

Biological Sciences, Biochemistry

## **Association of the Mediator complex with enhancers of active genes**

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*Abbreviations footnote:* ChIP, chromatin immunoprecipitation; pol II, RNA polymerase II; TAF, TBP-associated factors; TBP, TATA-binding protein; UAS, upstream activating sequence

## Abstract

The multiprotein Mediator complex has been shown to interact with gene-specific regulatory proteins and RNA polymerase II in vitro. Here we use chromatin immunoprecipitation (ChIP) to analyze the recruitment of Mediator to *GAL* genes of yeast in vivo. We find that Mediator associates exclusively with transcriptionally active and not inactive *GAL* genes. This association maps to the upstream activating sequence (UAS) rather than the core promoter, and is independent of RNA polymerase II, general transcription factors, and of core promoter sequences. These findings support the idea of Mediator as a primary conduit of regulatory information from enhancers to promoters in eukaryotic cells.

## Introduction

The activation of transcription is one of the major mechanisms of gene regulation in eukaryotes. It entails the binding of gene-specific activator proteins to enhancers (termed upstream activating sequences, or UASs, in yeast) and the recruitment of the RNA polymerase II (pol II) transcription machinery. Activator proteins are presumed to target one or more components of the transcription machinery, and two main targets have been proposed: the TBP-associated factors (TAFs), which are components of the multi-subunit TFIID complex (1, 2), and the 20-subunit Mediator complex, which associates with pol II to form a “holoenzyme” (3-5). The TAF hypothesis was challenged by genetic studies in yeast showing the destruction of TAFs without concomitant loss of transcriptional activation (6, 7), and by results of chromatin immunoprecipitation (ChIP) showing a lack of interaction of TAFs with many transcriptionally active yeast promoters (8, 9). A recent systematic analysis showed that no TAF is universally required for transcription (10). The Mediator hypothesis, on the other hand, has been supported by genetic studies – thirteen of the twenty subunits of isolated Mediator are products of genes identified in screens for mutations affecting transcriptional regulation in yeast (11-13). Moreover, at least two subunits (Srb4 and Srb6) are required for all pol II transcription (13, 14).

The purpose of the present work was to investigate Mediator interaction with yeast promoters and to compare the results with those obtained previously for TAFs. Our initial ChIP experiments were modeled after those on TAFs, which interact through TBP and the TFIID complex with core promoter elements (TATA box and transcription start site) (8, 9). As Mediator forms a complex with pol II, we expected it would similarly interact with core promoter elements through pol II. We found, however, for *GAL* genes, responsive to the Gal4 activator protein (15), that Mediator preferentially associates with UASs and not with core promoter elements. Pursuing this result, we analyzed the association of Mediator with

*GAL* genes in cells defective in assembly of the pol II transcription machinery, and with a truncated *GALI* gene lacking a TATA box. The results showed that Mediator associates with *GAL* UASs independently of the pol II transcription machinery, and in the absence of a functional core promoter.

## Materials and methods

**Strain and plasmid construction.** Strains used in this study derive from W303-1A (MATa *ade2 ura3 his3 leu2 trp1*) and were obtained through transformation or genetic crosses using standard procedures. TAP-tagged Mediator subunits and HA-tagged TBP were generated as described (16, 17). Strains containing the temperature sensitive TFIIB allele *sua7-1* and their isogenic wild-type (WT) counterparts were constructed as follows: (i) *SUA7* (encoding TFIIB) was disrupted by introducing a *sua7Δ::LEU2* fragment lacking most of *SUA7* open reading frame (nucleotide 207-972) and containing the *LEU2* marker (18) into diploid strains homozygous for *RGR1::RGR1-TAP-TRP1* or *SRB6::SRB6-TAP-TRP1* and *HA<sub>3</sub>-TBP*, *ade2*, *ura3*, *his3*, *leu2*, *trp1*. Disruption was confirmed by PCR and tetrad analysis. (ii) The disrupted strains were transformed with plasmids pRS413-SUA7 or pRS413-sua7-1 (kindly provided by S. Bhaumik and M. Green) and haploid cells containing a disrupted *SUA7* allele and plasmid pRS413-SUA7 or pRS413-sua7-1 were generated by sporulation and isolated by tetrad dissection. Strains containing *pGAL1-xylE* and *pGAL1Δ-xylE* chimeric genes were generated by one step integration at the *URA3* locus of two pRS306 (*URA3*) derivatives constructed by cloning into EcoRI/XbaI-digested pRS306 a EcoRI/BamHI PCR fragment containing *xylE* ORF from *Pseudomonas putida* together with a BamHI/XbaI PCR fragment containing either *GAL1* whole promoter (*pGAL1*, spanning position -13 to -537) or *GAL1* UAS (*pGAL1Δ*, spanning position -225 to -537).

**Chromatin immunoprecipitation.** Crosslinked chromatin was prepared essentially as described (17). Heat shocked cell cultures were cooled from 37°C to 28°C and crosslinked for 10-15 min with 1.4% formaldehyde. After extraction, crosslinked chromatin was collected by centrifugation at 17,400g for 20 min, solubilized by sonication (size of DNA fragments

ranging from 100 to 1000 bp with an average of 400 bp) and the remaining debris was eliminated by centrifugation at 12,100g for 15 min. TAP-tagged proteins were immunoprecipitated with rabbit IgG-agarose (Sigma) for 60 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. HA-tagged TBP and RNA pol II were immunoprecipitated with anti-HA (F7, Santa Cruz Biotechnology) and anti-RNA pol II (8WG16, Covance) mouse monoclonal antibodies coupled to protein A-Sepharose beads (Amersham Bioscience). Gal4 was immunoprecipitated with rabbit polyclonal antibodies against the Gal4 DNA-binding domain (Santa Cruz Biotechnology). Immune complexes were washed as described (17). Immunoprecipitated chromatin was eluted by heating for 20 min at 65°C in 25 mM Tris-HCl pH 7.5, 5 mM EDTA, 0.5% SDS. Formaldehyde crosslinkings were reversed by heating the eluate over night at 65°C in the presence of 1 mg/ml Pronase (Roche). DNA was purified using QIAquick DNA cleanup system (Qiagen) and analyzed by quantitative PCR as described (17). Reactions were carried out in 15 µl containing 1 µM of each primer, 0.2 mM of each dNTP and 1 µCi of [ $\alpha$ P32]dATP (specific activity, 3000 Ci/mmol). PCR products were separated in an 8% TBE polyacrylamide gel and quantified using a PhosphorImager with ImageQuant software (Molecular Dynamics). The IP/Tot ratio was calculated by dividing the amount of PCR product obtained with the IP DNA by the amount obtained with the total DNA, using for each time at least two dilutions confirmed to be in the linear range of the PCR (standard error less than 15%). 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 (19). Samples were digested with RNase-free DNase I (Sigma) and cDNA was generated by StrataScript Reverse Transcriptase (Stragene) using p(dN)<sub>6</sub> random hexamer (Roche). A primer specific for U4 small nuclear RNA (sequence: 5'-CACCGAATTGACCATGAG-3') was incorporated in the reaction to serve as internal reference. The cDNAs were quantified by real time PCR using the LightCycler instrument and LightCycler - FastStart DNA Master SYBR Green I reaction mix (Roche). Data were analyzed with the LightCycler data analysis software.

## Results

**Association of Mediator with upstream activating sequences.** We began by analyzing the association of Rgr1, an integral component of the Mediator complex, essential for cell growth (20), with *GAL1*, *GAL2*, and *GAL10* genes. CHIP analysis was performed with yeast cells expressing a TAP-tagged form of Rgr1. Cells grown under activating conditions, in the presence of galactose, or under non-activating conditions, in the presence of raffinose or glucose. The cells were cross-linked with formaldehyde, and chromatin was isolated and fragmented by sonication. Fragments bearing Rgr1 were precipitated with IgG-agarose, and DNA was detected in the precipitate by quantitative polymerase chain reaction (PCR) with primer pairs specific for the enhancer (upstream activating sequence, UAS<sub>G</sub>), the core promoter (TATA box and transcription start site), or the open reading frame (ORF) (Figure 1A). For comparison, CHIP analysis was performed in parallel on Gal4, the activator protein which binds to UAS<sub>G</sub> and activates the transcription of *GAL* promoters in the presence of galactose (15, 21), and also on TBP, whose binding to the TATA box correlates with transcriptional activity (17, 22). Gal4 protein was precipitated with anti-Gal4 antibody, while TBP was expressed with a haemagglutinin (HA) tag and precipitated with anti-HA antibody.

The results for Gal4 showed a strong association with all *GAL* promoters in the presence of galactose and raffinose, and a 4- to 5-fold weaker association in the presence of glucose (Figure 1B, lanes 18-20). This result is in agreement with previous studies showing that Gal4 is expressed in the absence of galactose, although its expression is diminished in glucose due to the general glucose-dependent repression system in yeast (15, 21). This result also confirms that Gal4 binds to UAS<sub>G</sub> *in vivo* in both the presence and the absence of galactose (15, 21). In contrast, TBP was associated with *GAL* promoters only in cells grown in galactose, where activation takes place, but not in cells grown in raffinose or glucose,



where activity of DNA-bound Gal4 is inhibited by Gal80 (Figure 1B, lanes 12-14). The results for Rgr1 showed a strong association with all *GAL* promoters in cells grown in galactose (Figure 1B, lanes 5-8). PCR signals were as much as 50-fold greater for the TAP-tagged Rgr1 strain than for the untagged strain (Figure 1B, compare lanes 5 and 8). In contrast, no association was observed in cells grown in raffinose or glucose. Thus Rgr1 specifically associates with transcriptionally active *GAL* promoters, but not with transcriptionally inactive ones.

Comparison of the ChIP results for Gal4 and TBP shows that the analysis could distinguish between association with UAS<sub>G</sub> and core promoter elements, despite their close proximity in some cases (only 150 bp apart for *GAL10*). For instance, the PCR signals for Gal4 association with the *GAL1-10* promoter and intergenic region formed a single peak centered on UAS<sub>G</sub>, while those for TBP formed two peaks over the core promoters, with a trough between over UAS<sub>G</sub> (Figure 1C). Similarly, the PCR signals for Gal4 and TBP association with the *GAL2* regulatory region formed two distinct peaks centered on UAS<sub>G</sub> and the core promoter, respectively. In all cases, the Rgr1 distribution was very similar to that of Gal4 but strikingly different from that of TBP (Figure 1C). For Rgr1 as well as for Gal4, the PCR signals from UAS regions were always at least two-fold greater than those from the core promoter regions, whereas for TBP, the PCR signals from UAS regions were always lower than those from the adjacent core promoter regions. Evidently, the series of overlapping DNA fragments produced by sonication, ranging in size from 100 to 1000 bp, with an overage of about 400 bp, is capable of resolving associations with sequences separated by few as 150 bp. We conclude that the Rgr1 subunit of Mediator preferentially associates with the UAS of transcriptionally active *GAL* genes.

The same experimental approach was applied to yeast strains expressing TAP-tagged forms of the Mediator subunits Srb6, Med7, and Nut1. Electron microscopy and image

processing has revealed a division of Mediator in three modules: “head,” which includes Srb6; “middle,” which includes Med7 and Nut1; and “tail,” with Rgr1 apparently straddling the junction between middle and tail (23). The ChIP results for Med7, Nut1, and Srb6 were essentially the same as those for Rgr1 (Figure 2). First, all four Mediator subunits were associated with *GAL* promoters in the presence of galactose and not in the presence of raffinose or glucose (Figure 2 and data not shown). Differences in PCR signals among the Mediator subunits were most likely due to differences in crosslinking efficiency and not to differences in gene activity among the four tagged strains. Indeed, TBP occupancy at *GALI*, *2* and *10* was the same in the four strains, indicating that *GAL* genes were equally expressed in all the strains. Second, all four subunits were preferentially associated with UAS regions, whereas TBP was preferentially associated with core promoter regions (Figure 2).

We conclude that the entire Mediator complex is associated with UAS<sub>G</sub> under activating conditions. In the absence of any reported Mediator-DNA interaction, we presume that Mediator subunits are cross-linked by formaldehyde to UAS DNA through Gal4 protein. The PCR signals obtained for Mediator subunits, and thus apparent cross-linking efficiencies, were several-fold lower than those for Gal4 protein or for TBP, consistent with indirect cross-linking of Mediator subunits to the DNA (data not shown). Moreover, Mediator was shown to interact with Gal4 in vitro (24).

It is noteworthy that Mediator is not associated with UAS<sub>G</sub> under non-activating conditions. Genetic studies have demonstrated a role of Mediator in repression as well as activation of transcription of many promoters (11), so Mediator might have been found at *GAL* genes in the presence of raffinose or glucose. Indeed, Gal4 is bound to UAS<sub>G</sub> and therefore in a position to recruit Mediator under these conditions. Instead, we detect Mediator at *GAL* genes only in cells grown in galactose, suggesting that repression in raffinose or glucose does not require Mediator.

**Mediator-UAS interaction is independent of the pol II transcription machinery.** The strongly preferential association of Mediator with UAS regions was unexpected, since Mediator is known to interact with pol II (5, 23, 25), which is found associated with the core promoters and ORFs of transcribed genes. It has been suggested that Mediator is recruited to promoters as part of an even larger complex, or “holoenzyme,” including not only pol II but also general transcription factors (12, 26). To investigate such possibilities, we performed ChIP analysis on a yeast strain bearing a temperature-sensitive allele (*sua7-1*) of the general transcription factor TFIIB, which prevents assembly of the pol II machinery at restrictive temperatures (22). Wild-type and *sua7-1* cells expressing TAP-tagged Rgr1 or TAP-tagged Srb6 were grown in the presence of galactose at a permissive temperature (24°C) and then raised to a restrictive temperature (37°C) for 40 min. Immunoprecipitates containing pol II and TBP were analysed with PCR primers specific for the core promoter region, and immunoprecipitates containing Mediator subunits and Gal4 were analyzed with primers for UAS<sub>G</sub> (Figures 3 and 4). The results showed an almost complete loss of pol II (20-fold decrease) and TBP (10-fold decrease) from *GAL* promoters in *sua7-1* cells raised to a restrictive temperature, compared with wild type cells (Figures 3 and 4, lanes 5-12). These results confirm that inactivation of TFIIB in *sua7-1* cells has a rapid and dramatic effect on assembly of the pol II machinery at promoters (22). In contrast, there was only a slight loss (less than two-fold) of Rgr1 (Figure 3, lanes 17-20) and Srb6 (Figure 4, lanes 17-20), and no loss of Gal4 (Figures 3 and 4, lanes 21-24). We conclude that Mediator is capable of association with transcriptionally active genes independently of pol II and general transcription factors.

**Mediator-UAS interaction in the absence of core promoter elements.** In the previous experiment, *sua7-1* was inactivated while *GAL* genes were already undergoing transcription. It might therefore be argued that pol II, TBP, TFIIB, and other general transcription factors are not required for the retention of Mediator at the UAS, but are nonetheless required for its recruitment upon the initiation of transcription. To investigate this possibility, we compared the association of Mediator with UAS<sub>G</sub> in a chimera containing the entire *GALI* promoter and regulatory region fused to the bacterial *xylE* gene (*pGALI-xylE*), and the association of Mediator with UAS<sub>G</sub> in the same chimera lacking the TATA element (*pGALIΔT-xylE*) (Figure 5A). Both chimeric genes were integrated at the *URA3* chromosome locus, leaving intact the native *GALI* gene. Measurement of mRNA levels by Reverse Transcriptase (RT)-PCR showed that transcription from *pGALI-xylE* was induced 100-fold upon addition of galactose to cells grown in raffinose, whereas removal of the TATA element prevented any induction at all (Figure 5B). As a control, transcription of *GALI* was induced to the same extent in cells containing *pGALI-xylE* and *pGALIΔT-xylE*.

Crosslinked chromatin was prepared from cells grown under activating conditions. Immunoprecipitates containing pol II, TBP, Nut1 or Rgr1, and Gal4 were analysed with PCR primers specific for *pGALI-xylE* and *pGALIΔT-xylE* (pairs C, D and E) and, as a control, with primer pairs specific for the native *GALI* gene (pairs A and B). The results showed that association of pol II and TBP with the core promoter was much diminished by removal of the TATA element (9.3- and 15.4-fold respectively) (Figure 5C, lanes 3-6 and lanes 13-16; compare results with probes D/E for *pGALI-xylE* and *pGALIΔT-xylE*; note that D and E are at the same distance from UAS<sub>G</sub>). By contrast, no significant change in association of Nut1 and Rgr1 subunits of the Mediator with UAS<sub>G</sub> was observed upon removal of the TATA element from *pGALI-xylE* (Figure 5C, lanes 7-8 and lanes 17-18; compare results with primer pair C for *pGALI-xylE* and *pGALIΔT-xylE*). We conclude that Mediator is recruited

to *GAL* promoters in the absence of a functional core promoter and without simultaneous recruitment of polIII and the general transcription factors. Therefore, Mediator recruitment to *GAL* promoters does not require Mediator to interact with the core promoter, nor does it require the presence of polIII and the general transcription factors. These results strongly suggest that Mediator is not recruited to *GAL* promoters or, in all likelihood, to other promoters, as part of a preassembled holoenzyme with the rest of the pol II transcription machinery.

## Discussion

The chief finding of this study is the distinction between Mediator interaction with UASs and core promoter sequences. The distinction is supported by three lines of evidence. First, Mediator was localized by ChIP analysis to *GAL* UASs. Although the distance from the UAS to the core promoter was as short as 150 bp (for *GAL10*), ChIP analysis with DNA fragments of, on average, about 400 bp was sufficient for localization to the UAS, as shown by the clear difference in ChIP profiles obtained for Mediator and TBP. Second, Mediator-UAS interaction was unaffected by a mutation preventing assembly of a transcription initiation complex at the core promoter, in contrast with pol II and TBP, whose interaction with the core promoter was abolished. Finally, Mediator interacted with a UAS in the absence of a key core promoter element, the TATA box, whereas pol II and TBP did not.

Our results have two major implications: they provide strong support for a central role of Mediator in transcriptional activation; and they contradict the proposal that a pol II holoenzyme, comprising pol II, Mediator, and general transcription factors, is recruited en bloc to the promoters of active genes (12). The primary association of Mediator is evidently with transcriptional activators rather than with the pol II machinery. Mediator does interact with pol II, as shown by biochemical evidence for a Mediator-pol II complex (5, 27), and by structural analysis of this complex (23). However, the interaction with pol II is transient, occurring only during transcription initiation (28), and is therefore less readily detected by ChIP analysis than the interaction of Mediator with activators and UASs, which is persistent.

Our spatial discrimination between interactions of Mediator with *GAL* UAS and core promoter elements complements a temporal discrimination among transcription protein interactions with *GAL* promoters published while this manuscript was in preparation (29). It was shown that Mediator interacts with *GAL1* promoter more rapidly than do pol II, TBP, TFIIE, or TFIIH, following the induction of transcription. Both spatial and temporal

discrimination between Mediator and pol II interactions were previously demonstrated for the *HO* endonuclease promoter (30, 31). The combined results from studies of four *GAL* promoters and the *HO* promoter points to the generality of the conclusions regarding Mediator interaction with UASs and the independent recruitment of Mediator and pol II.

The generality of the conclusions from studies in yeast extends to higher organisms. It was shown, at the resolution of indirect immunofluorescence from *Drosophila* polytene chromosomes, that heat shock transcription factor and Mediator colocalize to heat shock response elements rapidly following induction by heat shock (32). This colocalization occurred for heat shock response elements in the absence of associated core promoters. Neither TAFs nor pol II exhibited such behavior. The inference from this work, that Mediator interacts with heat shock transcription factor, is supported by biochemical studies of human transcription factors. For example, human thyroid hormone receptor protein was recovered from uninduced cells as an individual polypeptide, whereas it was isolated as a complex with human Mediator following hormonal induction (33). Vitamin D receptor, SREBP, and other inducible transcriptional activators were similarly isolated as tight complexes with Mediator from human cells grown under inducing conditions (34).

Our results thus contribute to the emerging picture of Mediator as the central molecule of transcriptional regulation. Mediator interacts with transcriptional activators and with enhancers of all genes so far analyzed, in contrast with TAFs, whose interactions are restricted to the core promoters of a limited number of genes. Mediator is in a position to orchestrate the entire series of events that ensues upon activator binding and culminates in the initiation of transcription.

## Footnotes

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## Figure legends

**Fig. 1.** Rgr1 association with *GAL* genes. (A) Schematic of *GALI-10* and *GAL2* promoters and regulatory regions showing location of primer pairs used in ChIP analysis. For *GALI-10*, A: -690/-481, B: -491/-322, C: -142/+81, D: +252/+439 (coordinates are given relative to *GAL1* ATG). For *GAL2*, E: -794/-524, F: -591/-538, G: -179/+70, H: +285/+444 (coordinates are given relative to *GAL2* ATG). The arrows indicate the position of transcription start sites. (B) Chromatin from cells expressing TAP-tagged (+) or untagged (-) Rgr1 and HA<sub>3</sub>-tagged TBP grown in YP medium containing glucose (D), raffinose (R) or galactose (G) was immunoprecipitated with IgG-agarose (Rgr1), anti-HA (TBP) or anti-Gal4 (Gal4) antibodies. Immunoprecipitated (IP) and input (Total) DNA were analyzed by radioactive PCR using primers shown in (A) and primers located in a transcriptionally inactive region (Control). PCR products were separated on a 8% TBE polyacrylamide gel and visualized by autoradiography. Signals shown in Total or IP derive from identical dilutions and were confirmed to be in the linear range of PCR. (C) Quantitation of the ChIP data obtained from the cells grown in galactose. Amounts of PCR products were quantified by PhosphorImager analysis, and IP/Tot ratios were calculated by dividing the amount of PCR product obtained with IP DNA by the amount obtained with the total DNA, using each time at least two dilutions confirmed to be in the linear range of the PCR (standard error less than 15%, data not shown). The highest value obtained in each immunoprecipitation was set to 100 and other values were expressed relative to this maximum.

**Fig. 2.** Mediator association with *GAL* genes. Equivalent amounts of crosslinked chromatin extracts prepared from cells grown in YP galactose medium and expressing either Rgr1-TAP, Nut1-TAP, Med7-TAP or Srb6-TAP and HA<sub>3</sub>-TBP were immunoprecipitated in parallel with

IgG-agarose (Mediator) or anti-HA antibodies (TBP). Immunoprecipitated (IP) and input (Total) DNA were analyzed with primer pairs described in Figure 1.

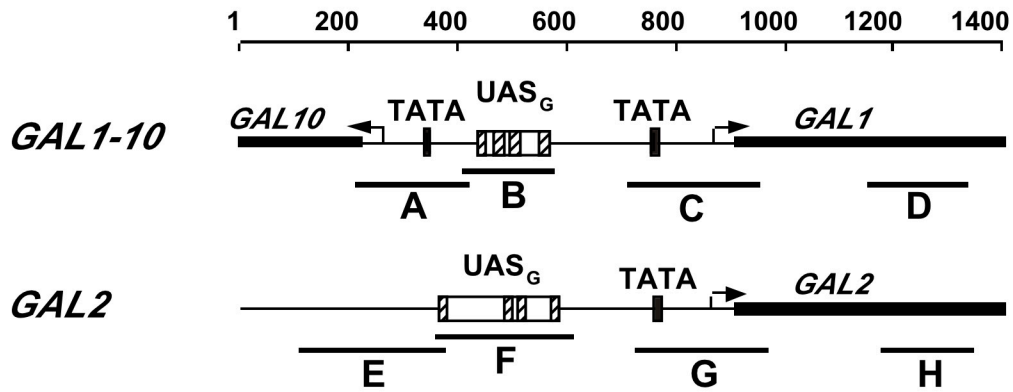
**Fig. 3.** Association of Rgr1 with *GAL* genes in TFIIB mutant cells. (A) TFIIB temperature sensitive (*sua7-1*) and isogenic wild-type (WT) cells, both expressing Rgr1-TAP and HA<sub>3</sub>-TBP, were grown in YP galactose medium at permissive temperature (24°C) and shifted for 40 min to restrictive temperature (37°C). Chromatin was immunoprecipitated with anti-pol II (Pol II), anti-HA (TBP), IgG-agarose (Rgr1) and anti-Gal4 (Gal4) antibodies. Immunoprecipitated and input (Total) DNA were analyzed with primers for *GAL1* and *GAL2* (TATA element region for pol II and TBP and UAS<sub>G</sub> region for Rgr1 and Gal4), and primers for a transcriptionally inactive region (Control). (B) Quantification by PhosphorImager analysis of the ChIP data obtained in the experiment presented in (A). Quantification was carried out as described in Figure 1.

**Fig. 4.** Association of Srb6 with *GAL* genes in TFIIB mutant cells. Same as in Figure 3 with cells expressing Srb6-TAP instead of Rgr1-TAP.

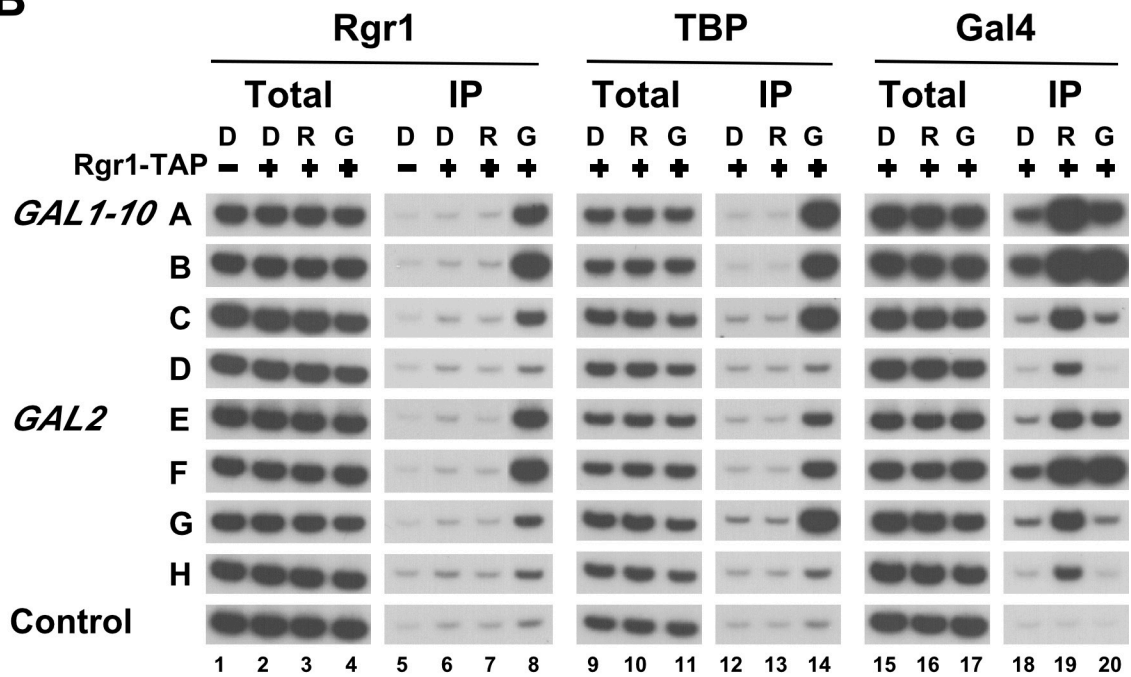
**Fig. 5.** Association of Mediator with an isolated UAS<sub>G</sub>. (A) Schematic of *GAL1-10* regulatory region and *pGAL1-xylE* and *pGAL1ΔT-xylE* chimeric genes showing location of primers used in the ChIP analysis. The arrow indicates the position of *GAL1* transcription start site. The chimeric genes contain a fragment of *GAL1* promoter spanning position -537 to -13 (*pGAL1-xylE*) and position -537 to -225 (*pGAL1ΔT-xylE*) fused to *xylE* ORF, and are integrated at the *URA3* locus. (B) Quantitation of *GAL1* and *xylE* RNA levels by RT-PCR in *pGAL1-xylE* and *pGAL1ΔT-xylE* cells grown in raffinose (R) and induced for 90 min with galactose (G). *GAL1* and *xylE* maximum RNA levels were arbitrarily set to 1000. (C) ChIP

analysis of galactose-induced pGAL1-xylE and pGAL1 $\Delta$ T-xylE cells expressing either Nut1-TAP or Rgr1-TAP. Crosslinked chromatin was immunoprecipitated with anti-pol II (Pol II), anti-HA (TBP), IgG-agarose (Rgr1) and anti-Gal4 (Gal4) antibodies. Immunoprecipitated and input (Total) DNA were analyzed by radioactive PCR with primers shown in (A) and primers specific for a transcriptionally inactive region (Control). Pair D was used specifically for samples deriving from pGAL1-xylE cells and pair E specifically for samples deriving from pGAL1 $\Delta$ T-xylE cells. PCR products were separated on a 8% TBE polyacrylamide gel and visualized by autoradiography (upper panel). Quantification in the lower pannel was carried out as described in Figure 1.

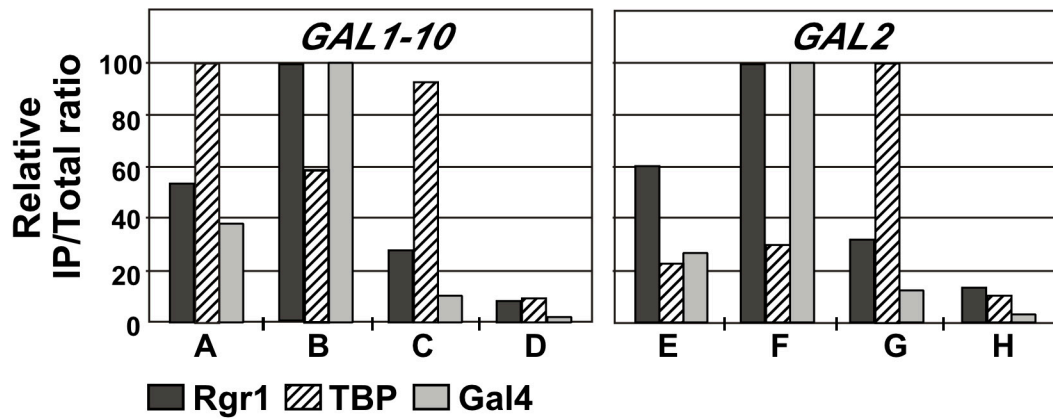
**A**



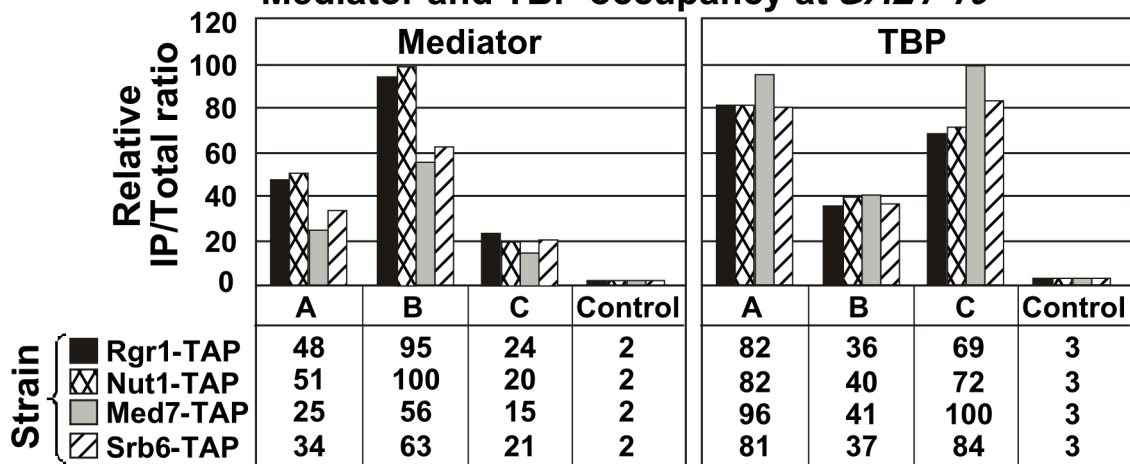
**B**



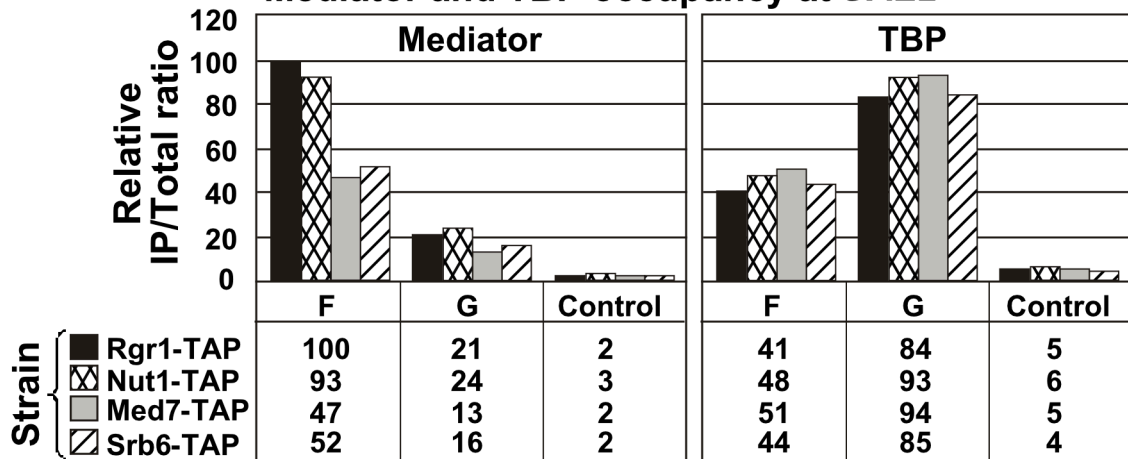
**C**



### Mediator and TBP occupancy at *GAL1-10*

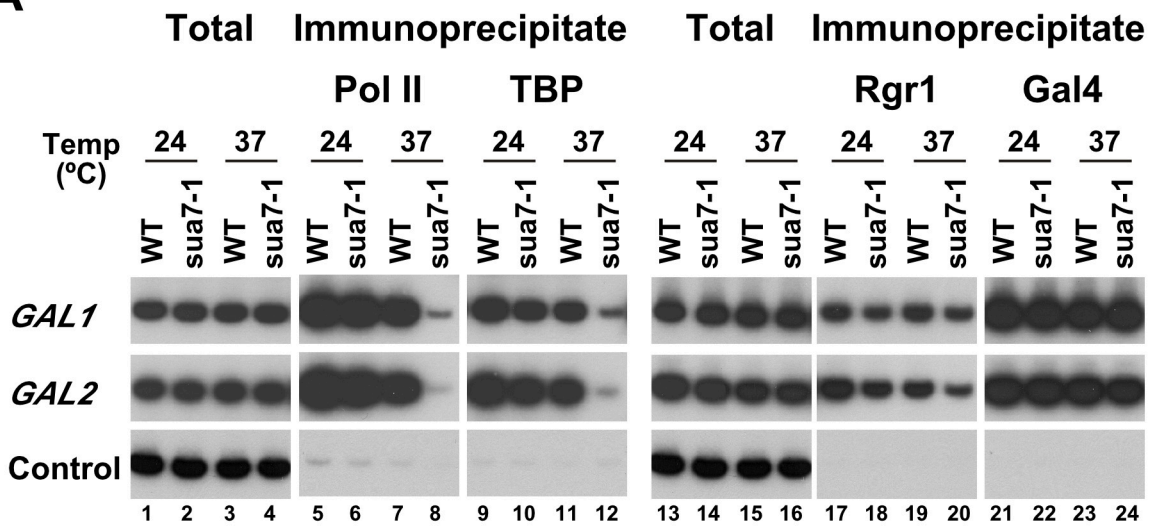


### Mediator and TBP occupancy at *GAL2*

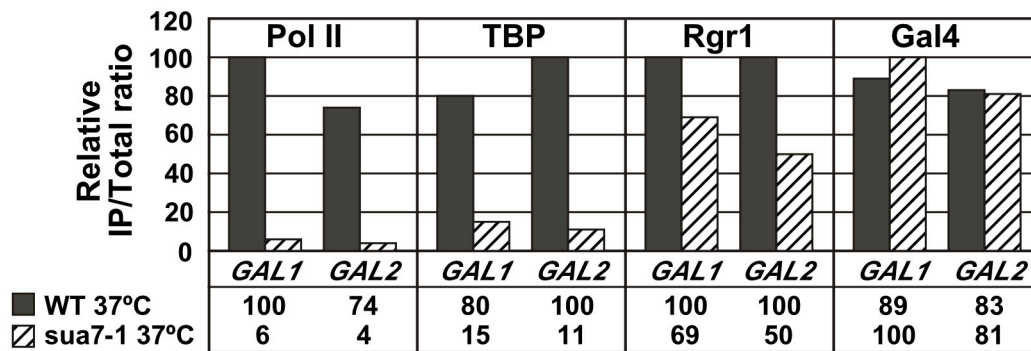




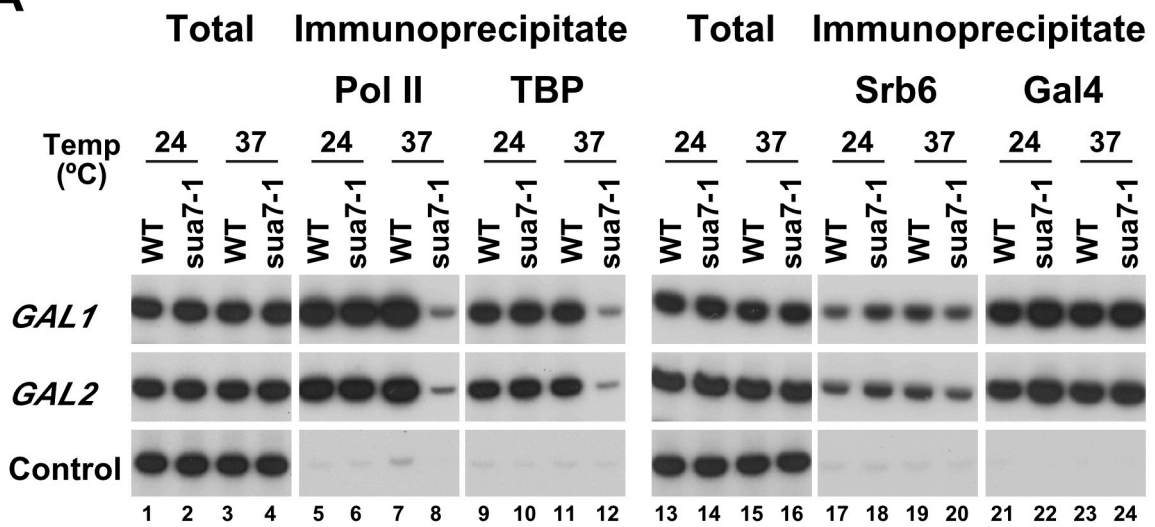
**A**



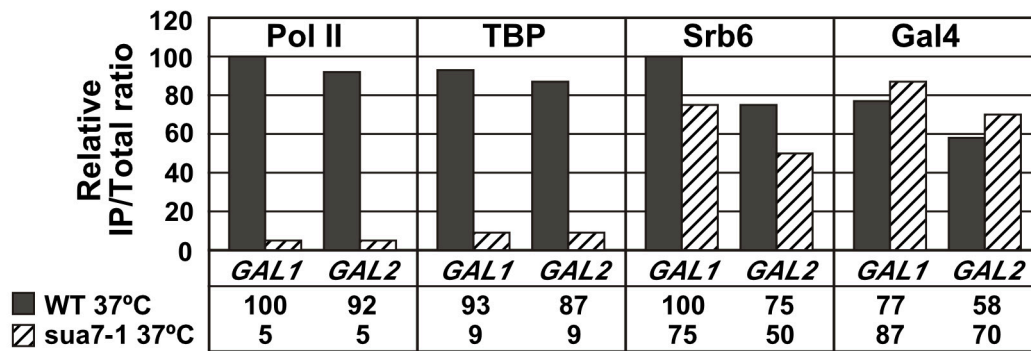
**B**



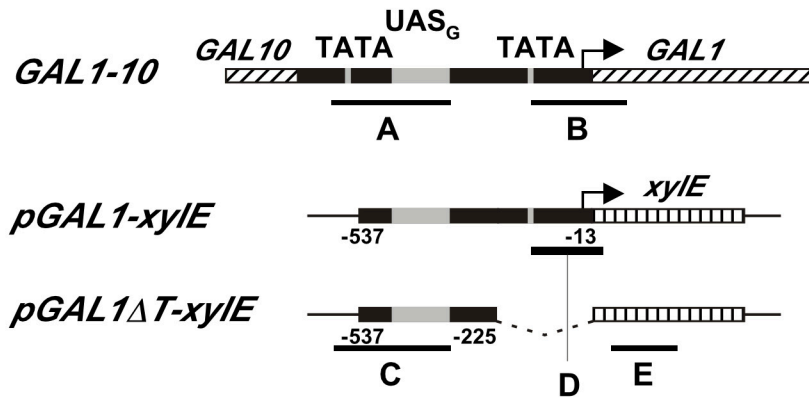
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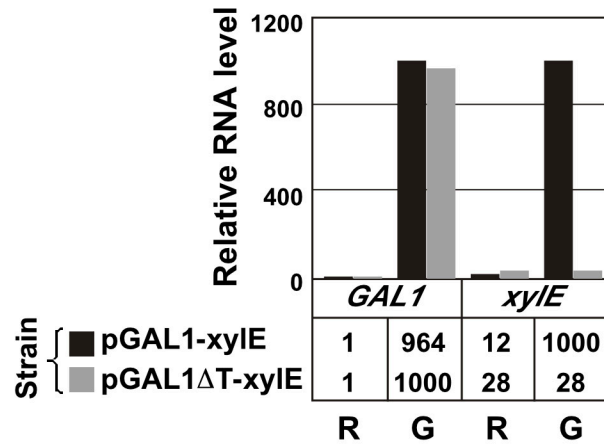
**B**



**A**



**B**



**C**

