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Facilitated Assembly of the Preinitiation Complex by Separated Tail and Head/Middle Modules of the Mediator

Luciano Galdieri, **Parima Desai**, and **Ales Vancura***
Department of Biological Sciences, St. John's University, Queens, New York 11439

Abstract

Mediator is a general coactivator of RNA polymerase II (RNA pol II) bridging enhancer-bound transcriptional factors with RNA pol II. Mediator is organized in three distinct subcomplexes: head, middle, and tail modules. The head and middle modules interact with RNA pol II and the tail module interacts with transcriptional activators. Deletion of one of the tail subunits *SIN4* results in derepression of subset of genes, including *FLR1*, by a largely unknown mechanism. Here we show that derepression of *FLR1* transcription in *sin4*Δ cells occurs by enhanced recruitment of the mediator, as well as Swi/Snf and SAGA complexes. The tail and head/middle modules of the mediator behave as separate complexes at the induced *FLR1* promoter. While the tail module remains anchored to the promoter, the head/middle modules are also found in the coding region. The separation of the tail and head/middle modules in *sin4*Δ cells is also supported by the altered stoichiometry of the tail and head/middle modules at several tested promoters. Deletion of another subunit of the tail module *MED2* in *sin4*Δ cells results in significantly decreased transcription of *FLR1*, pointing to the importance of the integrity of the separated tail module in derepression. All tested genes exhibited increased recruitment of the tail domain; however, only genes with increased occupancy of the head/middle modules displayed also increased transcription. The separated tail module thus represents a promiscuous transcriptional factor that binds to many different promoters and is necessary for derepression of *FLR1* in *sin4*Δ cells.

Keywords

SIN4; *FLR1* expression; transcriptional derepression; Gal11p and Srb4p recruitment; Swi/Snf and SAGA complexes

Introduction

Mediator is a highly conserved complex that plays an important role in transcription by RNA polymerase II (RNA pol II). The mediator subunits were identified by genetic screens and independently by biochemical approaches. Several genes encoding mediator subunits were identified as dominant and recessive suppressors of the cold sensitive phenotype associated with truncations in the carboxyl terminal domain (CTD) of RNA pol II.[1–4] Additional genetic screens identified other mediator subunits[5,6] and complemented a

*Corresponding author. Mailing address: Department of Biological Sciences, St. John's University, 8000 Utopia Parkway, Queens, NY 11439. Phone: (718) 990-6287. Fax: (718) 990-5958. vancuraa@stjohns.edu.

biochemical approach that resulted in purification of the mediator and identification of its subunits.[7] Three-dimensional reconstructions of electron micrographs combined with biochemical and genetic data suggest that mediator is roughly organized in three distinct subcomplexes: head module, middle module, and the tail module.[8–10] The head and middle modules interact with the CTD of RNA pol II[11] and the tail module interacts with transcriptional activators.[12–15] In addition, Srb8p, Srb9p, Srb10p, and Srb11p form a distinct subcomplex that contains the cyclin-kinase pair Cdk8 and CycC that phosphorylates the CTD at the serine 5 residue in the heptapetide repeat.[16] This subcomplex is involved in negative regulation of small subset of genes.[17]

The three-dimensional structure of the mediator suggests that in the presence of RNA pol II the mediator assumes an elongated shape and makes multiple contacts with RNA pol II through the head and middle domains.[8] The mediator appears to have a general role in transcription of majority of yeast genes, since inactivation of Med17p or Med22p resulted in almost complete loss of transcription, similar to inactivation of Rpb1p of RNA pol II.[17]

Mediator plays both positive and negative roles in transcription.[6,18–20] The positive role consists in activator-mediated recruitment of mediator and its interaction with RNA polymerase II. Mediator appears to function as a link between the gene-specific activator proteins and the basal RNA pol II transcription machinery. The gene-specific activators interact with subset of mediator subunits, typically involving the tail region. For example, Gal4p interacts directly with the tail subunit Gal11 and the head subunit Srb4,[12,13] and Gcn4p interacts with tail subunits Gal11, Pgd1, and Med2 and head subunit Srb2.[15]

Mediator can also function as a scaffold for repeated rounds of reinitiation by RNA pol II.[21,22] Mediator occupancy does not always parallel RNA pol II occupancy and mediator was found also upstream of inactive genes[23–25] and, conversely, some highly active promoters did not detectably recruit mediator.[23,26] Genome-wide location analysis also found mediator associated with coding region of many highly active genes.[24,25]

We have found previously that deletion of mediator subunit *SIN4* suppresses defect in transcription of the *FLR1* gene in *plc1*Δ cells.[27] Plc1p is a budding yeast phospholipase C that is required for the initial step of inositol polyphosphates (InsPs) synthesis. InsPs regulate activity of chromatin remodeling complexes *in vivo* and *in vitro*[28,29] and are required for induction of the phosphate-responsive *PHO5* gene. In this study, we elucidate the molecular mechanism by which *sin4*Δ mutation derepresses *FLR1* gene. Our results are consistent with the model in which *sin4*Δ mutation increases binding of the tail as well as head/middle modules of the mediator to the *FLR1* and other promoters.[15] The tail and head/middle modules of the mediator in *sin4*Δ cells facilitate recruitment of the Swi/Snf and SAGA complexes and assembly of the PIC complex. Interestingly, in induced state, the tail module remains bound to the *FLR1* promoter, while the head/middle module is found also in the *FLR1* coding region, suggesting that the tail and head/middle modules function as separate complexes in *sin4*Δ cells.

Results

FLR1 expression defect in swi2Δ cells is suppressed by sin4Δ mutation

We have described previously that expression of *FLR1* gene requires phospholipase C (Plc1p) – dependent pathway for synthesis of inositol polyphosphates (InsPs) and that the failure of *plc1*Δ cells to express *FLR1* is suppressed by *sin4*Δ mutation.[27] Since *plc1*Δ cells that are completely devoid of any InsPs[30] are not able to recruit Swi/Snf complex and to remodel chromatin of the *PHO5* promoter[28], we wanted to test whether the induction of the *FLR1* gene requires Swi2p, the catalytic subunit of the Swi/Snf chromatin remodeling

complex.[31,32] Our results show that *swi2*Δ cells, similarly to *plc1*Δ cells, are not able to induce *FLR1*, and this defect is partially suppressed by *sin4*Δ mutation (Fig. 1). Cells with *SIN4* deletion express *FLR1* even without induction with benomyl and when induced, the induction occurs faster than in the wild-type cells. However, the maximal expression at 3 h is higher in the wild-type cells than in the *sin4*Δ cells.

Expression of *FLR1* is regulated by several transcriptional factors, including Pdr1p, Pdr3p, Yrr1p, and Yap1p.[33–41] Correspondingly, *pdr1*Δ, *pdr3*Δ, *yrr1*Δ, and *yap1*Δ mutants are not able to express *FLR1*; however, the expression in *pdr1*Δ, *pdr3*Δ, and *yrr1*Δ mutants can be partially suppressed by *sin4*Δ mutation (Fig.1). It appears that Yap1p has a unique position in benomyl-induced activation of *FLR1* gene, since *yap1*Δ mutation is not suppressed by *sin4*Δ mutation (Fig. 1). This result suggests that the *sin4*Δ mutation does not act by increasing the recruitment of Pdr1p, Pdr3p, or Yrr1p to the *FLR1* promoter. However, increased expression of *FLR1* in *sin4*Δ cells before induction could be explained by increased recruitment of Yap1p to the *FLR1* promoter. To test this possibility, we determined by chromatin immunoprecipitation (ChIP) the occupancy of Yap1p at the *FLR1* promoter before and after induction. The results show that the *sin4*Δ mutation does not affect Yap1p occupancy at the *FLR1* promoter (Fig. 2A).

To better understand the mechanism by which *sin4*Δ mutation restores the expression of the *FLR1* gene in *plc1*Δ cells, we determined recruitment of Swi2p to the *FLR1* promoter in wild-type, *plc1*Δ, *sin4*Δ, and *plc1*Δ*sin4*Δ mutants during *FLR1* induction with benomyl. In wild-type and *sin4*Δ cells, Swi2p is recruited to the *FLR1* promoter in response to benomyl induction (Fig. 2B). This recruitment is almost completely eliminated in *plc1*Δ cells, however, introducing *sin4*Δ mutation in *plc1*Δ cells suppresses this defect and allows Swi2p recruitment (Fig. 2B). This result is thus consistent with the notion that InsPs play a role in recruitment of chromatin remodeling complexes[28] and suggest that the reduced *FLR1* expression in *plc1*Δ cells is caused by the defect in Swi2p recruitment. In addition, the recruitment of Swi2p in *sin4*Δ cells before induction and during induction is higher than in wild-type cells. To test the possibility that the assembly of the preinitiation complex (PIC) at the *FLR1* promoter is facilitated by *sin4*Δ mutation, we determined the occupancy of Ada2p, Spt15p, and RNA pol II at the *FLR1* promoter before and after induction (Fig. 2C, D, E). The results show an increased occupancy of Ada2p, Spt15p, and RNA Pol II, and suggest that *sin4*Δ mutation facilitates the PIC assembly at the *FLR1* promoter even under non-inducing conditions.

sin4Δ mutation derepresses FLR1 transcription by allowing recruitment of the tail and head/middle modules of the mediator

To explain the mechanism of derepression of *FLR1* transcription in *sin4*Δ cells, we considered a possibility that *sin4*Δ mutation causes separation of the tail domain of the mediator from the middle and head domains[15]. The tail module is anchored to the middle module of the mediator by Sin4p and deletion of *SIN4* results in separation of the tail module from the rest of the mediator composed of the middle and head domains. In *sin4*Δ mutant, the separated tail domain, composed of Med2p, Pgd1p, and Gal11p is recruited by Gcn4p to the *ARG1* promoter, where it stimulates transcription to the same level as intact mediator[15]. We hypothesized that in *sin4*Δ mutant even in uninduced state the separated tail domain would be recruited to the *FLR1* promoter, facilitate recruitment of the Swi/Snf complex and assembly of the PIC complex. To examine this possibility, we determined the recruitment of Gal11p and Srb4p to the *FLR1* promoter and coding region before and after induction. Gal11p and Srb4p are components of the tail and head modules of the mediator, respectively. The occupancy of both Gal11p and Srb4p at the *FLR1* promoter in the wild-type cells is at the background level before induction and is elevated about 2 fold after induction (Fig. 3A,B). The occupancy of both proteins in *sin4*Δ cells before induction is

increased approximately 2 fold in comparison to wild-type cells before induction (Fig. 3C, D). Interestingly, while the induction in *sin4*Δ cells results in increased Gal11p occupancy only at the promoter, the Srb4p occupancy is increased in both the promoter and the coding region (Fig. 3C, D).

These results suggest that upon induction, the mediator is recruited as a single complex in the wild-type cells and the occupancy at the *FLR1* promoter is increased about 2 fold above the background level. On the other hand, the occupancies of Gal11p and Srb4p suggest that in *sin4*Δ cells the tail and head/middle modules behave as separate complexes. While the tail remains at the *FLR1* promoter, the head/middle module is found also within the coding region. These results are consistent with the physical interactions of the mediator with components of the transcription machinery. The tail module interacts with transcriptional activators that are recruited to the promoter,[14] and the head and middle module interact with the CTD of the RNA Pol II.[11]

The induction of *FLR1* expression in *sin4*Δ cells results in a dramatic recruitment of the tail domain to the promoter. While in the wild-type cells Gal11p is not recruited to *FLR1* above background level before induction and the occupancy is increased 2 fold after induction, *sin4*Δ mutation causes about 2 fold increase in Gal11p recruitment before induction and about 9 fold increase after induction in comparison to wild-type cells before induction (Fig. 3A, C). To determine, whether this recruitment requires Swi/Snf complex or Yap1p transcriptional factor, we determined occupancy of Gal11p also in *sin4*Δ *swi2*Δ and *sin4*Δ *yap1*Δ strains. Deletion of *SWI2* in *sin4*Δ cells eliminates Gal11p recruitment before induction and reduces the Gal11p occupancy to a level 6 fold higher than in the wild type cells before induction. Deletion of *YAP1* completely eliminates Gal11p recruitment (Fig. 3E). These results suggest that Swi/Snf complex is not essential for recruitment of the tail module in *sin4*Δ cells and has only supportive role; however, Yap1p is essential for recruitment of the tail module.

The above results are consistent with the previous finding that *sin4*Δ mutation dissociates the tail module from the rest of the mediator[15]. Our results show that the tail, as well as the head/middle modules are recruited to the *FLR1* promoter in *sin4*Δ cells, and the increased occupancy is associated with elevated *FLR1* transcription. The finding that *sin4*Δ mutation results in elevated expression of HO and several other genes[42–47] lead to suggestion that the tail module represents an inhibitory module of the mediator. In this model, the *sin4*Δ mutation would remove this inhibitory domain and allow the tailless mediator to facilitate the *FLR1* transcription. To address this possibility, we determined *FLR1* expression in wild-type, *sin4*Δ, *med2*Δ, and *sin4*Δ*med2*Δ cells (Fig. 3F). Med2p is a subunit of the tail module and in the *sin4*Δ cells resides in separated tail module.[15] Since introducing the *med2*Δ mutation in *sin4*Δ cells reduces the *FLR1* expression, we conclude that the separated tail module is required for the *FLR1* expression in *sin4*Δ cells and does not represent the inhibitory domain, at least in the context of the *FLR1* promoter.

The tail module in sin4Δ cells is a promiscuous transcriptional activator

Deletion of *SIN4* gene affects transcription of relatively small fraction of the genome.[48] To determine, whether the genes with increased expression in *sin4*Δ cells display a particular pattern of recruitment of the tail and head/middle modules, we determined recruitment of Gal11p and Srb4p to several genes that display higher expression in *sin4*Δ cells than in the wild-type cells. Gene expression and recruitment of Gal11p and Srb4p were measured in cells grown in YPD medium at 30°C. Our results agreed with the genome-wide expression analysis[48] and showed higher expression of *TRX2*, *BNA1*, *HSP12*, and *HSP26* in *sin4*Δ cells than in the wild-type cells (Fig. 4A). For comparison, we also included *PHO5* and *GAL1*

genes that do not differ significantly in expression in wild-type and *sin4*Δ cells[48]. Again, our data (Fig. 4A) are in agreement with the genome-wide expression analysis[48].

The expression of Gcn4p-regulated *ARG1* gene is elevated in *sin4*Δ cells and the tail module is recruited independently of the rest of the mediator to the *ARG1* promoter.[15] We found that the expression of Yap1p-regulated *FLR1* gene is elevated and the tail module is dramatically recruited to the *FLR1* promoter in *sin4*Δ cells. Since Gcn4p and Yap1p play a major role in transcriptional regulation of *ARG1* and *FLR1*, respectively, we tested other genes that are regulated by these transcriptional factors. Recruitment of Gal11p to *TRX2*, another Yap1p-regulated gene,[40] was significantly elevated in *sin4*Δ cells (Fig. 4B). In addition, recruitment of Gal11p to Gcn4p-regulated *BNA1* gene was also elevated in *sin4*Δ cells. Surprisingly, however, the tail module in *sin4*Δ cells was also recruited to *PHO5*, *GAL1*, *HSP12*, and *HSP26* promoters that are not regulated by Gcn4p or Yap1p. While the expression of *TRX2*, *BNA1*, *HSP12*, and *HSP26* was higher in *sin4*Δ cells than in the wild-type cells, the expression of *PHO5* and *GAL1* was not significantly different in wild-type and *sin4*Δ cells (Fig. 4A). These results suggest that the tail module functions as a promiscuous transcriptional factor that does not require Gcn4p or Yap1p and is recruited to diverse genes that are regulated by different transcriptional factors. Surprisingly, all tested genes displayed increased recruitment of Gal11p in *sin4*Δ cells than in the wild-type cells; however, the recruitment of Srb4p in *sin4*Δ cells was elevated only at genes that display increased expression in *sin4*Δ cells (Fig. 4C). These results suggest that the tail module exhibits an increased recruitment to many different genes in *sin4*Δ cells and that it's increased recruitment in *sin4*Δ cells is not sufficient for the increased expression of the corresponding genes. On the other hand, genes that are upregulated in *sin4*Δ cells also display increased recruitment of Srb4p. These results suggest that both the tail and head/middle modules are recruited to the upregulated genes.

Discussion

The repressive role of the mediator at many genes was associated with the tail domain. The initial finding that mediator can repress transcription was based on mutation in *SIN4* that allowed Swi5-independent expression of HO.[42] Deletion of *SIN4* results in increased transcription of host of other genes.[43–47] These results suggest that the integrity of the tail module is required for proper transcriptional regulation of many genes and disruption of the tail module alleviates the repression. It appears that the tail domain plays specifically a role in repression of genes that are inactive because of low activity of the corresponding transcriptional activator or because they lack the UAS sequence.[42,47,49–53]

The mechanism for the repressive role of the tail domain was suggested by the finding that in the *sin4*Δ mutant the tail domain dissociates from the rest of the mediator and is recruited independently to the *ARG1* promoter by Gcn4p.[15] The isolated tail domain then facilitates recruitment of TBP and RNA pol II. Removal of the tail domain from the rest of the mediator in *sin4*Δ mutant resulted in significantly decreased recruitment of the tailless mediator, composed of the middle and head modules.[15]

Our results show that the recruitment of the tail module, measured as Gal11p occupancy, and the recruitment of the head/middle modules, measured as Srb4p occupancy, are significantly increased in *sin4*Δ cells in comparison to recruitment of the intact mediator in wild-type cells (Fig. 3). This can be explained by a structural change in the mediator in *sin4*Δ cells that either results in a complete separation of the tail and head/middle modules, or at least repositions the tail module with respect to the head/middle modules. We speculate that this structural change exposes more of the surface of the tail and head/middle modules

and allows for increased interaction with the transcriptional factors, RNA pol II, or components of the basal transcriptional machinery.

The expression of *FLR1* and recruitment of the tail module to the *FLR1* promoter in *sin4*Δ cells depend on Yap1p. This finding suggests that the isolated tail domain in *sin4*Δ mutant is not only target of Gcn4p as shown previously for the *ARG1* promoter,[15] but also target of other transcriptional factors, such as Yap1p. Indeed, we observed recruitment of the tail module also to *PHO5*, *GAL1*, *HSP12*, and *HSP26*, promoters that are not regulated by Gcn4p or Yap1p.

Our results with the *FLR1* promoter differ from the study of the *ARG1* promoter in the fact that both the tail and head/middle modules are recruited to the *FLR1* promoter, while only the tail module is recruited to the *ARG1* promoter. This difference can be explained by two possible mechanisms. The first mechanism assumes that individual promoters differ in their ability to recruit the separated tail module and the head/middle module; *ARG1* promoter recruits only the separated tail domain but both tail and the head/middle module are recruited to the *FLR1*, *TRX2*, *BNA1*, and *HSP26* promoters (Fig. 3,4). The second possibility is that in highly transcribed genes in *sin4*Δ cells the tail module remains bound to the promoter while the head/middle module binds to the elongating RNA pol II and its occupancy is highest within the coding region. This mechanism could explain that the head/middle module was not found at the *ARG1* promoter under inducing conditions in *sin4*Δ cells.[15]

The recruitment of the tail and head/middle modules to the *FLR1* promoter can occur by two mechanisms. Either the isolated tail module and head/middle modules are recruited as two separate complexes, or the tail module still remains associated with the middle/head module in *sin4*Δ cells and the mediator is recruited as a single complex in *sin4*Δ cells. The first possibility seems to be more likely, since after induction, the tail module remains associated only with the promoter, while the head/middle module is found also in the coding region. We do not know whether the tail and middle/head modules are recruited simultaneously or whether the tail module is recruited first by the transcriptional factor and subsequently recruits the basal machinery and the middle/head module. Regardless of the sequence of the recruiting steps, these results suggest a model in which the tail module is anchored to the promoter by interacting with the transcriptional factors and never leaves the promoter, while the head/middle module moves into the coding region, likely because of interaction with elongating RNA pol II. While our results do not allow conclusion whether the mediator in *sin4*Δ cells is recruited as a single complex or as two separate complexes, the data show that during active transcription of the *FLR1* gene, the tail and the head/middle modules function as two separate complexes. The tail module is recruited by interaction with the transcriptional factors that bind to the *FLR1* promoter (Pdr1p, Pdr3p, Yrr1p, and Yap1p). The head/middle module is likely recruited by the interaction with the RNA pol II or by residual interactions between the separated tail module and the head/middle module that might still exist in *sin4*Δ cells. Alternatively, the head/middle module might be recruited by the interaction with the basal transcription machinery. In this context it is tempting to speculate that the ability of the *sin4*Δ mutation to activate transcription when the upstream activation sequence (UAS) is far from the TATA box[54] is caused by the separation of the tail and head/middle modules of the mediator.

What do our results tell us about the function of the mediator? Mediator is involved in PIC assembly and typically binds to regulatory regions.[19] However, we found that in *sin4*Δ cells during active transcription of the *FLR1* gene, the tail module stays anchored to the promoter region while the head/middle module is found also in the coding region. Interestingly, the genome-wide location studies found intact mediator within coding regions of highly

transcribed genes.[24,25] It is possible that what we observed in *sin4*Δ cells is a reflection of the role of intact mediator in transcription of highly expressed genes. It is tempting to speculate that the intact mediator, bound to the transcription factor(s) in the promoter through its tail module, can be dislodged and dissociated from the promoter by its interaction with RNA pol II, and carried into the coding region (Fig. 5). This event presumes that the mediator is exposed to a "pulling" force exerted by RNA pol II when it leaves the promoter and enters the coding region. It is possible that the intact Mediator is thus partitioned between the transcription factor(s) in the promoter and RNA pol II in the coding region. If and how the mediator is translocated from the promoter to the coding region and the function of mediator within the coding region requires additional investigation.

Materials and Methods

Strains and media

All yeast strains are listed in Table 1. All the strains used in this study are isogenic to W303. Standard genetic techniques were used to manipulate yeast strains and to introduce mutations from non-W303 strains into the W303 background.[63] Cells were grown in rich medium (YPD; 1% yeast extract, 2% Bacto-peptone, 2% glucose) or under selection in synthetic complete medium containing 2% glucose and, when appropriate, lacking specific nutrients in order to select for a strain with a particular genotype. Meiosis was induced in diploid cells by incubation in 1% potassium acetate.

Chromatin immunoprecipitation and quantitative real-time PCR analysis

In vivo chromatin crosslinking and immunoprecipitation were performed as described previously[59,61,64] with the following antibodies: anti-myc polyclonal antibody A-14, anti-HA monoclonal antibody F7, anti-Yap1p polyclonal antibody (all Santa Cruz Biotechnology), and anti-RNA polymerase II monoclonal antibody 8WG16 (Covance). Primers used for real-time PCR analysis are as follows: *POL1* (5'-TCCTGACAAAGAAGGCAATAGAAG-3'and 5'-TAAAACACCC TGATCCACCTCTG -3'), *FLR1* promoter (5'-AATGGGCGGGATAATTAGTCAG-3' and 5'-GTGTGTCTGTACGTT GAAGTGTATACC-3'), *FLR1*, middle of the coding region (5'-GTTGGTTGTGCTACTGT GCATAAC-3' and 5'-AAACGAGAGGAACCATTTCTGG-3'), *FLR1*, 3' end of the coding region (5'-TACCCAAAGTATGTTGCATCCG-3' and 5'-CCATGCCACAGGATAGTTCTT AGT-3'), *PHO5* (5'-CCATTTGGGATAAGGGTAAACATC-3' and 5'-AGAGATGAAGCCATACTAACCTCG -3'), *GAL1* (5'-CGCTTAACTGCTCATTGCTATATTG-3' and 5'-TTGTTCGGAGCAGTGCGG-3'), *TRX2* (5'-ATACGACAGTGCTTTAGCATCTGG-3' and 5'-GTTCTGCAAACTTTTCAATCATTG-3'), *BNA1* (5'-GCGTCAGGATAAATTGTGAGTCAC-3' and CCTTCGTTCTCCTTCAACCATTTG-3'), *HSP12* (5'-AAGCACTCTAGACGGAGAGTAACTAG-3' and 5'-GAATCCTTTTCTACCTGCGTCAG-3'), *HSP26* (5'-CGGATCTATGCACGTTCTTGAGTG-3' and 5'-CCTTCACCCAGCAATCTGTTAAAG-3').

Real time RT-PCR analysis

Total RNA was isolated from cultures grown in YPD medium to optical density A_{600nm} = 1.0 by the hot phenol method, treated with RNase-free DNase (Qiagen) and purified with an RNeasy Mini Kit (Qiagen). Reverse transcription and real time PCR amplification were performed with iScript kit (BioRad) using 100 ng of RNA and the following primers: *ACT1* (5'-TATGTGTAAAGCCGGTTTTGC-3' and 5'-GACAATACCGTGTTCAATTGGG-3'), *FLR1* (5'-ACAAGAAAACCCGAGAATACCG-3' and 5'-

TGGGTTCTCAGGATCACTGG-3'), *PHO5* (5'-CAATTTTAGCCGCTTCTTTGG-3' and 5'-AAAGAGTAGTATGGTCCGGCAC-3'), *GAL1* (5'-CCTGAGTTCAATTCTAGCGCAA-3' and 5'-GCAACAAAATCCGGTTTAGCA-3'), *TRX2* (5'-ATACGACAGTGCTTTAGCATCTGG-3' and 5'-GTTCTGCAAACTTTTCAATCATTG-3'), *BNA1* (5'-TGGGCCTAATGAAAGAACCG-3' and 5'-GAACAGGACTGTGAGGAACATTTC-3'), *HSP12* (5'-AGTCATACGCTGAACAAGGTAAGG-3' and 5'-AGTCGTGGACACCTTGGAAGAC-3'), *HSP26* (5'-AAGACGTCAGTTAGCAAACACACC-3' and 5'-CATTGTCGAACCAATCATCTAAGG-3').

- Derepression of *FLR1* transcription in *sin4* cells depends on Yap1p.
- Assembly of the PIC is facilitated in *sin4* cells.
- The tail and head/middle modules of the mediator are separated in *sin4* cells.
- In *sin4* cells the tail is at the promoter and the head/middle module is at the orf.

Abbreviations

RNA pol II	RNA polymerase II
CTD	carboxyl terminal domain of RNA pol II
$InsP_s$	inositol polyphosphates
PIC	preinitiation complex
ChIP	chromatin immunoprecipitation

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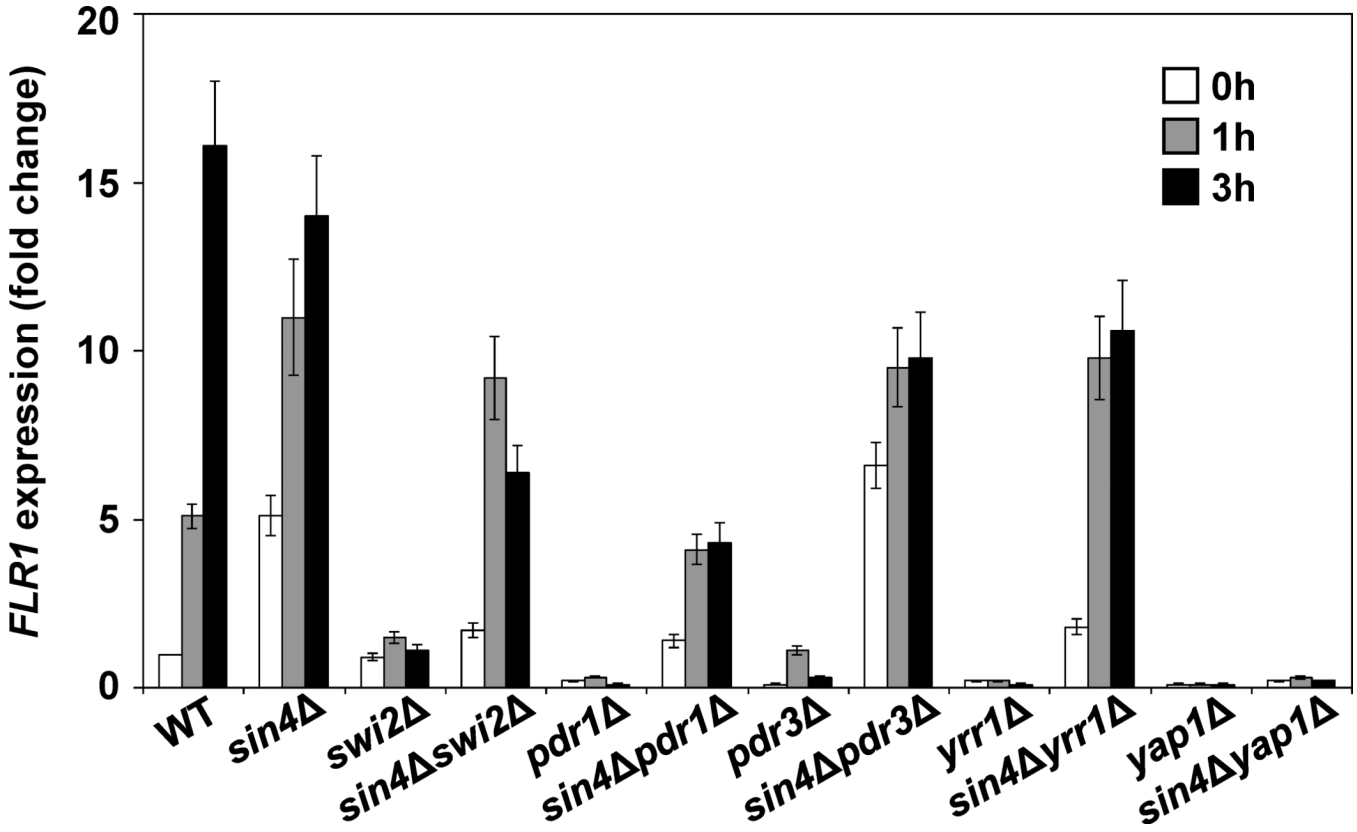

Figure 1.

Defective expression of *FLR1* in *swi2*Δ, *pdr1*Δ, *pdr3*Δ, and *yrr1*Δ cells is partially suppressed by *sin4*Δ mutation. Indicated trains were grown in YPD medium at 30°C to an A_{600} of 1.0. Benomyl was added to a final concentration of 5 μg/ml and samples were collected after 0, 1, and 3 h. Total RNA was isolated and assayed for *ACT1* and *FLR1* transcripts by real-time RT-PCR. The results were normalized to *ACT1* RNA and expressed relative to the value for the WT strain at 0 h. The experiment was repeated three times, and the results are shown as means ± SD.

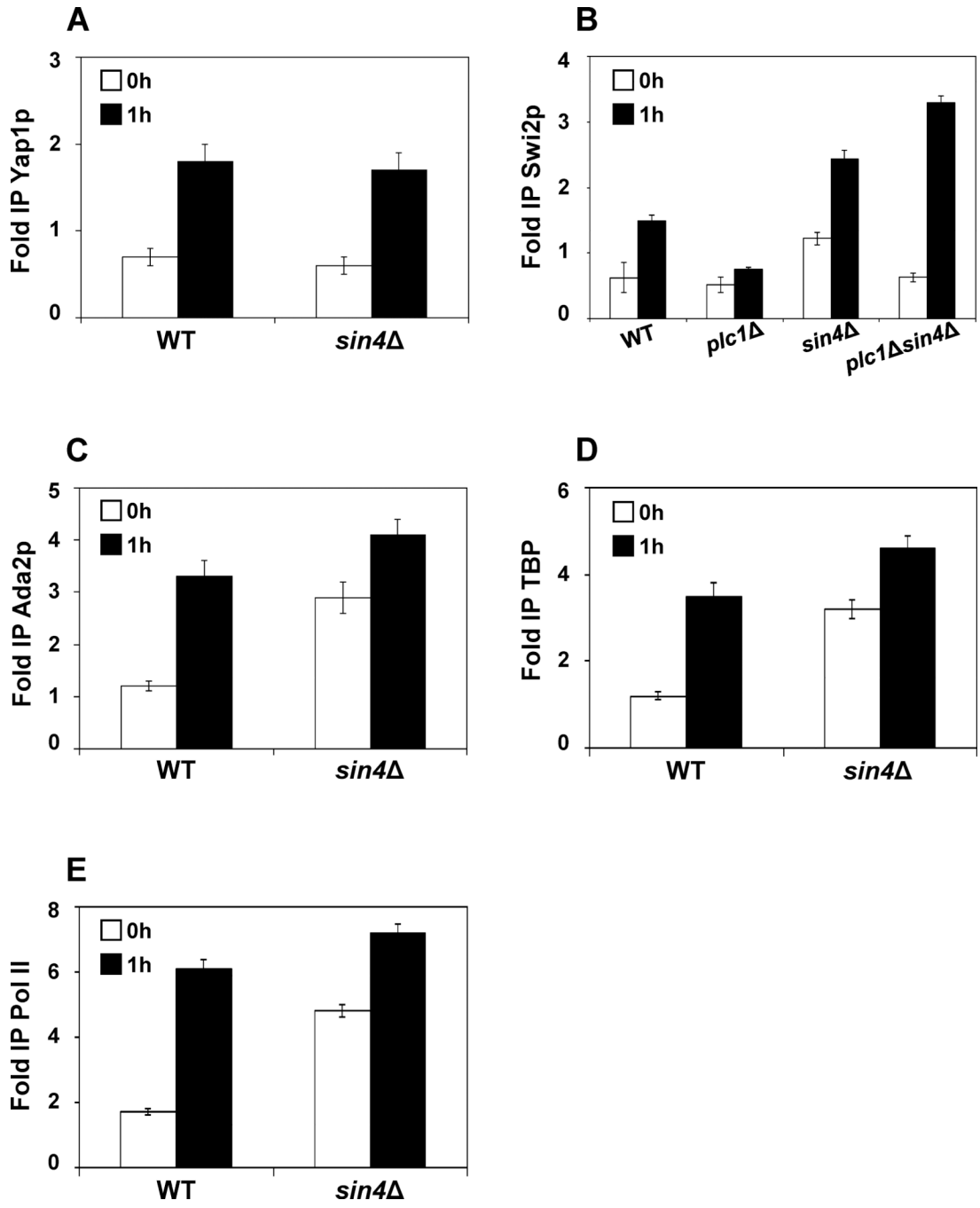

Figure 2. *sin4*Δ mutation facilitates PIC assembly

(A) Recruitment of Yap1p to the *FLR1* promoter is not altered in *sin4*Δ cells. Yap1p occupancy was determined with anti-Yap1p polyclonal antibody. (B) *plc1*Δ cells fail to recruit Swi2p to the *FLR1* promoter and the defect is suppressed by *sin4*Δ mutation. Swi2p occupancy was determined in strains expressing Swi2p-myc18 using anti-myc antibody. (C) *sin4*Δ cells display increased recruitment of Ada2p to the *FLR1* promoter. Ada2p occupancy was determined in strains expressing Ada2p-myc18 using anti-myc antibody. (D) *sin4*Δ cells display increased recruitment of TBP to the *FLR1* promoter. TBP occupancy was determined in strains expressing Spt15p-3HA using anti-HA antibody. (E) *sin4*Δ cells display increased recruitment of RNA Pol II to the *FLR1* promoter. RNA Pol II occupancy

was determined by immunoprecipitation of Rpb1p (the largest subunit of RNA polymerase II) with 8WG16 mAb. (A–E) ChIP was performed using chromatin from the corresponding cells grown in YPD medium (0 h) and treated with benomyl (5 μg/ml) for 1 h. Each immunoprecipitation was performed at least three times using different chromatin samples. The occupancy was calculated with *POL1* coding sequence as a negative control. The data are presented as fold occupancy over the *POL1* coding sequence control and represent means ± SD.

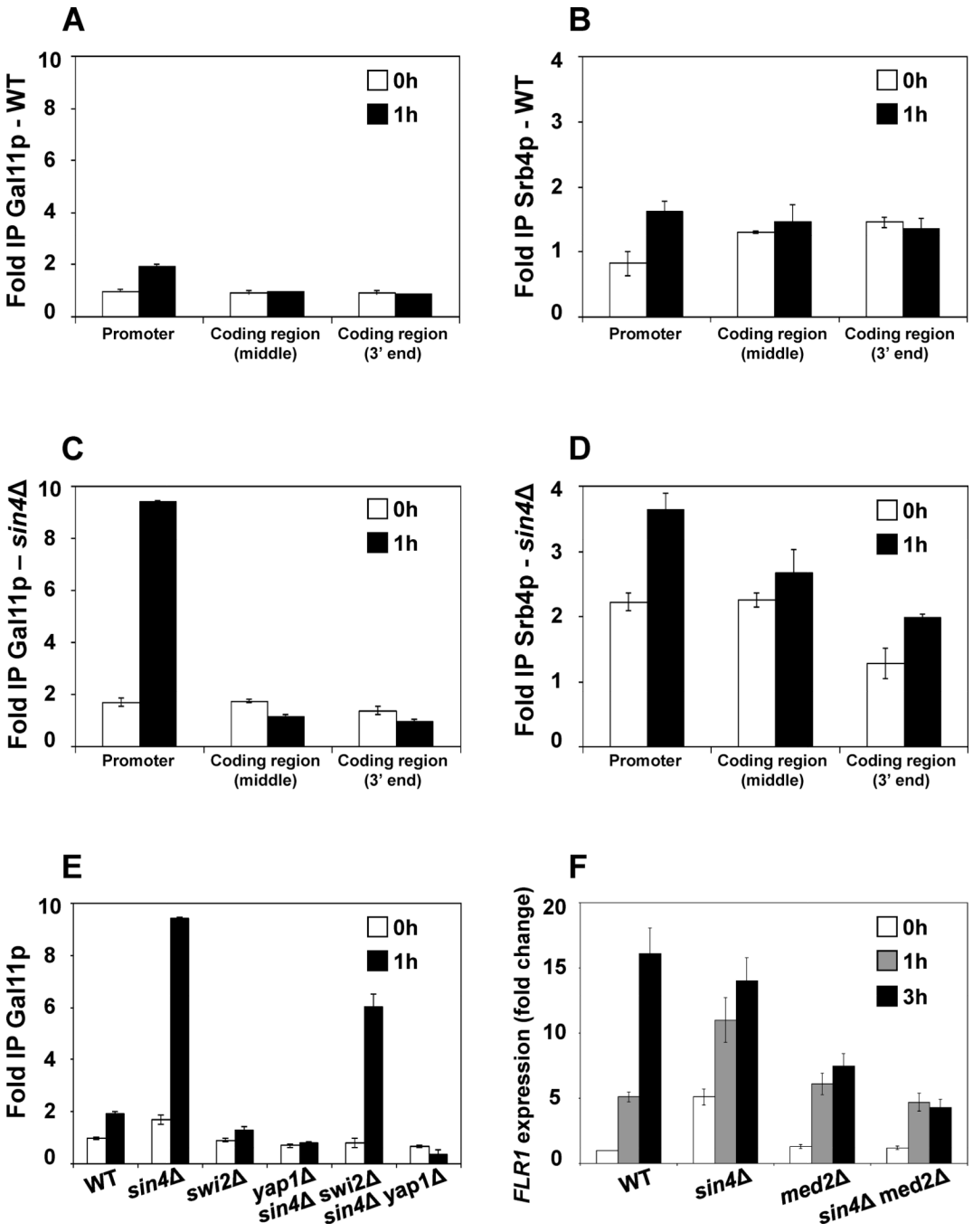

Figure 3. The tail and head/middle modules behave as separate complexes in *sin4*Δ cells

(A,B) Recruitment of Gal11p and Srb4p to the *FLR1* promoter and coding region in wild-type cells. (C,D) Recruitment of Gal11p and Srb4p to the *FLR1* promoter and coding region in *sin4*Δ cells. (E) Recruitment of Gal11p to the *FLR1* promoter in wild-type, *sin4*Δ, *swi2*Δ, *yap1*Δ, *sin4*Δ *swi2*Δ, and *sin4*Δ *yap1*Δ cells. Gal11p and Srb4p occupancies were determined in strains expressing Gal11p-3HA and Srb4p-myc9 using anti-HA and anti-myc antibodies, respectively. ChIP was performed using chromatin from the corresponding cells grown in YPD medium (0 h) and treated with benomyl (5 μg/ml) for 1 h. Each immunoprecipitation was performed at least three times using different chromatin samples. The occupancy was calculated with *POL1* coding sequence as a negative control. The data

are presented as fold occupancy over the *POL1* coding sequence control and represent means ± SD. (F) *FLR1* expression in *sin4*Δ cells requires *MED2*. Indicated trains were grown in YPD medium at 30°C to an A_{600} of 1.0. Benomyl was added to a final concentration of 5 μg/ml and samples were collected after 0, 1, and 3 h. Total RNA was isolated and assayed for *ACT1* and *FLR1* transcripts by real-time RT-PCR. The results were normalized to *ACT1* RNA and expressed relative to the value for the WT strain at 0 h. The experiment was repeated three times, and the results are shown as means ± SD.

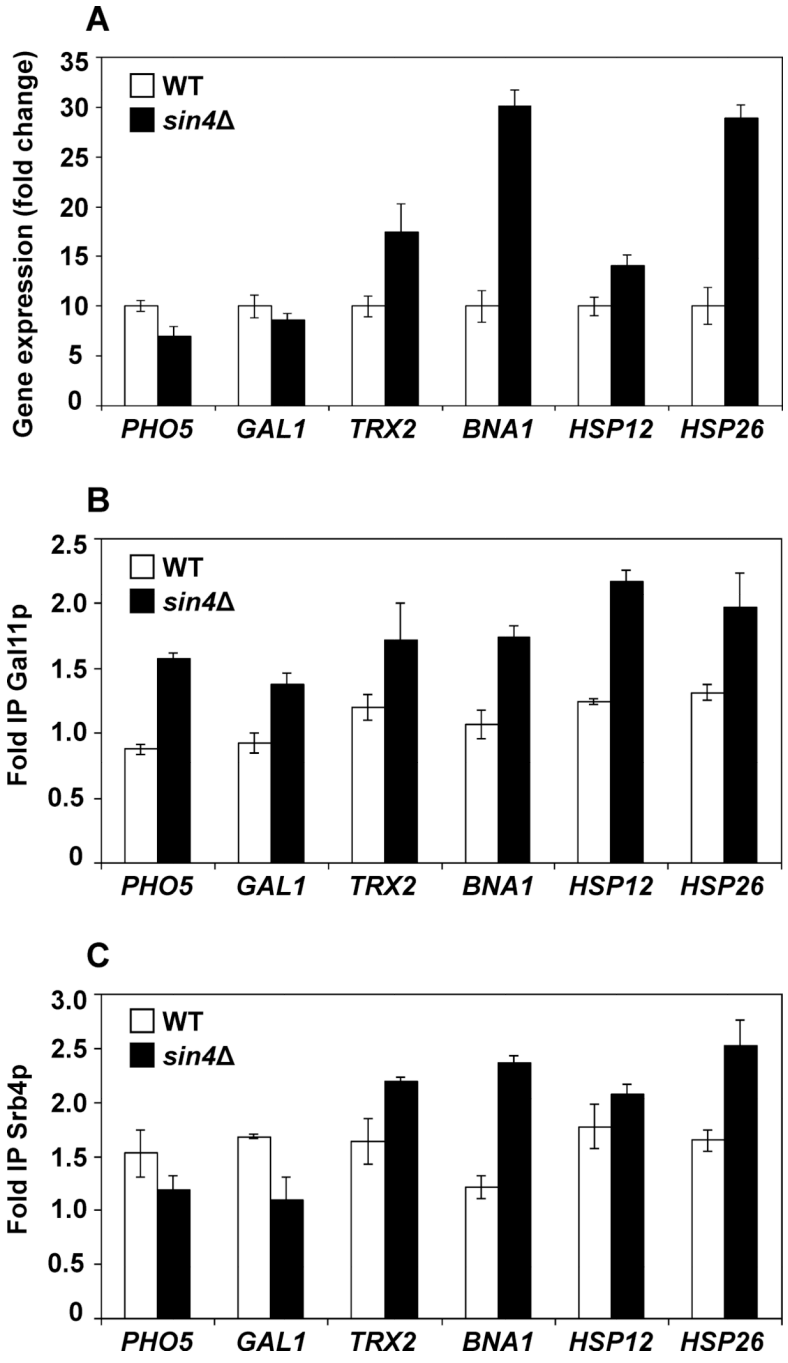

Figure 4. The tail module is recruited to various promoters in *sin4*Δ cells

(A) *sin4*Δ mutation affects expression of several genes. Indicated trains were grown in YPD medium at 30°C to an A_{600} of 1.0. Total RNA was isolated and assayed for *ACT1*, *PHO5*, *GAL1*, *TRX2*, *BNA1*, *HSP12*, and *HSP26* transcripts by real-time RT-PCR. The results were normalized to *ACT1* RNA and expressed relative to the value for the WT strain. The experiment was repeated three times, and the results are shown as means ± SD. (B, C) Gal11p and Srb4p occupancies at selected promoters. Gal11p and Srb4p occupancies were determined in strains expressing Gal11p-3HA and Srb4p-myc9 using anti-HA and anti-myc antibodies, respectively. ChIP was performed using chromatin from the corresponding cells grown in YPD medium. Each immunoprecipitation was performed at least three times using

different chromatin samples. The occupancy was calculated with *POL1* coding sequence as a negative control. The data are presented as fold occupancy over the *POL1* coding sequence control and represent means ± SD.

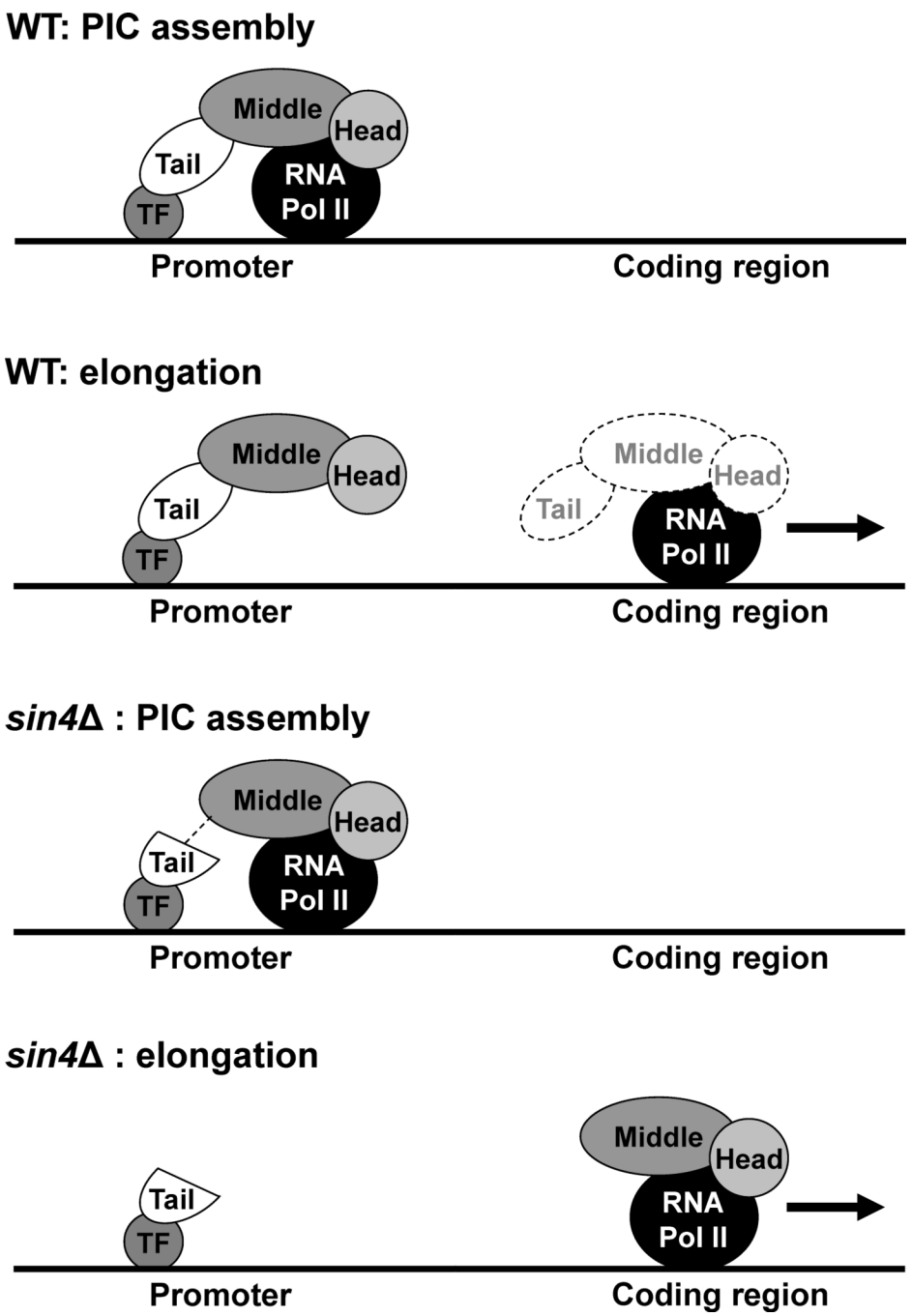

Figure 5.

Model depicting the comparison of transcription in wild-type cells expressing intact mediator and transcription in *sin4*Δ cells. In *sin4*Δ cells, we found the tail module anchored to the promoter of the *FLR1* gene, while the head/middle module was also found in the coding region. Interestingly, intact mediator was found not only in the promoters but also in the coding regions of number of strongly transcribed genes.[24] We speculate that in wild-type cells, the mediator partitions between the promoter and coding region, depending on the architecture of the promoter and interactions of the mediator with the DNA-binding transcriptional factors (TF), RNA Pol II, and other components of the transcriptional machinery.

Table 1

Yeast strains used in this study

Strain	Genotype	Source/reference
W303-1a	*MATa ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100*	R. Rothstein
W303-1α	*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 ssd1-d2 can1-100*	R. Rothstein
W303	*MATa/MATα ade2-1/ade2-1 his3-11,15/his3-11,15 leu2-3,112/leu2-3,112 trp1-1/ trp1-1 ura3-1/ ura3-1 can1-100/ can1-100*	R. Rothstein
HL1-1	W303-1α *plc1::URA3*	Ref. 55
HL1-3	W303-1a *plc1::URA3*	Ref. 56
DY1699	W303-1a *sin4::LEU2*	Ref. 57
PD169	W303-1a *sin4::LEU2*	This study
DY2348	W303-1a *swi2::HIS3*	Ref. 57
PD185	W303-1 *pdr1::kanMX*	This study
PD187	W303-1 *pdr3::kanMX*	This study
PD183	W303-1 *yrr1::kanMX*	This study
PD181	W303-1 *yap1::kanMX*	This study
DY5696	W303-1a *med2::TRP1*	Ref. 14
ND96	W303-1α *sin4::TRP1 plc1::URA3*	Ref. 27
PD176	W303-1a *sin4::LEU2 swi2::HIS3*	This study
PD184	W303-1a *sin4::LEU2 pdr1::kanMX*	This study
PD186	W303-1a *sin4::LEU2 pdr3::kanMX*	This study
PD182	W303-1a *sin4::LEU2 yrr1::kanMX*	This study
PD180	W303-1a *sin4::LEU2 yap1::kanMX*	This study
LG065	W303-1a *sin4::LEU2 med2::TRP1*	This study
K8135	W303-1a *ADA2-myc18::TRP1*	Ref. 58
PD171	W303-1a *sin4::LEU2 ADA2-myc18::TRP1*	This study
AD066	W303-1a *SPT15-HA3::URA3*	Ref. 59
PD152	W303-1a *sin4::TRP1 SPT15-HA3::URA3*	This study
DY6260	W303-1a *SRB4-myc9::TRP1*	Ref. 14
NG034	W303-1a *SRB4-myc9::TRP1*	This study
PD149	W303-1a *sin4::LEU2 SRB4-myc9::TRP1*	This study
DY6361	W303-1α *SWI2-myc18::TRP1*	Ref. 60
NG022	W303-1a *SWI2-myc18::TRP1*	Ref. 61
NG019	W303-1a *plc1::URA3 SWI2-myc18::TRP1*	Ref. 61
LG181	W303-1a *sin4::LEU2 SWI2-myc18::TRP1*	This study
YNYC108	*MATa ura3-52, his3Δ200 leu2Δ1 trp1Δ63 lys2Δ385 gal4Δ11 GAL11-3HA::HIS5*	Ref. 62
LG102	W303-1a *GAL11-3HA::HIS5*	This study
LG070	W303-1a *sin4::TRP1 GAL11-3HA::HIS5*	This study
LG173	W303-1a *plc1::URA3 sin4::LEU2 SWI2-myc18::TRP1*	This study
LG136	W303-1a *sin4::TRP1 swi2::kanMX GAL11-HA3::HIS5*	This study
LG076	W303-1a *sin4::LEU2 yap1::kanMX GAL11-HA3::HIS5*	This study