# A Role for Mediator Core in Limiting Coactivator Recruitment in *Saccharomyces cerevisiae*

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**ABSTRACT** Mediator is an essential, multisubunit complex that functions as a transcriptional coactivator in yeast and other eukaryotic organisms. Mediator has four conserved modules, Head, Middle, Tail, and Kinase, and has been implicated in nearly all aspects of gene regulation. The Tail module has been shown to recruit the Mediator complex to the enhancer or upstream activating sequence (UAS) regions of genes via interactions with transcription factors, and the Kinase module facilitates the transition of Mediator from the UAS/enhancer to the preinitiation complex via protein phosphorylation. Here, we analyze expression of the *Saccharomyces cerevisiae HO* gene using a *sin4* Mediator Tail mutation that separates the Tail module from the rest of the complex; the *sin4* mutation permits independent recruitment of the Tail module to promoters without the rest of Mediator. Significant increases in recruitment of the SWI/SNF and SAGA coactivators to the *HO* promoter UAS were observed in a *sin4* mutant, along with increased gene activation. These results are consistent with recent studies that have suggested that the Kinase module functions negatively to inhibit activation by the Tail. However, we found that Kinase module mutations did not mimic the effect of a *sin4* mutation on *HO* expression. This suggests that at *HO* the core Mediator complex (Middle and Head modules) must play a role in limiting Tail binding to the promoter UAS and gene activation. We propose that the core Mediator complex helps modulate Mediator binding to the UAS regions of genes to limit coactivator recruitment and ensure proper regulation of gene transcription.

**KEYWORDS** gene regulation; mediator; chromatin; coactivator.

**M**EDIATOR is a large multisubunit transcriptional coactivator complex that is conserved throughout eukaryotes. Mediator was first identified in budding yeast as a bridge between general transcription factors (GTFs) and RNA polymerase II (RNAPII) (Thompson *et al.* 1993; Kim *et al.* 1994; Koleske and Young 1994). Currently, Mediator has been implicated in nearly all facets of gene regulation: transcriptional initiation and elongation (reviewed in Malik and Roeder

(2000), Poss *et al.* (2013), Soutourina 2018), chromatin architecture (Allen and Taatjes 2015; Hsieh *et al.* 2015), messenger RNA (mRNA) processing and export (Huang *et al.* 2012; Schneider *et al.* 2015), and transcriptional memory (A. Zhang *et al.* 2013; D'Urso *et al.* 2016). Together, these activities allow Mediator to modulate activation and expression required for proper gene regulation.

In yeast, mediator contains 25 subunits that are organized into four conserved modules: Head, Middle, Tail, and Kinase. The Head and Middle modules are essential for viability, and thus make up the "core" Mediator (Jeronimo and Robert 2017). Both of these modules interact with RNAPII as well as with GTFs required for transcriptional initiation and elongation. The Tail module attaches to the core Mediator through the Med14 (Rgr1) scaffold subunit (Tsai *et al.* 2014), and interacts with sequence-specific transcription factors that recruit the Mediator complex to the upstream activating sequence (UAS) or enhancers of genes (Bhoite *et al.* 2001; Kagey *et al.* 2010; Jeronimo and Robert 2014).

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Transcriptional activators primarily interact with proteins in the Tail module (Jeronimo and Robert 2017), although there are examples where activators interact with Middle module subunits (Harms et al. 2015; Iida et al. 2015). The Tail module is also required for SWI/SNF recruitment to the yeast CHA1 gene (Ansari et al. 2016). Importantly, the Tail module is required for efficient recruitment of Mediator and RNAPII to promoters (Knoll et al. 2018). The Kinase module is freely dissociable and attaches to the Middle module of Mediator via an interaction with its Med13 subunit. As part of the Mediator complex, the Kinase/CDK8 module antagonizes the function of the Tail module (van de Peppel et al. 2005; Jeronimo and Robert 2017) and facilitates the transition of Mediator from the UAS to the preinitiation complex (Jeronimo et al. 2016; Petrenko et al. 2016).

Distinct forms of Mediator have been identified with and without the dissociable Kinase module (Poss et al. 2013), and additional stable forms have been identified through mutations of specific Mediator subunits. Deletion of the SIN4 (MED16) gene or a C-terminal truncation of the Med14 scaffold subunit each results in a stable tail-less Mediator complex and an independent Tail subcomplex composed of Med2, Pgd1 (Med3), and Gal11 (Med15) (Li et al. 1995). Yeast cells lacking the Mediator tail complex are viable but have pronounced defects in regulating a large subset of genes (Li et al. 1995; Larsson et al. 2013). This is likely due to the inability to recruit core Mediator and the CDK8/Kinase module to the UAS of these genes (Jeronimo et al. 2016; Petrenko et al. 2016). Changes in global chromatin structure (Macatee et al. 1997) and increased long-distance transcription factor activation (Dobi and Winston 2007) have also been reported during separation of the Tail subcomplex via disruption of SIN4.

Disruption of SIN4 has been studied extensively at the HO promoter (Stillman et al. 1994; Tabtiang and Herskowitz 1998; Yu et al. 2000; Li et al. 2005). HO expression is under substantial regulation (Stillman 2013) and typically requires sequential activation at two distinct upstream regulatory sequence (URS) regions, URS1 and URS2 (Nasmyth 1985; Cosma et al. 1999; Bhoite et al. 2001). Sequence-specific transcription factors Swi5 and SBF (Swi4/6 complex) bind to URS1 and URS2, respectively, and recruit the SWI/SNF, Spt-Ada-Gcn5 acetyltransferase (SAGA), and Mediator coactivator complexes. Loss of any of these factors results in defective HO activation. However, disruption of SIN4 suppresses the requirement for SBF binding to URS2, and allows robust HO expression even when the Gcn5 catalytic subunit of SAGA is mutated (Yu et al. 2000). Mediator is initially recruited to the HO promoter via an interaction between Swi5 and the Gal11 Tail subunit (Bhoite et al. 2001), and it is likely that disruption of SIN4 results in failure to recruit the core Mediator and/or the Kinase/CDK8, and/or Tail-less core Mediator, to the URS regions.

In this study, we further investigated the effects of disrupting SIN4 at the HO promoter, and we explored the mechanism behind the resulting suppression of key regulatory events. We found that disrupting SIN4 resulted in significant increases in both transcription factor and coactivator binding at the HO promoter, and that these increases are mostly due to prolonged persistence of these factors at the promoter. In agreement with this result, we also observed elevated and persistent nucleosome eviction during HO promoter activation. These results likely explain the suppression of multiple HO promoter mutants by sin4Δ. Surprisingly, we were unable to reproduce these results by mutating the catalytic subunit of the Kinase module, ruling out the simple model that the observed effects were due to loss of antagonistic effects of the Kinase module on Tail function. Rather, we found that elevated coactivator binding and suppression of promoter mutants were completely dependent on the presence of the Mediator Tail subcomplex. As we do not observe these effects with whole Mediator, we propose that the core Mediator must restrict the binding of either the Tail subcomplex or other transcription factors/coactivators to limit promoter activation and ensure proper regulation of gene transcription.

## Materials and Methods

### Experimental methods

All yeast strains used in this study are listed in Supplemental Material, Table S1 and are isogenic in the W303 background (Thomas and Rothstein 1989). Standard genetic methods were used for strain construction (Rothstein 1991; Sherman 1991). The 5X-sbf, +700, and +1300 HO promoter mutants were described in Yarrington et al. (2015), the ho(m-2700) mutation was described in Yu et al. (2016), and the HO-GFP-NLS-PEST reporter was described in Mitra et al. (2006). C-terminal epitope tags were added as described (Knop et al. 1999), using plasmids pZC03 (pFA6a-TEV-6xGly-V5-HIS3MX; #44073; Addgene plasmid) and pZC13 (pFA6a-TEV-6xGly-V5-HphMX; #44085; Addgene plasmid), provided by Zaily Connell and Tim Formosa, and plasmid pYM6 (Knop et al. 1999), provided by Elmar Schiebel. Strain YTT1722 with a SWI2:FLAG(3):KanMX tag (Kim et al. 2006) was provided by David Clark, and the marker swap method (Voth et al. 2003) was used to convert it to SWI2:FLAG(3):NatMX using plasmid pAG25 (Goldstein and McCusker 1999) provided by John McCusker.

Cell cycle synchronization was performed by galactose withdrawal and readdition with a GALp::CDC20 strain grown at 25° in yeast extract and peptone medium containing 2% galactose and 2% raffinose (Bhoite et al. 2001). A high degree of synchrony was confirmed by examination of budding indices and analysis of cycle-regulated mRNAs. In all other experiments, cells were grown at 30° in (yeast extract, peptone, adenine, and dextrose) medium (Sherman 1991).

Chromatin immunoprecipitations (ChIPs) were performed as described previously (Bhoite et al. 2001; Voth et al. 2007) using mouse monoclonal antibody to the V5 epitope (SV5-Pk1, Abcam), the FLAG epitope (M2; Sigma [Sigma Chemical], St. Louis, MO), the Myc epitope (4A6; Upstate), or
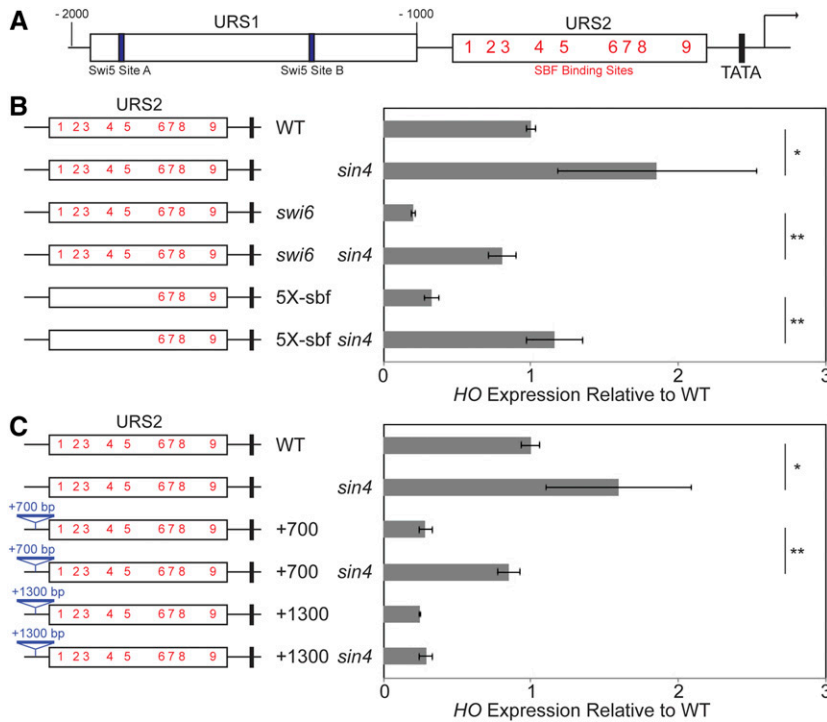
**Figure 1** A *sin4* mutation rescues expression of *HO* promoter mutants. (A) The diagram shows the structure of the *HO* promoter, including the two Swi5-binding sites at URS1 and the nine SBF-binding sites in URS2. (B) *HO* mRNA levels were measured for the various mutant *HO* promoters indicated on the left, in either WT, *sin4*, *swi6*, or *swi6 sin4* mutants. The error bars reflect the SD of three biological samples. (C) *HO* mRNA levels were measured for the various mutant *HO* promoters indicated on the left, in either WT or *sin4* mutants. The error bars reflect the SD of three biological samples. *$P < 0.05$, **$P < 0.01$. mRNA, messenger RNA; URS, upstream regulatory sequence; WT, wild-type.

anti-histone H3 (07-690, Upstate), and antibody-coated magnetic beads (rabbit and pan-mouse IgG beads, Life Technologies). Samples prepared for ChIPs were cross-linked in 1% formaldehyde overnight on ice. ChIP assays were analyzed by real time quantitative PCR (qPCR) as described previously (Eriksson *et al.* 2004). H3 samples were first normalized to the ChIP signal at the IGR-I gene-free reference region on chromosome I (Mason and Struhl 2005), while Swi4-V5 ChIP samples were first normalized to the *CLN1* or *CLN2* promoter, and then both types of ChIPs were normalized to their respective input DNA samples. Unless otherwise noted, error bars reflect the SD of at least three biological samples. *P*-values were calculated by paired Student's *t*-tests.

RNA was isolated from either synchronized or logarithmically growing cells, and *HO* mRNA levels were measured by RT-qPCR as described previously (Voth *et al.* 2007). For all logarithmically grown strains, RNA expression was normalized to *RPR1* expression and graphed relative to wild-type (WT) expression. For the synchrony experiment, RNA expression was normalized to *RPR1* expression and graphed relative to the peak WT expression. Unless otherwise noted, error bars reflect the SD of at least three biological samples. *P*-values were calculated by paired Student's *t*-tests.

Single-cell analysis of *HO* expression was performed by time-lapse fluorescence microscopy as described previously (Q. Zhang *et al.* 2013).

### Data availability

Strains are listed in Table S1 and are available upon request. Oligos used for RT-qPCR, for ChIP, and for strain construction are listed in Table S2. Supplemental material (including five supplemental figures and two supplemental tables) available at figshare: https://doi.org/10.25386/genetics.12111399

## Results

### Disruption of SIN4 can rescue HO promoter mutants that affect activation of URS2

We have previously demonstrated that *sin4*Δ can rescue *HO* expression when the Swi6 subunit of SBF is also disrupted (Yu *et al.* 2000). However, the SBF complex is an important transcriptional regulator with numerous binding sites at genes controlling the G1/S transition (Andrews and Herskowitz 1989), and our previous suppression result could have been impacted by alterations to the yeast cell cycle caused by the *swi6* mutation. To demonstrate the impact of *sin4*Δ specifically at the *HO* gene, we made use of *HO* promoter mutants that either eliminated SBF sites at the left-half of URS2 (URS2L) or increased the distance between URS1 and URS2 (Figure 1; Yarrington *et al.* 2015). Both of these mutation classes drastically reduce SBF binding to the entire URS2 region with corresponding decreases in *HO* expression (Yarrington *et al.* 2015).

*HO* expression results confirmed previous findings that SBF is essential for WT levels of *HO* expression and that *sin4*Δ is capable of suppressing a *SWI6* disruption (Figure 1B). Interestingly, we observed that *sin4*Δ alone resulted in elevated *HO* expression compared to WT; some of this increase is due to *HO* expression in daughter cells (see Figure 7C). Additionally, mutation of the five SBF sites at the left half of URS2 (the 5x-sbf construct, Figure 1B) or inserting 700 bp
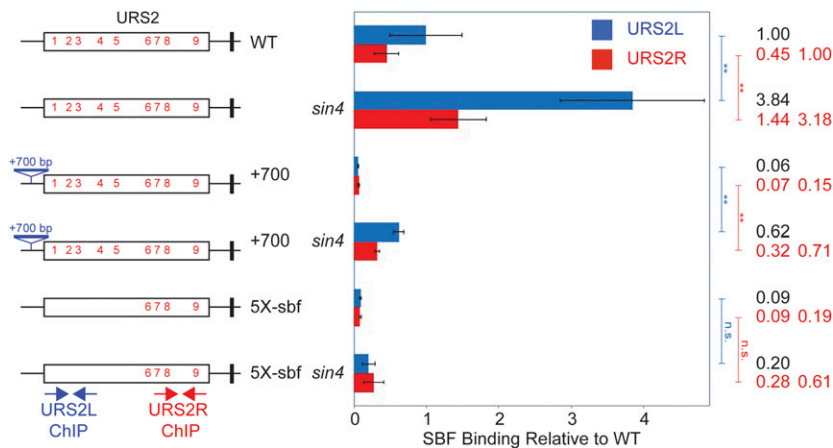
**Figure 2** A *sin4* mutation rescues SBF binding in *HO* promoter mutations. SBF binding was measured by ChIP assays of the Swi4-V5 subunit of SBF binding to the various mutant *HO* promoters indicated on the left, in either WT or *sin4* mutants. SBF binding to the left (blue) or the right (red) parts of URS2 was measured, using the primers indicated on the diagram. The first column on the right displays enrichment values relative to the native URS2L part of the promoter, and the second column on the right displays enrichment values relative to the native URS2R part of the promoter. The error bars reflect the SD of three biological samples. **$P$ < 0.01. ChIP, chromatin immunoprecipitation; URS, upstream regulatory sequence; WT, wild-type.

of *CDC39* exon sequence between URS1 and URS2 (+700, Figure 1C) resulted in decreases in *HO* expression, similar to those seen in a *swi6* mutant. However, in both cases disruption of *SIN4* rescued *HO* expression to levels similar to that of WT. As mutating the *HO* promoter does not affect cell cycle progression, these results indicate that suppression of *swi6*Δ by *sin4*Δ is not an artifact of cell cycle impairment. Furthermore, as SBF binding to the right half of URS2 (URS2R) is essential for *HO* expression (Yarrington *et al.* 2015), these results suggest that *sin4*Δ or Mediator Tail separation allows SBF binding to URS2R under circumstances when it is normally prohibited, such as mutation of SBF sites at URS2L, or insertion of 700 bp between URS1 and URS2. This suppression is not without limits as the reduced *HO* expression caused by a 1300-bp insertion between URS1 and URS2 (+1300) was not suppressed by *sin4*Δ (Figure 1C). Similarly, *sin4*Δ is unable to suppress mutations of the four SBF binding sites on the right side of URS2 (data not shown).

### Disruption of SIN4 rescues SBF binding to URS2 in HO promoter mutants

We next wanted to test the hypothesis that disruption of *SIN4* rescues SBF binding to URS2 in our *HO* promoter mutants. We have previously shown that mutation of SBF sites at URS2L or increasing the distance between URS1 and URS2 inhibits binding of SBF to the entire URS2 region (Yarrington *et al.* 2015). To test whether *sin4*Δ can rescue SBF binding, we performed a Swi4-V5 ChIP in strains with these *HO* promoter mutations and probed SBF binding at both URS2L and URS2R (Figure 2).

As expected, SBF enrichment at URS2L is over twofold higher than at URS2R in WT cells, despite similar numbers of SBF sites (Figure 2). Preferential enrichment to URS2L has been observed previously (Takahata *et al.* 2011; Yarrington *et al.* 2015) and is due to closer proximity to remodeling events initiated at the upstream URS1 region. Interestingly, a *sin4*Δ mutation results in an almost threefold increase in SBF binding compared to WT, at both URS2L and URS2R, and this elevated SBF binding likely explains the similar

increase in *HO* expression of this mutant. In the +700 and 5X-sbf *HO* promoter mutants, disruption of *SIN4* resulted in similar increases in SBF binding to URS2R to levels around two-thirds that of WT, and around fourfold higher than that of the single mutants without *sin4*Δ (Figure 2). In cell cycle synchrony experiments, we also see prolonged SBF binding to *HO* in the *sin4*Δ mutant compared to WT (Figure S1). These results support the model that *sin4*Δ or separation of the Mediator Tail subcomplex enhances SBF binding to URS2, even when combined with mutations that normally block SBF binding.

### sin4Δ-mediated suppression of HO is dependent on Swi5 and Gal11

We have shown that separation of the Mediator Tail subcomplex enhances SBF binding to URS2 and suppresses defects in *HO* expression in certain promoter mutants, but the mechanism behind this suppression remains largely unknown. To investigate this mechanism further, we combined *sin4*Δ with other mutations known to affect *HO* expression.

We first focused on the approximately two- to threefold increase in both *HO* expression and SBF binding observed in *sin4*Δ relative to WT. Similar increases occur when the gene for the daughter-specific inhibitor Ash1 factor is disrupted (Takahata *et al.* 2011; Stillman 2013). However, combining *sin4*Δ with an *ASH1* disruption revealed that these two mutations are additive for *HO* expression (Figure S2) and are therefore not working together in the same pathway.

We next investigated the Swi5 transcription factor that recruits Mediator to URS1 to initiate *HO* activation (Bhoite *et al.* 2001). Combining *sin4*Δ with a *SWI5* disruption blocked promoter activation similarly to that of *swi5*Δ alone, and *swi5*Δ *sin4*Δ failed to activate expression from the 5X-sbf *HO* promoter mutant with five SBF site mutations (Figure 3A). This result indicates that *sin4*Δ-mediated suppression does not bypass normal activation of the *HO* promoter and its dependence on Swi5. We further probed the relationship between *sin4*Δ and Swi5 by examining Swi5-V5 enrichment levels during logarithmic growth by ChIP at its two binding

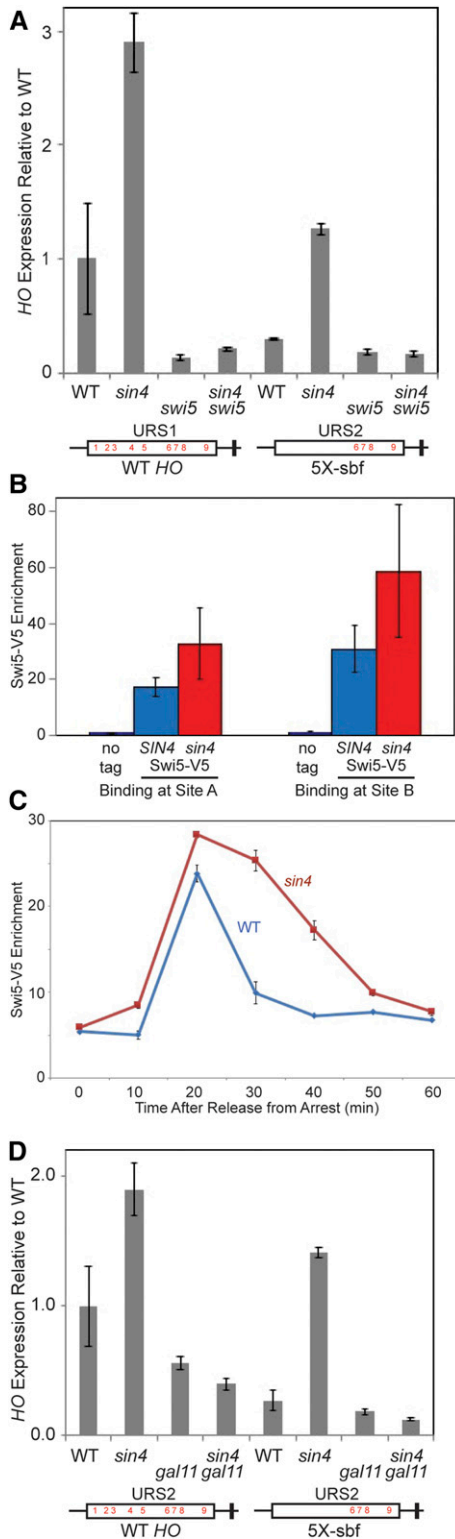**Figure 3** The *sin4* suppression of *HO* expression is dependent on Swi5 and Gal11. (A) The *sin4* suppression of *HO* expression is dependent on Swi5. *HO* mRNA levels were measured for the WT *HO* promoter (left four columns) or the 5X-sbf *HO* promoter mutant (right four columns), in either WT, *sin4*, *swi5*, or *sin4 swi5* strains. The error bars reflect the SD of two biological samples. (B) Swi5-V5 binding by ChIP is higher in *sin4* mutants in log phase cells at both site A (−1819) and site B (−1308); see diagram in Figure 1A. The error bars reflect the SD of three biological

samples. For the left panel, $P = 0.11$, and for the right panel, $P = 0.10$. (C) Swi5-V5 binding by ChIP at site B is higher in *sin4* mutants in cells synchronized with a *GALp:CDC20* arrest and release. The error bars reflect the SD of PCR replicates. (D) The *sin4* suppression of *HO* expression is dependent on Gal11. *HO* mRNA levels were measured for the WT *HO* promoter (left four columns) or the 5X-sbf *HO* promoter mutant (right four columns), in either WT, *sin4*, *gal11*, or *sin4 gal11* strains. The error bars reflect the SD of two biological samples. ChIP, chromatin immunoprecipitation; mRNA, messenger RNA; WT, wild-type.
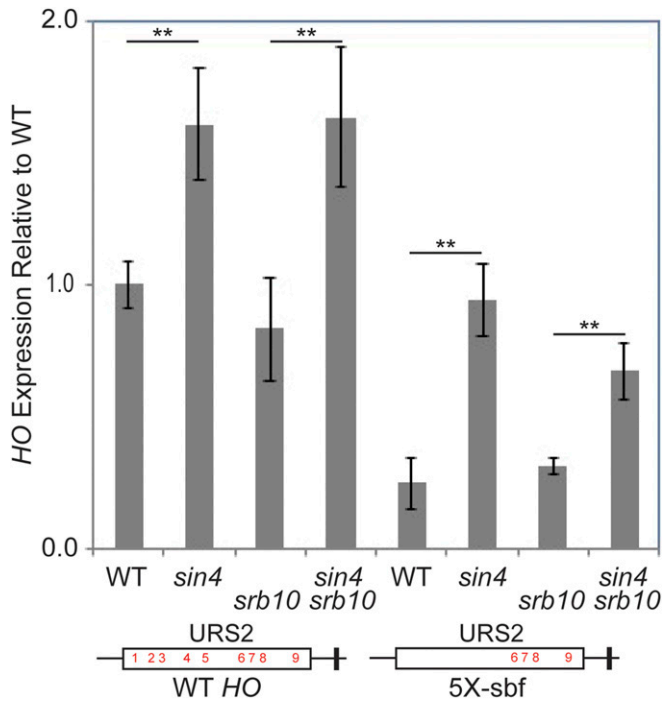
sites in URS1, sites A and B (Tebb *et al.* 1993). Comparing Swi5-V5 enrichment in WT and *sin4Δ* revealed an ∼50% increase in Swi5 binding at both sites when *SIN4* is disrupted (Figure 3B). Swi5 binding normally peaks 20 min after release from a G2/M arrest and quickly dissipates as the cell cycle progresses (Takahata *et al.* 2009b). To determine whether the increase in Swi5-V5 enrichment in *sin4Δ* was due to greater peak binding at 20 min after release or to a change in binding kinetics, we synchronized cells using *GALp:CDC20* arrest and examined Swi5-V5 binding after release. Total Swi5 binding at 20 min postrelease was largely similar between WT and *sin4Δ*, with the major difference being significant persistence of the Swi5 factor at URS1 in the *sin4* mutant (Figure 3C).

It has also been shown previously that Swi5 directly interacts with the Gal11 subunit of the Tail subcomplex, and that Mediator fails to bind when *GAL11* is disrupted (Bhoite *et al.* 2001). however, disruption of *SIN4* separates the Tail subcomplex from the rest of Mediator and it remains capable of binding independently of the rest of Mediator (Zhang *et al.* 2004; Ansari and Morse 2012). To address whether a *sin4* mutation affects binding of Mediator core to the *HO* promoter, we constructed WT and *sin4Δ* strains with epitope tags on three Mediator subunits: Gal11-V5, Nut1-Flag, and Srb4-Myc. Gal11(Med15) is a subunit of the Tail module, Srb4(Med17) is a subunit of the Head module, and Nut1(Med5) is a subunit of the Middle module of Mediator. ChIP experiments showed that while a *sin4Δ* mutation results in increased *HO* binding of Gal11-V5 from the Mediator Tail, binding of Nut1-Flag (Middle) and Srb4-Myc (Head) to the *HO* promoter are eliminated (Figure S3A). This ChIP result is consistent with the Tail module separating and binding independently from the rest of Mediator in a *sin4Δ* mutant. We previously showed that Srb4 transiently binds to the TATA region of the *HO* promoter at the time of expression (Bhoite *et al.* 2001). Binding of Nut1 and Srb4 to *HO* TATA is not reduced by a *sin4Δ* mutation (Figure S3B). This result is consistent with the report that core Mediator lacking the Tail associates with the TATA region of promoters via interactions with preinitiation complex components (Knoll *et al.* 2018).

To determine whether the Tail subcomplex was required for *sin4Δ*-mediated suppression, we next examined *HO* expression when *SIN4* and *GAL11* were disrupted either independently or together. The double mutant did not have elevated *HO* expression in the WT promoter and failed to

**Figure 4** Loss of the Kinase module with an *srb10* mutation does not affect *HO* expression. *HO* mRNA levels were measured for the WT *HO* promoter (left four columns) or the 5X-sbf *HO* promoter mutant (right four columns), in either WT, *sin4*, *srb10*, or *sin4 srb10* strains. The error bars reflect the SD of four biological samples. **$P < 0.01$. mRNA, messenger RNA; URS, upstream regulatory sequence; WT, wild-type.

rescue expression of the 5x-sbf site mutant *HO* promoter, indicating that *sin4*Δ-mediated suppression is dependent on functional Gal11 (Figure 3D). Furthermore, disruptions of other Tail subunits, *PGD1* and *MED2*, also blocked *sin4*Δ-mediated suppression (Figure S4). These results suggest that it is the independent recruitment of the Tail subcomplex, rather than the loss of core Mediator or its associated Kinase module, that is responsible for the observed suppression.

### sin4Δ-mediated suppression is not dependent on the mediator CDK module

The current model for Mediator-mediated promoter regulation proposes that the Kinase module antagonizes the function of the Tail module (van de Peppel *et al.* 2005; Jeronimo and Robert 2017). Disruption of *SIN4* leads to independent recruitment of the Tail subcomplex (Zhang *et al.* 2004), likely without the Kinase/CDK8 module (Tsai *et al.* 2014; Jeronimo *et al.* 2016; Petrenko *et al.* 2016), and our data are consistent with hyperactivation due to the loss of an inhibitor of promoter activation. Furthermore, the Srb10(Cdk8) catalytic subunit of the Kinase module has been shown to target Swi5 for degradation (Kishi *et al.* 2008), and failure to recruit the Kinase module might cause Swi5 protein levels to persist in a way congruent with our findings. However, this model is not consistent with the requirement of a functional Tail subcomplex for suppression. To better understand the role of the

Kinase module in *sin4*Δ-mediated hyperactivation of the *HO* promoter and suppression of promoter mutants, we examined *HO* expression when *SIN4* and *SRB10* were disrupted, either independently or together. As expected, disruption of *SIN4* resulted in elevated expression of the native promoter and suppression of the 5X-sbf promoter to WT levels (Figure 4). However, loss of Srb10 failed to show an appreciable effect on either the native or the mutant promoter, and *sin4*Δ *srb10*Δ double mutants produced expression results only modestly different from those of *sin4*Δ single mutants (Figure 4). These results indicate that loss of Kinase module activity has a minimal impact on *HO* expression. Additionally, these results further support the interpretation that *sin4*Δ-mediated hyperactivation of the *HO* promoter is due to independent recruitment of the Tail subcomplex rather than loss of the Kinase module associated with Mediator.

### Disruption of SIN4 results in elevated coactivator enrichment at the HO promoter

Swi5 recruits the SWI/SNF, SAGA, and Mediator coactivator complexes to URS1, and SBF recruits these same complexes to URS2 (Takahata *et al.* 2009b). Disruption of *SIN4* causes increased enrichment of both of these DNA-binding factors and may similarly affect their ability to recruit coactivators to the *HO* promoter. Elevated and/or persistent recruitment of coactivators to the *HO* promoter could explain the hyperactivation observed with disrupting *SIN4*. To examine this possibility, we performed ChIP to measure recruitment of Swi2-V5 of the SWI/SNF complex, Gcn5-V5 of the SAGA complex, and Gal11-V5 of the Mediator complex. As Gal11 is part of the Mediator Tail module, in the *sin4*Δ mutant we were only examining recruitment of the Tail subcomplex.

We first examined binding of the coactivator complexes to *HO* URS1 during logarithmic growth and found that disrupting *SIN4* caused coactivator enrichment to increase for all three complexes, ranging from 1.6- to twofold enhanced enrichment (Figure 5A). A functional Tail module was required for the hyperactivation and suppression by *sin4*Δ (Figure 3D), and we next investigated whether this Tail dependence held true for coactivator complex recruitment. To test this possibility, we examined Swi2-V5 binding when *SIN4* and *GAL11* were disrupted, either independently or together. Coactivator binding is interdependent (Takahata *et al.* 2011), and we observed an expected small decrease in Swi2-V5 enrichment in the *gal11*Δ single mutant. However, in the *gal11*Δ *sin4*Δ double mutant we saw complete loss of the near fourfold enrichment observed with the *sin4*Δ single mutant (Figure 5B). These results suggest that independent recruitment of the Tail subcomplex is necessary and sufficient to increase recruitment of SWI/SNF to the *HO* promoter. Interestingly, *sin4*Δ suppresses phenotypes caused by a *swi2*Δ mutation (Jiang and Stillman 1992; Roberts and Winston 1997).

Additionally, the Swi5 factor persists at the *HO* promoter in a *SIN4* disruption (Figure 3C), and this persistence may affect the binding kinetics of recruited coactivators. To
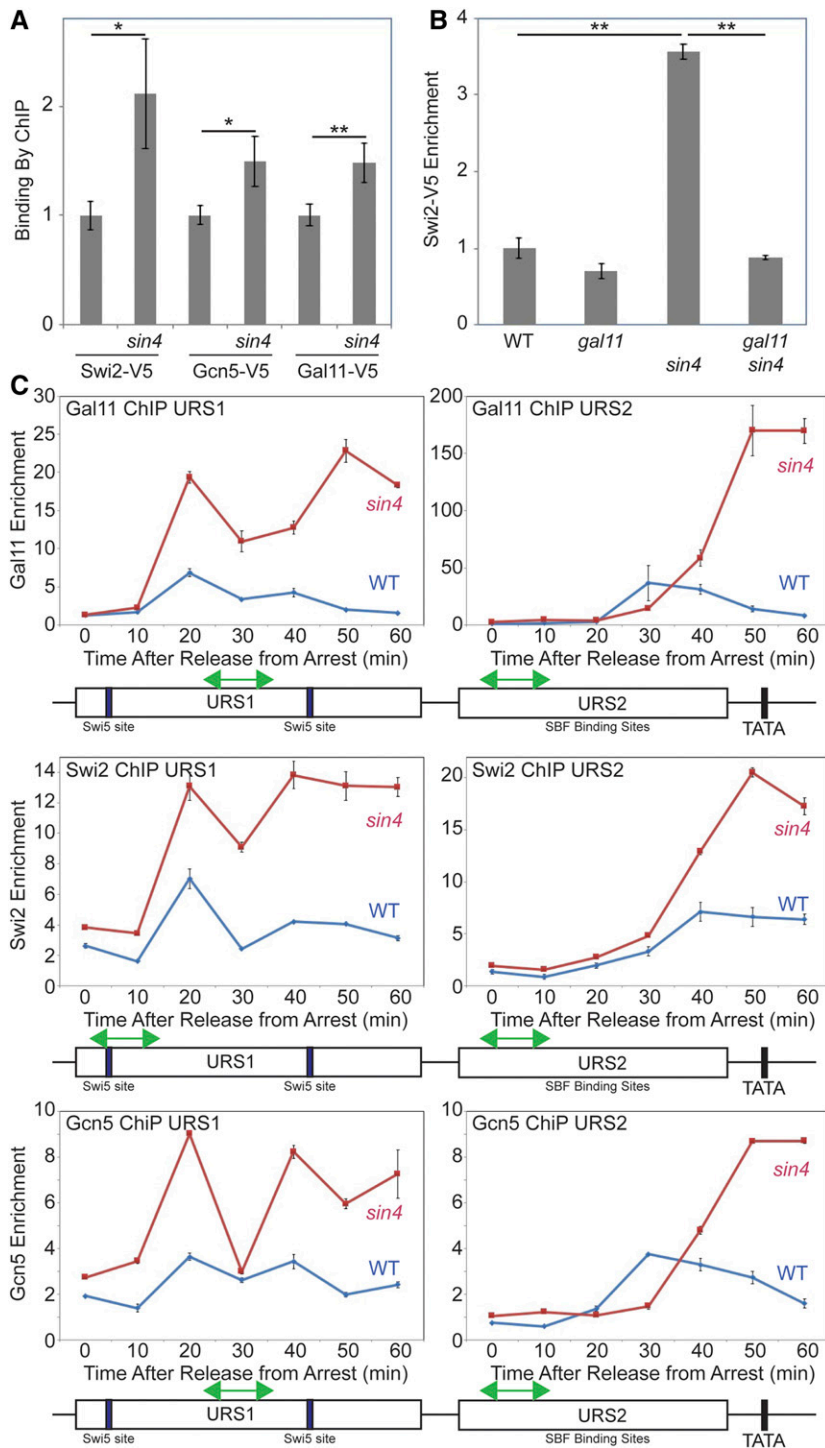
**Figure 5** A *sin4* mutation increases coactivator binding to the *HO* promoter. (A) The *sin4* mutation increases Swi2-V5, Gcn5-V5, and Gal11-V5 binding to the *HO* promoter as measured by ChIP. The error bars reflect the SD of three biological samples. *$P < 0.05$, **$P < 0.01$. (B) The increase in SWI/SNF binding measured by ChIP assays with Swi2-V5 is dependent upon Gal11. The error bars reflect the SD of three biological samples. **$P < 0.01$. (C) The increased coactivator binding in a *sin4* mutant persists during the cell cycle. WT (blue) and *sin4* (red) cells with a *GALp:CDC20* allele, and either a Gal11-V5, a Swi2-V5, or a Gcn5-V5 epitope tag, were synchronized by galactose withdrawal and readdition, and factor binding was measured by ChIP during the cell cycle. The ChIP signal is plotted as a function of time after release from the G2/M arrest. The left and right panels show binding at URS1 and URS2, respectively, with the positions of the PCR primers shown by green arrows. The error bars reflect the SD of PCR replicates. ChIP, chromatin immunoprecipitation; URS, upstream regulatory sequence; WT, wild-type.

examine this possibility, we synchronized cells using *GALp:CDC20* arrest and release, and performed ChIP on the V5-tagged coactivator subunits. For all three coactivator complexes, we observed greater and prolonged enrichment of URS1 (Figure 5C). Interestingly, coactivator enrichment at URS1 continues well past Swi5 binding at URS1 (compare Figure 3C and Figure 5C). We also found enhanced and persistent coactivator enrichment at URS2, and this recruitment appears to be in good agreement with altered SBF binding in *sin4Δ* (Figure S1).

### Disruption of SIN4 enhances nucleosome eviction at the HO promoter

*HO* experiences waves of nucleosome eviction along the length of its promoter during normal activation (Takahata *et al.* 2009b). However, these waves of nucleosome eviction
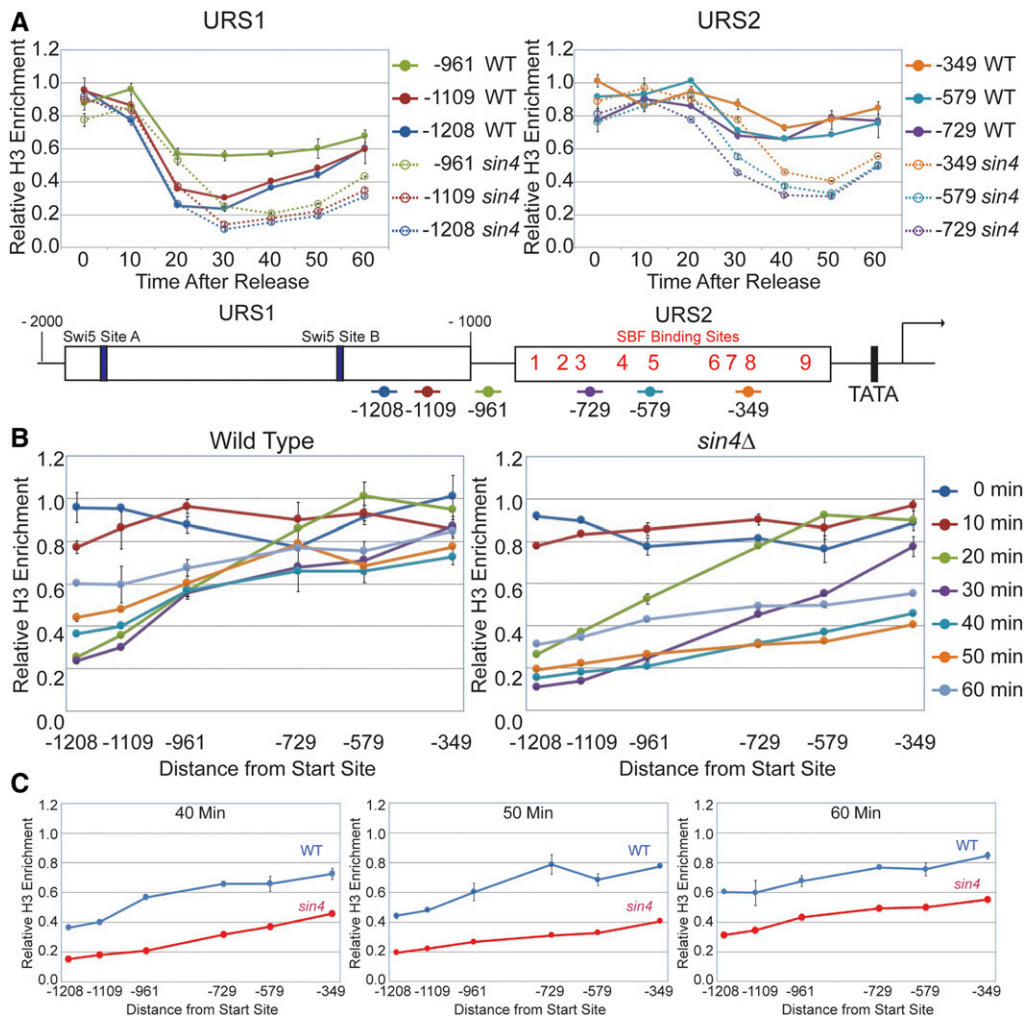
**Figure 6** A *sin4* mutation prolongs nucleosome eviction at the *HO* promoter. WT (left) and *sin4* (right) cells with a *GALp:CDC20* arrest allele were synchronized by galactose withdrawal and readdition, and H3 occupancy at the *HO* promoter was measured by ChIP during the cell cycle using primers spaced along the *HO* promoter. The error bars reflect the SD of PCR replicates. (A) The H3 ChIP is plotted as a function of time after release from the G2/M arrest, with the centers of the PCR intervals amplified listed on the right. The positions of the PCR amplicons are shown on the promoter map below. (B) The data from panel A are plotted as a function of the distance along the promoter, with the time points listed on the right. (C) The 50-min time points for WT and *sin4* are plotted as a function of the distance along the promoter. ChIP, chromatin immunoprecipitation; URS, upstream regulatory sequence; WT, wild-type.

are dependent on transient recruitment of transcription factors and coactivators whose binding kinetics are altered in a *SIN4* disruption. To determine whether *sin4*Δ affects nucleosome eviction at *HO*, we performed a H3 histone ChIP, examining H3 occupancy every 100 to 200 bp between URS1 and URS2R, after *GALp:CDC20* arrest and release.

Nucleosome eviction at URS1 was nearly identical between WT and *sin4*Δ for the first 20 min after release (Figure 6A). However, in agreement with altered coactivator recruitment (Figure 5C), nucleosome eviction appeared to continue beyond 20 min in *sin4*Δ leading to greater and more persistent eviction of nucleosomes. Typically, nucleosome occupancy at URS1 is mostly repopulated after 60 min postrelease (Takahata *et al.* 2009b) but repopulation in *sin4*Δ was significantly delayed (Figure 6B, compare WT and *sin4*Δ −1208 and −1109). Nucleosome eviction at URS2 was also affected, with much greater nucleosome depletion observed after 30 min postrelease in *sin4*Δ (Figure 6, A and B). For example, the H3 occupancy data clearly show a marked delay in nucleosome repopulation along the *HO* promoter in the *sin4* mutant at the later time points (Figure 6C). These results indicate that independent recruitment of the Tail subcomplex

leads to enhanced nucleosome eviction at the *HO* promoter, presumably due to increased and persistent recruitment of the SWI/SNF complex.

### Disruption of SIN4 can increase coactivator binding and increases the probability of HO promoter activation

Mutations in the catalytic subunits of SWI/SNF and SAGA greatly reduce *HO* expression and the recruitment of other coactivators (Cosma *et al.* 1999; Mitra *et al.* 2006; Takahata *et al.* 2011). As *sin4*Δ leads to *HO* hyperactivation and enhanced coactivator binding, we next wanted to investigate whether disrupting *SIN4* could also rescue *swi2* and *gcn5* coactivator mutants. To test this possibility, we examined Swi2-V5 and Gcn5-V5 binding when *SIN4*, and either *GCN5* or *SWI2*, were mutated, respectively.

As expected, disrupting *GCN5* alone resulted in a small decrease in Swi2-V5 at URS1, while disrupting *SIN4* alone resulted in a threefold increase in Swi2-V5 binding (Figure 7A). Disrupting *SIN4* and *GCN5* together yielded an approximately threefold increase in Swi2-V5 enrichment similar to that of the *sin4* single mutant. This result suggests that SWI/SNF binding at the *HO* promoter is no longer dependent on
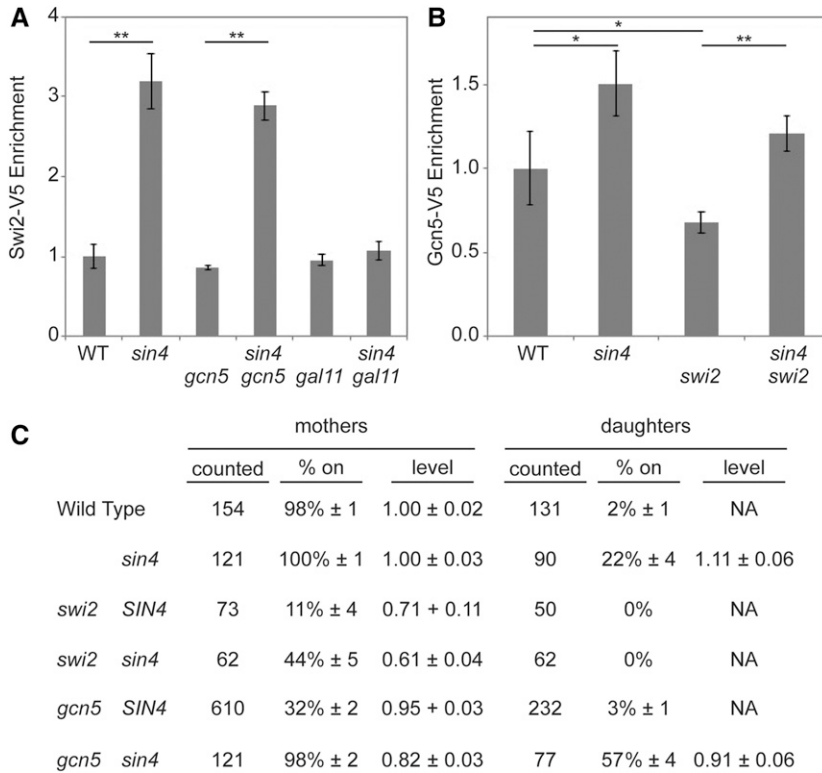
**Figure 7** A *sin4* mutation suppresses defects at *HO* caused by coactivator mutations. (A) A *sin4* mutation enhances SWI/SNF binding to *HO* despite a *gcn5* mutation. SWI/SNF binding to *HO* was measured by ChIP assays with Swi2-V5 in WT, *sin4*, *gcn5*, *sin4 gcn5*, *gal11*, and *sin4 gal11* strains. The error bars reflect the SD of three biological samples. **$P < 0.01$. (B) A *sin4* mutation enhances the Gcn5-V5 binding to *HO* despite a *swi2-314* mutation. Gcn5 binding to *HO* was measured by ChIP assays in WT, *sin4*, *swi2-314*, and *sin4 swi2-314* strains. The error bars reflect the SD of three biological samples. *$P < 0.05$, **$P < 0.01$. (C) A *sin4* mutation enhances *HO* expression despite coactivator mutations. Expression of an *HO-GFP* reporter was measured by single-cell time-lapse fluorescence microscopy, in both mother and daughter cells. The table lists the number of cells counted, the percentage of cells in which *HO-GFP* was expressed, and the relative level of expression. Expression levels were normalized so that the average expression level in WT mother cells is 1. The data for the wild-type and *gcn5* mutants are from Q. Zhang *et al.* (2013). ChIP, chromatin immunoprecipitation; WT, wild-type.

functional SAGA when *SIN4* is also disrupted. As a control, Swi2-V5 binding was similarly measured when *GAL11* was disrupted instead of *GCN5* and binding was found to be Tail-dependent (Figure 7A). We next examined Gcn5-V5 binding in a *swi2-314* (E843K) mutant that encodes a partially functional Swi2 protein (Mitra *et al.* 2006). Mutating *swi2* resulted in a large decrease in Gcn5-V5 enrichment, while disrupting *SIN4* resulted in a 50% increase in Gcn5-V5 binding over WT (Figure 7B). Combining *swi2-314* with *sin4*Δ rescued Gcn5-V5 binding to levels 20% higher than WT and nearly twofold higher than that found in the *swi2-314* strain alone. Taken together, these results indicate that when the Tail subcomplex is separated from the rest of Mediator, SWI/SNF and SAGA can be recruited to the *HO* promoter independently of the other coactivator.

The defect in *HO* activation observed in *gcn5* and *swi2-314* cells could be due to WT, or near-WT, levels of activity from a subset of cells or to low levels of promoter activity from a larger fraction of cells. Examining these two options and how *sin4*Δ suppresses these mutants at the single-cell level could provide additional information on the mechanism of its suppression. To address these possibilities, we combined *gcn5* and *swi2-314* mutations with an *HO-GFP* reporter, and analyzed expression using single-cell time-lapse fluorescence microscopy (Q. Zhang *et al.* 2013). Single-cell analysis revealed a significant reduction in the number of mother cells expressing at *HO-GFP* WT levels in both the *swi2* and *gcn5* single mutants, and this fraction increased significantly when a *sin4* mutation was introduced (Figure 7C; movies are shown in Figure S5). Disrupting *SIN4* did not result in cells expressing *HO* at levels surpassing WT, but rather increased the percentage of cells expressing *HO*. Therefore, independent recruitment of the Tail subcomplex increases the probability of promoter activation within a population of cells. *HO* is normally expressed only in mother cells, and not in daughters (Jensen *et al.* 1983; Nasmyth 1983). Interestingly, the *sin4*Δ *gcn5*Δ double mutant displayed expression in a significant fraction of daughter cells, a property not found in either of the single mutants. Mechanistically, it is not at all clear why *HO* is expressed in the *sin4*Δ *gcn5*Δ double mutant, and this requires further investigation.

### Disruption of SIN4 enables SWI/SNF binding under arrest conditions

A possible explanation for the effect of *sin4*Δ on coactivator recruitment is that the entire Mediator complex has both positive and negative roles in the binding of other coactivators. A similar argument has been proposed for SAGA, which is capable of stimulating SWI/SNF binding to chromatin via histone acetylation, but also facilitating SWI/SNF dissociation by direct Snf2 acetylation (Kim *et al.* 2010). By this model, the inhibitory roles of Mediator are limited to the core Mediator and the Kinase module, and are therefore absent when an independent Tail module alone is recruited to the promoter, thereby creating a permissive environment for other coactivators to bind. Elevated and persistent coactivator binding observed in *sin4*Δ (Figure 5C) supports this model.
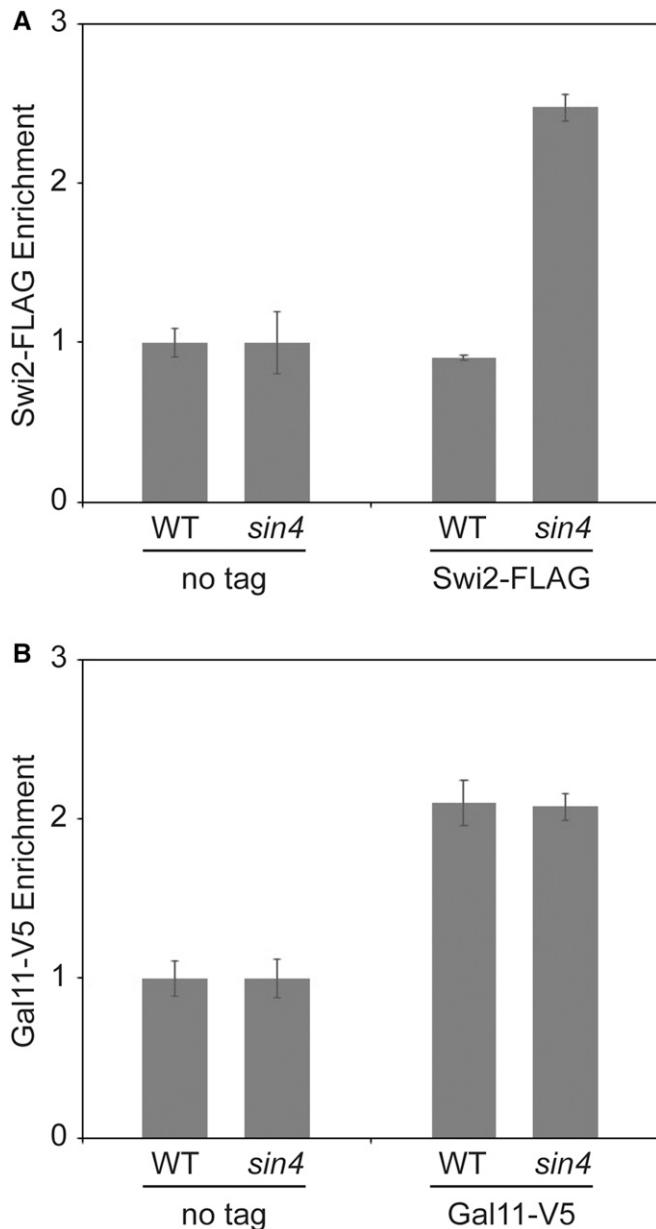
**Figure 8** A *sin4* mutation facilitates SWI/SNF binding at *HO* during G1 arrest. (A) SWI/SNF is normally not present at *HO* URS2 during a G1 arrest (Q. Zhang *et al.* 2013b). A ChIP experiment shows that a *sin4* mutation allows Swi2-FLAG to bind to *HO* URS2 during a G1 arrest. The error bars reflect the SD of two biological samples. (B) A ChIP experiment shows that a *sin4* mutation does not result in an increase in Gal11-V5 binding to *HO* URS2 during a G1 arrest. The error bars reflect the SD of two biological samples. ChIP, chromatin immunoprecipitation; URS, upstream regulatory sequence; WT, wild-type.

To further explore this proposed model of inhibition by the complete Mediator complex, we next examined *HO* under arrest conditions in which only SBF and Mediator are bound to the promoter (Q. Zhang *et al.* 2013; Yu *et al.* 2016). If our model is correct, the lack of SWI/SNF binding under arrest conditions is at least partly due to inhibitory pressure from

the Kinase module and core Mediator complex that would be absent in *sin4*Δ. To test this theory, we examined Swi2 enrichment by Swi2-V5 ChIP under α-factor arrest in WT and *sin4*Δ cells. Since α-factor arrest induces expression of a long noncoding RNA (lncRNA) that disrupts factor binding (Yu *et al.* 2016), the cells used in this experiment have an *HO* promoter mutation at the Ste12-binding site required for inducing this lncRNA. Interestingly, while WT cells had Swi2-V5 levels comparable to no tag controls, *sin4*Δ had elevated Swi2-V5 binding to URS2L with 2.5-fold enrichment over background (Figure 8A). Although it is possible that this persistent Swi2 binding is due to the three- to fourfold greater binding that was observed during logarithmic growth, we saw nearly identical levels of Gal11 binding in WT and *sin4*Δ cells when we probed similarly for Gal11-V5 instead of Swi2-V5 (Figure 8B). Thus, SWI/SNF is recruited at the arrest in *sin4*Δ cells, where only the Mediator Tail is present. In contrast, SWI/SNF is not recruited in WT cells where complete Mediator is present, suggesting that Mediator core can inhibit SWI/SNF recruitment under these circumstances. These results support a model in which Mediator can both positively and negatively regulate SWI/SNF binding to promoters, and, furthermore, that these two regulatory effects can be separated from one another by independent recruitment of only the Tail module to promoters.

## Discussion

Mediator consists of four conserved modules (Head, Middle, Kinase, and Tail), and previous work has implicated the Kinase module as the primary antagonist to Tail binding at the UAS elements of genes (van de Peppel *et al.* 2005; Jeronimo and Robert 2017). In this study, we probed the antagonist model at the *Saccharomyces cerevisiae HO* gene by utilizing a *SIN4* disruption that separates the Tail module from the Kinase module and core Mediator. In *sin4*Δ mutant cells we observed a significant increase in Tail module recruitment, elevated SWI/SNF and SAGA coactivator binding, persistent nucleosome eviction, and hyperactivation of the *HO* gene. These results are consistent with the Kinase module as the primary Tail antagonist, as the Kinase module would not be present at the UAS in the *sin4*Δ mutant to limit Tail module recruitment to the *HO* promoter. However, mutation of the catalytic subunit of the Kinase module failed to reproduce the hyperactivation of *HO* observed here with independent Tail recruitment. These results suggest that the activity and binding of the Tail is limited by other factors. One intriguing possibility is the core Mediator, which is also not present at the UAS during independent Tail module recruitment in the *sin4*Δ mutant, and thus core Mediator might function to limit Tail binding.

A *sin4*Δ mutation allows *HO* expression in the absence of the normally required SBF-binding sites at the left end of URS2, or when the spacing between URS1 and URS2 is increased by an additional 700 bp (Figure 1). ChIP experiments show that the *sin4*Δ mutation has this effect by increasing the
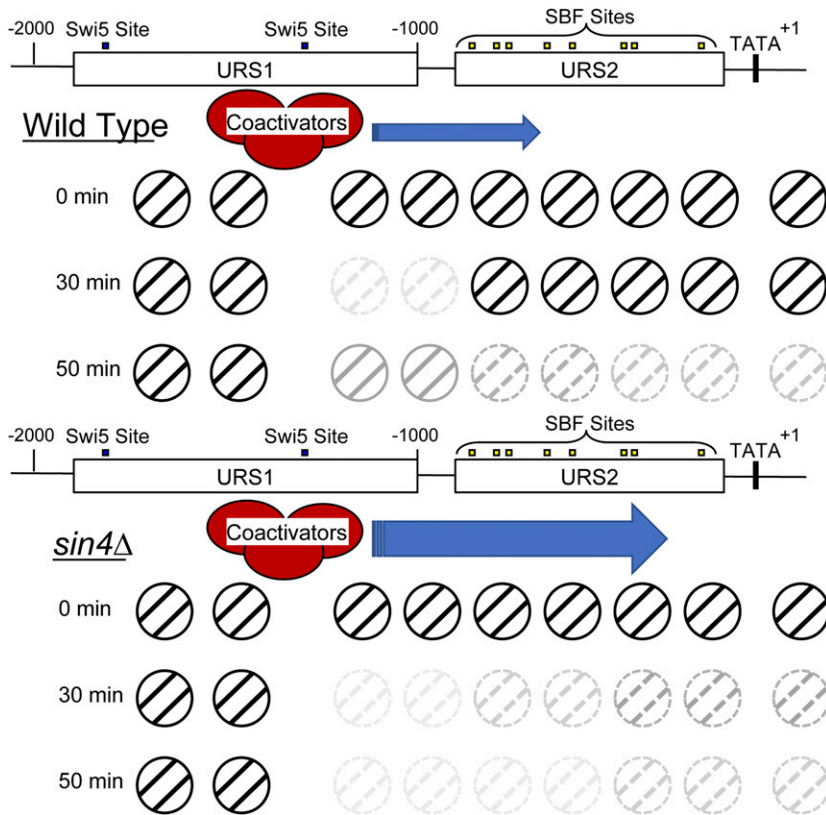
**Figure 9** Model for how a *sin4Δ* mutation increases coactivator recruitment, enhances nucleosome eviction, and increases SBF binding. The figure shows a diagram of the *HO* promoter and nucleosome along the promoter during the cell cycle in wild-type and *sin4Δ* cells, at three time points: G2/M arrest (0 min), 30 min after release, and 50 min after release. The changes in line color for the nucleosomes are an approximation for nucleosome density. Nucleosome density taken from Takahata *et al.* (2009b) and Figure 6. In wild-type cells, Swi5 and coactivators are at URS1 at 20 min, SBF and coactivators are at URS2 at 35 min, and the gene is transcribed at 40 min. In *sin4Δ* cells coactivator recruitment, SBF binding, and nucleosome eviction are all prolonged. The red ovals represent the coactivators and the blue arrows represent the activity of the coactivators, which act for a longer time and over a longer distance in the *sin4Δ* mutant. URS, upstream regulatory sequence.

amount of SBF bound to the *HO* promoter (Figure 2). We suggest that *sin4Δ* has this effect because the coactivators bound at URS1 can now act over a longer distance, and for a longer period of time (Figure 9). Thus, in *sin4Δ* the coactivators are more effective at evicting nucleosomes further away from URS1, increased nucleosome eviction allows more SBF to bind (Yarrington *et al.* 2015; Yarrington *et al.* 2016), and *HO* expression more likely (Figure 9). However, there are limits to this effect as *sin4Δ* is unable to suppress the transcriptional defect caused by a 1300-bp insertion between URS1 and URS2. This latter result raises the conundrum of why cannot *sin4Δ* suppress all of these promoter mutations when *sin4Δ* can suppress a *SWI6* disruption. Our best explanation is that the *swi6Δ* mutation is quite pleiotropic, including an altered cell cycle and secondary effects. Importantly, SBF also recruits the inhibitory Rpd3(L) histone deacetylase complex to the *HO* promoter, but Rpd3(L) is not present in the *swi6Δ* mutant (Huang *et al.* 2009; Takahata *et al.* 2009a, 2011; Wang *et al.* 2009). The evidence suggests that SBF bound to the URS2R is the proximal activator for *HO* expression (Yarrington *et al.* 2015). Since, SBF only recruits Mediator Tail in the *sin4Δ* mutant, how is transcription activated in the absence of core Mediator subunits that engage with the RNAPII? One answer comes from Petrenko *et al.* (2017), who suggest that the Tail module may be able to interact with a component of the basic RNAPII machinery, or that the Tail

may be able to help recruit Head and Middle subunits of Mediator to the transcription start site.

Our results suggest that the core Mediator may also limit the binding of the SWI/SNF and SAGA coactivators. When the Tail module was recruited independently of the rest of Mediator, we observed greater than WT levels of SWI/SNF and SAGA coactivators, even when the other coactivator or its activity was disrupted. Additionally, when core Mediator was absent, we were able to observe considerable enrichment of SWI/SNF at *HO* URS2 even under α-factor arrest conditions in which coactivators are typically evicted by transcribing RNAPII (Yu *et al.* 2016). These results suggest that Mediator has both positive and negative roles in the recruitment of other coactivators, similar to those of SWI/SNF in the recruitment of the SAGA complex (Kim *et al.* 2010). We propose that core Mediator limits the ability of other coactivators to bind while the Tail module facilitates their binding.

An alternative explanation for the increased coactivator recruitment in *sin4Δ* is that the Tail subcomplex stimulates the recruitment of other coactivators, while core Mediator, along with the Kinase module, simply limits Tail occupancy at promoters. When *SIN4* was disrupted, we saw an ∼50% increase in the enrichment of the Gal11 Tail subunit to *HO*. This increase in Tail occupancy in *sin4Δ* could be responsible for stimulating elevated recruitment of the SWI/SNF and SAGA coactivator complexes, and rescuing recruitment of either of

these coactivators when the other is impaired or disrupted. In this model, core Mediator would regulate coactivator binding by limiting the availability of the Tail module to the UAS or enhancers of genes.

Why would Mediator limit its own recruitment and the binding of other coactivators? The *S. cerevisiae* genome is very compact, with inter-ORF distances ranging from 150 to 400 bp (Pelechano *et al.* 2006). The increased coactivator recruitment and nucleosome eviction that we observe at the *HO* promoter when the Tail module is recruited independently has profound effects on *HO* transcriptional regulation, and it is possible that these effects are occurring genomewide. Although microarray analysis of *sin4Δ* does not reveal drastic changes in global transcript levels (van de Peppel *et al.* 2005), changes in global chromatin structure and hypersensitivity to micrococcal nuclease have been reported in a *sin4Δ* mutant (Macatee *et al.* 1997). We have shown previously that nucleosomes act as gates to regulate both activation and timing of expression (Yarrington *et al.* 2016), and coactivator recruitment and nucleosome eviction must be precise to limit promoter activation, and ensure proper regulation of gene transcription.

Disruption of *SIN4* has been previously implicated in the long-range activation of genes (Dobi and Winston 2007). In this report, the authors identified *sin4Δ* as capable of enabling transcriptional activation at normally nonpermissive distances of ≥ 800 bp in *S. cerevisiae*. Interestingly, this effect was specific to *sin4Δ* as the authors were unable to reproduce long-range activation with other Mediator mutations affecting all four modules. These results are therefore consistent with the effect being due to an independent Tail subcomplex. Although the authors were unable to provide a mechanism for this altered activation by *sin4Δ*, based on our results with *HO*, it is likely that the observed long-range activation is due to independent Tail recruitment with associated elevated and persistent transcription factor and coactivator binding.

Lastly, it is important to note that we find no evidence that disrupting *SIN4* has altered the function of Mediator, only the recruitment of its four modules. Binding of the Tail subcomplex to *HO* still requires both the Gal11 Tail subunit and the Swi5 transcription factor, and *HO* expression requires SBF bound to URS2R. Furthermore, single-cell analysis demonstrates that independent recruitment of the Tail subcomplex has not altered the mechanism of *HO* activation, but rather the probability of activation. These results are consistent with whole Mediator working in concert with, and regulating the recruitment of, other coactivators to achieve proper gene regulation.

## Acknowledgments

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