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Independent Recruitment of Mediator and SAGA by the Activator Met4

Christophe Leroy, Laëtitia Cormier, and Laurent Kuras*

Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique,
91198 Gif-sur-Yvette, France

**Corresponding author.* Mailing address : Centre de Génétique Moléculaire, Centre National de la Recherche Scientifique, 91198 Gif-sur-Yvette, France. Phone: +33(0)169823162. Fax: +33(0)169824372. E-mail: laurent.kuras@cgm.cnrs-gif.fr

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ABSTRACT

Mediator is a key RNA polymerase II (pol II) cofactor in the regulation of eukaryotic gene expression. It is believed to function as a coactivator linking gene-specific activators to the basal pol II initiation machinery. In support to this model, we provide evidence that Mediator serves *in vivo* as a coactivator for the yeast activator Met4, which controls the gene network responsible for the biosynthesis of sulfur-containing amino acids and S-adenosylmethionine. In addition, we show that SAGA (Spt-Ada-Gcn5-acetyltransferase) is also recruited to Met4 target promoters where it participates in the recruitment of pol II by a mechanism involving histone acetylation. Interestingly, we find that SAGA is not required for Mediator recruitment by Met4, and *vice versa*. Our results provide a novel example of functional interplay between Mediator and coactivators involved in histone modification.

INTRODUCTION

Synthesis of eukaryotic messenger RNA requires the assembly at the promoter of a basal transcription machinery comprising RNA polymerase II (pol II) and a defined set of general transcription factors (GTFs), including TATA-binding protein (TBP), TFIIB, IIE, IIF, and IIH.

5 The regulation of this process in response to environmental signals involves a number of additional factors termed coactivators, which are recruited to enhancers or upstream activating sequences (UAS) by gene-specific activators and operate by several distinct mechanisms involving alteration of the structure of chromatin and direct interaction with pol II and GTFs. Mediator has emerged in recent years as a prominent coactivator linking activators with the basal
10 transcription machinery from yeast to man (reviews in 7, 11, 28, 29, 46).

The *S. cerevisiae* core Mediator complex comprises 21 subunits and is found in free form and as a holoenzyme in association with pol II (20, 27, 44, 59). Electron microscopy and image processing revealed that *S. cerevisiae* Mediator presents an extended conformation divided in three distinct submodules (termed head, middle and tail domains) in association with pol II (1,
15 15). The head and middle domains establish multiple contacts with pol II whereas the tail domain extends away from pol II (14). The tail domain contains subunits involved in interactions with several activators, including Gal4 and Gcn4 (42, 48). In addition to supporting activated transcription *in vitro*, Mediator has the capacity to stimulate basal transcription as well as TFIIH-dependent phosphorylation of pol II carboxy-terminal domain (CTD) (27).

20 The general requirement of Mediator for pol II transcription *in vivo* was shown in genome-wide transcription analysis with a yeast temperature-sensitive mutant of Med17 (Srb4) (23). This analysis revealed that genome-wide transcription is as dependent on Med17 (Srb4) as it is on pol II largest subunit Rpb1; however, this analysis did not distinguish whether Med17 (Srb4) was required for basal transcription or interaction between activators and the basal transcription

machinery. In fact, only a limited number of activators have been shown to recruit Mediator *in vivo* (6, 9, 17, 51, 56), and the question whether Mediator is generally required as a coactivator remains unanswered.

The *S. cerevisiae* SAGA complex is an example of coactivator that targets the chromatin. SAGA is a multisubunit complex that contains Gcn5, a histone acetyltransferase (HAT) protein which preferentially acetylates nucleosomal histone H3 (19). Histone acetylation is thought to destabilize chromatin higher-order structure and, as a result, improve accessibility to DNA for transcription factors. Alternatively, histone acetylation may provide binding sites for bromodomain-containing proteins, such as the Swi2/Snf2 subunit of the chromatin remodelling complex Swi/Snf (22). At certain promoters, SAGA has been proposed to operate independently of its HAT activity by directly interacting with TBP through its Spt3 and Spt8 subunits (3, 4, 16, 38).

Met4 is the transcriptional activator which controls the gene network responsible for the biosynthesis of the sulfur-containing amino-acids, methionine and cysteine (58). Met4 is also involved in the regulation of the genes needed for the biosynthesis of S-adenosylmethionine (34), a sulfur-containing compound widely used as methyl donor in methylation of proteins, nucleic acids and lipids. Met4 activity is tightly regulated by the intracellular concentration of methionine, cysteine and S-adenosylmethionine (58). Under conditions of excess, Met4 is inactivated by ubiquitination *via* the SCF^{Met30} ubiquitin-ligase (49, 53). The consequence of Met4 ubiquitination depends on the environmental growth conditions. When cells are grown in minimal medium, ubiquitination targets Met4 for degradation by the 26S proteasome (53). In contrast, when cells are grown in rich medium, ubiquitination does not affect Met4 stability but impairs its recruitment to DNA (26, 34). Under conditions of limitation in sulfur-containing amino-acids, Met4 is tethered to its target genes through interaction with the DNA-binding

factors Cbf1 and Met31/32, which bind two distinct DNA elements found, individually or in combination, upstream of all Met4-responsive genes (8, 31). In addition to its role in the recruitment of Met4, Cbf1 is also required for the function of centromeres (2, 10). Because Cbf1 is partly dispensable for *MET* gene regulation, Met31/32 provide the main platform for the
5 recruitment of Met4 to DNA (8, 33, 37). Cbf1 and Met31/32 possess no intrinsic capacity to activate transcription and they are thought to be mainly dedicated to the recruitment of the activator Met4 (8, 33, 57).

In this study, we present evidence that Met4 uses Mediator as a coactivator, reinforcing the notion that Mediator is a prevalent interface between enhancer-bound activators and pol II
10 transcription machinery in yeast. In addition, we find that SAGA is also recruited to Met4 target genes and catalyses acetylation of histone H3 through its Gcn5 HAT subunit. Interestingly, the recruitment of Mediator by Met4 does not require SAGA, and *vice versa*. These results provide a novel instance of interplay between Mediator and SAGA.

MATERIALS AND METHODS

Yeast strains, plasmids and media. Yeast strains used in this study are listed in Table 1.

TAP-tagged Mediator subunits and hemagglutinin (HA)-tagged TBP were generated as described (32). Gene deletions and HA-tagged strains were generated using PCR-based, one-step integration strategies (45). Plasmids *pGAL1-oplexA-lacZ*, *plexA-MET4* and *plexA-MET4Δ12* were described previously (36). B minimal medium is a synthetic medium lacking organic and inorganic sulfur sources (37). YPD medium contains 1% yeast extract, 2% bacto-peptone and 2% glucose. YNB medium contains 0.7% yeast nitrogen base, 0.5% ammonium sulfate and 2% glucose.

Chromatin immunoprecipitation method. ChIP experiments were carried out essentially as previously described (32, 35). Cells (culture of 100 ml at a density of $1-2 \times 10^7$ cells/ml) were fixed with 1.4% formaldehyde for 15 min at 28°C. Fixation was stopped by addition of glycine to a final concentration of 0.4 M. Cells were disrupted in FA-lysis buffer (50 mM Hepes-KOH, pH7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% deoxycholic acid sodium salt, 0.1% SDS) containing protease inhibitors using a FastPrep instrument (Qbiogene). The crosslinked chromatin was collected by centrifugation for 15 min at 16100g, 4°C in a microcentrifuge, resuspended in 2 ml FA-lysis buffer containing protease inhibitors, and subjected to sonication yielding DNA fragments in a size range between 100 to 1000 bp with an average of 400 bp. The insoluble debris was eliminated by centrifugation for 10 min at 16100g, 4°C.

TAP-tagged proteins were immunoprecipitated by incubating chromatin from $2-3 \times 10^8$ cells with 15 μ l of rabbit IgG-agarose (Sigma) or human IgG-Sepharose (Amersham Pharmacia) for 60 to 90 min at room temperature. Immune complexes were washed twice in FA lysis buffer

containing 1M NaCl, once in 10 mM Tris-HCl pH 8.0, 0.25 M LiCl, 1 mM EDTA, 0.5% NP-40, 0.5% Na-deoxycholate, once in 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.150 M urea and once in 10 mM Tris-HCl pH 8.0, 1 mM EDTA. Other proteins were immunoprecipitated by incubating for 60 to 90 min at room temperature chromatin from 2-3 10^8 cells with specific antibodies

5 prebound to 15 μ l of protein A-Sepharose (Amersham Pharmacia). The antibodies used in this study include monoclonal antibodies to HA (F7, Santa Cruz Biotechnology; 20 μ l per IP) and pol II CTD (8WG16, Covance; 5 μ l per IP) and polyclonal antibodies to Met4 (kindly provided by Mike Tyers, Samuel Lunenfeld Research Institute; 10 μ l per IP), Gal4 DBD (Santa Cruz Biotechnology; 20 μ l per IP), H3 carboxy terminus (Abcam; 2 μ l per IP), acetyl K9 and acetyl

10 K14 (both from Abcam or Upstate Biotechnology; 2 μ l per IP). Immune complexes were washed three times in FA lysis buffer containing 0.5 M NaCl, once in 10 mM Tris-HCl pH 8.0, 0.25 M LiCl, 1 mM EDTA, 0.5% NP-40, 0.5% Na-deoxycholate and once in 10 mM Tris-HCl pH 8.0, 1 mM EDTA. Immunoprecipitates (IP) were eluted from the beads by heating for 20 min at 65°C in 125 μ l of 25 mM Tris-HCl pH7.5, 5 mM EDTA, 0.5%. Formaldehyde crosslinks were

15 reversed by heating the eluates over night at 70°C in the presence of 1 mg/ml Pronase (Roche). Aliquots of total chromatin input were processed in parallel for subsequent normalization. DNA was purified using QIAquick DNA cleanup system (Qiagen). Final elution was performed with 100 μ l of TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA). Amounts of specific DNA targets present in input and IP samples were measured by real-time PCR using the LightCycler instrument and

20 the FastStart DNA Master SYBR Green I mix (Roche). Measures were calculated using two separate aliquots or two independent dilutions of the sample with a standard deviation less than 15%. Primers used are listed in Table 2. A standard curve was generated for each run using a dilution series of input sample. This standard curve was used to assess the PCR efficiency and

determine the relative concentration of target DNA in other samples. The specificity of the PCR products was assessed by performing a melting curve analysis. Data were analyzed with the LightCycler software, version 3. The IP/Tot ratio corresponds to the concentration of target DNA in the IP sample relative to that in the corresponding input sample. For each immunoprecipitation, the highest value obtained was set to 100 and other values were expressed relative to this maximum.

Analysis of mRNA levels. Total RNA was isolated by extraction with hot acidic phenol (25). cDNA was generated by SuperScript II Reverse Transcriptase (Invitrogen) using p(dN)₆ random hexamers (Roche) and anchored oligo(dT)₂₃ primers (Sigma). Oligonucleotides specific for U4 small nuclear RNA or 25S ribosomal RNA (sequence in Table 2) were incorporated in the reaction to serve as internal reference. Individual cDNAs were quantified by real-time PCR using the LightCycler instrument and the FastStart DNA Master SYBR Green I reaction mix (Roche). Data were analyzed with the LightCycler data analysis software.

RESULTS

Mediator is required for transcription of sulfur-containing amino-acid biosynthesis genes. To determine whether Mediator is required for the regulation of the genes responsible for cysteine and methionine biosynthesis (referred to as *MET* genes), we first tested the ability of yeast strains containing null alleles of all non-essential subunit of core Mediator to metabolize inorganic sulfur sources. We tested in parallel a strain containing a viable mutation in the essential subunit Med14 (Rgr1) (*med14-100*) (13). As shown in figure 1A, mutation of Med2, Med3, Med14 (Rgr1) and Med15 (Gal11) led to a severe loss of viability on medium containing sulfate compared to methionine. Remarkably, the phenotype of the *med14-100* (*rgr1-100*) and *med15* Δ (*gal11* Δ) strains was very similar to that of the *met4* Δ strain which lacks the activator controlling the *MET* genes, consistent with the possibility that Mediator plays a central role in expression of Met4 target genes.

To further define this role, we examined the effect of mutations in Mediator on *MET* gene transcription (figure 1B). The results showed that mutation of the tail domain subunits Med2 or Med3 and the middle subunits Med14 (Rgr1) or Med9 caused a marked decrease (two- to sevenfold) in mRNA levels of all five *MET* genes examined. Interestingly, deletion of the tail subunit Med15 (Gal11) caused a decrease by a factor of two or more in mRNA levels of *MET3*, *MET17* and *CYS3* but not *MET2* and *MET30*. Conversely, deletion of the middle subunit Med1 caused a decrease by a factor of two or more in mRNA levels of *MET2* and *MET30* but not *MET3*, *MET17* and *CYS3*. In contrast to other tail and middle subunit mutants, the *med16* Δ (*sin4* Δ) and *med5* Δ (*nut1* Δ) mutants showed only a weak or no defect in transcription of *MET* genes. Deletion of the two non-essential subunits of the head domain Med18 (Srb5) and Med20

(Srb2) had no major effect either. To extend this analysis to essential subunits of the head domain, we measured mRNA levels in yeast strains bearing temperature-sensitive alleles of Med6, Med11 and Med17 (Srb4) (figure 1C). The results showed that inactivation of Med11 upon incubation at non-permissive temperature had no effect whereas inactivation of Med6 and Med17 (Srb4) caused a major decrease (five- to fiftyfold) in mRNA levels of all *MET* genes examined. These results, summarized in the schematic figure 1D, demonstrate as a whole that Mediator is required for full activation of *MET* genes. In addition, individual subunits in each domain are differentially required and some subunits seem to be required at only a subset of *MET* genes.

10 Transcription activation is a complex process with several successive steps, including activator binding, pol II recruitment, transcription initiation, elongation and termination. Defects in each step can have an impact on final mRNA levels. To clarify the function of Mediator in Met4-mediated activation, we performed ChIP experiments to measure pol II and Met4 recruitment to *MET* promoters in cells containing mutations in the subunits Med3, Med14 (Rgr1) and Med15 (Gal11) (figure 2). The results showed that pol II association with *MET2*, *MET3*, *MET17*, *MET30* and *CYS3* promoters is decreased by a factor of two to five in the mutants compared to the wild-type. In contrast, Met4 association did not show any decrease. These results strongly suggest that Mediator operates in the first place during the recruitment of pol II to Met4 target promoters, although they do not exclude additional roles in subsequent steps.

20 **Mediator associates with *MET* genes under activating conditions.** We next asked whether Mediator is physically recruited to Met4 target genes and whether its recruitment correlates with transcriptional activity. To address these questions, we first performed ChIP analysis with a strain expressing TAP-tagged Med14 (Rgr1) and HA-tagged TBP. Analysis of the immunoprecipitates showed association of Med14 (Rgr1) with *MET* promoters under inducing

conditions but not under repressing conditions (figure 3A). To assess transcriptional activity, we measured in parallel TBP and RNA pol II association. Comparison of the results for Med14 (Rgr1), TBP and RNA pol II revealed a strong correlation between Med14 (Rgr1) recruitment and transcriptional activity (note that the apparent high TBP occupancy at *MET17* in repressing conditions is due to the presence of a tRNA gene just upstream *MET17* promoter). Similar results were obtained with strains expressing TAP-tagged derivatives of the middle subunits Med5 (Nut1) and the head subunits Med18 (Srb5) (figure 3B and C). Additional ChIP experiments showed also association with *MET* genes of the tail subunit Med2, the middle subunits Med10 and Med21 (Srb7), as well as the head subunits Med6 and Med19 (Rox3) (figure 3D). We conclude that the entire Mediator associates with transcriptionally active but not inactive Met4 target promoters.

The Met4 activator recruits Mediator through its activation domain. We next sought to establish Mediator localization along the prototype *MET2* promoter. Like most Met4 target genes, *MET2* upstream regulatory region contains binding sites for the DNA-binding factors Cbfl and Met31/32, which serve as anchorage platforms for Met4 (figure 4A). Med14 (Rgr1) and TBP immunoprecipitates from cells incubated in inducing conditions were analyzed with PCR primer pairs flanking Cbfl and Met31/32 binding sites (fragment A) or flanking the TATA box (fragment B). Comparison of the results showed that fragment A was significantly more abundant than fragment B in Med14 (Rgr1) and Met4 immunoprecipitates. By contrast, fragments A and B were equally represented in the TBP immunoprecipitate (note that the TATA box is at the 5' end of fragment B and very close to fragment A). Similar results were obtained with cells expressing TAP-tagged versions of the middle subunit Med5 (Nut1) and the tail subunit Med18 (Srb5) (data not shown). Therefore, Mediator shows preferential association with the upstream regulatory

region and not to the core promoter at *MET2*. This observation is consistent with the idea that Mediator is targeted to *MET* genes primarily through interaction with Met4 and its cofactors.

To assess the respective contributions of Met4 and its cofactors Cbf1 and Met31/32 in recruitment of Mediator, we performed ChIP analysis on a *met4Δ* mutant (figure 4B). The results showed that association of Med14 (Rgr1) with *MET2*, *MET17* and *CYS3* was completely abolished in the *met4Δ* mutant, indicating that Met4 is essential for recruitment of Mediator to *MET* genes whereas Met4 cofactors are not able to recruit Mediator on their own. We next asked whether Met4 would be able to recruit Mediator independently of its cofactors. To answer this question, ChIP analysis was performed on a strain expressing Met4 fused to the DNA-binding domain of LexA and containing a *lacZ* reporter gene driven by LexA operators integrated at the chromosome (figure 4C). The results showed that Med14 (Rgr1) is efficiently recruited to the reporter gene in cells expressing LexA-Met4 compared to cells expressing no LexA-Met4. Therefore, Met4 can recruit Mediator in the absence of its cofactors Cbf1 and Met31/32. To assess the requirement for Met4 activation domain, we performed ChIP analysis on a strain expressing a derivative of LexA-Met4 lacking the region spanning the activation domain. The results showed that deletion of Met4 activation domain completely abolished the recruitment of Med14 (Rgr1) without affecting the binding of LexA-Met4.

We conclude that Met4 has the intrinsic ability to target Mediator to DNA. Moreover, recruitment of Mediator by Met4 requires a functional activation domain.

SAGA serves as a coactivator for transcriptional activation by Met4. During the course of this work, Bhaumik et al (5) found that recruitment of Mediator by Gal4 required SAGA, leading to the proposal that SAGA might function at some promoters as an “adaptor” that enables DNA-bound activators to recruit Mediator. To determine whether this model would apply to the

MET gene network, we examined whether SAGA was involved in Met4-activated transcription. To this end, we first performed quantitative RT-PCR on mutant strains lacking Gcn5, Spt3 and Spt20, three distinct subunits of SAGA involved in histone acetylation, TBP recruitment and structural integrity, respectively (3, 19, 38, 54). As shown in figure 5A, mRNA levels for *MET* genes were reduced as much as tenfold in the *gcn5Δ* and *spt20Δ* mutants compared to the wild-type cells. By contrast, *MET* gene transcription is only mildly affected in the *spt3Δ* mutant. These results demonstrate that some functions of SAGA, but not all, are required for transcriptional activation by Met4. We next performed ChIP analysis on strains expressing HA-tagged forms of Gcn5, Spt3 and Spt20 (figure 5B). The results showed association of the three subunits of SAGA with *MET2*, *MET17* and *CYS3* promoters under inducing conditions but not under repressing conditions. Additional ChIP experiments revealed that Gcn5 association with *MET* genes is reduced to background level in a *met4Δ* strain (figure 5C). Altogether, our results suggest that SAGA serves as a coactivator for transcriptional activation by Met4.

Comparison of transcription levels among the different SAGA mutants (figure 5A) showed that elimination of Gcn5 had a similar effect on *MET* mRNA levels as elimination of Spt20, which is required for structural integrity, suggesting that the function of SAGA in Met4-activated transcription might involve Gcn5-mediated histone acetylation. To test this possibility, we examined the levels of histone acetylation at *MET* genes at different times upon activation in parallel in the wild-type and *gcn5Δ* strains (figure 6A). The results showed higher levels of H3 acetylation at *MET17* and *MET2* in the wild-type compared to the *gcn5Δ* mutant. Moreover, following the induction, H3 acetylation was increased in the wild-type but not in the *gcn5Δ* mutant. These results argue for a Gcn5-dependent H3 acetylation at Met4 target genes upon transcriptional induction. Note that the difference in acetylation levels between the wild-type and

gcn5Δ strains at the 0 time point is certainly due to the fact that Met4 is not completely absent from *MET17* and *MET2* in complete medium and is still able to weakly activate transcription (34). This difference might also reflect Gcn5 role in global, untargeted histone acetylation (30, 60). To directly assess the importance of Gcn5 HAT activity in Met4 dependent activation, we next examined the recruitment of pol II at *MET* genes in a strain containing substitution mutations in Gcn5 that impairs its HAT activity (figure 6C). The results showed a defect in pol II recruitment to *MET17* and *MET2* in the catalytic mutant compared to the wild-type, supporting the hypothesis that function of SAGA in Met4 mediated activation depends, at least in part, on the HAT activity of its subunit Gcn5.

A remarkable feature of the results in figure 6A was that H3 acetylation in the wild-type strain peaked very rapidly following induction (within 4 min) and then diminished. To discriminate between removal of acetyl groups by deacetylases and loss of histone as a whole, the crosslinked chromatin was immunoprecipitated with an antibody specific for the carboxy terminal end of histone H3. The results in figure 6B showed a substantial decrease with time in histone H3 at *MET17* and *MET2* in the wild-type. Therefore, the apparent diminution in acetylation subsequent to the initial burst is certainly due to the loss of H3 and not to deacetylation. The results with the *gcn5Δ* mutant showed also a decrease in H3 association with *MET17* and, to a lesser extend, *MET2*. These results indicate that eviction of histone H3 from *MET17* and *MET2* promoters can occur in the absence of Gcn5 HAT activity.

Altogether, our results suggest that SAGA is recruited to *MET* promoters through interaction with Met4 and then acetylates histone H3 through its Gcn5 HAT subunit.

Independent recruitments of Mediator and SAGA by Met4 To characterize the functional relationship and respective mechanisms of action of Mediator and SAGA at *MET*

genes, we performed ChIP analysis on mutant cells containing null alleles of SAGA and Mediator subunits. The results with the *gcn5Δ* and *spt20Δ* mutants showed a decrease in pol II association with *MET17* and *MET2* in the mutants compared to the wild-type (by a factor of 2.4 to 6.7), but no significant decrease in Met4 association (figure 7A). Thus, SAGA facilitates the assembly of pol II basal transcription machinery at *MET* genes at a post-activator-binding step. Moreover, inactivation of Gcn5 and Spt20 did not cause any decrease in Med14 (Rgr1) association either, strongly suggesting that the recruitment of Mediator by Met4 is independent of SAGA. The results with the *med2Δ* and *med3Δ* mutants showed a decrease in Med14 (Rgr1) and pol II association with *MET17* and *MET2* in the mutants compared to the wild-type (by a factor of 2.8 to 4.8), but no decrease in Met4 association (figure 7B). Thus, inactivation of the tail subunits Med2 and Med3 has no significant effect on the binding of the Met4 but impairs the recruitment of Mediator and prevents formation of pol II basal transcription machinery. Moreover, inactivation of Med2 and Med3 did not affect Gcn5 and Spt3 association with *MET17* and *MET2* (figure 7B and C), suggesting that the recruitment of SAGA by Met4 is independent of Mediator.

To further explore the functional interdependence between SAGA and Mediator in Met4-activated transcription, we compared the growth of *med2Δ*, *gcn5Δ* and *med2Δ gcn5Δ* mutants on minimal medium containing either sulfate alone as sulfur source or with additional methionine (figure 8). The results showed that inactivation of Med2 and Gcn5 together had a more severe effect on growth in the absence of methionine than inactivation of Med2 and Gcn5 separately, suggesting that Mediator and SAGA can operate independently from each other and perform additive functions in the regulation of the *MET* gene network. Moreover, the growth of the *med2Δ* mutant was more severely affected than that of the *gcn5Δ* mutant, suggesting that the set

of *MET* genes regulated by SAGA does not completely overlap with that regulated by Mediator.

It is also possible that transcription of a *MET* gene particularly important for methionine biosynthesis is more affected in the *med2Δ* mutant compared to the *gcn5Δ* mutant.

DISCUSSION

In this report, we provide direct evidence that Mediator serves *in vivo* as a coactivator for transcriptional activation by the yeast activator Met4. First, mutations in several Mediator subunits impair transcription of the gene network regulated by Met4, leading ultimately to a defect in biosynthesis of methionine and cysteine. Second, Mediator associates with Met4-target promoters *in vivo*. This association maps to the Met4 binding region and happens only under inducing conditions. Third, artificial binding of Met4 to an ectopic site leads to recruitment of Mediator. Thus, Met4 provide an additional example of yeast activator that direct Mediator recruitment to their target promoters during transcriptional activation, alongside with Gal4 (51), Gcn4 (56), Swi5 and Swi4/Swi6 (6, 12) and Pdr1 (17).

Differential requirement of Mediator subunits for Met4-activated transcription. Our results provide new insights into the role of individual Mediator subunits in transcription activation. As already observed with Gal4 and Gcn4, elimination of Mediator subunits has various consequences on transcription activation by Met4, ranging from a severe decrease in mRNA to no visible defect. Remarkably, among the non-essential subunits, those which are the most critically required for Met4-activated transcription belong to the tail domain which includes Med2, Med3, Med15 (Gal11) and Med16 (Sin4). The fact that elimination of Med2 and Med3 leads to the loss of Med14 (Rgr1) from *MET2* and *MET17* promoters (figure 7) suggests that the tail domain is required for Mediator recruitment to Met4 target genes. This supports the proposal that the tail domain serves as binding surface for recruitment by gene-specific activators (48). The similarities in the transcriptional defects of the *med2Δ*, *med3Δ* and, at some point, *med15Δ* (*gal11Δ*) mutants is probably due to the fact that elimination of one of the subunits causes dissociation of the others (43, 47, 61). Alternatively, it is also possible that Med2, Med3 and

Med15 (Gal11) do not work individually but make concerted contribution in Mediator recruitment by Met4.

Contrary to Med2, Med3 and Med15 (Gal11), inactivation of Med16 (Sin4) has no significant effect on *MET* gene transcription. This is surprising considering that Mediator isolated from *med16Δ (sin4Δ)* cells lacks the three other subunits (15, 43, 47). One explanation may be that elimination of Med16 (Sin4) destabilizes the tail domain but does not lead to its complete dissociation from the rest of Mediator within the cells. Consistently, Med2 and Med3 were observed to comigrate with other Mediator components during the first purification steps from *med16Δ (sin4Δ)* cells (47). Alternatively, Med2, Med3 and Med15 (Gal11) might be required for Mediator recruitment by Met4 only in the presence of Med16 (Sin4) but not in its absence. This hypothesis implies the existence of other binding sites for Met4 and suggests furthermore that the Mediator tail domain could be a regulatory module rather than an activator-binding surface. Finally, similarly to what was observed in the case of some Gcn4-regulated genes (61), it is also possible that, in the *med16Δ (sin4Δ)* mutant, Med2/Med3/Med15 (Gal11) might be recruited to *MET* genes as a distinct functional entity able to function as a coactivator independently of the rest of Mediator. Further investigation will be necessary to decide between these possibilities.

There is strong evidence that the two yeast activators Gal4 and Gcn4 also recruit Mediator to their target genes *in vivo* (9, 32, 56). Comparison of the requirement in Mediator subunits for Gal4, Gcn4 and Met4 clearly indicates that the three activators function through overlapping but distinct sets of subunits. Indeed, inactivation of Med2, Med3 and Med15 (Gal11) impairs transcription activation by all three activators (figure 1C) (47, 50, 55, 56, 61). By contrast inactivation of Med6 severely affects activation by Gal4 and Met4 but not Gcn4 (figure 1C and ref. 41). On the other hand, inactivation of Med18 (Srb5) affects activation by Gcn4 (56) but not

Gal4 and Met4 (figure 1B and data not shown). Finally, inactivation of the middle subunit Med9 impairs activation by Met4 (figure 1B) but not Gal4 and Gcn4 (21). Thus, Met4, Gal4 and Gcn4 activate transcription through Mediator by different mechanisms. The exact role of Med6, Med9 or Med18 (Srb5) is still unknown. It cannot be excluded that these subunits serve as binding sites for specific activators in concert with the tail subunits; however, we favor the hypothesis that they function at a post-recruitment stage. Additional experiments are in progress to address this point.

Contrary to other Mediator subunits, deletion of Med15 (Gal11) and Med1 has differential effects on *MET* gene transcription. Interestingly, the requirement for these two subunits seems to vary in an opposite direction: *MET17* and *CYS3* transcription is impaired in the *med15Δ* (*gal11Δ*) mutant but not in the *med1Δ* mutant whereas *MET2* and *MET30* transcription is impaired in the *med1Δ* mutant but not in the *med15Δ* (*gal11Δ*) mutant (figure 1B). These results suggest that the requirement for individual Mediator subunits depends primarily on the activator but can also be influenced by the intrinsic structural characteristics of the promoter bound by the activator. It is tempting to speculate that some Mediator subunits may be essentially dedicated to accommodate the differences that may exist among promoters co-regulated by a same activator.

Interplay between Mediator and SAGA at Met4-activated genes. We found that SAGA is physically present at promoters of *MET* genes and is required for full transcriptional activity, demonstrating that *MET* genes are SAGA-dependent (figure 5). Moreover, SAGA is required for pol II, but not Met4, association with *MET* promoters, suggesting a coactivator role for SAGA during transcription activation by Met4. Our results support a model in which SAGA would function during activation by Met4 primarily by modifying the structure of nucleosomes at *MET* promoters in order to promote formation of pol II basal transcription machinery. Indeed,

elimination of SAGA HAT subunit causes similar defects in *MET* gene transcription as disruption of the whole complex (figure 5). Moreover, transcription activation by Met4 is accompanied by a Gcn5-dependent increase in acetylation of histone H3 at the promoter and 5' end of the coding region of *MET* genes and, in addition, mutation of Gcn5 HAT activity impairs recruitment of pol II at the promoter (figure 6 and data not shown). The weak transcriptional defect resulting from Spt3 inactivation (figure 5A) makes unlikely a model where the function of SAGA at Met4 target promoters would be involved, as in the case of Gal4-activated promoters (16, 38, 54) recruitment of TBP via interaction with Spt3 and Spt8.

Our result that disruption of SAGA impairs substantially pol II association with *MET* promoters but has no effect on Med14 (Rgr1) association with *MET* regulatory regions (figures 7A) has several important mechanistic implications. First, Mediator can associate with *MET* promoters independently of pol II. Analyses of Mediator association with *HO*, *GAL1,10* and *ARG1* promoters have led to similar conclusions, supporting the notion that Mediator and pol II are recruited separately and not as a preassembled holoenzyme complex in yeast (6, 9, 12, 32, 52). Secondly, the presence of Mediator at *MET* regulatory regions is not sufficient by itself to achieve high levels of pol II association. The intervention of SAGA, and more particularly Gcn5, is certainly required to help removing nucleosomes likely to obstruct the access of pol II and GTFs to the promoter. Finally, these results dismiss a role for SAGA in the recruitment of Mediator by Met4. In this respect, Met4 differentiates itself from Gal4 and Gcn4, which are also known to recruit both Mediator and SAGA (4, 9, 38, 51, 56) (see model in Figure 9). Indeed, recruitment of Mediator by Gal4 and Gcn4 is, at least in part, dependent on SAGA (5, 39, 52). Note however that there are conflicting reports regarding the actual contribution of SAGA in Mediator recruitment by Gal4. Indeed, monitoring Med15 (Gal11) and Med17 (Srb4) in a *spt20Δ*

mutant, Bryant and Ptashne (9) reported that SAGA and Mediator were recruited to *GALI* promoter independently from each other; however, no direct comparison of the amount of bound Mediator in wild-type and *spt20Δ* strains was presented in the report, making it difficult to appreciate whether Mediator recruitment is optimal or not. On the other hand, Bhaumik *et al* (5) reported a sharp diminution of Med17 (Srb4) association with *GALI* UAS in several mutants of SAGA, including a *spt20Δ* mutant, leading to the proposal that SAGA might serve as an “adaptor” that recruit Mediator to Gal4 activation domain. A possible explanation to account for this discrepancy might be differences in strain backgrounds and/or experimental conditions. We found under our own experimental conditions that Mediator association with *GALI* UAS is substantially impaired in the absence of SAGA (Supplementary figure S1), supporting the model that SAGA would indeed play a role in the recruitment of Mediator to Gal4-activated genes. Similarly, recruitment of Mediator was shown to involve an important contribution of SAGA at the Gcn4 target genes *ARG4* and *SNZI* (52). However, at *ARG1*, Mediator recruitment by Gcn4 is largely independent of SAGA, possibility because this promoter possesses different structural features or binds additional transcription factors that render SAGA dispensable (18, 52).

As a whole, our results indicate in combination with results from other laboratories that the interplay between SAGA and Mediator can vary greatly depending on the gene network and the nature of the activator (figure 8). As mentioned above, SAGA is required for optimal recruitment of Mediator at genes activated by Gal4 and Gcn4 but not at genes activated by Met4. In the other way, Mediator is required for optimal recruitment of SAGA at the Gcn4-activated genes *ARG1*, *ARG4* and *SNZI* (52) but not at genes activated by Gal4 (5) and Met4. The molecular bases which underlie these mechanistic differences remain to be elucidated.

Altogether, our results emphasize the variety of mechanisms of action of coactivators and underline the necessity to diversify the model systems studied.

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FIGURE LEGENDS

FIG. 1. Role of Mediator in transcription of the methionine biosynthesis genes. (A) Phenotypic analyses. Wild-type, *med1Δ*, *med2Δ*, *med3Δ*, *med9Δ*, *med5Δ* (*nut1Δ*), *med20Δ* (*srb2Δ*), *med18Δ* (*srb5Δ*), *med14-100* (*rgr1-100*), *med15Δ* (*gal11Δ*), *med16Δ* (*sin4Δ*) and *met4Δ* strains were grown to exponential phase, collected, washed and suspended in water. Serial fivefold dilution was spotted on B medium containing 0.05 mM ammonium sulfate (SO_4^{2-}) or 0.05 mM L-methionine (Met). The most concentrated spot corresponds to a dilution at a density of 0.2×10^7 cells/ml. Plates were incubated at 28°C for 3 days. (B) Analysis of mRNA levels for selected Met4 target genes in strains used in (A). Cells were grown in YPD medium, collected, washed and incubated for 60 min into B medium. RNA levels were quantified by reverse transcription followed by real-time PCR and normalized using U4 snRNA. Values represent the average of two independent experiments and error bars indicate standard deviation. (C) Analysis of mRNA levels in cells containing temperature-sensitive mutations in Med6, Med17 (Srb4) and Med11. Strains containing wild-type or temperature-sensitive alleles of *MED6* (40), *MED17* (*SRB4*) (23) and *MED11* (21) were grown in YPD medium at permissive temperature (25°C), shifted at restrictive temperature (38°C) for 90 min, collected, washed and incubated in B medium at 38°C for 30 min. RNA levels were quantified as in (B) except that 25S rRNA was used for normalization. Values represent the average of two independent experiments. Error bars indicate standard deviation. (D) Summary of the effects of Mediator mutations on *MET* gene transcription. The model of topological organization of Mediator is adapted from (20). The Mediator subunits analyzed in this analysis are marked in bold. Subunits essential for viability are underlined.

FIG. 2. Association of pol II and Met4 with *MET* genes in Mediator mutants. ChIP was performed on wild-type, *med3Δ*, *med14-100 (rgr1-100)* and *med15Δ (gal11Δ)* using antibodies against Pol II CTD or Met4 and PCR primers for the indicated Met4 target promoters and *IME2* ORF as control. Cells were grown as described in figure 1B. Values represent the average of two independent experiments. Error bars indicate standard deviation.

FIG. 3. Association of Mediator with selected Met4-activated genes. (A) Med14 (Rgr1), TBP and pol II association. ChIP was performed on a strain expressing TAP-tagged Med14 and HA₃TBP using IgG to immunoprecipitate Med14-TAP and antibodies to HA and pol II CTD. Cells were grown in B medium containing 0.05 mM L-methionine until early exponential phase, filtered, washed and transferred into B medium. After 1 hour incubation (activating conditions, A), half of the culture was crosslinked with formaldehyde and the other half was supplemented with 1 mM L-methionine and incubated an additional 40 min (repressing conditions, R) prior to formaldehyde addition. Immunoprecipitates (IP) were quantified using PCR primers for the indicated *MET* promoters and *IME2* or *POL1* ORF as control. Values represent the average of two independent experiments. Error bars indicate standard deviation. (B) and (C) Med5 (Nut1) and Med18 (Srb5) association, respectively. ChIP was performed on strains expressing TAP-tagged Med5 or Med18 and HA₃TBP as described in (A). The results for TBP and pol II were essentially the same as those obtained in (A) (data not shown). (D) Med2, Med14 (Rgr1), Med10, Med21 (Srb7), Med6 and Med19 (Rox3) association. ChIP was performed on strains expressing TAP-tagged Mediator subunits and HA₃TBP as described in (A), except that the entire culture was crosslinked after one hour incubation in B medium. Immunoprecipitates (IP) were quantified using PCR primers for the *CYS3* and *MET2* promoters and *POL1* ORF as control. Values represent the average of two independent experiments. Error bars indicate standard deviation.

FIG. 4. Mediator is recruited through Met4 activation domain. (A) Localization of Med14 (Rgr1), Met4 and TBP along *MET2* promoter. Top: schematic of *MET2* representing Cbf1 and Met31/32 binding sites (grey boxes), TATA element (black box) and open reading frame (open rectangle). Bottom: ChIP on a strain expressing TAP-tagged Med14 and HA₃TBP using IgG to immunoprecipitate Med14 and antibodies to Met4 and HA. Cells were grown as in figure 1B. Immunoprecipitates (IP) were quantified using PCR primers for fragment A, fragment B and *POLI* ORF as control. Values represent the average of two independent experiments and error bars indicate standard deviation. (B) Association of Med14 (Rgr1) with selected Met4 target genes in wild-type (WT) and *met4Δ* strains. ChIP was performed as described in (A) on WT and *met4Δ* strains expressing TAP-tagged Med14. Immunoprecipitates (IP) were quantified using PCR primers for the indicated *MET* promoters and *POLI* ORF as control. Values represent the average of two independent experiments and error bars indicate standard deviation. (C) A *met4Δ* strain expressing TAP-tagged Med14 (Rgr1) and bearing a *GALI-lexAop-lacZ* chimeric gene (which contains four LexA operators in place of *GALI* UAS) integrated at the *URA3* locus was transformed with a plasmid expressing LexA DNA-binding domain fused to either wild-type Met4 or a derivative lacking the activation domain (Met4Δ12, deleted for residues 79 to 180). As a control, the strain was also transformed with an empty plasmid (pRS313). Cells were grown in YNB medium, collected, washed, incubated for 60 min into B medium and subjected to ChIP using IgG to immunoprecipitate Med14 and antibodies against Met4. Immunoprecipitates (IP) were quantified using PCR primers for the *GALI-lexAop-lacZ* promoter. Values represent the average of two independent experiments and error bars indicate standard deviation.

FIG. 5. Role of SAGA in transcription of Met4-activated genes. (A) Analysis of mRNA levels in cells lacking Gcn5, Spt3 and Spt20. Wild-type, *gcn5Δ*, *spt3Δ*, *spt20Δ* strains were grown

in YPD medium, collected, washed and incubated for 60 min into B medium. RNA levels were quantified by RT-PCR and normalized using 25S rRNA. Values represent the average of two independent experiments and error bars indicate standard deviation. (B) Gcn5, Spt3 and Spt20 association with Met4 target promoters under activating and repressing conditions. ChIP was performed on strains expressing HA-tagged Gcn5, Spt3 or Spt20 as described in Figure 3A. Immunoprecipitates (IP) were quantified using PCR primers for the indicated *MET* promoters and *IME2* ORF as well as *GALI* promoter as control. Values represent the average of two independent experiments and error bars indicate standard deviation. (C) Gcn5 association with Met4 target promoters in wild-type (WT) and *met4Δ* strains. ChIP was carried out on WT and *met4Δ* strains expressing HA-tagged Gcn5 using antibodies to HA and PCR primers for the indicated Met4 target promoters and *POL1* ORF as control. Cells were grown in YPD medium, collected, washed and incubated for 60 min into B medium prior to crosslinking. Values represent the average of two independent experiments and error bars indicate standard deviation.

FIG. 6. Levels of histone acetylation at Met4-activated genes. (A) Effect of Gcn5 inactivation on histone H3 acetylation at *MET17* and *MET2*. ChIP on wild-type and *gcn5Δ* strains using antibodies specific for histone H3 acetylated at K9 or K14 and PCR primers for the 5' end of *MET17* and *MET2* ORF. Cells were grown in YPD medium, filtered and suspended into B medium. Aliquots were crosslinked at different times. Values represent the average of two independent experiments and error bars indicate standard deviation. (B) Status of histone H3 at *MET* genes upon induction. Same as in (A) except that an antibody specific for the carboxy terminal end of histone H3 was used. (C). Recruitment of pol II to *MET17* and *MET2* in a Gcn5 HAT mutant. ChIP on strains containing wild-type GCN5 or *gcn5^{hat}* possessing the KQL mutation (24) using antibodies for pol II CTD and PCR primers for *MET17* and *MET2* promoters.

Cells were grown and crosslinked as in (A). Values represent the average of two independent experiments and error bars indicate standard deviation.

FIG. 7. Functional relationship between Mediator and SAGA in Met4-activated transcription. (A) Med14 (Rgr1), pol II and Met4 association with *MET* promoters in cells lacking Gcn5 and Spt20. ChIP on wild-type, *gcn5Δ* and *spt20Δ* strains expressing Med14-TAP using IgG to immunoprecipitate Med14-TAP and antibodies to pol II CTD and Met4. Cells were grown in YPD medium, collected, washed and incubated for 60 min into B medium prior to crosslinking. Immunoprecipitates (IP) were quantified using PCR primers for *MET2* and *MET17* promoters and *POLI* ORF as control. Values represent the average of two independent experiments and error bars indicate standard deviation. (B) Gcn5, Med14 (Rgr1), pol II and Met4 association with *MET* promoters in cells lacking Med2 and Med3. ChIP on wild-type, *med2Δ* and *med3Δ* cells expressing Med14-TAP and Gcn5-HA₃ using IgG to immunoprecipitate Med14-TAP, antibodies to HA to immunoprecipitate Gcn5-HA₃ and antibodies to pol II CTD and Met4. Cells were grown in YPD medium, collected, washed and incubated for 60 min into B medium prior to crosslinking. Immunoprecipitates (IP) were quantified using PCR primers for *MET2* and *MET17* promoters and *POLI* ORF as control. Values represent the average of two independent experiments and error bars indicate standard deviation. (C). Spt3 association with *MET* promoters in cells lacking Med2 and Med3. ChIP on wild-type, *med2Δ* and *med3Δ* cells expressing Spt3-HA₃ using antibodies to HA. Cells were grown in YPD medium, collected, washed and incubated for 60 min into B medium prior to crosslinking. Immunoprecipitates (IP) were quantified using PCR primers for *MET2* and *MET17* promoters and *POLI* ORF as control. Values represent the average of two independent experiments and error bars indicate standard deviation.

FIG. 8. Growth phenotype on plates. Wild-type, *met4Δ*, *med2Δ*, *gcn5Δ* and *med2Δ gcn5Δ* strains were grown to exponential phase, collected, washed and suspended in water. Serial fivefold dilution was spotted on YNB minimal medium containing or not 0.05 mM L-methionine. The most concentrated spot corresponds to a dilution at a density of 0.2×10^7 cells/ml. Plates were incubated at 28°C for 3 and 4 days.

FIG. 9. A model for Mediator and SAGA interplay in transcriptional activation. Like Met4, Gal4 and Gcn4 are able to direct Mediator and SAGA recruitment to their target promoters (see text for references). Recruitments of SAGA and Mediator are independent at Met4 target promoters and interdependent at Gcn4 target promoters. In contrast, at Gal4 target genes, optimal Mediator recruitment requires SAGA but SAGA recruitment does not require Mediator (see text for details and references). The function of SAGA involves acetylation of histone H3 at Gcn4 (30) and Met4 target promoters, and recruitment of TBP via interactions with Spt3/Spt8 at Gal4 target promoters (16, 38, 54). Mediator functions primarily by promoting recruitment of pol II to the promoter but roles at subsequent steps are not excluded.

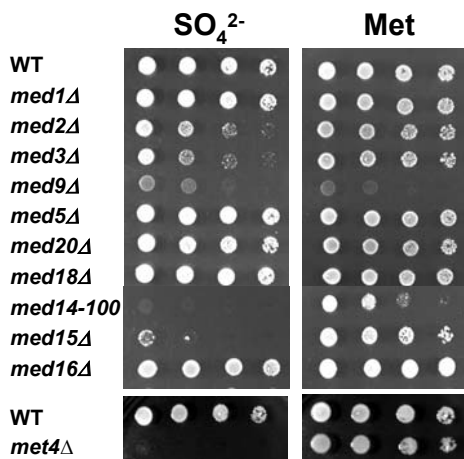
TABLE 1. *Saccharomyces cerevisiae* strains used in this study

Strain	Relevant genotype	Reference/Source
W303-1A	Mata <i>his3 leu2 ura3 ade2 trp1</i>	R. Rothstein
Y62	Mata <i>pep4::HIS3 prb1::LEU2 prc1::hisG SPT15::HA3-SPT15-URA3 MED18::MED18-TAP-kl TRP1</i>	This study
Y69	Mata <i>pep4::HIS3 prb1::LEU2 prc1::hisG SPT15::HA3-SPT15 MED5::MED5-TAP-kl TRP1</i>	Kuras et al. 2004
Y70	Mata <i>pep4::HIS3 prb1::LEU2 prc1::hisG SPT15::HA3-SPT15 MED14::MED14-TAP-kl TRP1</i>	Kuras et al. 2004
Y84	Mata <i>his3 leu2 ade2 ura3 trp1 SPT15::HA3-SPT15 MED14::MED14-TAP-kl TRP1</i>	This study
Y86	Mata <i>his3 leu2 ade2 ura3 trp1 SPT15::HA3-SPT15 MED14::MED14-TAP-kl TRP1</i>	This study
CL7-1	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 med3Δ::HIS3 MX6</i>	This study
CL8-1	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 med1Δ::HIS3 MX6</i>	This study
CL9-1	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 med9Δ::HIS3 MX6</i>	This study
CL10-1	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 med5Δ::HIS3 MX6</i>	This study
CL11-1	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 med18Δ::HIS3 MX6</i>	This study
CL12-1	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 med20Δ::HIS3 MX6</i>	This study
CL18-1	Mata <i>his3 leu2 ura3 ade2 trp1 med2Δ::HIS3 MX6</i>	This study
DY3168	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 lys2 med14-100</i>	D. Stillman
DY1700	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 lys2 med16Δ::LEU2</i>	D. Stillman
CC939-1A	<i>his3 leu2 ura3 ade2 trp1 can1-100 lys2 med15Δ::LEU2</i>	This study
YCL10	Mata <i>his3-Δ200 leu2-Δ1 ade2-101 ura3-52 lys2-801 trp1-Δ63 GAL med6::LEU2 [pRS313-MED6, HIS3]</i>	Lee and Kim, 1998
YCL8	Mata <i>his3-Δ200 leu2-Δ1 ade2-101 ura3-52 lys2-801 trp1-Δ63 GAL med6::LEU2 [pRS313-med6ts2, HIS3]</i>	Lee and Kim, 1998
YSJ7	Mata <i>his3-Δ200 leu2-Δ1 ade2-101 ura3-52 lys2-801 trp1-Δ63 GAL med11::TRP1 [pRS313-MED11, URA3]</i>	Han et al. 1999
YSJ8-4	Mata <i>his3-Δ200 leu2-Δ1 ade2-101 ura3-52 lys2-801 trp1-Δ63 GAL med11::TRP1 [pRS313-med11ts, URA3]</i>	Han et al. 1999
Z579	Mata <i>his3 leu2 ura3 med17Δ2::HIS3 [MED17, LEU2, CEN]</i>	Holstege et al. 1998
Z628	Mata <i>his3 leu2 ura3 med17Δ2::HIS3 [med17-138, LEU2, CEN]</i>	Holstege et al. 1998
Y217	Mata <i>his3 leu2 ura3 ade2 trp1 SPT15-3HA::SPT15 MED6::MED6-TAP-kl TRP1</i>	This study
Y221	Mata <i>his3 leu2 ura3 ade2 trp1 SPT15-3HA::SPT15 MED19::MED19-TAP-kl TRP1</i>	This study
Y225	Mata <i>his3 leu2 ura3 ade2 trp1 SPT15-3HA::SPT15 MED2::MED2-TAP-kl TRP1</i>	This study
Y229	Mata <i>his3 leu2 ura3 ade2 trp1 SPT15-3HA::SPT15 MED21::MED21-TAP-kl TRP1</i>	This study
Y233	Mata <i>his3 leu2 ura3 ade2 trp1 SPT15-3HA::SPT15 MED10::MED10-TAP-kl TRP1</i>	This study
CL26-A	<i>MED14::MED14-TAP-kl TRP1 GCN5::GCN5-3HA KAN MX6 med2Δ::HIS3 MX6</i>	This study
CL27-j	<i>MED14::MED14-TAP-kl TRP1 GCN5::GCN5-3HA KAN MX6 med3Δ::HIS3 MX6</i>	This study
CL15-1	Mata <i>his3 leu2 ura3 ade2 trp1 can1-100 spt3Δ::HIS3 MX6</i>	This study
CL16-1	<i>his3 leu2 ura3 ade2 trp1 spt20Δ::HIS3 MX6</i>	This study
CL17-1	Mata <i>his3 leu2 ura3 ade2 trp1 gcn5Δ::HIS3 MX6</i>	This study
CL29-25	Mata <i>MED14::MED14-TAP-kl TRP1 GCN5::GCN5-3HA KAN MX6</i>	This study
Y404	Mata <i>his3 leu2 ura3 ade2 trp1 GCN5::GCN5-3HA-HIS3 MX6</i>	This study
Y421	Mata <i>his3 leu2 ura3 ade2 trp1 SPT20::SPT20-3HA-HIS3 MX6</i>	This study
Y282	Mata <i>his3 leu2 ura3 ade2 trp1 SPT3::SPT3-3HA-HIS3 MX6 MED14::MED14-TAP-kl TRP1</i>	This study
Y431	Mata <i>his3 leu2 ura3 ade2 trp1 SPT3::SPT3-3HA-HIS3 MX6 MED14::MED14-TAP-kl TRP1 med3Δ::KAN MX6</i>	This study
Y432	Mata <i>his3 leu2 ura3 ade2 trp1 SPT3::SPT3-3HA-KAN MX6 MED14::MED14-TAP-kl TRP1 med2Δ::HIS3 MX6</i>	This study
CL19-2	Mata <i>SPT15::HA3-SPT15 MED14::MED14-TAP-kl TRP1 spt3Δ::HIS3 MX6</i>	This study
CL21-B	Mata <i>SPT15::HA3-SPT15 MED14::MED14-TAP-kl TRP1 gcn5Δ::HIS3 MX6</i>	This study
yJS6	Mata <i>his3 leu2 ura3 trp1 taf1Δ gcn5Δ::KanR psW104-TAF1 pJW215-GCN5</i>	Huisinga et al. 2004
yJS7	Mata <i>his3 leu2 ura3 trp1 taf1Δ gcn5Δ::KanR psW104-TAF1 pKQL4-gcn5^{hat}</i>	Huisinga et al. 2004
CL33-4	Mata <i>SPT15::HA3-SPT15 MED14::MED14-TAP-kl TRP1 spt20Δ::HIS3 MX6</i>	This study
Y364	Mata <i>his3 leu2 ura3 ade2 trp1 med2Δ::HIS3 MX6 gcn5Δ::HIS3 MX6</i>	This study
CC849-8A	Mata <i>his3 leu2 ura3 trp1 met4Δ::TRP1</i>	Rouillon et al. 2000
CL37-B	Mata <i>met4Δ::TRP1 MED14::MED14-TAP-kl TRP1 GCN5::GCN5-3HA KAN MX6</i>	This study
CL23-2	Mata <i>met4Δ::TRP1 ura3::LexAop-LacZ::URA3 SPT15::HA3-SPT15 MED14::MED14-TAP-kl TRP1</i>	This study

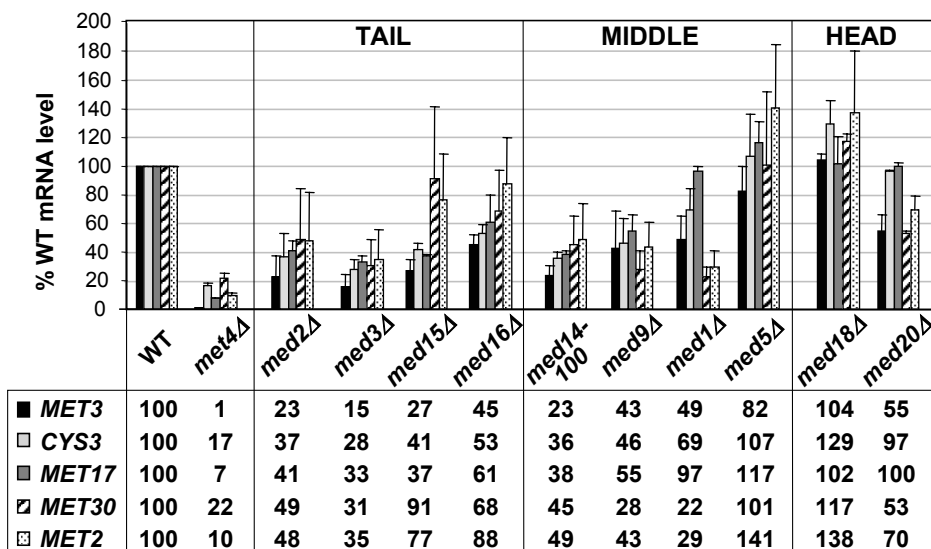
TABLE 2. Sequence and position of primers used in this study

Gene	position	Sequence	Experiment
<i>MET2</i> -promoter fwd	-395	ATTTCTTGCTATTGTTAGTGGCTCC	ChIP
<i>MET2</i> -promoter rev	-167	CAACGAAGCGGAAGCTCATCTATT	ChIP
<i>MET2</i> -UAS (A) fwd	-395	ATTTCTTGCTATTGTTAGTGGCTCC	ChIP
<i>MET2</i> -UAS (A) rev	-242	GGTGTGTGCCAAATCCAAACGATTA	ChIP
<i>MET2</i> -core (B) fwd	-250	GCACACACCCACAAATATACACATTAC	ChIP
<i>MET2</i> -core (B) rev	-51	AAACTTTAGACGGACCCTGTGACT	ChIP
<i>MET2</i> -ORF fwd	+205	GTAATTTGTGCATGCCTTGACTGGGTC	ChIP/RT-PCR
<i>MET2</i> -ORF rev	+379	ATCTAACGCCCGTCTCCTCATTAT	ChIP/RT-PCR
<i>MET3</i> -promoter fwd	-402	ACGGATTGCTGACAGAAAAAAGG	ChIP
<i>MET3</i> -promoter rev	-191	AGAAAGAGCCTCTATTTCTCATTGGT	ChIP
<i>MET3</i> -ORF fwd	+220	TTAGCAGACGGCACATTGTGG	RT-PCR
<i>MET3</i> -ORF rev	+424	TGGCTGGATGTTCTGGGTCA	RT-PCR
<i>MET17</i> -promoter fwd	-329	AGGTCACATGATCGCAAATGG	ChIP
<i>MET17</i> -promoter rev	-73	GAAAAGACAAGAGAGCAAGAAAAAGG	ChIP
<i>MET17</i> -ORF fwd	+59	ATGCTCACAGATCCAGAGCT	ChIP/RT-PCR
<i>MET17</i> -ORF rev	+309	TGTCACCAGTGTGTGCCAAA	ChIP/RT-PCR
<i>MET30</i> -promoter fwd	-231	GTGTTGGCGTGTGTGGTACAATGT	ChIP
<i>MET30</i> -promoter rev	+30	ACTCATCATCCTTTGCCTCTCTCT	ChIP
<i>MET30</i> -ORF fwd	+819	TTGGAAAGTCATCTACAGAGAACGGT	RT-PCR
<i>MET30</i> -ORF rev	+996	CCCCGTGAATAAGTCCCATATACCTA	RT-PCR
<i>CYS3</i> -promoter fwd	-300	GACCCCATACCACTTCTTTTTGTT	ChIP
<i>CYS3</i> -promoter rev	-36	AGGTGCAAATGTCTATGTGTATAGGC	ChIP
<i>CYS3</i> -ORF fwd	+512	GCCAAGACGTGATCTTGGTTGTCG	RT-PCR
<i>CYS3</i> -ORF rev	+697	TTTGTAAGAAGTGCAGACGCTCGTA	RT-PCR
<i>GAL1</i> -UAS fwd	-295	AGAGGAAAAATTGGCAGTAACCTGG	ChIP
<i>GAL1</i> -UAS rev	-156	TAGATCAAAAATCATCGCTTCGCTG	ChIP
<i>GAL1</i> -core fwd	-144	ATAAATGGAAAAGCTGCATAACCAC	ChIP
<i>GAL1</i> -core rev	-9	TTTCTCCTTGACGTTAAAGTATAGAGG	ChIP
<i>IME2</i> -ORF fwd	+1173	ATCCCAAGTAGACGCAAGAGGCAAT	ChIP
<i>IME2</i> -ORF rev	+1377	TTCTTGATTTAATGTTGGTGAGCACA	ChIP
<i>POL1</i> -ORF fwd	+2499	TGCACCAGTTAATTCTAAAAAGGCA	ChIP
<i>POL1</i> -ORF rev 2	+2717	AAAACACCCTGATCCACCTCTGAA	ChIP
U4 fwd	+1	ATCCTTATGCACGGGAAA	RT-PCR
U4 rev	+110	CACCGAATTGACCATGAG	RT-PCR
25S fwd	+3875	GGTTATATGCCGCCCGTCTTGA	RT-PCR
25S rev	+4051	CCCAACAGCTATGCTCTTACTC	RT-PCR

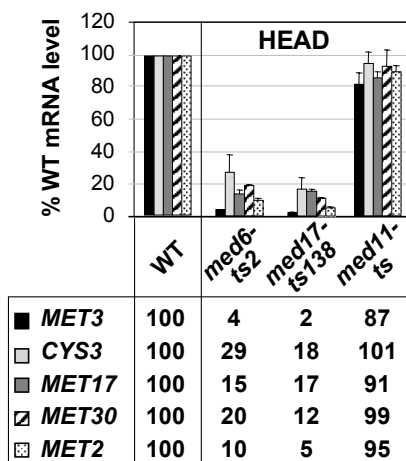
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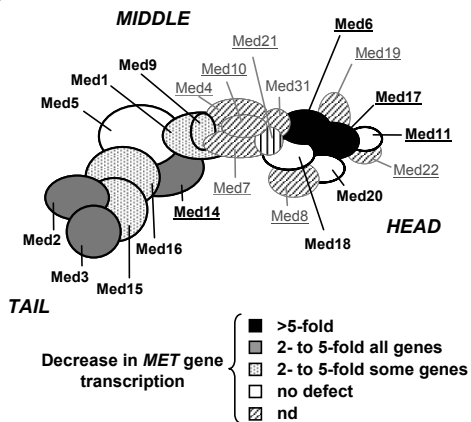
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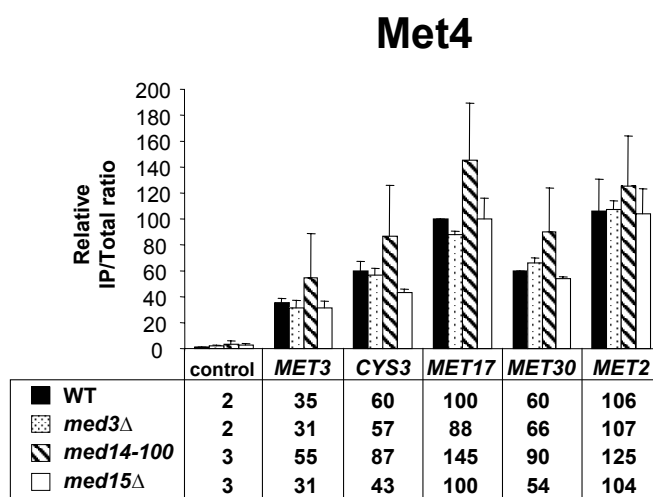
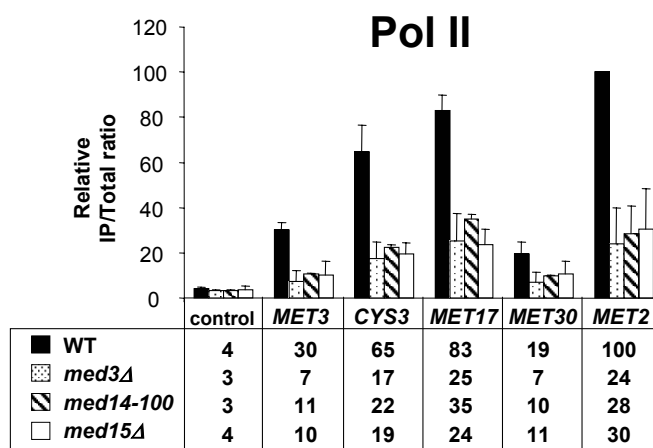


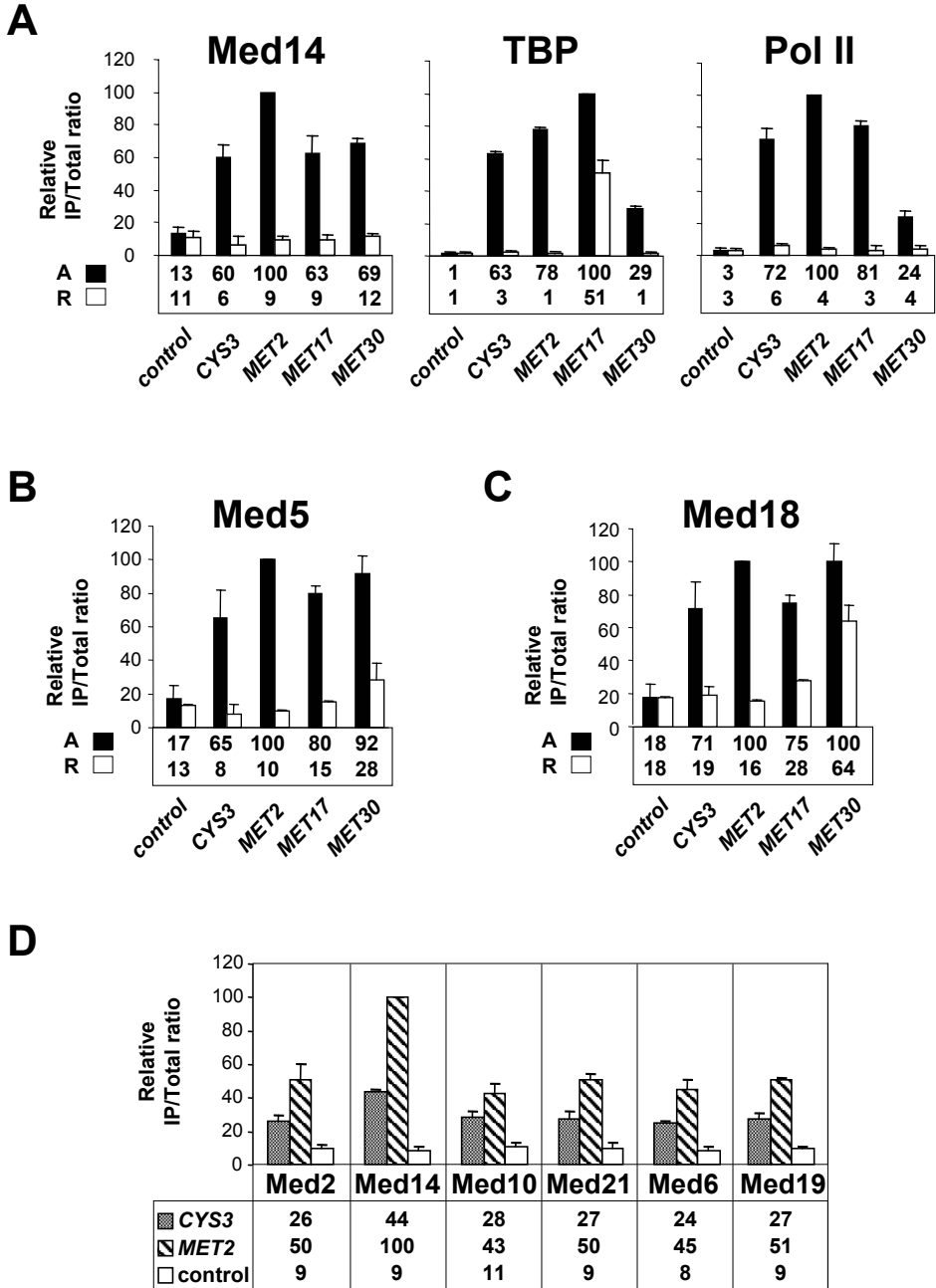
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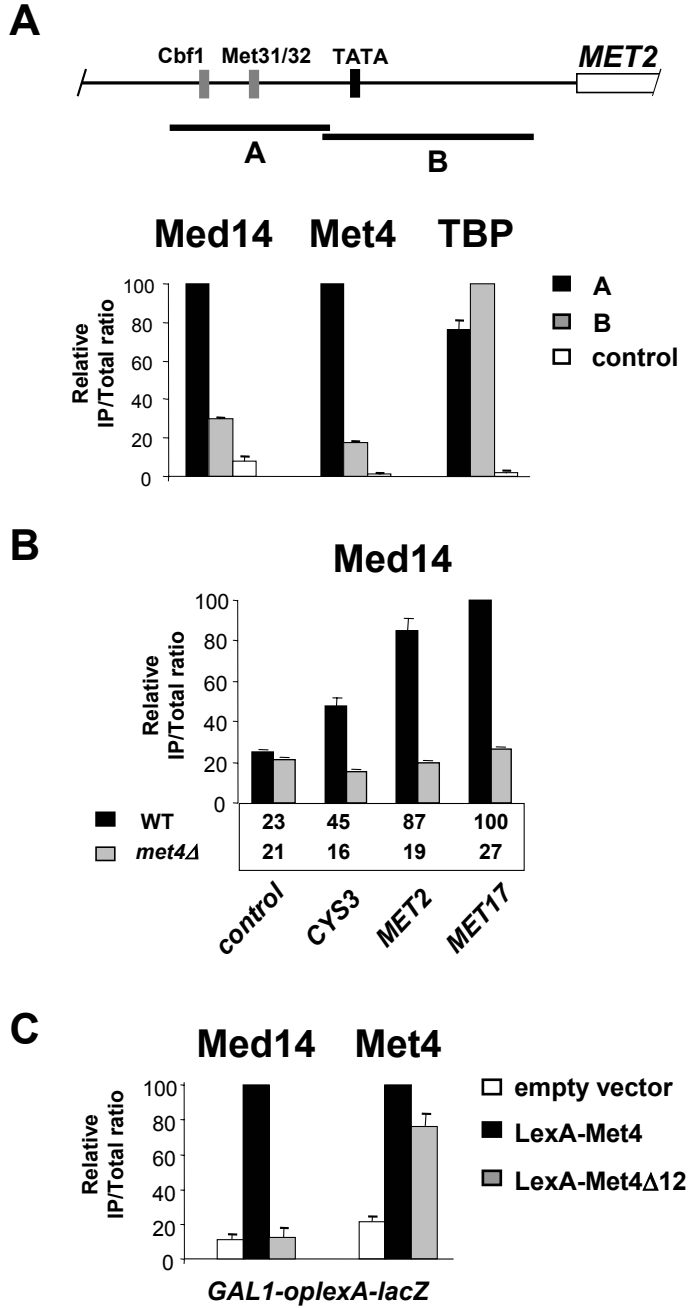


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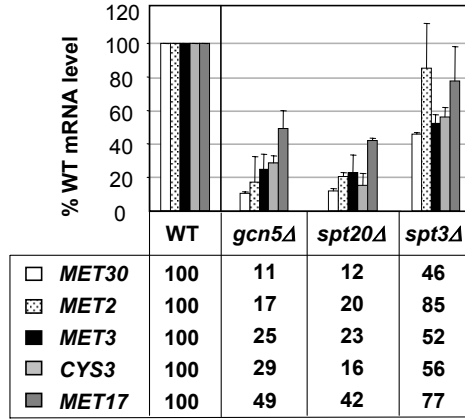




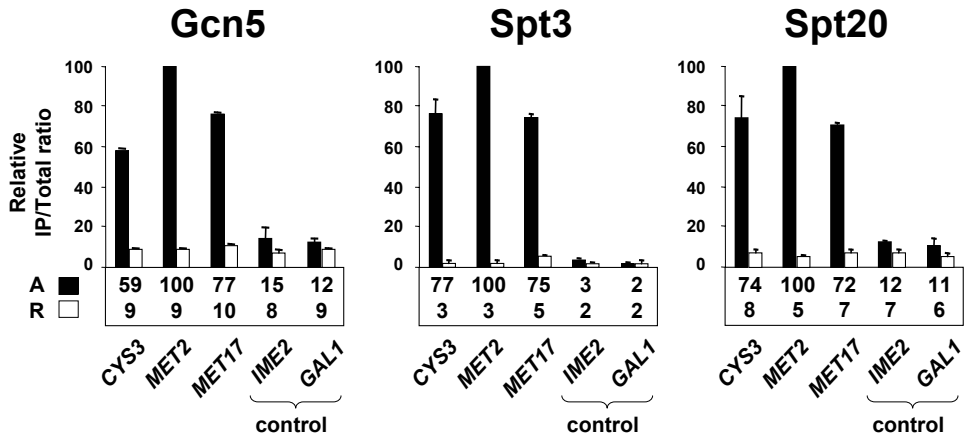




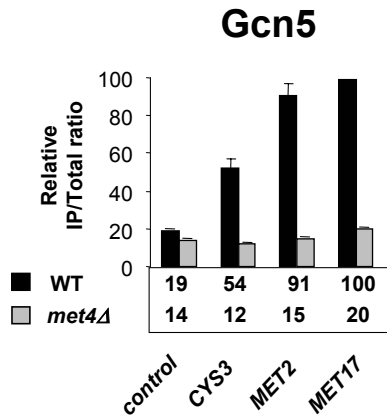
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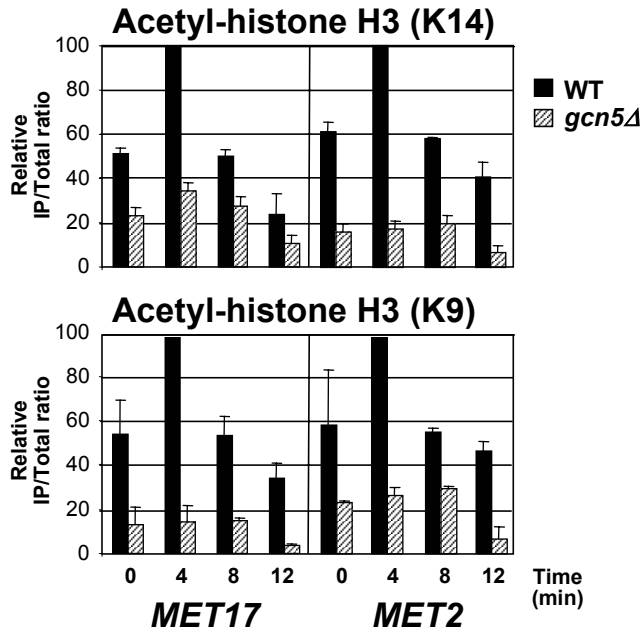
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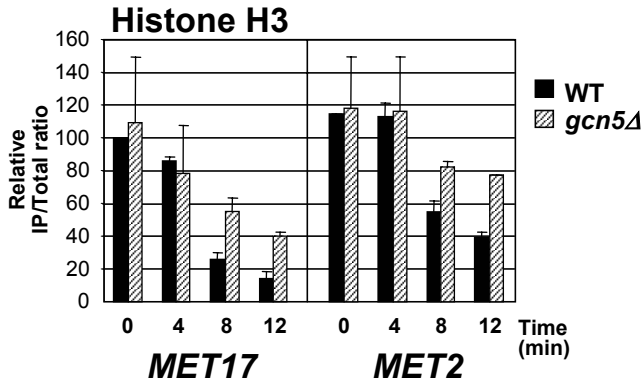
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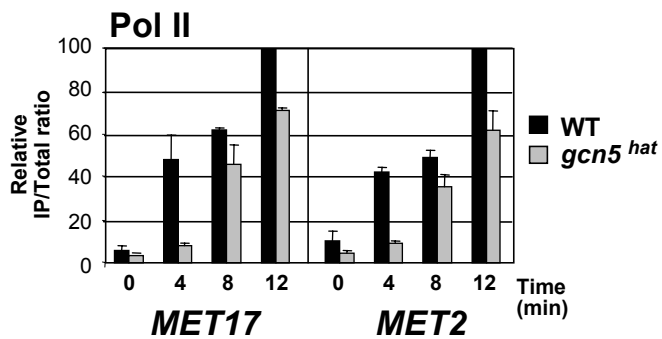
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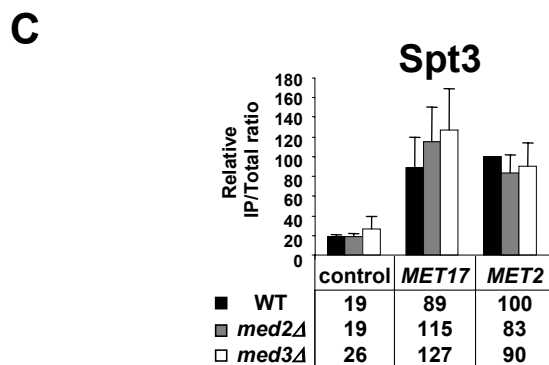
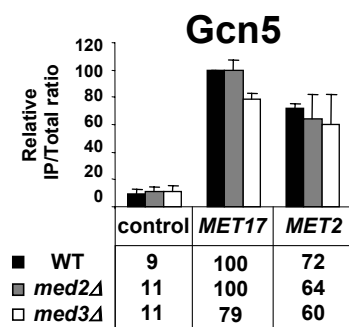
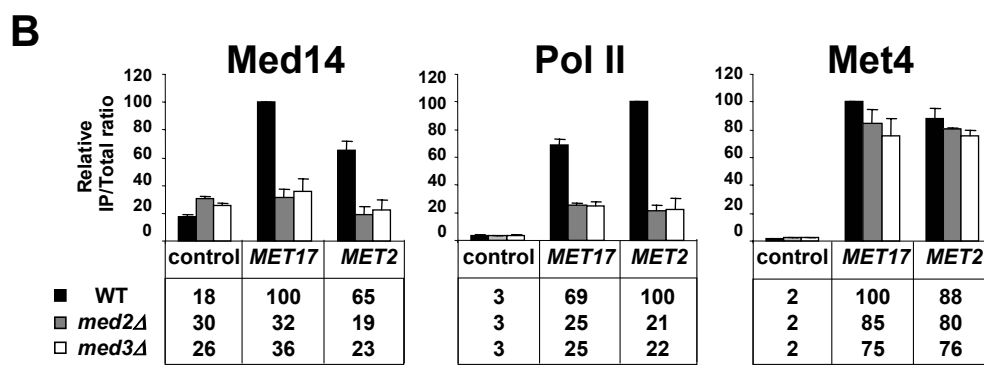
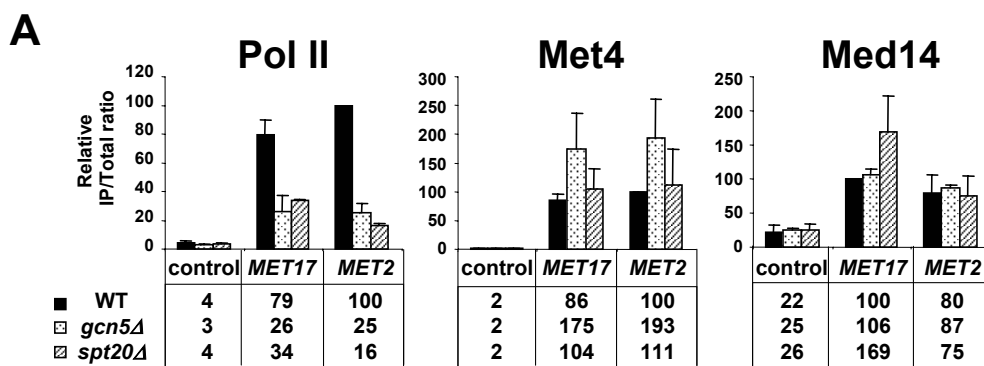


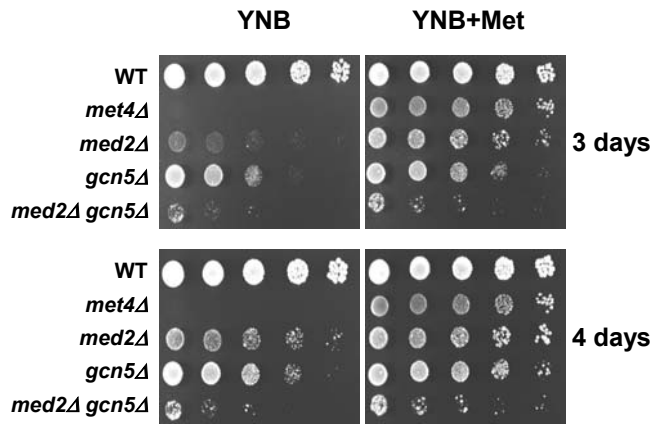
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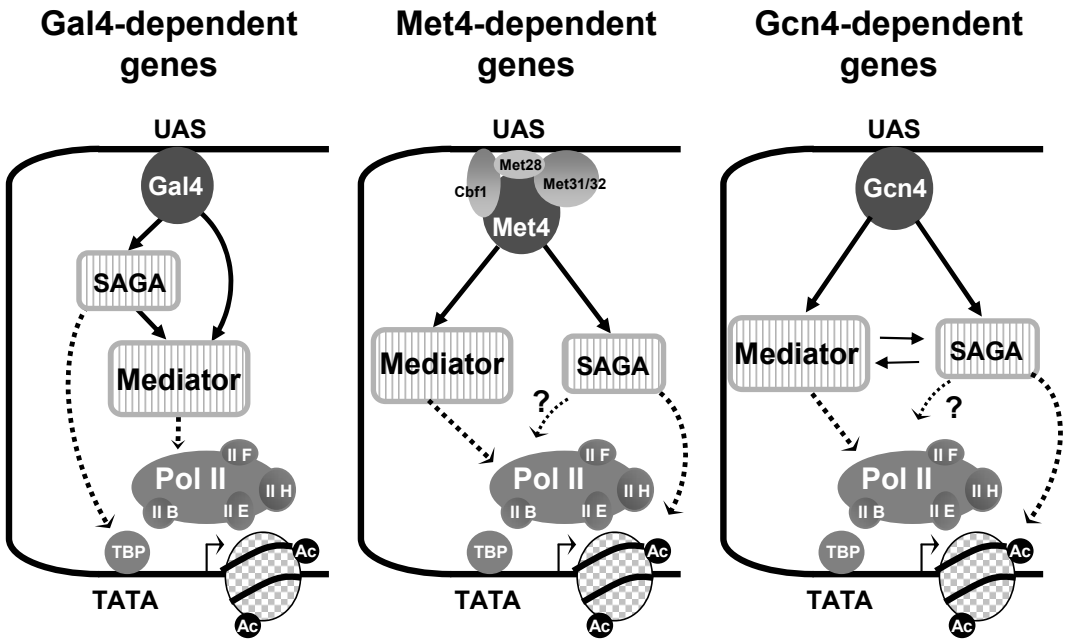


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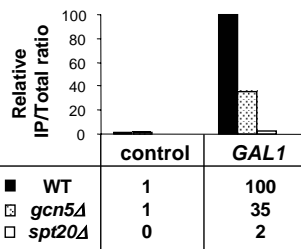
SUPPLEMENTAL MATERIAL

Independent recruitment of Mediator and SAGA by the activator Met4

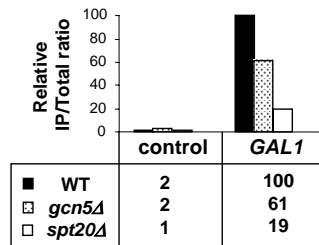
Christophe Leroy, Laëtitia Cormier, and Laurent Kuras

FIG. S1. Mediator recruitment by Gal4 in SAGA mutants. Med14 (Rgr1), pol II and TBP association with *GALI* in cells lacking Gcn5 and Spt20 was measured by ChIP using IgG to immunoprecipitate Med14-TAP and antibodies to pol II CTD and Gal4 DNA-binding domain. Wild-type, *gcn5Δ* and *spt20Δ* cells (same strains as in figure 7A) were grown in YPD medium, collected, transferred to YPGal medium (at a density of 0.2×10^7 cells/ml) and incubated for 2-3 cell divisions prior to formaldehyde addition. Immunoprecipitates (IP) were quantified using PCR primers for *GALI* UAS (Med14 and Gal4) and core promoter region (pol II) and *IME2* ORF as control. The histogram shows the results from one representative experiment. Similar results were obtained from several independent assays. Note that Med14 (Rgr1) and pol II association was less affected in the *gcn5Δ* mutant compared to the *spt20Δ* mutant. This result is in agreement with previous studies showing that Gcn5 and Spt20 are differentially required for SAGA activity at *GALI* (Bhaumik and Green, 2001; Larschan and Winston, 2001).

Pol II



Med14



Gal4

