

# Mot1-mediated control of transcription complex assembly and activity

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**Mot1 is an essential Snf2/Swi2-related ATPase and TATA-binding protein (TBP)-associated factor (TAF). *In vitro*, Mot1 utilizes ATP hydrolysis to disrupt TBP–DNA complexes, but the relationship of this activity to Mot1's *in vivo* function is unclear. Chromatin immunoprecipitation was used to determine how Mot1 affects the assembly of preinitiation complexes (PICs) at Mot1-controlled promoters *in vivo*. We find that the Mot1-repressed *HSP26* and *INO1* promoters are both regulated by TBP recruitment; inactivation of Mot1 leads to increased PIC formation coincident with derepression of transcription. For the Mot1-activated genes *BNA1* and *URA1*, inactivation of Mot1 also leads, remarkably, to increased TBP binding to the promoters, despite the fact that transcription of these genes is obliterated in *mot1* cells. In contrast, levels of Taf1, TFIIB, and RNA polymerase II are reduced at Mot1-activated promoters in *mot1* cells. These results suggest that Mot1-mediated displacement of TBP underlies its mechanism of repression and activation at these genes. We suggest that at activated promoters, Mot1 disassembles transcriptionally inactive TBP, thereby facilitating the formation of a TBP complex that supports functional PIC assembly.**

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## Introduction

Transcriptional control of RNA polymerase II (Pol II)-dependent genes results from the interplay of a large number of regulatory factors, many of which target TATA-binding protein (TBP; Matangkasombut *et al*, 2004). Interaction of TBP with promoter DNA is the rate-limiting step for transcription preinitiation complex (PIC) assembly for many genes *in vivo* (Kuras and Struhl, 1999; Li *et al*, 1999; Kim and Iyer, 2004). Mot1 is an essential, conserved, *Saccharomyces cerevisiae* transcriptional regulator that interacts with TBP and has global effects on Pol II transcription *in vivo* (reviewed in

Pereira *et al*, 2003). The Snf2/Swi2-related ATPase activity of Mot1 can drive dissociation of the TBP–DNA complex *in vitro* (Auble *et al*, 1994), explaining in principle how Mot1 represses transcription. Consistent with this, promoter-bound Mot1–TBP complexes do not contain the general transcription factors TFIIA, TFIIB or RNA polymerase II, suggesting that under normal growth conditions, the Mot1–TBP complex is transcriptionally inactive (Geisberg and Struhl, 2004).

On the other hand, Mot1 can also activate transcription directly *in vivo* by an ATPase-dependent mechanism that is not readily explained by the known biochemical activity of Mot1 (Andrau *et al*, 2002; Dasgupta *et al*, 2002; Geisberg *et al*, 2002). Furthermore, Mot1 co-occupies promoters with TFIIB and RNA Pol II in response to environmental stress, suggesting that Mot1 provides a coactivator function at some promoters under specific conditions (Geisberg and Struhl, 2004). How is ATP hydrolysis used by Mot1 to activate transcription? Transcriptional activation of the *GAL1* promoter was shown to require Mot1, which localizes to the promoter (Topalidou *et al*, 2004). Activation of *GAL1* involves remodeling of promoter-associated nucleosomes, and this remodeling failed to occur in *mot1* cells (Topalidou *et al*, 2004). This led to the suggestion that Mot1 can activate transcription by providing ATP-dependent chromatin remodeling at *GAL1* (Topalidou *et al*, 2004), an entirely different biochemical activity compared to the TBP–DNA-dissociating activity that had been previously characterized for this enzyme (Auble *et al*, 1994). Mot1 also facilitates TBP recruitment to the *HXT2* and *HXT4* promoters (Andrau *et al*, 2002), but whether this activity is mechanistically coupled to chromatin remodeling is not known.

To define mechanisms by which Mot1 controls transcription and to examine the relationship between Mot1's TBP–DNA-dissociating activity and its function *in vivo*, we determined how Mot1 affects transcriptional activity and PIC assembly at selected Mot1-activated and Mot1-repressed promoters identified by previous microarray analysis (Dasgupta *et al*, 2002). We report that transcription driven by each of two prototypical Mot1-repressed promoters is regulated by TBP recruitment. Inactivation of Mot1 led to increased TBP occupancy of the promoter and increased PIC assembly. Thus, Mot1-mediated repression can be explained by Mot1's TBP–DNA-dissociating activity. Surprisingly, inactivation of Mot1 also led to an increase in TBP occupancy at each of two Mot1-activated promoters, despite the fact that transcription was dramatically reduced. In contrast, promoter occupancy by Taf1, TFIIB and Pol II was reduced. Mot1 therefore appears to destabilize TBP from Mot1-activated as well as repressed promoters. Chromatin structure of the Mot1-activated *URA1* gene was indistinguishable in wild-type and *mot1* cells. These results strongly suggest that Mot1 is not required at these genes to create a chromatin environment that is permissive for TBP binding, nor is Mot1 required as a coactivator to facilitate recruitment of TBP to Mot1-activated promoters. Instead, the data support a model

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in which Mot1 activates transcription by facilitating establishment of an active PIC via disassembly of inactive TBP-containing complexes. Both Mot1-mediated activation and repression of these target genes can be explained by Mot1's TBP recycling activity, which we suggest can prevent PIC formation at repressed promoters or bias the assembly of a functional PIC at activated promoters.

## Results

### **Mot1 represses HSP26 transcription during heat shock adaptation**

A mutation in *MOT1* was reported to result in elevated levels of TBP binding to several promoters *in vivo*, leading in some cases to transcriptional derepression (Li *et al*, 1999). To extend these observations, analysis of *HSP26* was undertaken. We reported previously that *HSP26* is one of a number of stress response genes that are repressed transcriptionally by Mot1 (Dasgupta *et al*, 2002). In that study, temperature-sensitive alleles of *MOT1* (*mot1-14* or *mot1-42*) were inactivated by a 45 min heat shock, a condition that was expected to activate *HSP26* transcription in wild-type cells. The induction of *HSP26* transcription that occurred in *mot1* cells under these conditions was therefore greater than the normal heat shock response seen in wild-type cells. To investigate the role of Mot1 in *HSP26* transcription further, wild-type or *mot1* cells were heat-shocked, and *HSP26* message levels were analyzed by Northern blotting at various times following temperature shift. As shown in Figure 1A, *HSP26* transcription was not induced in the *mot1* strains in the absence of heat shock, despite the fact that *mot1-14* cells display extremely slow growth and possess very little Mot1 protein when grown at 30°C (Darst *et al*, 2003). Instead, *HSP26* transcription was rapidly induced in wild-type or *mot1* strains following temperature shift, with a peak of *HSP26* message accumulation occurring after 20 min at 35°C. This burst of heat shock gene transcription was followed by adaptation to heat stress resulting in a return in *HSP26* message to the pre-heat shock level. *HSP26* message levels were similar throughout the time course in both *mot1-14* and *mot1-42* strains, and were significantly elevated two- to four-fold compared to the levels of message in wild-type cells. Thus, mutation of *MOT1* resulted in an increased level of *HSP26* transcription following heat shock, and a delayed adaptive response to the heat shock stimulus. This delayed adaptive response explains why *HSP26* was originally identified as a Mot1-repressed gene.

To determine how Mot1 represses the *HSP26* activated state, chromatin immunoprecipitation (ChIP) was performed to determine the extent of TBP, TFIIB and RNA Pol II binding to the *HSP26* promoter prior to and during heat shock in wild-

type and *mot1* cells. As shown in Figure 1B, TBP was bound to the *HSP26* promoter prior to heat shock. However, a 5 min heat shock resulted in an increase in TBP binding to the promoter in wild-type cells, and by 45 min the TBP level was still elevated compared to non-heat-shocked cells. A similar pattern was observed for TFIIB and RNA Pol II (Figure 1C and D). These results indicate that activation of the *HSP26* promoter coincides with increased recruitment of TBP, TFIIB and Pol II to the promoter. In *mot1-42* cells, there was more *HSP26*-associated TBP, TFIIB and Pol II compared to their levels in wild-type cells, and the relative differences in ChIP levels over time mirrored the pattern seen in *HSP26* message levels. To further validate the ChIP analysis, the extent of PIC formation at the well-characterized *GAL1* promoter was determined. As expected, very robust recruitment of these factors to *GAL1* was observed in response to galactose, demonstrating that our ChIP assay has a high degree of sensitivity (Supplementary Figure 1; Bhaumik and Green, 2001; Larschan and Winston, 2001). These results suggest that *HSP26* is regulated in part by TBP recruitment, and Mot1 sets the level of *HSP26* activation and determines the kinetics of the adaptive response. Importantly, *HSP26* is not induced in *mot1* cells in the absence of heat shock, indicating that Mot1 antagonizes the activated state rather than repressing basal transcription. The levels of TBP, TFIIB and Pol II associated with the *HSP26* promoter were significant in the absence of heat shock, indicating that a post-recruitment mechanism also exists for controlling heat shock transcription. The idea that *HSP26* transcription is controlled by both recruitment and postrecruitment mechanisms is in good agreement with analyses of the yeast *HSP82* promoter (Giardina and Lis, 1995; Erkin *et al*, 1996).

To determine how heat shock influences Mot1 occupancy of the *HSP26* promoter, Mot1 ChIP was performed prior to and following heat shock. As shown in Figure 1E, Mot1 occupancy increased following heat shock, and then returned to non-heat shock levels by 45 min, paralleling the transient induction of the heat shock response and the transient binding of TBP, TFIIB and Pol II to the promoter. The level of Mot1 binding to promoters has previously been observed to correlate with promoter activity (Geisberg *et al*, 2002); in this case, the increased level of Mot1 may be required for the timely inactivation of *HSP26* following heat shock-mediated activation.

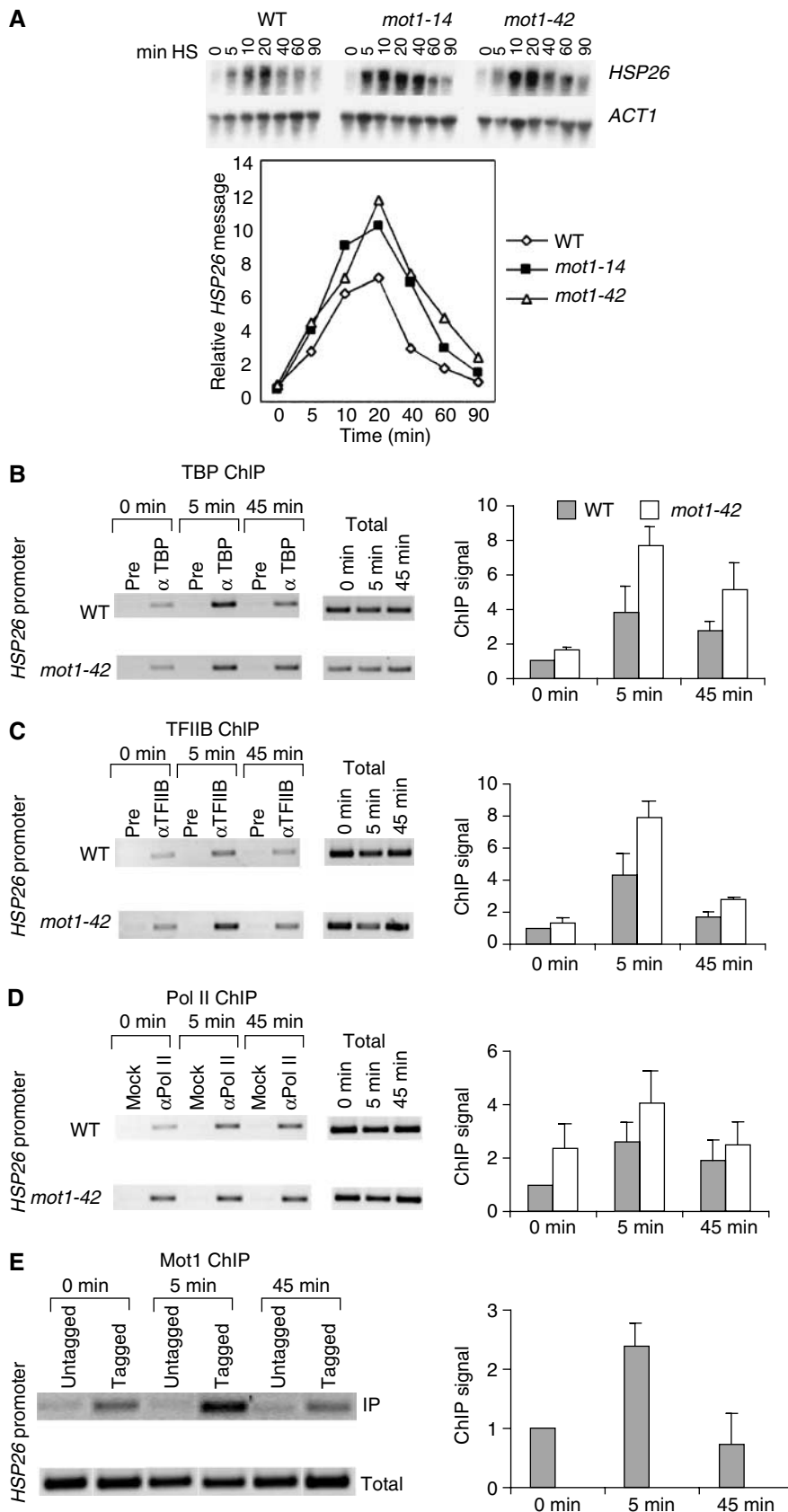
### **Mot1 represses *INO1* transcription by antagonizing the activator *Ino2***

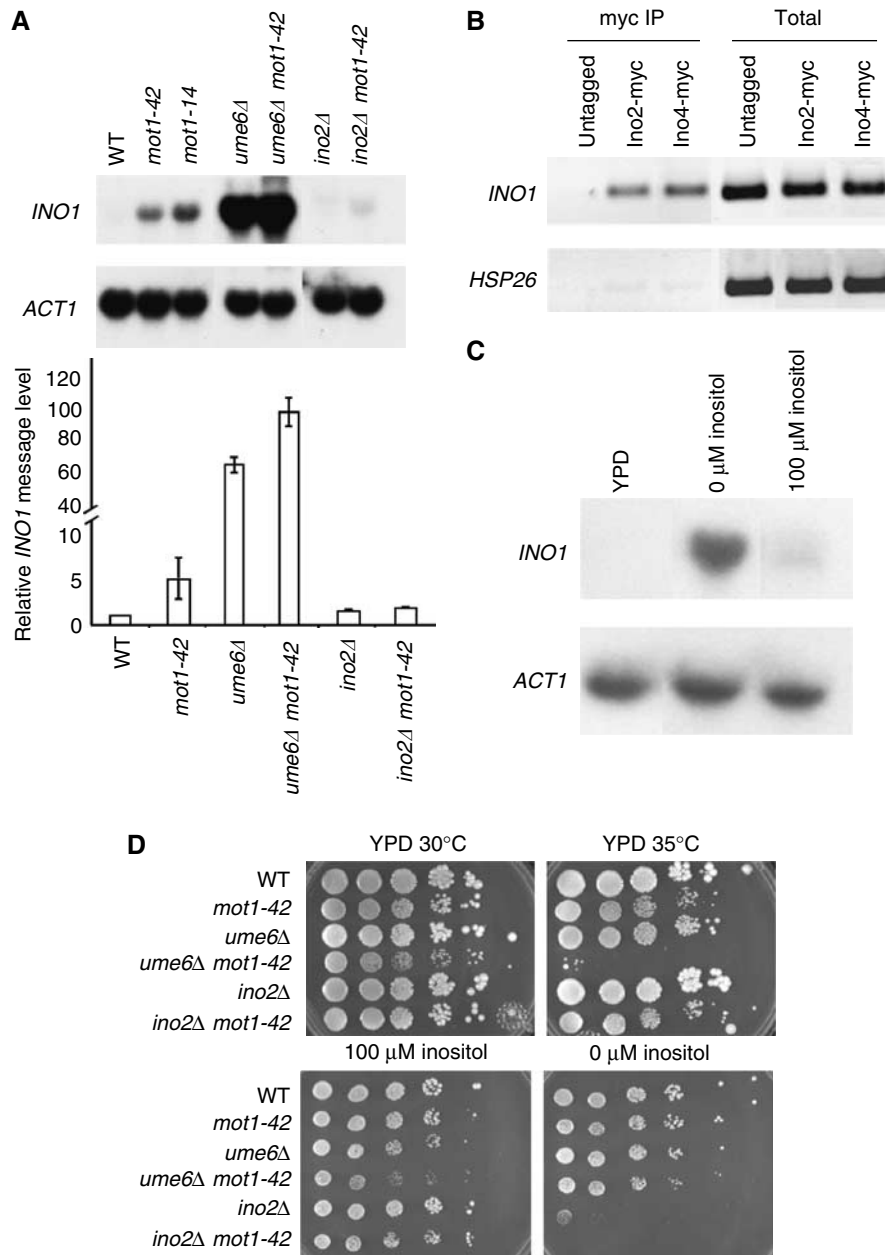
To determine if features of the *HSP26* repression mechanism are shared with another Mot1 repressed promoter, the *INO1*

**Figure 1** Mot1 affects the magnitude of induction and rate of shut-off of *HSP26*. (A) Northern blot analysis of *HSP26* message in wild-type and *mot1* mutant cells following 35°C heat shock for the indicated times (min). *ACT1* message levels were used as a loading control. The graph shows quantitation of the results. The increase in *HSP26* message in *mot1* cells was statistically significant: for example, at 40 min, message levels were elevated  $2.8 \pm 0.5$ -fold in *mot1-14* cells compared to wild type. See also Dasgupta *et al* (2002). (B) Levels of TBP associated with the *HSP26* promoter were determined by ChIP. Wild-type or *mot1-42* cells were harvested following 0, 5 or 45 min of heat shock. TBP levels were normalized to the input chromatin (labeled 'total'), and assigned values relative to the TBP level in wild-type, non-heat-shocked cells. (C) Levels of TFIIB associated with the *HSP26* promoter were determined by ChIP following heat shock as described in panel B. (D) Levels of RNA Pol II large subunit associated with the *HSP26* promoter were determined by ChIP as described in panel B. 'Mock', control in which no antibody was added. (E) ChIP for Mot1-TAP associated with the *HSP26* promoter was performed as described in panel B. The untagged strain contained untagged Mot1, whereas the tagged strain contained Mot1-TAP. In panels B–D, the graphs indicate the mean relative ChIP signal (normalized to the value for wild-type cells at time zero), with standard deviations derived from at least three independent experiments. Western analysis (not shown) indicated that TBP and TFIIB levels were indistinguishable in extracts from wild-type and *mot1* cells. Levels of Pol II large subunit were modestly reduced (~3-fold) in *mot1* extracts compared to wild-type extracts.

promoter was analyzed. As shown in Figure 2A, inactivation of *MOT1* led to derepression of *INO1* transcription. Ume6 represses *INO1* transcription by interacting with the *INO1*

URS element and recruiting histone deacetylase and chromatin remodeling activities to the promoter (Rundlett *et al*, 1998; Kent *et al*, 2001; Sugiyama and Nikawa, 2001). As expected,





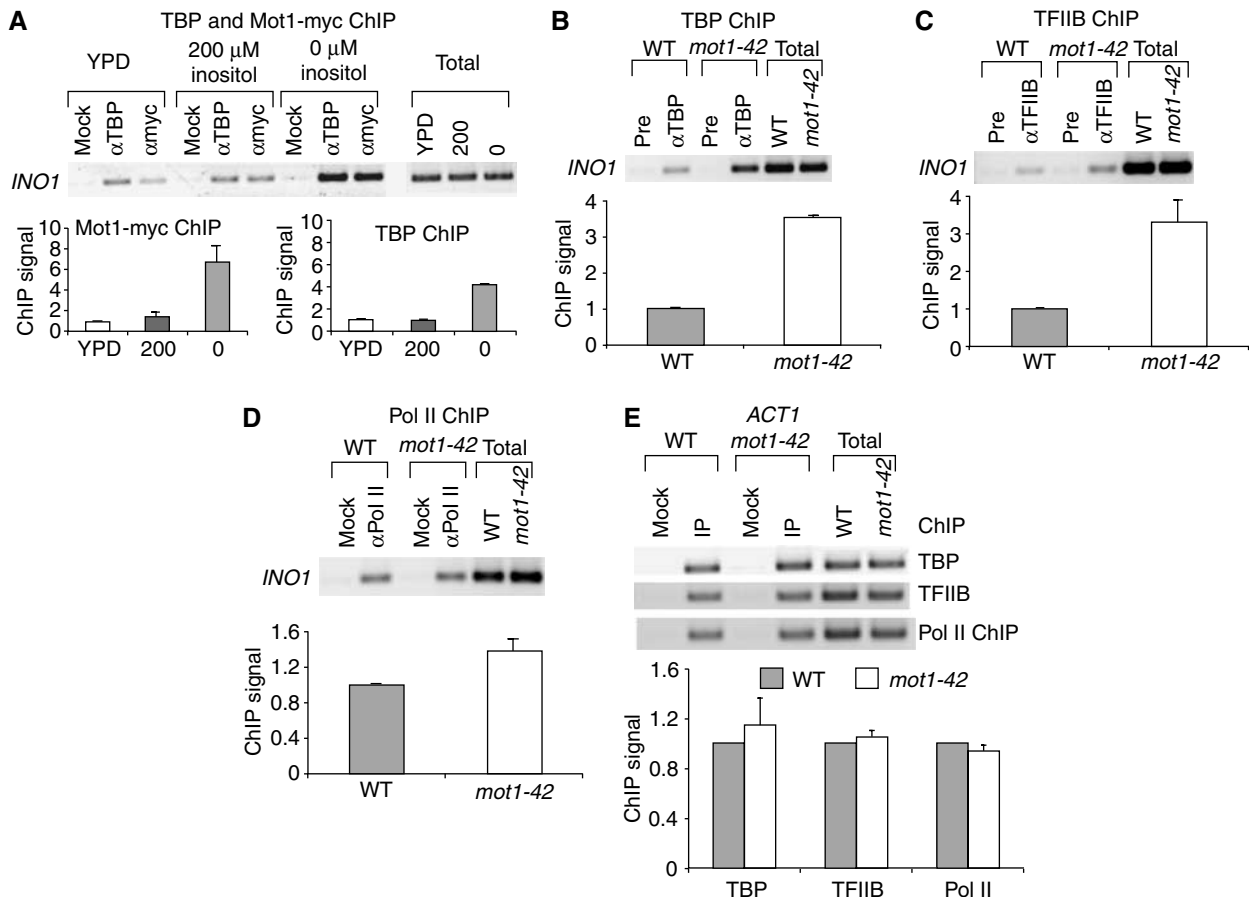
**Figure 2** Mot1-mediated derepression of *INO1* requires Ino2 but not Ume6. **(A)** Northern blots of RNA from the indicated strains were probed for *INO1* or *ACT1* (loading control). Cells were grown in YPD and then heat-shocked for 45 min at 35°C to inactivate *mot1-42*. Quantitation of the results is shown in the bar graph below; error bars represent the standard deviation obtained from three independent experiments. **(B)** ChIP analysis of Ino2-myc or Ino4-myc binding to the *INO1* promoter or the *HSP26* promoter (which served as a negative control). Chromatin was prepared from cells grown in YPD. **(C)** Northern blot analysis of the *INO1* message from cells grown in YPD or inositol starvation media with or without 100 μM inositol. *ACT1* message levels were used as a loading control. **(D)** Serial dilution spot assay of the indicated strains. 'WT' is wild type.

deletion of *UME6* led to a large induction of *INO1* message, but inactivation of Mot1 led to additional derepression of *INO1* transcription, indicating that Ume6 is not required for recruitment of Mot1 to the *INO1* promoter. On the other hand, loss of the *INO1* activator Ino2 erased the induction of *INO1* seen in *mot1-42* cells (Figure 2A). The above experiments were performed using cells grown in rich media in which inositol is not limiting. *INO1* is induced in response to inositol deprivation (Figure 2C), and this induction depends on Ino2 and its heterodimeric partner Ino4 (Henry and Patton-Vogt, 1998). Consistent with the Northern blot in Figure 2A, serial dilution spot assays demonstrated that mutation of *MOT1* did

not suppress the growth defect of *ino2Δ* cells on media without added inositol (Figure 2D). Interestingly, *ume6Δ mot1-42* cells grew very slowly on all media tested; as *MOT1* and *UME6* regulate largely distinct sets of genes (data not shown), this growth defect may be due to deregulation of a much larger set of genes than in either single mutant alone.

#### ***INO1* promoter is regulated by TBP recruitment**

ChIP experiments demonstrated that Ino2 and its heterodimeric partner Ino4 occupied the *INO1* promoter under both repressing and inducing conditions (Figure 2B and data not



**Figure 3** *INO1* promoter is regulated by TBP recruitment. (A) TBP and Mot1 ChIPs were performed using chromatin from Mot1-myc cells by immunoprecipitation with myc antibody, rabbit polyclonal TBP antiserum or no added antibody (mock). TBP or Mot1 occupancy of the *INO1* promoter was determined in cells grown in the indicated media. TBP and Mot1 levels were normalized to the input chromatin levels (total), and the levels of TBP and Mot1 associated with *INO1* in YPD were arbitrarily assigned the value of 1.0. (B) ChIP was performed to determine the level of TBP bound to the *INO1* promoter in wild-type (WT) or *mot1-42* cells. Cells were grown in YPD and then heat-shocked at 35°C for 45 min to inactivate *mot1-42* prior to harvest. (C) ChIP was performed as in panel B to determine the level of TFIIB associated with the *INO1* promoter in wild-type or *mot1-42* cells. (D) ChIP was performed as in panel B to determine relative Pol II association with the *INO1* promoter in wild-type and *mot1-42* cells. Pol II ChIP signal in *mot1-42* cells was significantly greater than that in wild-type cells ( $1.4 \pm 0.1$ ). (E) TBP, TFIIB and Pol II association with the *ACT1* promoter, determined as in panel B. In all panels, the graphs show the mean relative ChIP signals (normalized to the wild-type value) with standard deviations determined from at least three independent experiments.

shown). Thus, the *INO1* promoter is constitutively bound by Ino2/Ino4, and the function of Mot1 is apparently to antagonize Ino2/Ino4-mediated activation that would otherwise occur under repressing conditions. To determine how Mot1 represses *INO1* transcription, ChIP experiments were performed as was done for analysis of *HSP26* transcription. As shown in Figure 3A, induction of *INO1* transcription in wild-type cells by limiting inositol resulted in an increase in the levels of both TBP and Mot1, as was seen for *HSP26* during induction. The TBP occupancy of *INO1* was also increased in *mot1-42* cells grown in rich (repressing) media (Figure 3B), and was accompanied by increases in the levels of TFIIB and Pol II (Figure 3C and D). The increase in Pol II occupancy was modest but was observed in each of three independently performed ChIP experiments, indicating that the change is significant. As expected, a mutation in *MOT1* did not affect the levels of TBP, TFIIB or Pol II bound to the *ACT1* promoter, which is not under Mot1 control (Figure 3E). The observed effects on promoter occupancy also depended on inactivation of *mot1-42* because there was little effect on TBP, TFIIB or Pol II occupancy in *mot1-42* cells in the absence of heat shock

(Supplementary Figure 2). We conclude that *INO1* transcription is regulated by TBP recruitment, and the reduced levels of TBP bound to the *INO1* promoter in the repressed state are due at least in part to the TBP–DNA-dissociating activity of Mot1. Inactivation of *MOT1* apparently led to increased *INO1* transcription by eliminating a barrier to TBP binding and thereby allowing PIC formation to occur to a greater extent than in wild-type cells. Absence of Mot1 activity *in vivo* led to levels of TBP, TFIIB and Pol II on the *INO1* promoter that were equivalent to the levels of these factors in wild-type cells grown under conditions that activate *INO1* transcription (Supplementary Figure 3), indicating that Mot1 imposes a major barrier to PIC formation at *INO1*.

#### Mot1 facilitates shut-off of *INO1* transcription

As shown in Figure 1, Mot1 affected the rate at which *HSP26* transcription adapted to heat shock. One possibility is that Mot1 functions generally to de-activate promoters once their inducing signal has been terminated. To test this idea, the rate of shut-off of the *INO1* promoter was determined in wild-type and *mot1-42* cells following addition of inositol to cells in

which *INO1* transcription was fully induced. As shown in Figure 4A, *INO1* promoter activity was shut-off with a half-life of about 8 min in wild-type cells. In contrast, shut-off of the *INO1* promoter was delayed in *mot1-42* cells, with a half-life for shut-off of about 15 min. We conclude that Mot1 expedites the shut-off of *INO1* transcription during the normal response to an increase in inositol in the medium. To determine if Mot1 might function similarly on another regulated gene that is not otherwise *MOT1* controlled, the rate of decay of the *MET15* message was determined in wild-type and *mot1-42* cells following promoter shut-off by addition of methionine. As shown in Figure 4B, *MET15* message decayed with similar half-lives of 5–6 min in both strains, indicating that Mot1 does not function in this way at all de-activated promoters.

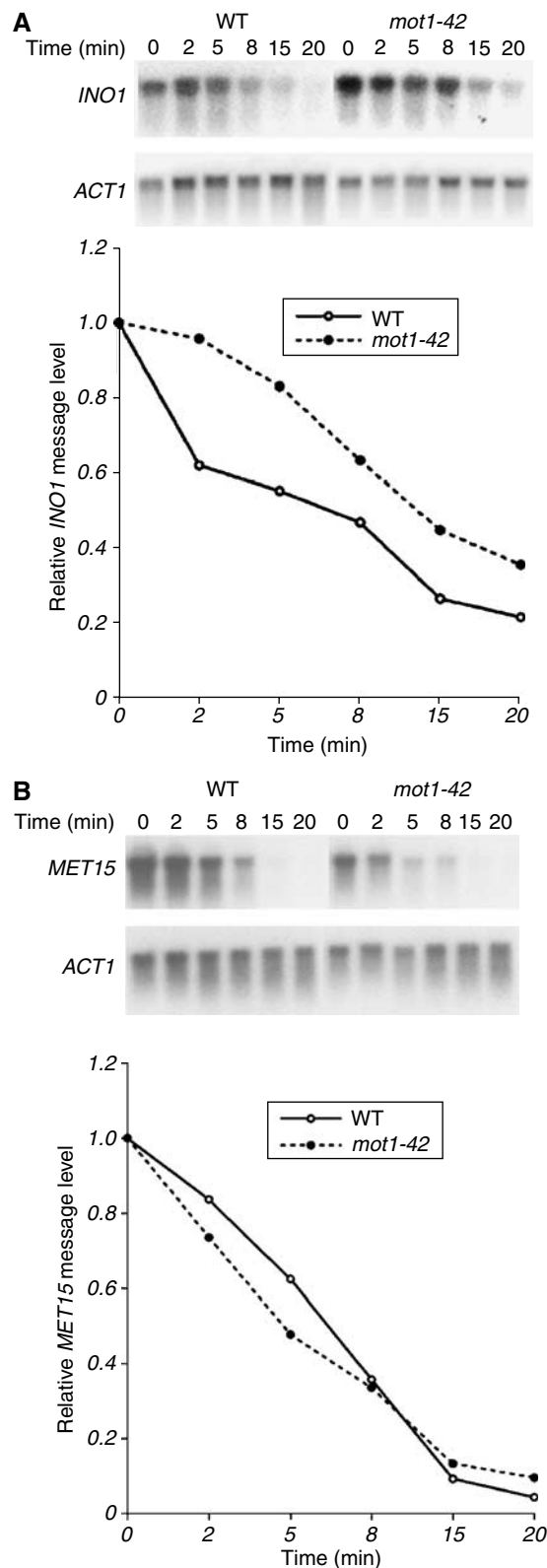
#### Mot1-mediated activation limits TBP occupancy

The above results demonstrate that Mot1's repressive effect on *HSP26* and *INO1* transcription can be explained by its TBP–DNA-dissociating activity, which reduces TBP levels at promoters that are controlled by TBP recruitment. To better understand Mot1's activation function, we analyzed transcription of *BNA1* and *URA1*, two Mot1-activated genes identified previously by microarray analysis (Dasgupta *et al*, 2002). As shown in Figure 5A, *BNA1* and *URA1* message levels were reduced in *mot1-42* cells, and Mot1 was associated with their promoters (Figure 5B), as expected. Remarkably, these decreases in message occurred despite increases in promoter-bound TBP as determined by ChIP (Figure 5C). To determine why transcription was defective at these promoters, the levels of TFIIB and Pol II were also determined by ChIP. In contrast to TBP, TFIIB and Pol II levels were significantly decreased in *mot1-42* cells compared to wild-type cells (Figure 5D and E). To estimate the aggregate effect of increasing the occupancy by TBP but reducing the occupancy by TFIIB and Pol II, TFIIB:TBP and Pol II:TBP occupancy ratios were calculated. Mutation of *MOT1* led to large decreases in both ratios (Figure 5F). These results indicate that Mot1 is not required for loading TBP onto these promoters, but rather directs the formation of a TBP-containing platform that is competent for assembly of an active PIC.

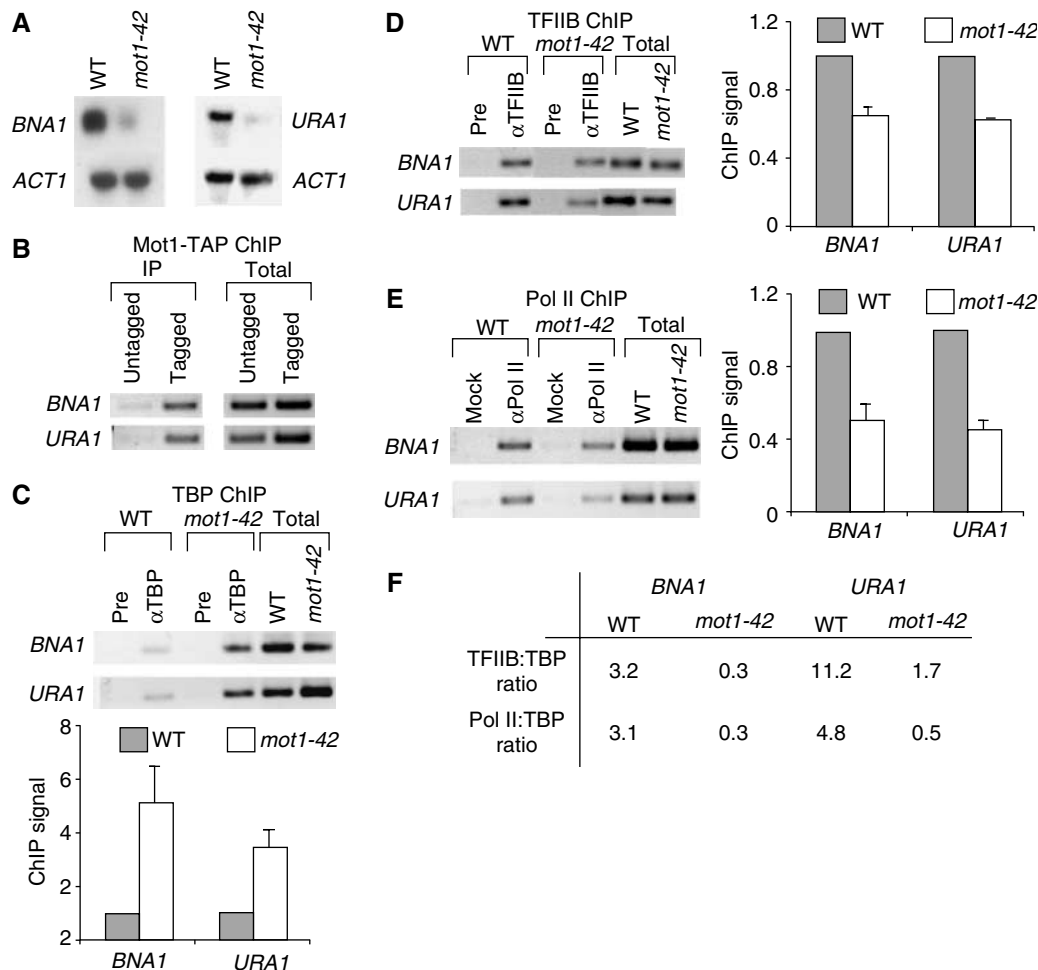
#### Effect of Mot1 on chromatin-bound TAFs

Northern blot analysis (Figure 6A) showed that Mot1-activated *BNA1* and *URA1* genes were dependent on the TFIID-specific subunit Taf1. *ACT1* was also Taf1-dependent, and *HSP26* and *PHO84* were Taf1-independent, as expected (Kuras *et al*, 2000; Li *et al*, 2000; Basehoar *et al*, 2004). To determine if the effect of Mot1 on *BNA1* and *URA1* was mediated by an

effect on TFIID, we compared the total and chromatin-bound levels of Taf1 in wild-type and *mot1-42* cells. In parallel, we also examined the levels of the TFIID-specific subunit Taf4. In wild-type cells, ChIP analysis showed detectable association of Taf1 and Taf4 with nearly all promoters tested (Figure 6C and D), in good agreement with the broad distribution of Tafs



**Figure 4** Mot1 accelerates the rate at which the *INO1* promoter is inactivated. (A) Wild-type (WT) and *mot1-42* cells were grown at 30°C in inositol starvation medium and then shifted to 35°C for 15 min. Following this, 100 μM inositol was added and cells were harvested at the indicated times. *INO1* and *ACT1* message levels at each time point were determined by Northern blotting. The results are shown in the graph, in which RNA levels were normalized to *ACT1* and are plotted relative to the message level present in cells at time zero. (B) Wild-type and *mot1-42* strains were grown at 30°C in synthetic media lacking methionine and cysteine. The cells were shifted to 35°C for 15 min and methionine and cysteine were then added and *MET15* and *ACT1* message levels were measured as in panel A. The results are quantified in the graph.



**Figure 5** Mot1 catalyzes the formation of a transcriptionally active form of TBP. (A) Northern blot analysis of *BNA1* and *URA1* message levels in wild-type (WT) and *mot1-42* cells. Blots were stripped and reprobed for *ACT1* as a loading control. (B) Mot1-TAP binding to *BNA1* and *URA1* promoters *in vivo* was detected by ChIP using chromatin from wild-type cells with untagged or TAP-tagged Mot1, as indicated. (C) TBP binding to *BNA* and *URA1* promoters *in vivo* was determined by ChIP using chromatin from wild-type (WT) or *mot1-42* mutant cells. Mean relative ChIP signals are shown in the graph, with standard errors. (D) TFIIB binding to *BNA1* and *URA1* promoters *in vivo* was determined by ChIP using chromatin from wild-type (WT) or *mot1-42* mutant cells. The bar graph shows the mean relative ChIP signal, normalized to the wild-type signal for each gene, with error bars representing standard deviations from multiple independent experiments. (E) RNA Pol II binding to *BNA1* and *URA1* promoters *in vivo* was determined by ChIP using chromatin from wild-type (WT) or *mot1-42* mutant cells. The bar graph shows the mean relative ChIP signals, normalized to the wild-type value. Error bars are standard deviations. (F) ChIP data in panels C–E were used to calculate the ratio of TFIIB to TBP and RNA Pol II to TBP at the *BNA1* and *URA1* promoters; ratios are shown in the table.

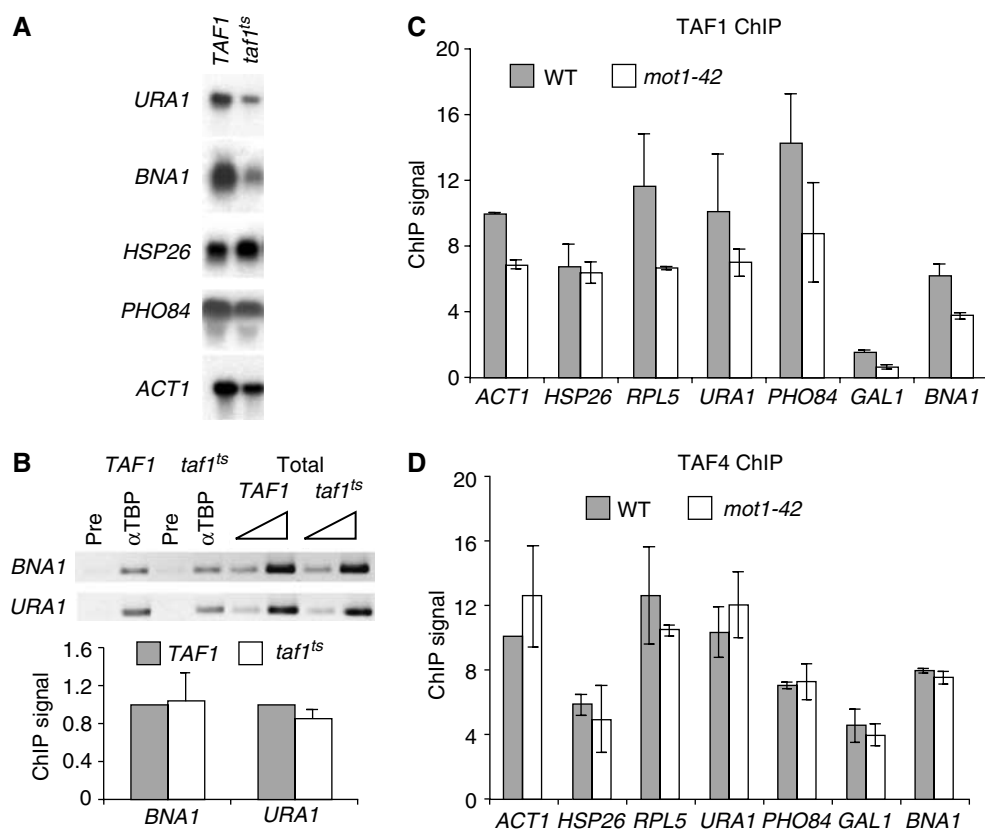
following heat shock that has been reported (Kuras *et al*, 2000; Zanton and Pugh, 2004). Western analysis of whole-cell extracts showed that Taf1 and Taf4 protein levels were reduced 10-fold or more in extracts from *mot1* cells (not shown). This effect of *mot1* on overall Taf levels is consistent with the observation that Taf levels were decreased in cells entering stationary phase (Walker *et al*, 1997) and Mot1's role in repression of diauxic shift and stationary-phase genes (Dasgupta *et al*, 2002). Despite reduced overall levels of Taf4, chromatin-associated levels of Taf4 were very similar in wild-type and *mot1-42* cells (Figure 6D). In contrast, chromatin-associated Taf1 levels were reduced in *mot1-42* cells at several sites tested. While this may be due to reduced overall levels of Taf1 in *mot1-42* cells rather than a direct effect of Mot1 on TFIID assembly, these results show that a defective form of TFIID was present on Mot1-activated promoters in *mot1-42* cells. Moreover, despite the fact that Mot1 and TFIID do not coassociate at promoters *in vivo* (Geisberg and Struhl, 2004), these results show that both factors are

required for *BNA1* and *URA1* transcription and argue for a direct effect of Mot1 and TFIID on their promoters.

To determine if increased levels of TBP at *BNA1* and *URA1* promoters in *mot1* cells were due to a defect in Mot1 or a defect in TFIID, TBP ChIP experiments were performed in wild-type and *taf1* cells. As shown in Figure 6B, TBP levels were unaffected in *taf1* cells, indicating that the enhancement in TBP binding seen in *mot1* cells is not an indirect effect of a defect in TFIID. The simplest interpretation of these results is that Mot1 functions as a TBP recycling factor at Mot1-activated promoters, disassembling inactive TBP-containing complexes and thereby facilitating assembly of an active form of TFIID.

#### Differential effect of SAGA on Mot1-regulated gene expression

Several reasons prompted a consideration of the relationship between Mot1 and SAGA (see Discussion). As shown in Figure 7A and B, Mot1-controlled genes were differentially



**Figure 6** Taf1 and Taf4 chromatin distribution in wild-type and *mot1-42* cells. (A) Wild-type and *taf1* cells were grown in rich medium at 30°C and then heat-shocked at 35°C for 45 min. Cells were harvested and Northern analysis was carried out on total RNA isolated from the cells using radiolabeled probes to detect the indicated transcripts. (B) Wild-type (*TAF1*) and *taf1* cells were grown and heat-shocked as in panel A. TBP binding to the *BNA1* and *URA1* promoters was then determined by ChIP. Quantitations of the results are shown by the bar graph with standard errors. (C) ChIP was performed to detect chromatin-bound Taf1-myc in wild-type (WT) or *mot1-42* cells. Cells with untagged Taf1 were used as controls. Quantitative PCR was performed using the indicated promoter primers; band intensities were normalized to the input and untagged signals and values are shown relative to the *ACT1* PCR signal in wild-type cells, which has been arbitrarily set to 10. Quantitations of the results with standard errors are represented in the bar graph. (D) ChIP was performed and quantified as in panel C to detect chromatin-bound Taf4 at the indicated promoters. Cells with untagged Taf4 were used as controls and relative ChIP signals were calculated as in panel C.

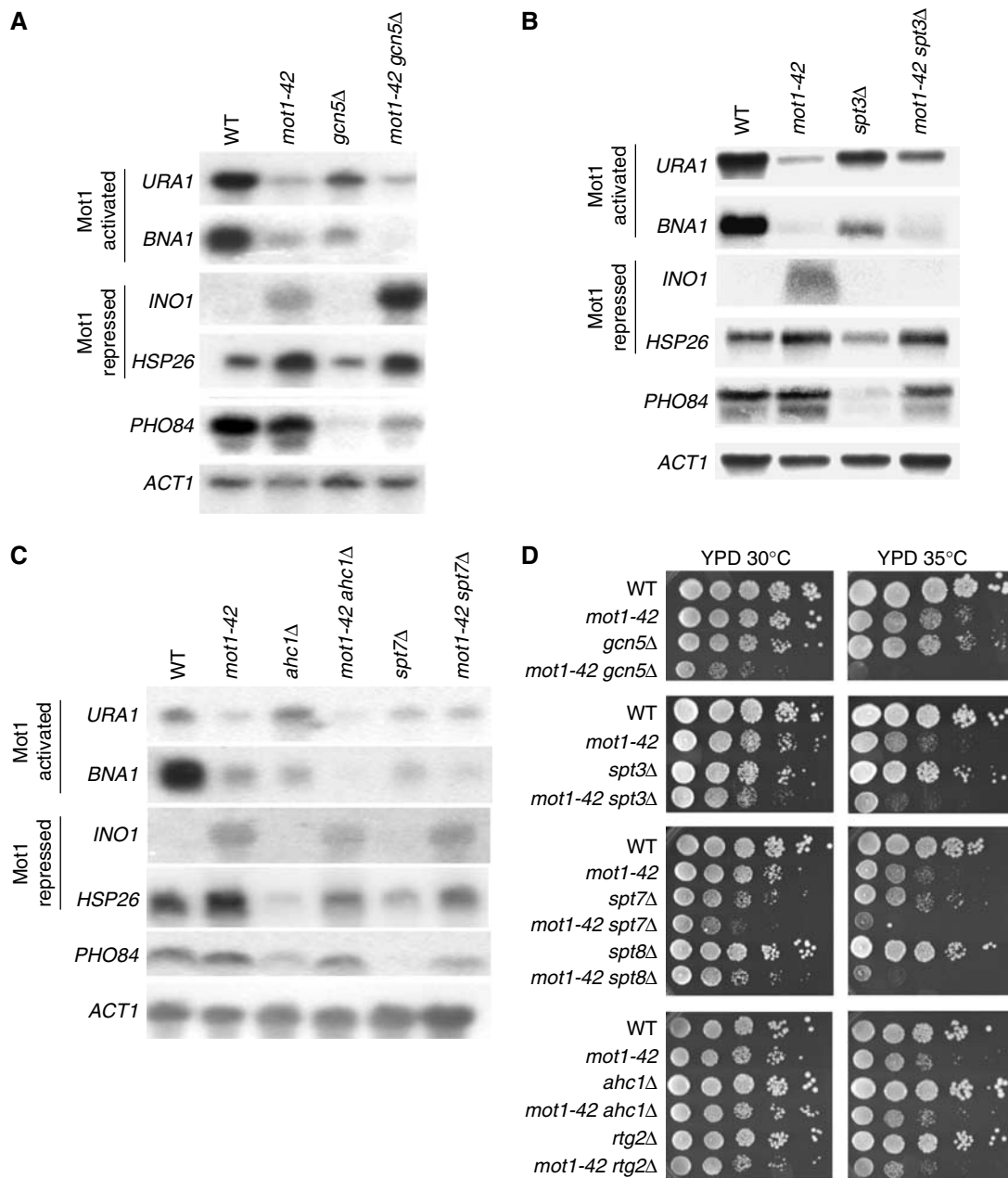
dependent on the SAGA subunits Gcn5 and Spt3. Mot1-repressed *HSP26* remained off in *gcn5Δ* or *spt3Δ* cells. On the other hand, under repressing conditions, *INO1* transcription was markedly more induced in *mot1-42 gcn5Δ* cells than in the single mutants. These results suggest a novel role for Gcn5 in repression of *INO1*, an effect reminiscent of SAGA-mediated effects on *ARG1* transcription (Ricci *et al*, 2002). Mot1-activated *BNA1* and *URA1* transcription was modestly reduced by deletion of *SPT3*, and *URA1* transcription was also only weakly affected by *gcn5Δ*. *BNA1* transcription was clearly dependent on *GCN5*, however (Figure 7A). Spt7 is an integral component of both SAGA and SLIK/SALSA complexes (Pray-Grant *et al*, 2002; Sterner *et al*, 2002). Transcription of *BNA1*, *URA1* and *HSP26* was reduced in *spt7Δ* cells (Figure 7C). The broader requirement for Spt7 compared to Gcn5 and Spt3 is consistent with Spt7's role in stabilizing two related complexes with partially overlapping function. Interestingly, *BNA1* and *HSP26* transcription was markedly reduced in *ahc1Δ* cells (Figure 7C), suggesting previously unreported roles for the Gcn5-containing ADA complex (Eberharter *et al*, 1999) in expression of these genes. As anticipated based on published results, transcription of *PHO84* was dependent on Gcn5, Spt3 and Spt7, and

*GAL1* transcription was dependent on *SPT3* (Figure 7B; data not shown; Bhaumik and Green, 2001; Larschan and Winston, 2001).

A synthetic growth defect was previously reported for *mot1 spt3* cells (Madison and Winston, 1996). In addition to this effect, synthetic growth defects were also observed in *mot1-42 gcn5Δ*, *mot1-42 spt7Δ* and *mot1-42 spt8Δ* cells (Figure 7D). In contrast, no synthetic growth defect was observed in *mot1-42 ahc1Δ* or *mot1-42 rtg2Δ* cells. As Ahc1 is a subunit of the ADA complex, and Rtg2 is a subunit of SLIK, these results suggest that defects in Mot1 and both SAGA and SLIK are responsible for these synthetic growth defects (Eberharter *et al*, 1999; Pray-Grant *et al*, 2002; Sterner *et al*, 2002).

#### Chromatin structure of the *URA1* gene is unaltered in *mot1-42* cells

The ChIP results in Figure 5 show that TBP binding to Mot1-activated promoters is elevated in *mot1* cells, despite the fact that the promoters are poorly transcribed. This strongly suggests that Mot1 functions differently at these promoters than at *GAL1*, where Mot1 is thought to facilitate PIC formation by remodeling chromatin (Topalidou *et al*, 2004). To test



**Figure 7** Differential effects of Mot1 and SAGA. (A–C) Northern blot analysis of RNA from the strains indicated above each lane. ‘WT’, wild-type cells. *BNA1* and *URA1* message levels were reduced ~10- and ~5-fold, respectively, in *mot1-42* cells. Message levels for these genes were reduced 2- to 2.5-fold in *spt3Δ* cells; a similar intermediate effect was observed for the *URA1* message level in *gcn5Δ* cells. *BNA1* message was reduced ~4-fold in *ahc1Δ* cells compared to wild type, and both *BNA1* and *URA1* message levels were reduced (~3- and ~10-fold, respectively) in *spt7Δ* cells. (D) Serial dilution spot assays of the indicated strains. Cells were grown in rich media at 30°C and then spotted in 10-fold dilutions on YPD and incubated at 30 or 35°C for 2–3 days.

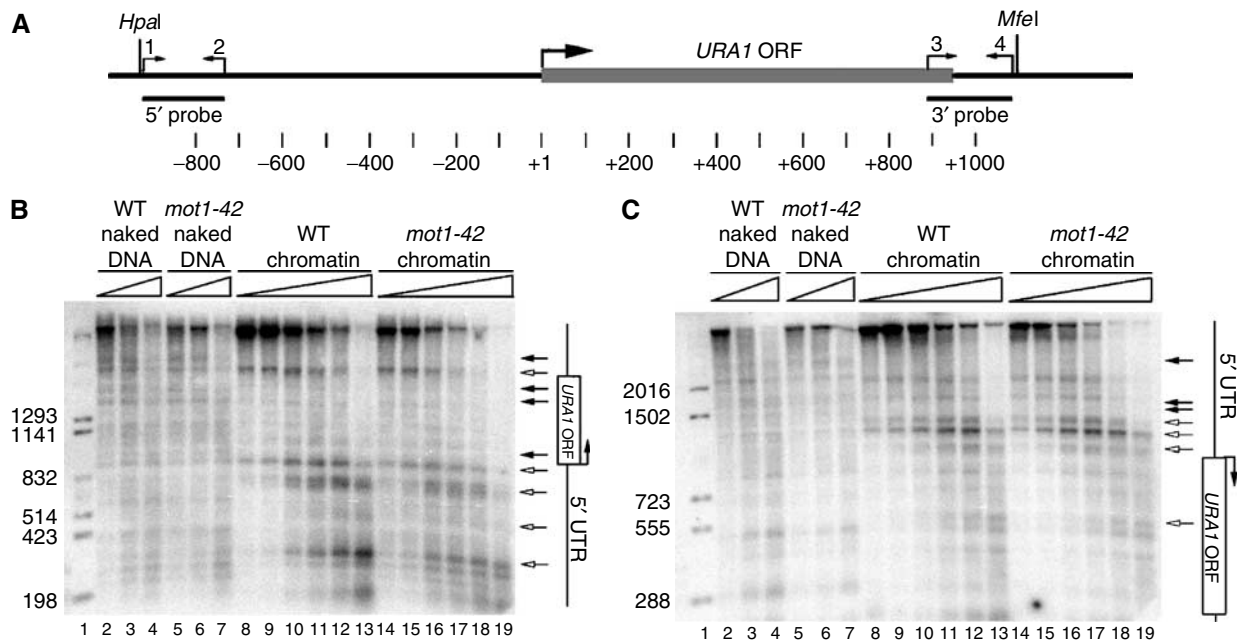
directly the idea that Mot1 can activate transcription without altering chromatin structure, micrococcal nuclease (MNase) was used to analyze the chromatin structure of *URA1*. As shown in Figure 8, the digestion pattern of *URA1* chromatin resembled that of naked DNA at many sites throughout the promoter and open reading frame (ORF), although a few positions were observed at which chromatin was modestly more resistant or more susceptible to MNase cleavage. The digestion patterns do not provide support for the presence of stably positioned nucleosomes anywhere on this gene. No major differences in the MNase digestion pattern were observed when chromatin from wild-type and *mot1-42* cells was

compared, consistent with the conclusion that Mot1 does not function as a chromatin remodeling enzyme at *URA1*.

## Discussion

### **Mot1-mediated repression via displacement of TBP from chromatin**

*HSP26* transcription is induced by environmental stress, an effect mediated by the activators Hsf1 and Msn2/4 (Estruch, 2000; Amoros and Estruch, 2001). Hsf1 displays both constitutive and heat-inducible binding to heat shock promoters (Giardina and Lis, 1995; Erkinen *et al*, 1999; Hahn *et al*, 2004).



**Figure 8** Chromatin structure of *URA1* is similar in wild-type and *mot1-42* cells. (A) Map of the *URA1* locus, indicating the 5' and 3' probes used for indirect end labeling. (B) Naked genomic DNA (lanes 2–7) or chromatin from wild-type or *mot1-42* cells (lanes 8–19) was digested with MNase and digestion products spanning the *URA1* 5' probe shown in panel A were detected by Southern blotting as described in Materials and methods. Naked genomic DNA isolated from wild-type or *mot1-42* cells was treated with 10, 17.5 or 35 U/ml MNase (increasing amounts symbolized by the ramps above the lanes). Chromatin from these cells was treated with doubling concentrations of MNase ranging from 1.875 to 75 U/ml. Markers were created by digestion with restriction endonucleases with sites at the indicated distances from the probe. The positions of the *URA1* ORF and 5' untranslated region are indicated and the arrow indicates the direction of transcription. Closed arrows denote sites in chromatin protected from MNase digestion compared to naked DNA controls. Open arrows denote chromatin sites with increased sensitivity to MNase compared to naked DNA. (C) Southern blot of MNase-digested naked DNA or chromatin as in panel B but probed with the 3' probe depicted in panel A.

*In vivo* footprinting analysis indicated that TBP is bound to the *HSP82* promoter under non-heat shock conditions and increases following heat shock (Giardina and Lis, 1995). These results are consistent with the results reported here for the *HSP26* promoter using ChIP, and imply that Hsf1 facilitates TBP recruitment in response to heat stress. Importantly, *HSP26* transcription is not induced in *mot1* cells in the absence of heat shock, despite the fact that Mot1 function is severely impaired. This indicates that Mot1 acts to antagonize the *HSP26* activated state rather than to repress basal transcription. Mot1 apparently limits the extent and duration of *HSP26* activation by limiting TBP recruitment without affecting other regulatory mechanisms involved in controlling *HSP26* transcription. This is consistent with conclusions reported recently by Zanton and Pugh (2004) employing genome-wide analysis of Mot1 occupancy and transcription. *INO1* transcription is also controlled by TBP recruitment, and like *HSP26*, Mot1 antagonizes activator function rather than repressing basal transcription. For both promoters, Mot1-mediated repression can be explained by an inhibition of TBP binding, in good agreement with Mot1's *in vitro* TBP–DNA-dissociating activity.

Previous ChIP analysis demonstrated that the level of Mot1 closely correlated with the level of TBP at the promoter (Geisberg *et al*, 2002). A similar correlation was observed during induction of *HSP26* (Figure 1). Impairing Mot1 function led to a greater level of *HSP26* induction and a delay in adaptation to the heat shock response. A similar delay in the shut-off of *INO1* transcription in response to inositol was also

observed (Figure 4A). These results suggest general functions for Mot1 in setting the level of activated transcription and in disassembling PICs during the return of an activated promoter to the transcriptional 'ground state'. Whether Mot1 affects the rate of shut-off of a particular promoter probably depends on the cofactor requirement for the promoter, which in turn dictates the inherent stability of the PIC and the accessibility of TBP to Mot1 action.

#### **Mot1-mediated establishment of an active TBP-containing complex**

At the Mot1-activated *BNA1* and *URA1* promoters, mutation of *MOT1* led to a decrease in transcription but an increase in TBP bound to the promoter. An increase in TBP occupancy is consistent with a role for TBP–DNA dissociation by Mot1, even at Mot1-activated promoters. A general increase in TBP crosslinking to DNA in *mot1* cells was observed using both polyclonal antisera against native TBP and immunoprecipitation using an epitope tag on TBP's C-terminus (Supplementary Figure 1), ruling out the idea that TBP–DNA complexes are recovered more efficiently from *mot1* cells for trivial reasons. The increased crosslinking of TBP may not reflect an increase in the proportion of promoters bound by TBP, but instead a change in the residency time of TBP bound to chromatin *in vivo*. TBP that is bound stably to these promoters may be more likely to be crosslinked to DNA compared to TBP in wild-type cells that is undergoing cycles of DNA binding and dissociation catalyzed by Mot1. In contrast to the increase in the TBP ChIP signals at

Mot1-activated promoters, there is apparently less TFIIB and Pol II bound to these promoters in *mot1* cells. We suggest that these results mean that Mot1 is required for establishment of a transcriptionally active form of TBP at the promoter. *BNA1* and *URA1* transcription is *TAF1* dependent and Taf1 and 4 are bound to their promoters. The large changes in TFIIB:TBP, Pol II:TBP and TAF:TBP ratios indicate that the putative inactive form of TBP results from depletion of promoter-bound TAFs, TFIIB and Pol II. It is also possible that one or more of these components is missing from a subset of individual promoters or that these factors bind to TBP in an inactive conformation. A function for Mot1 in establishing an active form of TBP is consistent with data showing that Mot1-TBP complexes are not associated with TFIIA, TFIIB or Pol II under normal growth conditions (Geisberg and Struhl, 2004). While the combined data suggest a role for Mot1 in TBP recycling, we cannot exclude a model in which Mot1 activates transcription by recruiting TFIIB and Pol II, either directly or via another factor. If this recruitment is a distinct function from Mot1's ATP-dependent activity, then loss of Mot1 function could lead to higher TBP occupancy and reduced levels of TFIIB and Pol II at promoters. Additional tests of the TBP recycling model for Mot1-mediated activation await a system for analysis of transcription factor dynamics at specific promoters *in vivo*.

### Interplay between Mot1 and SAGA

The relationship between Mot1 and SAGA was investigated for several reasons. First, the Mot1-repressed *INO1* gene is under SAGA control (Lo *et al*, 2001) and we sought to better understand how both factors control *INO1* transcription. Second, SAGA and Mot1 have global roles in control of stress response gene transcription (Dasgupta *et al*, 2002; Huisinga and Pugh, 2004; Zanton and Pugh, 2004). Finally, a recent report indicates that Mot1 and SAGA cooperate to activate *GAL1* transcription (Topalidou *et al*, 2004). The Spt3 subunit of SAGA is required for recruitment of Mot1 to the *GAL1* promoter and *vice versa*. Moreover, remodeling of the promoter depended on Mot1, leading to the suggestion that Mot1 activates *GAL1* by providing a chromatin remodeling function that facilitates recruitment of TBP and PIC establishment (Topalidou *et al*, 2004). This mechanism does not explain how Mot1 activates *BNA1* and *URA1* transcription, because not only is the chromatin of these promoters accessible for TBP binding in the absence of Mot1, but also they do not display the dependence on SAGA as was observed with *GAL1*. Neither *BNA1* nor *URA1* transcription depends strongly on the SAGA subunit Spt3, indicating that Spt3 is not required for recruitment of Mot1 or TBP to these promoters. *BNA1* transcription requires the Gcn5 subunit of SAGA, but *URA1* does not, and there is no defect in either TBP or Mot1 recruitment to the *BNA1* promoter in *gcn5Δ* cells (data not shown). Other studies have shown that SAGA has both positive and negative effects on transcription, and promoters display a differential requirement for the TBP-interacting Spt3 subunit and the Gcn5 HAT subunit (Dudley *et al*, 1999; Belotserkovskaya *et al*, 2000; Bhaumik and Green, 2002; Barbaric *et al*, 2003; Huisinga and Pugh, 2004; Warfield *et al*, 2004). The results presented here are in good agreement with these studies. As different Mot1-regulated genes display different dependencies on SAGA subunits, we suggest that the synthetic growth phenotypes observed in *mot1 sga* double-

mutant strains may result from combined deregulation of different sets of genes as much as combined effects of specific genes that require both factors.

## Materials and methods

### Yeast strains and growth conditions

*S. cerevisiae* strains used in the study are listed in Supplementary Table I. All strains are derived from YPH499 (Sikorski and Hieter, 1989) unless otherwise noted. Strains were constructed by single-step gene replacement using PCR-generated DNA fragments. Appropriate targeting of the disruption cassettes was confirmed by PCR and in most cases by standard genetic crosses and tetrad analysis. Double-mutant strains were constructed by sequential gene disruption in haploid cells or by mating the appropriate single-deletion strains constructed in the same strain background. Strains expressing C-terminal epitope-tagged proteins were obtained by transformation with PCR-generated DNA cassettes containing the epitope tag in-frame with the gene of interest. Appropriate integration events were confirmed by PCR and Western blotting.

For Northern blotting and ChIP experiments comparing wild-type cells to *mot1-14* or *mot1-42* cells, strains were grown in YPD at 30°C to an OD<sub>600</sub> of about 1.0. Then, cells were shifted to 35°C for 45 min. The cells were then harvested for isolation of total RNA or formaldehyde treated for ChIP experiments (see below). For the *INO1* shut-off experiment shown in Figure 4, wild-type and *mot1-42* cells were grown in inositol starvation medium at 30°C to an OD<sub>600</sub> of about 1.0. Cells were then shifted to 35°C for 15 min with addition of prewarmed inositol starvation medium. A portion of the cells was harvested (time zero) and inositol (100 μM) was added to the remainder of the culture, and cells were harvested at the indicated times thereafter. For the *MET15* shut-off experiment, wild-type and *mot1-42* cells were grown at 30°C in synthetic media plus glucose without methionine and cysteine to an OD<sub>600</sub> of about 1.0. Cells were then shifted to 35°C for 15 min and a portion of the cells was harvested (time zero). Cysteine and methionine were added to the remaining culture (0.1 mg/ml final concentration) and cell aliquots were harvested at the indicated times thereafter. TBP and Mot1 occupancy of the *INO1* promoter (Figure 3) was performed using chromatin from cells that were grown to an OD<sub>600</sub> of about 1.0 in YPD or inositol starvation medium supplemented with 200 μM inositol. Cells grown in inositol starvation medium with zero inositol were obtained by transferring cells from 200 μM inositol cultures to media containing zero inositol for 4 h prior to harvesting.

Spot assays were performed by growing cells overnight at 30°C in YPD, appropriate selective media, or the media indicated in the figure legends. Normalized 10-fold serial dilutions of the cells were then spotted on the indicated plates, which were incubated at the indicated temperatures for 2–3 days prior to photography.

### RNA isolation and Northern blotting

Total RNA was isolated using a hot acid-phenol extraction protocol. For Northern blots, 5–20 μg total RNA was separated by electrophoresis, transferred to Nytran N membrane (Schleicher & Schell) and probed with random-primed DNA probes obtained from cloned genes or by PCR amplification of portions of the ORFs of the genes of interest. The blots were hybridized overnight at 42°C in 50% formamide and washed twice in 0.1 × SSC and 0.1% SDS at room temperature for 15 min each followed by washing twice in the same buffer at 50°C for 15 min each. The bands were detected by autoradiography and quantitated by PhosphorImager analysis. Details of this procedure were described by Dasgupta *et al* (2002).

### Chromatin immunoprecipitation

Cells were grown as described above and then treated with 1% formaldehyde for 15 min. Glycine was added to a final concentration of 125 mM and the cultures were further incubated for 5 min. The cells were then washed once with cold TBS (20 mM Tris-HCl pH 7.4, 150 mM NaCl) with or without 125 mM glycine. Cells were frozen in liquid nitrogen and stored at –80°C for later analysis. Cell pellets were resuspended in 600 μl ChIP lysis buffer (50 mM Hepes-KOH, pH 7.5, 140 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, 1% Triton X-100, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin A). The resuspended cell suspension was then mixed with

an equal volume of acid-washed glass beads (425–600 µm) and the cells were disrupted at 4°C using a FastPrep™ FP120 device (Bio Savant) at 4°C. Cell lysates were then sonicated to yield an average DNA fragment size of 500bp, and the sonicated material was clarified by centrifugation at 14 000 r.p.m. for 30 min in a microfuge. ChIP of Mot1-TAP was carried out as described by Dasgupta *et al* (2002). For ChIP analysis of other components, chromatin protein was measured by BioRad protein assay using BSA as the standard, and equal amounts of protein (1–2 mg) were immunoprecipitated overnight with 2 µl of TBP or TFIIB rabbit polyclonal antiserum or with 5 µg of 9E10 anti-myc monoclonal antibody. ChIP for Pol II was performed using the RNA Pol II monoclonal antibody 8WG16 (Thompson *et al*, 1989; Bhaumik and Green, 2001).

The reactions were then incubated with 60 µl of protein A Sepharose beads equilibrated in FA lysis buffer (50 mM Hepes, pH 7.5, 140 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, 1% Triton X-100). The bead-bound immune complexes were recovered by centrifugation and washed twice each with 1.0 ml of FA lysis buffer, 1.0 ml of FA lysis buffer with high salt (50 mM Hepes-KOH, pH 7.5, 500 mM NaCl, 1 mM EDTA, 0.1% sodium deoxycholate, 1% Triton X-100), 1.0 ml LiCl wash buffer (10 mM Tris-HCl, pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, 1 mM EDTA) and TE (10 mM Tris, pH 8.0, 1 mM EDTA). The immunoprecipitated material was eluted twice with 190 µl of 2% SDS, 0.1 M NaHCO<sub>3</sub> and 250 mM NaCl. Alternatively, the immunoprecipitated material was eluted twice with 50 mM Tris, pH 8.0, 1% SDS and 10 mM EDTA. The eluted material was incubated at 65°C overnight and the immunoprecipitated DNA was treated with proteinase K and phenol-chloroform extraction or was purified using a PCR purification kit (Qiagen) following the instructions of the manufacturer. Quantitative PCR was performed using 1/100–1/500 of the material recovered after the immunoprecipitation or 1/5000–1/10 000 of the input DNA. In all cases, titrations were performed to ensure that the yield of PCR product was linearly related to the amount of added template (see Supplementary Figure 4). Primers for PCR of Mot1-regulated promoters were described by Dasgupta *et al* (2002); other primer sequences are available upon request. PCR products were resolved on 2% agarose gels, stained with ethidium bromide, and visualized and quantified using an Alphamager. Band intensities obtained for the immunoprecipitated samples were corrected for the signal obtained from the untagged or preimmune control samples and normalized to the band intensities obtained using the input samples. For each experiment shown, ChIP analysis was performed at least three times using independently prepared batches of chromatin. TBP ChIP was performed using rabbit polyclonal antisera or using a strain harboring myc-tagged TBP. Mot1 ChIP was performed using a Mot1-TAP tagged strain or a myc-tagged Mot1 strain. The results were indistinguishable regard-

less of the antibody or epitope tag used, arguing that the results are not influenced by accessibility of epitopes on TBP or Mot1, but instead reflect true differences in promoter occupancy. The specific ChIP signals were at least two-fold higher than the signals obtained with untagged or preimmune controls, and typically ranged from ~5- to 14-fold greater than the negative controls.

#### Micrococcal nuclease analysis

MNase digestion of lyticase-permeabilized spheroplasts was performed as described (Kent *et al*, 1993; Kent and Mellor, 1995) with minor modifications. In brief, *MOT1* and *mot1-42* cells were grown at 30°C in 100 ml YPD to an OD<sub>600</sub> of 1.0, and were then heat-shocked at 35°C for 45 min. Cells were pelleted and resuspended in 0.95 ml of 10 mg/ml *Arthrobacter luteus* lyticase (Sigma) in 1 M sorbitol and 5 mM 2-mercaptoethanol. Incubation was carried out for 15 min at 22°C. Spheroplasts were pelleted, washed twice in 1 M sorbitol (without disturbing the pellet) and then resuspended in 1.2 ml Spheroplast Digestion Buffer (1 M sorbitol, 50 mM NaCl, 10 mM Tris-HCl, pH 7.5, 5 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 1 mM 2-mercaptoethanol, 0.5 mM spermidine, 0.075% NP-40). Samples were divided into 0.2 ml aliquots, and MNase (1.375–75 U/ml) or 5% SDS plus 250 mM EDTA (final concentrations) were added to each sample and incubated at 37°C for 4 min. Following digestion, DNA was purified by RNase treatment at 37°C for 30 min, phenol-chloroform extraction and ethanol precipitation. Purified, naked DNA was then digested with MNase in the same buffer as was used for digestion of chromatin. DNA was then digested with *HpaI* (for the 5' probe) or *MfeI* (for the 3' probe), followed by resolution on 1.5% agarose gels. DNA was transferred to an uncharged nylon membrane (Osmonics), and hybridized to 5' or 3' probes as indicated in Figure 8. The 5' probe was made by random-prime labeling of a 186 bp PCR fragment obtained using oligonucleotides #1 (5'-GCAAGATGGTAGATTACTGTG-3') and #2 (5'-CCATTCAGTCAAGAACCAA-3'). The 3' probe was made by labeling a 197 bp PCR fragment obtained using oligonucleotides #3 (5'-GAAGCTAAGGGT TATACATCC-3') and #4 (5'-GAATGCACTACCGAAAA-3').

#### Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

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