

## RESEARCH COMMUNICATION

## Different upstream transcriptional activators have distinct coactivator requirements

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**Activated transcription by RNA polymerase II (Pol II) requires coactivators, one of which is the SRB/mediator. Whereas *Srb4*, an essential subunit of the SRB/mediator, is broadly required for Pol II transcription in yeast, we have shown that it is dispensable for the transcriptional activation of some genes. Here, we show that transcriptional activation by different natural activators, and by artificial recruitment of various transcription factors, have very different degrees of *Srb4* independence. These data, and the analysis of an *rgr1* mutant, point to an *Rgr1* subcomplex of the SRB/mediator as the mechanistic route of activation by *Srb4*-independent activators in vivo.**

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The eukaryotic genome contains thousands of genes that show varied and sophisticated regulation. For example, transcriptional activation by upstream activator proteins requires not only general transcription factors (GTFs), but also coactivators that include TFIID (Verrijzer and Tjian 1996), histone acetyltransferase complexes such as SAGA (Grant et al. 1998a), and the SRB/mediator (Bjorklund and Kim 1996). TFIID is composed of TATA-binding protein (TBP) and TBP-associated factors (TAFs) (Verrijzer and Tjian 1996). Although the model of the TAFs having a general coactivator function has been challenged (Moqtaderi et al. 1996; Walker et al. 1996; Oelgeschlager et al. 1998), the in vivo importance of the TFIID-specific TAFs for transcription of about one-sixth of all yeast genes and of some developmentally regulated genes in higher organisms has been clearly demonstrated (Walker et al. 1997; Holstege et al. 1998; Zhou et al. 1998).

The yeast SAGA complex contains Gcn5, the catalytic subunit of HAT activity, Ada proteins, Spt proteins, and several TAFs (for review, see Grant et al. 1998b). A mutant of *GCN5* resulted in altered transcription of <5% of yeast genes, whereas a mutation in the SAGA subunit, *Taf17* (which is also a subunit of TFIID), affected tran-

scription of over two-thirds of yeast genes (Holstege et al. 1998).

The SRB/mediator complex appears to be a more generally required coactivator. Mediator binds tightly to the carboxy-terminal domain (CTD) of the largest subunit of RNA polymerase II (Pol II), mediates activated transcription in vitro (Kim et al. 1994), and can physically interact with at least some activators (Hengartner et al. 1995; Koh et al. 1998). These results, coupled with the observation that artificial recruitment of mediator results in a robust transcriptional activation both in vitro (Gaudreau et al. 1998) and in vivo (Barberis et al. 1995), suggest that the SRB/mediator is a primary target of activators in vivo. The general requirement of the SRB/mediator for Pol II transcription in vivo was shown in studies with temperature-sensitive mutants of essential subunits of the SRB/mediator, such as *Srb4* and *Srb6* (Thompson and Young 1995; Holstege et al. 1998). However, these experiments did not distinguish whether *Srb4* and *Srb6* are required for basal transcription or the transmission of upstream activation signals to the core transcription machinery. We and others have shown that the *Ace1*-driven transcription of the *CUP1* gene, and *Hsf*-driven transcription of heat shock genes, *SSA4* and *HSP82*, can be activated independently of *Srb4* or *Srb6* in vivo, whereas activation by *Gal4* is severely reduced (Lee and Lis 1998; McNeil et al. 1998). The unique nature of the upstream activators *Ace1* and *Hsf* was further revealed by studies of *taf17<sup>ts</sup>* mutants. *Taf17* is required for the transcription of many genes, but not the *Ace1* and *Hsf*-regulated *CUP1* and *SSA4* genes (Apone et al. 1998; Moqtaderi et al. 1998). These observations support the hypothesis that different upstream activators activate transcription by different mechanisms.

In this work, we explore the mechanism of *Srb4* (and *Taf17*)-independent *CUP1* transcription by examining the differential effects of *Srb4* or *Taf17* on transcriptional activation by different upstream activation domains, and on the activation caused by artificially recruiting various subunits of the SRB/mediator to the *CUP1* promoter.

### Results and Discussion

We have reported previously that the induced transcription of *CUP1* and *SSA4* genes can occur even after *Kin28*, *Srb4*, or *Srb6* have been inactivated (Lee and Lis 1998). These results suggested that the transcription of these genes can be activated independently of the essential TFIID kinase and the SRB/mediator. More recently, using transcription run-on assays, we demonstrate that the *Srb4*-independent increase in *CUP1* mRNA occurs at a step that leads to an increase in the density of transcription complexes on the gene (data on our web site). These results agree with McNeil et al. (1998) and extend their studies to provide a direct measurement of the recruitment of Pol II on the *CUP1* gene after copper induction.

The molecular basis for the *Srb4*-independent transcription must be specified by features of the promoter.

[Key Words: *Srb4*; mediator; copper inducibility; *CUP1*; *Rgr1*; coactivator]

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By testing a series of hybrid promoters that contain the upstream activating sequence (UAS) from the *Srb4*-independent *CUP1* gene and the core promoter from an *Srb4*-dependent gene (*ADH1* and *GAL1*), and vice versa, we determined that *Srb4*-independent transcription is specified by the UAS, not by the core promoter (data on our web site). The same conclusion was derived independently with a different set of hybrid promoters by McNeil et al. (1998).

The UAS of the *CUP1* gene contains known binding sites for two transcription factors, Ace1 (Evans et al. 1990) and Hsf (Tamai et al. 1994), that allow the *CUP1* gene to be activated, respectively, by copper and by heat shock. One might assume that the *Srb4*-independent copper induction is mediated by the transcription factor Ace1 alone; however, Hsf shows some binding to its DNA sites in yeast even under non-heat shock conditions (Giardina and Lis 1995). Also, because transcription of other heat shock genes has been shown to be moderately heat inducible in yeast strains depleted of *Srb4* (Lee and Lis 1998; McNeil et al. 1998), Hsf on its own could potentially specify the *Srb4* independence. Therefore, we tested directly the role of the Hsf-binding site in the copper induction of the *CUP1* gene, using a *CUP1-LacZ* reporter containing a point mutation in the HSE (HSEM) (Fig. 1A). The HSEM construct was defective in heat shock induction (Fig. 1A). However, in the *srb4<sup>ts</sup>* mutant, the HSEM construct was highly inducible by copper after *Srb4* inactivation (Fig. 1A), demonstrating that the HSE is dispensable for *CUP1* transcription in *srb4<sup>ts</sup>* cells, and that the Ace1 transcription factor is sufficient for *Srb4*-independent *CUP1* activation by copper.

Is the activation domain of Ace1 qualitatively different from other activation domains that drive *Srb4*-dependent transcription? To answer this, we first deleted the endogenous *ACE1* gene from *SRB4* wild-type and *srb4<sup>ts</sup>* strains, and then transformed the resulting strains with plasmids expressing the Ace1 DNA-binding domain fused to various activation domains. The DNA-binding domain of Ace1 undergoes a conformational change on copper addition, allowing it to bind to the DNA sequence element within the *CUP1* UAS (Furst et al. 1988). Therefore, the hybrid proteins should bind DNA in the same way wild-type Ace1 binds in response to copper (Fig. 1B). As expected, strong induction of the *CUP1* gene is observed in both *SRB4 ace1Δ* and *srb4<sup>ts</sup> ace1Δ* strains expressing the wild-type Ace1 (Fig. 1C, ACE1). In contrast, whereas the Ace1-VP16 hybrid activated the *CUP1* gene in the *SRB4* strain, it failed to activate in the *srb4<sup>ts</sup>* strain at the nonpermissive temperature (Fig. 1C, VP16). Ace1-VP16 activated transcription normally in the *srb4<sup>ts</sup>* strain at the permissive temperature (data not shown). The Ace1-Gal4 construct, which contains the carboxy-terminal activation domain of Gal4, is also very sensitive to the inactivation of *Srb4* (Fig. 1C, GAL4). These data clearly show that the Ace1 activation domain operates in a mechanistically distinct manner from VP16 and Gal4 activation domains *in vivo*.

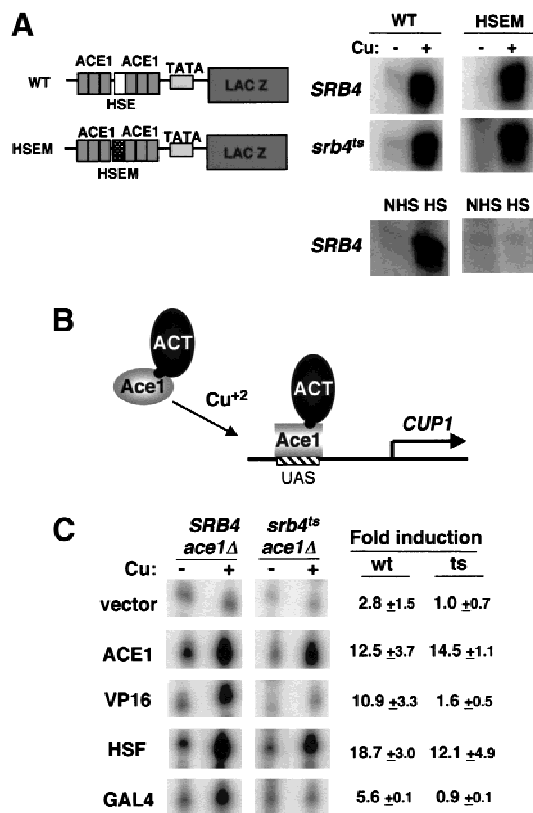
It should be noted that although the ratio between uninduced and the Ace1-induced *CUP1* gene transcrip-

tion was similar in the *SRB4* wild type and mutant, the uninduced level of *CUP1* mRNA was two- to threefold less in the *srb4<sup>ts</sup>* mutant even at the permissive temperature. This lowered, uninduced level of *CUP1* mRNA in the *srb4<sup>ts</sup>* mutant is likely to be a secondary effect, because the temperature shift to inactivate *Srb4* did not further reduce the *CUP1* mRNA level (data not shown). Additionally, this lowered uninduced level may be why the *CUP1* gene was scored as *Srb4* dependent in another study (Holstege et al. 1998). Nonetheless, it is clear that the *CUP1* gene shows the normal fold inducibility in yeast that has an inactivated *Srb4* protein.

Because some heat shock genes can be activated after inactivating *Srb4*, we anticipated that a fusion between the DNA-binding domain of Ace1 and the carboxy-terminal activation domain of Hsf (Ace1-Hsf) might activate the *CUP1* gene transcription in the *srb4<sup>ts</sup>* mutant. Ace1-Hsf activated transcription in the *srb4<sup>ts</sup>* mutant (Fig. 1C, HSF). These results reinforce the proposal that the *Srb4* requirement in transcription activation depends on the nature of the particular activation domain.

Activation domains may exert their effect on transcription through interactions with coactivators or components of the general transcription machinery. The artificial recruitment of these target proteins to the promoter, for example, via their fusion to a LexA DNA-binding domain, leads directly to high-level expression of promoters that have LexA sequence elements in the absence of any natural activator (Barberis et al. 1995; Gaudreau et al. 1998, and references therein). We reasoned that the particular target contacted by the Ace1 activation domain should activate transcription in an *Srb4*-independent manner, when it is directly recruited to a promoter. We first tested TBP and TAF17. Recruiting either of these could conceivably provide a platform for the assembly of a preinitiation complex with core Pol II (i.e., Pol II lacking *Srb4*/mediator). Tethering TBP or TAF17 to the Ace1 DNA-binding domain resulted in the copper-induced activation of the *CUP1* reporter gene in the *SRB4* wild type (Fig. 2A). In the *srb4<sup>ts</sup>* mutant, the fold induction by these hybrid proteins reached 50%–70% of that observed in wild type (Fig. 2A). Therefore, activation by recruiting TBP or TAF17 is partially independent of *Srb4*. Recent genetic studies have shown that the loss-of-function mutations in the negative regulators of TBP, such as NC2 and MOT complexes, can suppress the temperature-sensitive phenotype of the *srb4<sup>ts</sup>* mutant (Lee et al. 1998). This suggests that an activation mechanism that utilizes the recruitment and/or stabilization of TBP to the promoter may be independent of *Srb4*. It is possible that the artificial recruitment of TBP mimics such a situation at least partially. TAF17 is a subunit of both TFIID and SAGA complex. Therefore, the artificial recruitment of TAF17 may result in recruitment of either TFIID or SAGA, or both, and these complexes may function in a manner that is partially independent of *Srb4*.

We then tested the ability of artificially recruited subunits of *SRB*/mediator to activate transcription. It was proposed that the inactivation of the *Srb4* subunit would



**Figure 1.** SrB4 independence of the native *CUP1* gene is activation domain specific. (A) S1 nuclease assays showing the heat shock element (HSE) is dispensable and Ace1-binding sites in the UAS of the *CUP1* gene are sufficient for the SrB4-independent transcription. (Left) Reporter constructs used in the experiment. The binding sites for Ace1 (ACE1) and a single heat shock element (HSE), and TATA box (TATA) are shown. The HSE with a point mutation that destroys Hsf binding is designated as HSEM. (Top, right) The 30-min copper induction of wild-type (WT) and HSE mutant *CUP1* promoter (HSEM) in *SRB4* wild-type or *srB4<sup>ts</sup>* mutant. *SRB4*wt and *srB4<sup>ts</sup>* cells were grown until OD = 0.3–0.5 at 25°C, then cells were moved to a 37°C water bath and incubated for 1.5 hr; 8-ml cells were harvested (–Cu sample). Then, 1 mM CuSO<sub>4</sub> was added and cells were incubated for 30 min at 37°C and again, 8-ml cultures were harvested (+Cu sample). (Bottom, right) The heat inducibility of each construct. Cells were grown to mid-log phase at 25°C and an aliquot was taken (NHS, non heat shock) or cells were transferred to a 39°C water bath for 15 min (HS, heat shock). RNAs were isolated and assayed by S1 nuclease protection. (B) Design of the experiment in part C. (C) Transcriptional activation of the *CUP1* gene by Ace1-hybrid activators. Isogenic DLY981 (*SRB4ace1Δ*) and DLY982 (*srB4<sup>ts</sup>ace1Δ*) strains are transformed with the following plasmids: pRS316 (vector), p316:ACE1 (ACE1), p316:ACVP (VP16), pAC–HSF (HSF), and pAC–GAL4 (GAL4). Cell growth and copper induction was done as in Fig. 1A. Fold induction of the endogenous *CUP1* gene in wild-type and temperature-sensitive mutants are shown next to the S1 assay raw data. Standard deviation is shown for each value. Each value is an average of at least three experiments except for GAL4 (two).

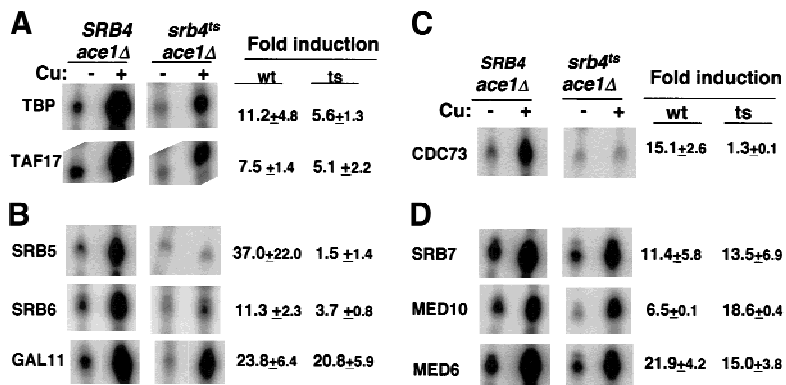
result in an inactive SRB/mediator-holoenzyme (Thompson and Young 1995). Consistent with this hypothesis, the recruitment of either SrB5 or SrB6, two sub-

units of SRB/mediator, gave either very little activation (Fig. 2B, SrB5) or severely reduced activation (Fig. 2B, SrB6) in the *srB4<sup>ts</sup>* mutant. Surprisingly, however, the recruitment of Gal11, another subunit of SRB/mediator, resulted in a robust activation in the *srB4<sup>ts</sup>* mutant at the nonpermissive temperature (Fig. 2B, Gal11). One explanation for this result is that there exists another Gal11-containing complex, and activation through the recruitment of this complex is SrB4 independent. Recent work has suggested that there exists another form of RNA Pol II holoenzyme, which includes factors such as Cdc73, Paf1, and Gal11, but is devoid of SRBs (Shi et al. 1997). If the recruitment of Gal11 also recruits the Cdc73/Paf1/Gal11 holoenzyme, the transcription by this holoenzyme should be independent of SrB4. However, transcription activation by the Ace1–Cdc73 construct, which provides another way of recruiting the Cdc73/Paf1/Gal11 holoenzyme, is completely dependent on SrB4 (Fig. 2C). Therefore, the recruitment of the Cdc73/Paf1/Gal11 holoenzyme cannot explain the SrB4 independence of the Ace1–Gal11 activator. This result also suggests that the function of Cdc73/Paf1/Gal11 holoenzyme requires functional SrB4.

Another explanation of the SrB4-independent activation by Ace1–Gal11 derives from the observation that the SRB/mediator complex consists of two biochemically separable modules, an SrB4 subcomplex and an Rgr1 subcomplex that contains Gal11 (Lee and Kim 1998). The mediator lacking the SrB4 subcomplex may function to activate transcription when recruited to a promoter. More recently, Gu et al. (1999) purified a mammalian complex that consists of several homologs of yeast SRB/mediator subunits such as Rgr1, SrB7, Med6, and Med10, but is devoid of the SrB4 subcomplex. This complex, named SMCC, contains several homologs of the subunits of the Rgr1 subcomplex. This complex is identical to the human thyroid hormone receptor-associated TRAP complex (Ito et al. 1999). Considering the similarity between SMCC and the Rgr1 subcomplex, we speculate that the yeast Rgr1 subcomplex might also be able to act as a coactivator on its own. Recruiting Gal11 may then recruit the Rgr1 subcomplex, thereby activating transcription independently of functional SrB4, or even a functional SrB4 subcomplex. We tested the possibility by tethering another component of the Rgr1 subcomplex to the promoter to see if this results in an SrB4-independent activation. Ace1–SrB7 activated transcription in the *srB4<sup>ts</sup>* mutant to a level comparable with wild type (Fig. 2D, SrB7). Artificial recruitment of Med10 or Med6, both present in the SMCC complex (Gu et al. 1999), and at least one (Med10) proposed to be present in the yeast Rgr1 subcomplex (Han et al. 1999), also resulted in strong activation in the *srB4<sup>ts</sup>* mutant. These results support the idea that the transcriptional activation through a SMCC-like Rgr1 subcomplex is SrB4 independent in vivo.

Further support for the role of an Rgr1 subcomplex in the SrB4-independent activation of *CUP1* comes from the analysis of the *rgr1-Δ2* mutant, a strain that grows slowly and is temperature sensitive (Jiang et al. 1995).





**Figure 2.** Transcriptional activation by artificial recruitment in the *srb4<sup>ts</sup>* mutant. DLY981 (*SRB4ace1Δ*) and DLY982 (*srb4<sup>ts</sup>ace1Δ*) strains were transformed with following plasmids: pAC-TBP (TBP), pAC-TAF17 (TAF17), pAC-SRB5 (SRB5), pAC-SRB6 (SRB6), pAC-GAL11 (GAL11), pAC-CDC73 (CDC73), pAC-SRB7 (SRB7), pAC-MED10 (MED10), and pAC-MED6 (MED6). Cell growth and copper induction was done as in Fig. 1A. Fold induction of the endogenous *CUP1* gene in wild-type and temperature-sensitive mutants were shown next to the S1 assay raw data. Standard deviation is shown for each value. Each value is an average of at least three experiments, except for CDC73 and MED10 (two). pAC-SRB5 and pAC-CDC73 activated *CUP1* gene transcription normally in the *srb4<sup>ts</sup>* strain at the permissive temperature (data not shown). The TAF17 samples were run at the edge of a smiling gel. (A) Transcriptional activation by artificial recruitment of TBP and TAF17. (B) Activation by recruiting different subunits of SRB/mediator have different dependence on *Srb4*. (C) Activation by the artificial recruitment of *Cdc73* is *Srb4* dependent. (D) Subunits of the SMCC-like yeast Rgr1 subcomplex mediate *Srb4*-independent transcription.

The mediator complex isolated from this strain is missing not only a portion of Rgr1 but also several subunits of the Rgr1 subcomplex including Sin4p, Gal11p, Med2, and Hrs1p (Li et al. 1995). Figure 3 shows that the *CUP1* gene is poorly inducible by copper in this mutant line, providing additional support for the role of an Rgr1-containing complex in the Ace1 activation of *CUP1*.

Finally, we applied our hybrid activators to investigate the role of another broadly required coactivator, Taf17, in the activation of *CUP1*. It has been shown that, whereas Taf17 is required for most Pol II transcription *in vivo* (Apone et al. 1998; Michel et al. 1998; Moqtaderi et al. 1998), transcriptional activation of *CUP1* and *SSA4* in *taf17<sup>ts</sup>* mutants is normal. Also, like the *Srb4*-independent *CUP1* activation (McNeil et al. 1998; this work), the UASs of *CUP1* and *SSA4* promoters specify the Taf17 independence, suggesting a mechanism of activation by Ace1 and Hsf that is different from other gene-specific activators, such as Gcn4 (Moqtaderi et al. 1998). Here, we show that the activation domains of Ace1 and Hsf resulted in normal activation of the *CUP1* gene in a *taf17<sup>ts</sup>* mutant when fused to the Ace1 DNA-binding domain (Fig. 4A, ACE1 and HSF). In contrast, the activation domain of Gcn4 was defective in activating transcription in the *taf17<sup>ts</sup>* mutant (Fig. 4A, GCN4). The basal level of *CUP1* was not affected in this *taf17<sup>ts</sup>* mutant (unlike the *srb4<sup>ts</sup>* mutant). Therefore, the absolute levels after copper induction are similar between the wild type and *taf17<sup>ts</sup>* for both Ace1 and Hsf-mediated activation of the *CUP1* gene and are reported in Figure 4

quantitatively as percent-induced level in mutant relative to wild type.

If the activation pathway through the Rgr1 subcomplex is utilized by Ace1 and/or Hsf, then it too should be independent of not only *Srb4* but also Taf17. To test this hypothesis, we measured whether the Ace1-Gal11 can also activate transcription in a *taf17<sup>ts</sup>* mutant. As shown in Figure 4B, the recruitment of Gal11 resulted in strong activation in both wild type and mutant. Therefore, the transcriptional activation by Ace1-Gal11, like native Ace1 activation, is independent of both *Srb4* and Taf17.

How do cells with inactive *Srb4* still maintain the ability to mediate activation by Ace1 and Hsf? It was proposed that the activation domains of Ace1 or Hsf have the ability to interact with any of a number of targets including *Srb4* (Moqtaderi et al. 1998). Alternatively, it is possible that distinct activation pathways exist *in vivo*, and some pathways, which can be utilized by Ace1 and Hsf, are independent of *Srb4* and Taf17, but others, which are utilized by other activators such as Gal4, are not.

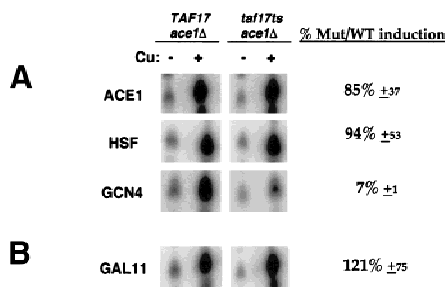
Our result that Ace1-Med6 can activate transcription in the *srb4<sup>ts</sup>* mutant indicates that Med6 behaves like other members of the Rgr1 subcomplex of the mediator. Yet, biochemical data show yeast Med6 is more tightly associated with the *Srb4* subcomplex than with the Rgr1 subcomplex (Lee and Kim 1998).

In contrast, hMed6 is present in a distinct coactivator complex, SMCC (Gu et al. 1999), along with human homologs of Rgr1, *Srb7*, and Med10. The coactivator activity of SMCC lacks any homologs of the yeast *Srb4* subcomplex. Could an SMCC-like complex exist in yeast? The Rgr1 subcomplex is, to date, the best candidate for a yeast SMCC homolog on the basis of the similarity of subunit composition and the ability to mediate *Srb4*-independent activation. Our results do not distinguish whether the activation pathway we utilized by artificial recruitment of Gal11/Med6/Med10/*Srb7* is through the Rgr1 subcomplex within the SRB/mediator, or through an SMCC-like complex (or a free Rgr1 subcomplex) that exists separately from the *Srb4* subcomplex in yeast. Nonetheless, each of the tested yeast SRB/mediator subunits that



**Figure 3.** *CUP1* induction is compromised in an *rgr1* mutant strain. The copper inducibility of the *CUP1* gene was measured by S1 nuclease protection assays in the *rgr1-Δ2* yeast mutant and the isogenic *RGR1* control. The average quantified fold inducibility and standard deviations for three experiments are shown.

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**Figure 4.** Transcriptional activation in a *taf17<sup>ts</sup>* mutant. DLY983 (*TAF17ace1Δ*) and DLY984 (*taf17<sup>ts</sup>ace1Δ*) strains were transformed with following plasmids: p316:ACE1 (ACE1), pAC-HSF (HSF), pAC-GCN4 (GCN4), and pAC-GAL11 (GAL11). Cell growth, copper induction, and S1 assays of *CUP1* RNA were done as in Fig. 1A. The average induced levels in mutant relative to wild type are shown as percentages with standard deviations from three experiments (except two for HSF). (A) Activation domain of Ace1 and HSF, but not Gcn4, can activate Taf17-independent transcription. (B) Activation by artificial recruitment of Gal11 is Taf17 independent.

have homologs in the human SMCC succeeded in mediating Srb4-independent activated transcription, whereas those subunits that fail are not found in the SMCC. These results are compatible with the existence of a functional SMCC-like complex in yeast.

The Rgr1 subcomplex has characteristics of an activation target for Ace1 and Hsf, in that the activation produced by recruiting components of this subcomplex is, like the activation produced by Ace1 and HSF, independent of Srb4 or Taf17. This finding and our observation that a mutation that disrupts the Rgr1 protein and its interaction with components of the Rgr1 complex dramatically reduces the activation of *CUP1* strongly implicate an Rgr1 subcomplex (or a yeast SMCC) as the target of this activation.

In the broader perspective, our results show that by studying the coactivator requirements of natural activators and comparing them with the coactivator requirements of activation by artificial recruitment, one can gain insight into the activation mechanism of the natural activators in vivo. We propose that our strategy, applied with many different transcription factor mutants that already exist (e.g., Holstege et al. 1998), will help identify the coactivators and general transcription machinery required by particular upstream activators in vivo. Our results also strongly support the view that, in vivo, no single unified pathway of transcriptional activation exists. Rather, it is likely that many different activators target different coactivators (or GTFs), that is, different activation pathways, to achieve gene-specific activation. This view is consistent with the large number and complexity of transcriptional coactivators and the numerous interactions of activator proteins with coactivators and GTFs identified so far. The use of multiple pathways could accommodate the specificity of activation of many thousands of genes responding to a broad spectrum of signals.

## Materials and methods

### Construction of strains and plasmids

DLY981 (*SRB4wt, ace1Δ:hisG*) and DLY982 (*srb4<sup>ts</sup>, ace1Δ:hisG*) were derived from CTY233 (*SRB4wt*) and CTY271 (*srb4-138*), respectively (Thompson and Young 1995). To generate DLY981 and 982, CTY233 and CTY271 strains were transformed with *Xba*I-*Afl*III-digested pACE1Δ:HISG.URA3 (Pena et al. 1998) and Ura<sup>+</sup> colonies were scored. Ura<sup>+</sup> transformants were then streaked on SCUra plates with 500 μm CuSO<sub>4</sub>. Those transformants that did not grow on CuSO<sub>4</sub> plates were then grown overnight in YPD and plated on 5-FOA plates to lose the URA3 gene. Again, the sensitivity on CuSO<sub>4</sub> was checked after 5-FOA selection. pACE1Δ:HISG.URA3 is a gift from Dennis Thiele (University of Michigan Medical School, Ann Arbor). DLY983 (*TAF17* wild-type, *ace1Δ:hisG*) and DLY984 (*taf17<sup>ts</sup>, ace1Δ:hisG*) were derived from YSB380 (*TAF17* wild-type) and YSB463 (*taf17<sup>ts</sup>*), respectively (Michel et al. 1998), by the same procedure as above. The *taf17* mutant we have used is the allele (*taf17-1*) that was shown to dramatically reduce the global transcription on temperature shift. DY881 (*RGR1* wild-type) and DY2587 (*rgr1-Δ2*) strains were gifts from David Stillman (University of Utah Health Science Center, Salt Lake City) (Jiang et al. 1995).

The HSEM construct, which is a 2-μ-based CUP1-LacZ reporter with a point mutation in the heat shock element within the *CUP1* promoter, is a gift from Dennis Thiele (Tamai et al. 1994).

Details of construction of plasmids for run-on transcription and ACE1-hybrid activators can be found on the Lis laboratory website <http://www.mbg.cornell.edu/lis/lis.html>.

### Run-on transcription assay

Run-on transcription assays were done with a modified procedure from Elion and Warner (1986). The detailed description of the run-on method can be found on the Lis laboratory website.

### S1 nuclease assay

S1 assay was done as described (Ausubel et al. 1992), except 0.1 pmole of oligos were used per reaction. The sequences of oligos used can be found on the Lis laboratory website.

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## Differential requirement for PolII coactivators

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