*his3AI* plasmid (Fig 5C), we could not determine whether Ty1i*his3AI* RNA levels were
350 higher when expressed from LTR-Ty1*his3AI* versus P_{TEF1}-Ty1*his3AI*. Nonetheless, derepression of
351 retromobility in tail module triad mutants when Ty1*his3AI* expression is under control of the TFIID-
352 dependent *TEF1* promoter is consistent with repression of Ty1 mobility in tail module triad mutants
353 being dictated by loss of an interaction between Mediator tail and the TATA-containing, SAGA-
354 dependent LTR promoter.

355

356 *Ty1i RNA Is Repressed by the Mediator Tail Module Triad Independently of Full Length Ty1*
357 *Transcription*

358 Our results indicate that the Mediator tail module triad acts at the Ty1 promoter to repress
359 expression of Ty1i RNA. We considered two mechanisms by which this might occur. First, recruitment
360 of Mediator to the Ty1 promoter could lead to enhanced Ty1 transcription, and this could repress the
361 downstream Ty1i promoter by readthrough effects. In this mechanism, the polymerase moving from the
362 Ty1 TSS could disrupt transcription factor or PIC binding to the Ty1i promoter [61–63]. In the second
363 mechanism, the Mediator tail could act as a direct repressor of the Ty1i promoter.

364 To distinguish between these two mechanistic possibilities, we wished to determine whether
365 increased Ty1i expression would still be observed in tail module triad deletion mutants under conditions
366 where transcription from full length Ty1 was strongly repressed. To this end, we employed a plasmid-
367 based Ty1 element under control of the *GAL1* promoter (Fig 6) [64]. When grown in glucose-containing
368 media that represses the *GAL1* promoter, expression of Ty1 RNA is assumed to be repressed, but Ty1i

RNA is expressed [25]. The 2µM-plasmid-borne *GAL1*-Ty1 element (pGTy1) that we used also has an internal deletion within the *POL* ORF, which facilitates resolution of Ty1ΔPOL RNA from full-length Ty1 mRNA, thus permitting a direct comparison between endogenous Ty1 RNA and plasmid-derived Ty1ΔPOL RNA by northern blot or single strand cDNA synthesis followed by PCR analysis.

We first sought to verify that Ty1ΔPOL RNA expression was repressed and Ty1iΔPOL RNA was preferentially expressed in glucose. To distinguish Ty1ΔPOL from Ty1iΔPOL transcripts, the same reverse primer was used with one of two forward primers located 250bp upstream or 250bp downstream of the Ty1i TSS (Fig 6, top). Use of these primers precluded use of real time PCR; instead, aliquots from the reactions were removed at two cycle intervals for analysis by gel electrophoresis. Using this strategy, we found that Ty1iΔPOL cDNA amplified four to five cycles before Ty1ΔPOL cDNA in strains grown in glucose (Fig 6). This verifies preferential expression of Ty1i RNA from this element by about 16- to 32-fold in cells grown in glucose, even in the hypermobile *med20*Δ genetic background.

To examine the effect of Mediator subunit deletions on Ty1i RNA expression from pGTy1ΔPOL, levels of Ty1 and Ty1i RNA from endogenous Ty1 elements and Ty1ΔPOL and Ty1iΔPOL RNA from pGTy1ΔPOL were measured by northern blotting (Fig 7A & B). Levels of endogenous Ty1 RNA were not significantly affected by deletion of different Mediator subunits. Ty1i and Ty1iΔPOL RNA levels also remained nearly equivalent in the wild-type strain and head and middle subunit deletion strains; however, Ty1i and Ty1iΔPOL RNAs were markedly increased in strains lacking the tail module subunit, *MED2* or *MED3* (Fig 7A & B). Therefore, disruption of the Mediator tail increases Ty1iΔPOL RNA expression, even when expression from the upstream Ty1 promoter is suppressed. This indicates that the tail module triad does not suppress expression of Ty1i RNA via readthrough inhibition from the upstream Ty1 promoter. Taken together with the altered occupancy of Pol II over Ty1 seen in yeast lacking tail module triad subunits (Fig 4B), these results suggest that the

392 Mediator tail module triad may act to direct PIC formation preferentially to the Ty1 TSS over the Ty1i
393 TSS.

394 Chromosomal Ty1*his3AI* retromobility in strains carrying pGTy1ΔPOL is decreased relative to
395 strains of the same genotype containing no plasmid (compare Fig 7C to Fig 2B), consistent with an
396 increase in Ty1i RNA. We could not determine whether expression of pGTy1ΔPOL increases repression
397 of Ty1*his3AI* mobility in tail module triad gene deletion strains, since retromobility is already
398 undetectable in the absence of the plasmid. Deletion of head and middle module genes still increased
399 retromobility in the presence of the pGTy1ΔPOL plasmid as it did in its absence (Fig 2B), although less
400 robustly in the middle subunit gene deletion strains, *med1*Δ and *med31*Δ, for reasons we cannot explain.
401
402 *Mediator Tail and Head Module Subunits Direct Mediator Association with Ty1 and Ty1i Proximal*
403 *Promoters, Respectively*

404 Results presented so far indicate that the opposing effects of deletion of subunits from the
405 Mediator head module (*med18*Δ and *med20*Δ) and tail module triad (*med2*Δ, *med3*Δ, and *med15*Δ) on
406 Ty1 mobility occur via mechanisms that operate at a similar stage in the Ty1 life cycle (Fig 3).
407 Decreased mobility in mutants lacking subunits from the tail module triad is accompanied by increased
408 expression of Ty1i, and similarly decreased occupancy of Pol II in the first 1kb of Ty1, suggesting a
409 causal mechanism (Fig 4). However, Ty1i RNA and p22-Gag levels are very low in wild type yeast, and
410 we have not been able to detect any decrease of this already low level in *med18*Δ or *med20*Δ mutants.

411 To gain additional insight into how these mutants give rise to the observed hypermobility
412 phenotype, and into the mechanism by which both kinds of deletions affect Ty1 mobility, we used ChIP-
413 seq to examine Mediator association with the proximal promoter regions of Ty1 and Ty1i in wild type
414 yeast and Mediator mutants. For this purpose, we used *kin28* Anchor Away (*kin28-AA*) strains harboring
415 appropriate deletions of Mediator subunits. The *kin28-AA* conditional mutation allows eviction of Kin28

from the nucleus to the cytoplasm by addition of rapamycin [65,66]. Eviction of Kin28 prevents phosphorylation of Ser5 of the Pol II C-terminal domain (CTD). This impedes release of engaged Pol II from the proximal promoter of active genes, thus stabilizing association of Mediator, allowing robust ChIP signals to be observed at gene promoters [66,67].

ChIP-seq against myc-tagged Med17 from the head module or Med15 from the tail module reveals peaks of Mediator association at both Ty1 and Ty1i TSS's in wild type yeast (Fig 8 and S1 Fig). In a *med2*Δ *med3*Δ *med15*Δ mutant, Med17 association with Ty1 is decreased relative to its association with Ty1i, consistent with increased Ty1i mRNA levels in tail module triad deletion mutants. In contrast, occupancy by Med17 and Med15 at Ty1i is virtually eliminated in *med18*Δ (S1 Fig) and *med20*Δ (Fig 8) mutants, while Ty1 occupancy remains robust. These results strongly suggest that the relative occupancy levels by Mediator, and presumably by the PIC, at Ty1 and Ty1i proximal promoters are dictated by Mediator subunits, and play a critical role in governing Ty1 mobility.

Discussion

This work provides a systematic analysis of the role of the Mediator complex in Ty1 retrotransposition. We describe a novel mechanism in which subunits from the Mediator tail and head modules preferentially direct transcription from the Ty1 and Ty1i promoters, respectively, thereby affecting the balance of Ty1 and Ty1i RNA and, consequently, of retrotransposition. These findings are reminiscent of a recent report that retrotransposition of the *Tf2* retrotransposon in *S. pombe* is increased in stressed cells by a mechanism involving altered TSS selection [68]. The mechanism in this latter study appears to be very different from that described here, as the altered TSS usage occurring in stressed cells depends on a reduction in nucleosome occupancy under stress conditions at the TSS for the full length, functional *Tf2* transcript. Nucleosome occupancy at the *Tf2* TSS depends on the activity of Fun30 chromatin remodelers, and there was no evident relation to Mediator function. However,

Mediator is subject to stress-dependent phosphorylation that affects gene expression [69], suggesting a possible mechanism for regulating Ty1 retromobility during stress.

Previous reports provided evidence that loss of various non-essential Mediator subunits affected Ty1 mobility [31–36,50], but they did not systematically characterize the role of Mediator in this process, nor did they provide an explanation for how a transcriptional activator appeared to be acting post-transcriptionally. By examining the effects of deletion of all non-essential Mediator subunits on Ty1 retromobility, we show that Mediator functions as both an activator and repressor of Ty1 retromobility (Fig 2B). Deletions of subunits from different, structurally defined domains exhibited distinct effects, consistent with genetic data [41,55,70]. Deletion of subunits from the kinase module had little effect on Ty1 mobility, while loss of subunits from the Mediator tail module triad reduced mobility by at least 100-fold, and deletion of subunits from the head and middle modules increased retromobility about 100-fold (Fig 2B). Changes in unintegrated Ty1 cDNA levels were strongly correlated with the changes in retromobility, while levels of Ty1 RNA and of Gag protein showed little alteration in any of the Mediator deletion mutants (Fig 3). These results suggest that increased retromobility in head or middle module mutants and decreased retromobility in tail module triad mutants, are opposing effects that transpire through the same pathway.

Our finding that deletion of kinase module genes has a modest effect on Ty1 mobility relative to changes seen in the head or tail subunit deletion strains (Fig 2B) is not entirely unexpected, as the kinase module has been shown to be involved in the regulation of a smaller subset of genes than the Mediator core and is not necessary for the function of the core Mediator complex [43,71]. The finding that deletion of *MED12* increases Ty1 retromobility modestly and deletion of *MED13* has a minimal effect is inconsistent with previous work that implicated *SRB8/MED12* and *SSN2/MED13* in activation of Ty1 retromobility [32,35]. However, this disparity could be the result of differences in the retrotransposition assays and other experimental conditions.

464 A key insight into the mechanism by which tail module triad gene deletions cause decreased Ty1
465 mobility was obtained by northern analysis of Ty1 polyA$^+$ transcripts, which revealed increased levels
466 of the internally generated, inhibitory Ty1i transcript in these deletion mutants (Fig 4). Increased Ty1i
467 RNA was accompanied by elevated levels of p22-Gag, which interferes with VLP formation and
468 production of Ty1 cDNA [25,30]. The increased expression of Ty1i is caused by a direct effect on
469 transcription, as opposed to Ty1i RNA processing or stability, based on the altered occupancy of Pol II
470 in a *med3*Δ *med15*Δ yeast mutant (Fig 4). These findings strongly suggest that the tail module triad
471 supports Ty1 retrotransposition by inhibiting transcription of Ty1i RNA relative to transcription of Ty1
472 RNA.

473 If head and middle module subunits of Mediator regulate Ty1 retromobility through the same
474 pathway as tail module triad subunits, albeit in opposite fashion, their deletion ought to result in
475 *decreased* levels of Ty1i RNA. We did not find evidence for such a decrease from northern analysis of
476 Ty1i transcripts, or by monitoring p22-Gag expression by western blot (Fig 4). However, levels of Ty1i
477 mRNA and p22-Gag are very low in wild-type cells, making detection of any decrease inherently
478 difficult. Furthermore, the product of Ty1i is a potent inhibitor of retrotransposition [25], and subtle
479 changes in transcript levels or the relative translatability of transcripts could conceivably produce
480 substantial changes in mobility levels. Adding to these difficulties, retrotransposition is a rare event, and
481 it is not currently feasible to determine the molecular characteristics of cells that are undergoing a
482 mobility event against the massive background of cells that are not.

483 In spite of these limitations, we have obtained evidence that both tail module triad and head
484 module subunits control Ty1 mobility by regulating the relative levels of Ty1 and Ty1i RNA through a
485 promoter competition mechanism (Fig 9). First, we showed that levels of Ty1i RNA expressed from a
486 pGTy1ΔPOL reporter increase in *med2*Δ and *med3*Δ mutant yeast grown in glucose, where the *GAL1*
487 promoter driving Ty1ΔPOL has very low activity (Fig 6 & 7). This finding indicates that repression of

488 the Ty1i promoter by the tail module triad is unlikely to occur through a mechanism depending on
489 readthrough transcription from the Ty1 promoter. Next, we used ChIP-seq to compare Mediator
490 occupancy at Ty1 and Ty1i promoters in wild type yeast and Mediator mutants. We observed ChIP
491 signals for Mediator (Med17 and Med15) at both Ty1 and Ty1i proximal promoter regions in wild type
492 yeast (Fig 8). In *med2*Δ *med3*Δ *med15*Δ yeast, which completely lack the tail module triad, Med17
493 occupancy is still observed at Ty1i, while it is greatly reduced at Ty1, consistent with our northern
494 analyses showing increased Ty1i mRNA levels in yeast lacking individual tail module triad subunits. In
495 contrast, yeast lacking head subunits Med18 or Med20, which exhibit increased Ty1 retromobility, show
496 reduced Mediator occupancy at the Ty1i promoter and continued occupancy at the Ty1 promoter.
497 Taken together, these findings lead us to propose a mechanism in which competition between
498 PIC formation at the Ty1 and Ty1i promoters, governed by the Mediator complex, regulates Ty1
499 mobility (Fig 9). As discussed earlier, the Ty1 promoter belongs to the family of TATA-containing,
500 SAGA-regulated promoters, while Ty1i appears to fall into the category of TATA-lacking, TFIID-
501 regulated promoters [57]. Genes whose activation depends on the Mediator tail module triad are
502 relatively enriched for TATA-containing, SAGA-regulated family members [55]; in accordance with
503 this observation, we propose that mutants lacking tail module triad subunits have reduced efficiency of
504 PIC formation at the Ty1 promoter, thereby allowing increased utilization of the TATA-less Ty1i
505 promoter. Conversely, we propose that the TATA-less Ty1i promoter depends on head and middle
506 module subunits for Mediator association and PIC formation, consistent with our Mediator ChIP-seq
507 results. Finally, we suggest that replacing the TATA-containing Ty1 promoter with the strong, TFIID-
508 dependent *TEF1* promoter allows Ty1 transcription to successfully compete against the Ty1i promoter
509 even when tail module triad subunits are deleted, so that no increase in Ty1i is observed for this reporter
510 (Fig 5).

Although this model provides, in our view, a reasonable explanation for results presented here, it also illuminates gaps that remain in our understanding. First, the model and our ChIP-seq results indicate substantial Mediator occupancy at both the Ty1 and the Ty1i promoter in wild-type yeast (Fig 8), despite the apparent differences in transcriptional output from these two promoters (Fig 4A). Likewise, we cannot confirm that reduced Mediator occupancy at the Ty1i promoter in *med18*Δ and *med20*Δ yeast is accompanied by reductions to Ty1i RNA or p22-Gag levels, due to their inherently low levels in the wild-type strain. One possibility to explain Mediator occupancy at the Ty1i promoter in the absence of detectable Ty1i RNA is that Ty1i RNA is substantially less stable than Ty1 RNA. Indeed, Ty1 RNA is known to be unusually stable [72,73]. A second possibility is that transcription from Ty1i is inefficient in spite of the apparent presence of Mediator at the Ty1i proximal promoter. This may be altered in Mediator head and middle module mutants, or it may be that a minor change in Ty1i RNA abundance leads to a significant change in the small population of cells that undergo transposition. It is also possible that the head and middle additionally influence other steps in the retrotransposition life cycle. For example, loss of head and middle module subunits may alter Ty1 RNA localization in such a way as to prevent retrotransposition. This possibility is supported by observed increases in ribonucleoprotein foci known as retrosomes in *med20*Δ, *med18*Δ, *med9*Δ, and *med1*Δ mutants [34].

Another question raised by this model is what gives rise to the postulated competition between the Ty1 and Ty1i promoters, which are about 1 kb apart. We cannot answer this question at present, but it seems likely to involve Ty1 promoter elements that are present upstream of the Ty1i proximal promoter. Ty1 possesses an unusual promoter architecture that includes transcription factor binding sites both upstream of the Ty1 TSS and sites within the ORF, but upstream of the Ty1i TSS (Fig 1C) [18]. The latter region includes sites for Ste12, Tec1, Tye7, Mcm1, Rap1, and Tea1; intriguingly, Med15 has been shown to negatively regulate activation of a reporter gene by this intra-ORF region via the Mcm1 site [74]. Conceivably, interaction of Mediator with this region may be sufficient to inhibit PIC

formation at the Ty1i proximal promoter, in a manner dependent on the tail module triad, even when the Ty1 proximal promoter is inactive, as is the case for pGTy1ΔPOL in yeast grown in glucose medium. Further work will be necessary to test the proposed promoter competition model and determine its molecular basis.

An unanticipated finding of this study was that an LTR-driven Ty1*his3AI* element has nearly the same retrotransposition frequency as a P_{TEF1}-driven Ty1*his3AI* element when located on a low copy *CEN*-plasmid (Fig 5B). These frequencies are over 30 times higher than that of the active chromosomal element, Ty1*his3AI*-3114 (Fig. 2B). Moreover, Ty1*his3AI* RNA levels are only three times higher when expressed from the P_{TEF1} promoter versus the LTR (Fig 5C). Because the promoters of chromosomal Ty1 elements have been reported to be weak, whereas the *TEF1* promoter is strong [55,59,60], we expected a marked difference in LTR-Ty1*his3AI* and P_{TEF1}-Ty1*his3AI* retromobility. A possible explanation of these data is that the LTR promoter is more strongly repressed in the chromosome, possibly by a high level of nucleosome occupancy at the LTR that is altered on the *CEN*-plasmid [60,75]. A second possibility that could be a consequence of, or independent of, the first, is that less Ty1i RNA is expressed from the plasmid-based Ty1*his3AI* element than a chromosomal element. Indeed, Ty1i RNA expressed from LTR-Ty1*his3AI* is not detectable (Fig 5C), and the modest increases in retromobility in head and middle subunit deletion mutants carrying LTR-Ty1*his3AI* supports the idea that expression LTR-Ty1*his3AI* reduces the Ty1i/Ty1 RNA ratio (Fig 5B). Potential differences in Ty1 or Ty1i RNA expression between chromosomal and plasmid elements warrants further investigation, as this might be one reason why different screens for Ty1 regulators yield largely non-overlapping gene sets [18].

Consistent with its role in Ty1 retromobility, Mediator also plays an important part of the host cell response to retroviruses such as HIV-1 [13,14,76]. Human Med4, and Med7 (orthologs to yeast Med4, and Med7, respectively), and Med28 (no known yeast orthologs) were identified as HIV-1

559 dependency factors [13,14]. Additionally, Med6, Med8, Med11, Med17, Med19, Med20, Med26,
560 Med27, and Med31 (all of which have yeast orthologs of the same name with the exception of Med19,
561 Med26, and Med27) were shown to be important activators of HIV-1 transcription [14,76]. While the
562 evidence of Mediator's role in Ty1 TSS selection contrasts mechanistically with the role that Mediator
563 takes in regulating HIV-1 transcription, the results presented here provide a novel mechanism by which
564 Mediator can influence retroviral transcripts [76]. Further work is needed to determine whether other
565 human retroviruses or retrotransposons encode internal transcripts and whether Mediator plays a role in
566 regulating TSS selection in these elements.
567 The relationship between altered Ty1 retromobility and altered Ty1i regulation reported here and
568 the CNC phenomenon, in which increased Ty1 copy number leads to increased Ty1i expression[22,25],
569 remains to be explored. It will be interesting to determine the molecular mechanism underlying CNC
570 and the specific role that promoter competition and Mediator complex plays in this phenomenon.
571

572 **Materials and Methods**

573 *Yeast Strains and Plasmids*

574 Strains used in this study are derivatives of BY4741. Genotypes of each strain are provided in S1
575 Table. Strains containing a chromosomal *his3AI-[Δ1]*-marked Ty1 element (Ty1*his3AI*-3114) were
576 described previously [19,77]. Recombination of the *his3AI-[Δ1]* allele with the *his3Δ1* allele present in
577 strain BY4741 derivatives does not result in a functional *HIS3* allele. Strains containing Mediator
578 subunit gene deletions were constructed via lithium acetate transformation with a *KanMX* allele as
579 described [78,79].
580 Plasmid pBDG633 is a *URA3-CEN* plasmid containing a Ty1*his3AI-[Δ1]* element, kindly
581 provided by Dr. David Garfinkel [80]. Plasmid pBJC1250 is a *LEU2- CEN* vector containing a
582 Ty1*his3AI-[Δ1]* element wherein the U3 region of the 5′ LTR was replaced with a *TEF1* promoter

583 (herein referred to as P_{TEF1}-Ty1*his3AI*; see Fig 5). Plasmid pBJC80, herein referred to as pGTy1ΔPOL,
584 has been described previously [64].
585
586 *Transposition Frequency Assay*
587 Ty1 mobility was determined as previously described [19]. Individual colonies from strains were
588 grown in triplicate in liquid YPD at 30°C overnight. Each culture was then diluted in quadruplicate by a
589 factor of 1000 in YPD broth and grown at 20°C to an optical density beyond log growth phase. 1μL of a
590 1:1000 dilution of each of the resulting 12 cultures was plated on YPD agar to provide an accurate
591 representation of the cell density. In parallel, 100μL to 1mL of each culture was plated on SC-HIS agar.
592 All plates were grown at 30°C for 3-4 days. Mobility frequency was calculated as a ratio of the number
593 of HIS+ colony forming units to the number of colony forming units in each culture as represented by
594 the number of colonies growing on YPD agar. For strains for which no HIS+ prototrophs were observed,
595 mobility was reported as an upper limit equal to the ratio of (1/the total number of colony forming units
596 in all three biological replicates).
597 For strains containing a plasmid, the above protocol was modified such that cultures were grown
598 in their respective selective media (SC-URA, or SC-LEU) at 30°C until confluent, diluted 1:1000 and
599 grown at 20°C in YPD until confluent, and plated on their respective dropout media (SC-URA, or SC-
600 LEU) as well as the corresponding media lacking histidine (SC-URA-HIS or SC-LEU-HIS).
601
602 *RNA Purification*
603 Cells were grown in YPD broth at 20°C to mid-log phase. Total cellular RNA was extracted
604 using a hot phenol/chloroform extraction protocol [81]. PolyA^+ RNA was purified from 250μg to 1mg
605 of total cellular RNA using the Magnetic mRNA Isolation Kit (New England Biolabs) following the
606 manufacturer's protocol.

607

608 *Northern Blot*

609 A 20µg aliquot of total cellular RNA was separated on a 1% Seakem GTG agarose gel as
610 previously described [82]. Three to six µg of poly A^+ RNA was separated on an 0.8% Seakem GTG
611 agarose gel as previously described [82]. Separated RNA was transferred to a Hybond XL membrane
612 (GE Healthcare) using a gradient of 6X SSC to 10X SSC overnight at room temperature. Synthesis of
613 ^{32}P-labeled RNA riboprobes was carried out in vitro using SP6 or T7 polymerase (New England
614 Biolabs). Membranes were incubated with probes in NorthernMax PreHyb Buffer (Ambion) at 65°C
615 overnight. Images were scanned using a Typhoon 9400 scanner, and quantified using ImageQuant
616 software (Molecular Dynamics, Sunnyvale, CA).

617

618 *Western Blot*

619 Cultures were grown at 20°C in YPD for one cell doubling (OD_{600} 0.3 to OD_{600} 0.6) after
620 dilution from overnight cultures grown at 30°C. Protein was extracted from total cell lysates as
621 previously described [83] and resolved on a 10% SDS-PAGE gel. When resolving p18- and p22-Gag, a
622 15% SDS-PAGE gel was used. Protein was then transferred to a polyvinylidene difluoride (PVDF)
623 membrane. Membranes were blocked in a 5% nonfat milk solution dissolved in phosphate buffered
624 saline (PBS) with 0.1% TWEEN 20. Membranes were then incubated in 0.5% nonfat milk in PBS with
625 0.1% TWEEN 20 with a 1:7500 dilution of affinity-purified anti-VLP antisera [50] to detect p49-Gag, a
626 1:5000 dilution of a polyclonal antibody specific to p18-Gag (a gift from David Garfinkel, described in
627 [25]), a 1:7500 dilution of anti GAPDH monoclonal antibody (Thermo Fisher Scientific), or a 1:5000
628 dilution of anti actin monoclonal antibody (Abcam). Membranes were subsequently incubated with
629 horseradish peroxidase (HRP)-conjugated secondary antibodies (Millipore). Following terminal washes,
630 membranes were incubated with SuperSignal West Pico chemiluminescence substrate (Pierce, Thermo

631 Fisher Scientific), and exposed to film (Kodak). Antibody was stripped from membranes as described
632 previously [84]. Images were developed on film using a Model SRX-101A Medical Scanner (Konica
633 Minolta) and scanned using a Cannon MP480 scanner. Protein bands were quantified using ImageJ
634 (NIH).
635
636 *Southern Blot*
637 Cultures were grown past log growth phase at 20°C in YPD broth. Total genomic DNA was
638 isolated as previously described [15,85], and digested with *Sph I* endonuclease. Digested genomic DNA
639 was then fractionated by gel electrophoresis on a 1% GTG agarose gel and subjected to Southern blot
640 analysis with a ^{32}P-labeled riboprobe specific for *POL* as described previously [15,31].
641
642 *cDNA Synthesis*
643 Polyadenylated RNA was used to synthesize cDNA using the First Strand cDNA Synthesis Kit
644 (Affymetrix). 100ng of polyA^{+} mRNA was used per reaction, as were 0.5µM concentration each of
645 primers specific to the Δ*POL* region of pGTy1ΔPOL (5′-
646 CCACCCATAATGTAATAGATCTATCGATTCTAGAC-3′) and to *ACT1* (5′-
647 ATCGTCCCAGTTGGTGACAATACC-3′). Reactions were performed according to manufacturer
648 protocols, and run at 44°C for 1 hour, followed by incubation at 92°C for 10 minutes. For comparison of
649 levels of full-length Ty1 and Ty1i transcripts generated from pGTy1ΔPOL (Fig 7), cDNA was PCR
650 amplified using primers binding 250 bp upstream of the Ty1i TSS (5′-
651 GATTCATCCTCAGCGGACTCTG-3′), or 250 bp downstream of Ty1i TSS (5′-
652 AGAAGAATGATTCTCGCAGC-3′), and the Δ*POL* region of the element (5′-
653 CCACCCATAATGTAATAGATCTATCGATTCTAGAC-3′). PCR product was isolated at different
654 cycle numbers and compared with amplification of *ACT1* (forward primer: 5′-

GGTTCTGGTATGTGTAAAGCCGGT-3′; reverse primer: 5′-ATCGTCCCAGTTGGTGACAATACC-3′) to control for relative cDNA abundance.

ChIP-seq

For analysis of Mediator occupancy at Ty1 elements (Fig 8), chromatin immunoprecipitation followed by high throughput sequencing (ChIP-seq) was performed using strains in which Mediator subunits Med15 or Med17 carried 13-myc epitope tags and which were engineered to allow Kin28 inactivation by the anchor away technique (S1 Table) [65]. For anchor away experiments, yeast were grown in YPD to an OD_{600} of 0.8. Rapamycin was then added to a concentration of 1 μg/mL (from a 1 mg/mL stock in ethanol stored at -20˚C for not more than one month) and cultures allowed to grow one hr at 30˚C prior to cross-linking. ChIP against epitope-tagged Mediator subunits was carried out as described previously [56], using 2 μg of anti-myc antibody (clone 9E10, Sigma) and protein G sepharose beads for capture (GE Healthcare). Library preparation for Illumina sequencing was performed using the NEBNext Ultra II library preparation kit (New England Biolabs) according to manufacturer's directions. Libraries were bar-coded using NEXTflex barcodes (BIOO Scientific) and sequenced on the Illumina NextSeq platform at the Wadsworth Center, New York State Department of Health.

Unfiltered paired-end sequencing reads were aligned to the *S. cerevisiae* reference genome (Saccer3) by using BWA [86]. Up to one mismatch was allowed for each aligned read; reads mapping to multiple locations were retained and randomly assigned. Duplicate reads were removed based on paired end information. Occupancy profiles for Ty1 elements were generated by averaging the signal of all the 31 Ty1 elements. The occupancy is plotted on the window from 2kb upstream of TSS to 2kb downstream of TES (Fig 4 & 8). In each Ty1 element ORF, from TSS+1kb to TES-1kb, the region is

678 divided into 100 bins and the average occupancy of each bin was calculated. For the flanking regions,
679 the occupancy was calculated for each base pair.
680
681 *Data Deposition*
682 ChIP-seq data has been deposited in Arrayexpress under accession number E-MTAB-5824. Data
683 used in Figure 4B is available from the NCBI Sequence Read Archive under accession number
684 SRP047524.
685
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691
692
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Figure Legends

Fig 1: The Ty1 retrotransposon lifecycle and transcriptional regulation (adapted from [18]). (A) The Ty1 mobility lifecycle. Following transcription, Ty1 RNA is exported to the cytoplasm where it is localizes co-translationally to a microscopically distinct cytoplasmic focus known as the retrosome. The retrosome is the site of assembly of the VLPs, which serve as the sites for Ty1 protein maturation and reverse transcription of Ty1 cDNA. Ty1 cDNA is then transported back into the nucleus and integrated into the host genome. (B) Ty1 contains two ORFs (*GAG* and *POL*) flanked by a 5′ and a 3′ LTR. Ty is transcribed from the 5′ untranslated region (R-U5) in the 5′ LTR, and it terminates within the 3′ LTR. In addition to the primary Ty1 transcript, the Ty1i transcript initiates within the *GAG* ORF to produce a 5.0 kb truncated transcript. Antisense (AS) RNA is also transcribed from within the *GAG* ORF to the 5′ LTR. (C) The Ty1 5′ LTR as well as the first 1 kb of the *GAG* ORF contain numerous transcription factor binding sites, as well as two TATA elements (T1 and T2). The transcription termination sites in the 3′ LTR (TS1 and TS2) are located in the R-U5 region.

Fig 2: Mediator subunit deletions influence Ty1 mobility in a module-specific manner. (A) The Mediator transcriptional coactivator complex is composed of head (red), middle (blue), tail (yellow), and kinase (grey) modules (figure adapted from [37]). Individual subunits investigated in this study are labeled. (B) The frequency of retrotransposition of the chromosomal Ty1*his3AI*-3114 element was measured in congenic WT, *spt3*Δ and Mediator subunit deletion strains that each lack an individual, non-essential Mediator subunit. Error bars represent standard deviation for three biological replicates. Asterisks denote values that represent upper limit retrotransposition estimates in strains with no retrotransposition events among the total number of cells assayed. Bars are color-coded to match structural organization as shown in (A).

Fig 3: Mediator subunits influence Ty1 cDNA levels without altering levels of Ty1 RNA or Gag protein. (A) Quantitative northern blot analysis of sense-strand (Ty1) and antisense-strand (Ty1 AS) RNA. Northern membranes were probed for Ty1 AS RNA, followed by stripping and probing for Ty1 RNA using strand-specific riboprobes, and for 18S rRNA as a loading control. Image quantification is representative of two biological replicates. The graph on the right shows the quantitation of Ty1 RNA and Ty1 AS RNA levels, each relative to the 18S subunit rRNA level. (B) Western blot of total cell lysates probed for Gag using anti-VLP antibodies. Asterisks indicate values derived from two biological replicates. All other values represent those measured from three biological replicates. (C) Quantitative Southern blot analysis to determine the level of unintegrated Ty1 cDNA (cDNA) relative to the amount of DNA in bands representing two genomic Ty1 elements (G1 and G2). Image quantification is representative of three biological replicates, only one of which is shown. All experiments were performed using congenic WT, *spt3*Δ and Mediator subunit deletion strains harboring Ty1*his3AI*-3114. Bars are color coded as in Figure 2.

Fig 4: Deletion of the Mediator tail increases polyA$^+$ Ty1i RNA and p22-Gag translation. (A) Northern blot probed for Ty1, Ty1i and *PYK1* RNA, the latter as a loading control. Image is representative of three biological replicates. Quantification of Ty1 or Ty1i RNA relative to *PYK1* RNA is an average of three biological replicates, unless marked with an asterisk, which denotes two biological replicates. (B) Pol II occupancy in the first 1kb of the Ty1 element is reduced in *med3*Δ *med15*Δ yeast. ChIP-seq data from [56] was analyzed to obtain Pol II occupancy averaged over all 31 genomic Ty1 elements. (C) Western blot of total cell lysate measuring levels of p22- and p18-Gag relative to the loading control, GAPDH. The p22-Gag and p18-Gag were measured using p22-polyclonal antibody. Image is representative of three biological replicates, and quantitation is the average ratio of p22-Gag or p18-Gag to GAPDH signal from three biological replicates of each strain. In panels (A) and (C),

quantitation was performed on RNA or protein samples from the WT strain and congenic *spt3*Δ strain as a negative control (grey bars), Mediator head subunit gene deletion strains (red bars), middle subunit gene deletion strains (blue bars) and tail subunit gene deletion strains (yellow bars).

Fig 5: The Mediator tail acts on Ty1 mobility in an LTR promoter-dependent manner. (A) Schematic of the P_{TEF1}-Ty1*his3AI* element relative to a Ty1*his3AI* element with the standard LTR promoter. P_{TEF1}-Ty1*his3AI* has a *TEF1* promoter in place of Ty1 promoter elements in the U3 region of the 5′ LTR (See Fig 1C). P_{TEF1}-Ty1*his3AI* retains the Ty1 TSS and R-U5 region of the 5′ LTR. (B) Retrotransposition frequency for a plasmid-based P_{TEF1}-Ty1*his3AI* (right) or LTRTy1*his3AI* (left) element in the WT and *spt3*Δ negative control strains (grey bars), Mediator head subunit gene deletion strains (red bars), middle subunit gene deletion strains (blue bars) and tail subunit gene deletion strains (yellow bars). Retrotransposition frequency is derived from three biological replicates. Asterisks denote values that represent upper limit mobility estimates in strains that had no His^+ prototrophs per total number of Ura^+ or Leu^+ cells analyzed. (C) Northern blot performed using 6 µg of $polyA^+$ RNA from strains containing a plasmid-based *his3AI*-marked Ty1 under control of the native promoter (LTRTy1*his3AI*) or the *TEF1* promoter (P_{TEF1}-Ty1*his3AI*). The blot was probed with a sense-strand *HIS3* riboprobe to detect Ty1*his3AI* and Ty1i*his3AI* RNA. All lanes shown are from a single gel. The values reported in the graph are the average ratio of Ty1*his3AI* RNA relative to *PYK1* RNA in two biological replicates. Bars are color-coded as in (B).

Fig 6: Internal Ty1iΔPOL transcript is expressed at higher levels than full-length Ty1ΔPOL RNA from pGTy1ΔPOL in yeast grown in glucose medium. Top: Schematic of the *GAL1*:Ty1ΔPOL cassette in pGTy1ΔPOL showing forward primer locations for detection of Ty1 RNA (blue) versus Ty1i RNA (red). Both amplifications utilized the same reverse primer (purple), that crosses the deletion

979 junction and contains sequences unique to the pGTy1ΔPOL element. A reverse primer specific for
980 ACT1 was also used to synthesize cDNA used as a template for the PCR amplification. No PCR product
981 was detected using the ΔPOL reverse primer when RNA from yeast lacking pGTy1ΔPOL was used as
982 template (data not shown). Bottom: Reverse Transciption-PCR reactions using polyA$^+$ RNA isolated
983 from strains of the indicated genotype bearing plasmid pGTy1ΔPOL, and grown in glucose-containing
984 broth. Aliquots were taken from reactions at the indicated number of cycles and analyzed by agarose gel
985 electrophoresis. RT-PCR amplification products of Ty1, Ty1i, and *ACT1* RNA are indicated. The same
986 WT samples were used for all panels; results were similar for a second biological replicate of all three
987 samples (WT, *med20*Δ, and *med3*Δ).
988
989 **Fig 7: Increased internal Ty1iΔPOL transcript levels in Mediator tail mutant triad deletion**
990 **mutants.** (A) Northern blot probed with a Ty1 antisense riboprobe that hybridizes to Ty1, Ty1i,
991 Ty1ΔPOL and Ty1iΔPOL RNA. Note that the full-length Ty1ΔPOL transcript cannot be distinguished
992 from the rRNA band. Image is representative of three biological replicates. (B) Quantification for Ty1,
993 Ty1i, and Ty1iΔPOL bands from (A). Quantification is representative of three biological replicates.
994 Graph bar colors correspond to WT strain and the congenic *spt3*Δ strain as a negative control (grey
995 bars), Mediator head subunit gene deletion strains (red bars), middle subunit gene deletion strains (blue
996 bars) and tail subunit gene deletion strains (yellow bars). (C) Retrotransposition frequency of the
997 chromosomal Ty1*his3AI*-3114 element in WT, congenic *spt3*Δ, and Mediator subunit deletion strains
998 bearing a plasmid containing pGTy1ΔPOL. Asterisks denote an upper limit mobility estimate in strains
999 with no His+ prototrophs among total cells plated. Graph bars are color-coded as in (B).
1000
1001 **Fig 8: Mediator occupancy at the Ty1 and Ty1i proximal promoters.** Occupancy of Med17-myc and
1002 Med15-myc from the Mediator head and tail modules, respectively, was determined by ChIP-seq and

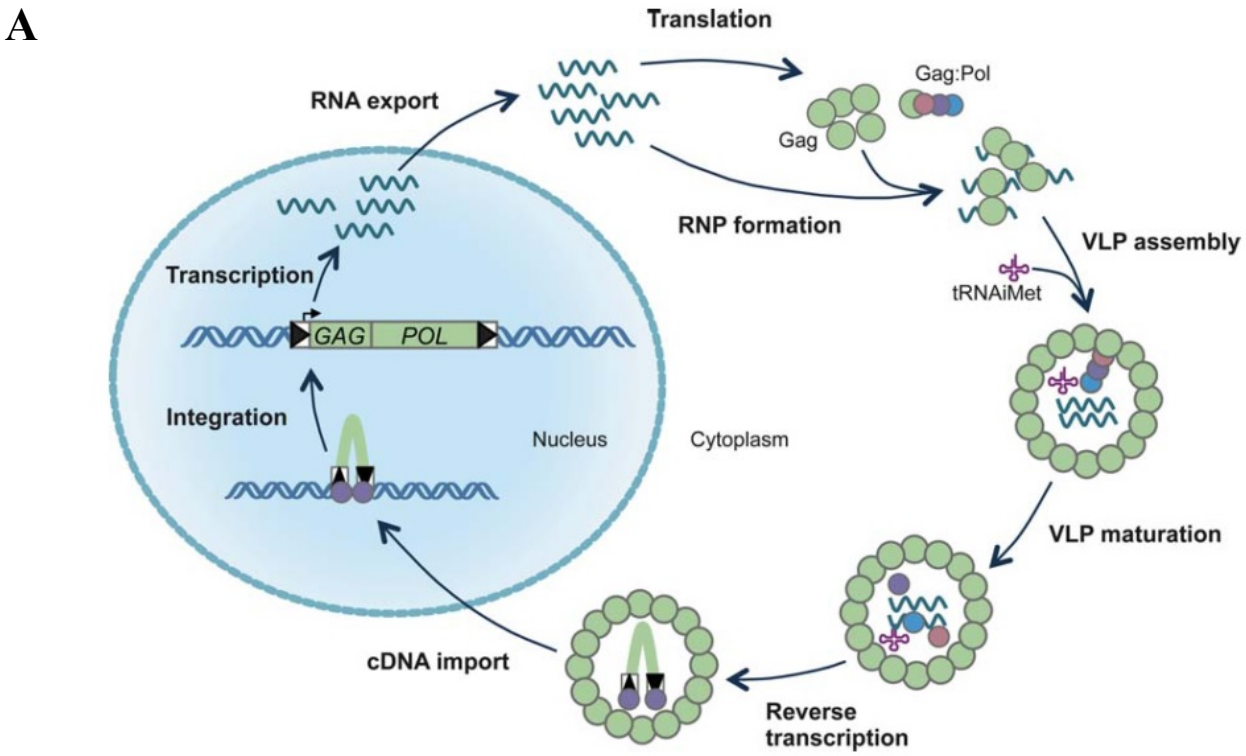
summed over all Ty1 elements in *kin28-AA* yeast that were otherwise wild type (WT), *med20*Δ, or carried the triple deletion *med2*Δ *med3*Δ *med15*Δ. ChIP-seq signals were normalized to an untagged *kin28-AA* control (S1 Fig). The upstream occupancy peak is slightly downstream of the Ty1 TSS, likely reflecting the location of Ste12 and Tec1 binding sites that are required for Ty1 transcription. Reads deriving from the Ty1 TSS, and therefore in the LTR, are unavoidably also assigned to the 3' LTR, leading to the observed signal in that region.

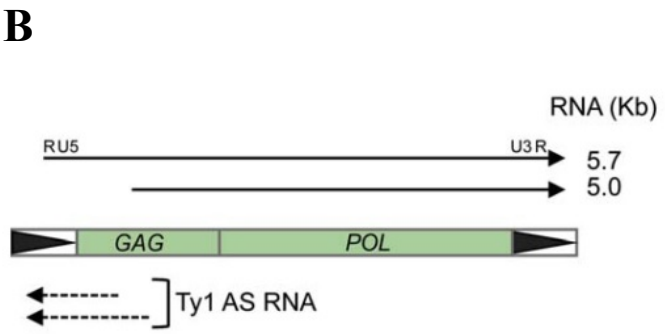
Fig 9: Promoter competition model for Mediator influence on Ty1 mobility. In wild type yeast, the Mediator complex acts at both the Ty1 and Ty1i proximal promoters, stimulating robust transcription of Ty1 and a small amount of Ty1i expression that is sufficient to restrict mobility. Deletion of subunits from the tail module triad increases Mediator activity at the Ty1i promoter, thus increasing Ty1i production and reducing retromobility. Conversely, when a subunit from the head module is deleted, the association between Mediator and the Ty1i promoter is disrupted, permitting increases in retromobility. Finally, when the SAGA-dependent Ty1 promoter is swapped for the strong, TFIID-dependent *TEF1* promoter, the complex preferentially associates with the Ty1 promoter, irrespective of the influence of the tail module.

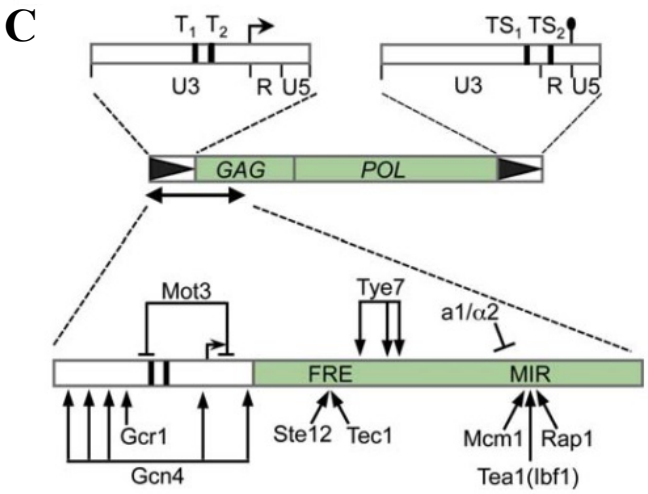
S1 Figure. Occupancy of Mediator over Ty1 elements determined by ChIP-seq. Top, untagged control subjected to ChIP-seq using anti-myc antibody. Bottom, occupancy of myc-tagged Med15 and Med17 over summed Ty1 elements in *med18*Δ *kin28-AA* yeast. Note the absence of any Mediator peak at the Ty1i TSS.

S1 Table. Yeast strains used in this work.

1027







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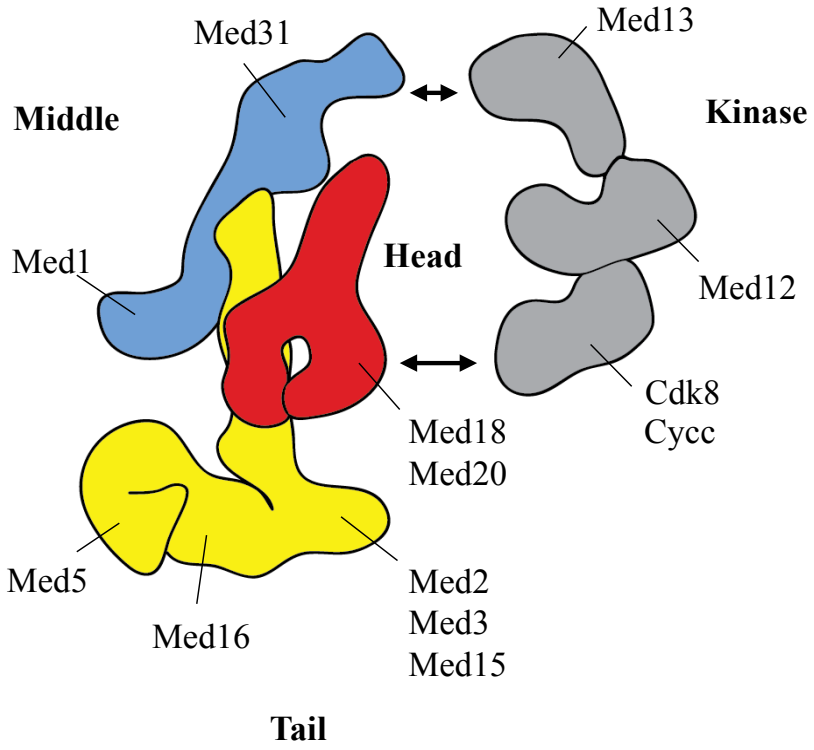

B