The Swi5 activator recruits the Mediator complex to the *HO* promoter without RNA polymerase II

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Regulation of *HO* gene expression in the yeast *Saccharomyces cerevisiae* is intricately orchestrated by an assortment of gene-specific DNA-binding and non-DNA binding regulators. Binding of the early G₁ transcription factor Swi5 to the distal URS1 element of the *HO* promoter initiates a cascade of events through recruitment of the Swi/Snf and SAGA complexes. In late G₁, binding of transcription factor SBF to promoter proximal sequences results in the timely expression of *HO*. In this work we describe an important additional layer of complexity to the current model by identifying a connection between Swi5 and the Mediator/RNA polymerase II holoenzyme complex. We show that Swi5 recruits Mediator to *HO* by specific interaction with the Gal11 module of the Mediator complex. Importantly, binding of both the Gal11 and Srb4 mediator components to the upstream region of *HO* is independent of the SBF factor. Swi/Snf is required for Mediator binding, and genetic suppression experiments suggest that Swi/Snf and Mediator act in the same genetic pathway of *HO* activation. Experiments examining the kinetics of binding show that Mediator binds to *HO* promoter elements 1.5 kb upstream of the transcription start site in early G₁, but this binding occurs without RNA Pol II. RNA Pol II does not bind to *HO* until late G₁, when *HO* is actively transcribed, and binding occurs exclusively to the TATA region.

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Regulated expression of some genes requires carefully choreographed binding by multiple transcription factors with distinct roles. In addition to sequence specific DNA-binding proteins, there are a variety of multiprotein complexes whose actions control gene expression, such as the Swi/Snf chromatin remodeling complex and the SAGA histone acetyltransferase complex. Cosma et al. (1999) used chromatin immunoprecipitation (ChIP) to show that activation of the yeast *HO* gene is characterized by the sequential recruitment of factors. The first step, at the end of mitotic anaphase, is the binding of the Swi5 zinc finger protein to distal sequences at the *HO* promoter. Swi5 facilitates binding of the Swi/Snf complex, and then the unstable Swi5 protein is degraded. After Swi5 disappears, the SAGA complex binds to the promoter. Finally, the SBF DNA-binding factor, composed of the Swi4/Swi6 factors, binds, and it is believed that SBF ultimately activates *HO* transcription. Importantly, the sequential binding of Swi5, Swi/Snf, SAGA, and then SBF are causally linked, as mutation in any one factor eliminates subsequent binding events. Changes in

histone acetylation at *HO* occur at the time of SAGA binding (Krebs et al. 1999). A mutation in the *GCN5* histone acetyltransferase blocks *HO* expression, suggesting that acetylation of the chromatin template is required.

RNA polymerase II is found in a large holoenzyme complex containing several general transcription factors and the Mediator (for reviews, see Hampsey and Reinberg 1999; Lee and Young 2000; Malik and Roeder 2000; Myers and Kornberg 2000). The 20-protein Mediator complex functions as an interface between sequence-specific transcription factors and the general transcriptional apparatus. Genetic analysis of yeast strains with Mediator mutations show defects in both transcriptional activation and repression, suggesting that Mediator functions to transduce both positive and negative regulatory information from promoter elements to RNA polymerase II. Mediator may also play a role in transcription reinitiation (Yudkovsky et al. 2000).

Genetic and biochemical experiments suggest that Mediator contains subcomplexes with distinct functions. Some Mediator subunits are required for the transcriptional regulation of specific genes, whereas others are necessary for general transcription in vivo. Urea treatment leads to the dissociation of Mediator subunits into stable modules, whose members are also function-

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ally related by genetic analysis (Lee and Kim 1998). The Srb subcomplex (Srb2, Srb4, Srb5, Srb6) is required generally for transcriptional activation because conditional mutations in either the essential *SRB4* or *SRB6* genes similarly result in a rapid loss of all Pol II transcription at the nonpermissive temperature (Holstege et al. 1998). The Gal11 subcomplex contains the Gal11, Pgd1, Sin4, Med2, and Rgr1 proteins, and mutations in the genes encoding these proteins can affect either transcriptional activation or repression, depending on the promoter (Myers and Kornberg 2000). For example, a *gal11* mutation results in reduced expression of *SUC2*, *CTS1*, and mating type genes, and increased expression of *GAL1* and *Ty1* genes (Fassler and Winston 1989; Vallier and Carlson 1991; Chen et al. 1993; Sakurai and Fukasawa 1997). Gal11 functions as a strong activator when artificially recruited to DNA (Barberis et al. 1995), and Gal11 interacts with TFIIE (Sakurai and Fukasawa 1997).

Sequence-specific DNA-binding transcription factors can recruit transcription complexes to promoters. Activation domains from Gal4, VP16, Gcn4, and Swi5 interact with the Swi/Snf complex (Natarajan et al. 1999; Neely et al. 1999; Yudkovsky et al. 1999). The Gcn4 activation domain also interacts with SAGA and Mediator (Drysdale et al. 1998; Utley et al. 1998; Natarajan et al. 1999). The Gal4 activation domain binds Srb4 (Koh et al. 1998), and the Gal4, Gcn4, and VP16 activation domains interact directly with the Gal11 protein in Mediator (Lee et al. 1999; Park et al. 2000). The idea that gene-specific activators directly bind Mediator to recruit RNA Pol II to the promoter is supported by genetic experiments showing that mutations in specific Mediator components block the activity only of certain activators (Piruat et al. 1997; Han et al. 1999; Myers et al. 1999; Park et al. 2000).

Our analysis of regulation of *HO* gene expression through cooperative promoter binding by Swi5 and Pho2 identified point mutations in Swi5 within a 24-amino-acid region that specifically affect *HO* activation (Bhoite and Stillman 1998). Although most of these Swi5 mutations reduce the ability of Swi5 to bind DNA cooperatively with Pho2, two Swi5 mutants, V494A and S497P, interact normally with Pho2, suggesting that this region of Swi5 has an additional function. We therefore searched for other proteins that interact with this domain of Swi5, and identified the Gal11 component of the Mediator complex. Here we show that Swi5 interacts directly with Gal11, and that Mediator is recruited to *HO* by Swi5. This binding of Mediator is an early event in the sequence of events at *HO*, and Mediator binding occurs without the concomitant binding of RNA polymerase II.

Results

Gal11 interacts with Swi5 in a one-hybrid assay

Swi5 and Pho2 bind to *HO* cooperatively (Brazas and Stillman 1993), and we identified mutations between amino acids 482 and 505 of Swi5 that affect activation of

a reporter gene (Bhoite and Stillman 1998). Most of these Swi5 mutations affect its ability to bind DNA cooperatively with Pho2. However, two mutations showed a significant defect in activation of *HO*, although this region lacks an activation domain and these mutant Swi5 proteins showed normal cooperative binding with Pho2. These results suggested that this region of Swi5 has an unidentified role in *HO* activation in addition to cooperative DNA-binding with Pho2. To analyze the role of this region, we performed a two-hybrid screen using a fusion of the LexA DNA-binding domain to Swi5 amino acids 398–513. Two plasmids were isolated from a library of yeast protein fusions to the Gal4 activation domain. One plasmid contained *PHO2*, as expected because this region of Swi5 interacts with Pho2. The other plasmid contained a truncated version of Gal11, containing amino acids 1–441 (full-length Gal11 is 1081 amino acids). The Gal11 protein is part of the RNA polymerase II Mediator complex and has been shown to contain a strong activation domain (Kim et al. 1994; Barberis et al. 1995).

Surprisingly, the *GAL11* clone we recovered did not express an in-frame fusion to the Gal4 activation domain, but contained the native *GAL11* promoter, driving expression of the truncated gene. To further analyze the interaction of Swi5 with Gal11, Gal11(1–441), as well as full-length Gal11(1–1081), was cloned into YEp plasmids, each with the native *GAL11* promoter. We have previously shown that amino acids 471–513 of Swi5 are necessary and sufficient to interact with Pho2 (Brazas et al. 1995). However, activation by this minimal LexA–Swi5(471–513) fusion is not stimulated by either YEp–*GAL11* construct. In contrast, a LexA fusion construct with a larger region of Swi5, LexA–Swi5(398–513), shows a modest but reproducible 1.7-fold stimulation by YEp–Gal11(1–441) and a 2.5-fold stimulation by YEp–Gal11(1–1011). These results suggest that Swi5 possesses a Gal11 interacting domain within amino acids 381–513, and that the 471–513 region of Swi5 is insufficient for this interaction.

Direct physical interactions between Swi5 and Mediator

Because Gal11 overexpression stimulates the transcriptional activity of LexA–Swi5(381–513), we asked whether Swi5 physically interacts with Mediator. Neely et al. (1999) have shown that purified GST–Swi5 can interact with purified Swi/Snf complex in a GST pull-down assay. We modified this assay, using whole cell lysates instead of purified complexes, which should be more stringent. We constructed strains where the *SWI2*, *SRB4*, and *GAL11* chromosomal loci were each tagged with Myc epitopes at their C termini (Fig. 1A, lanes 1–3). All three proteins resolved well on a gel, and a single strain was constructed that expressed all three tagged proteins (Fig. 1A, lane 4).

An extract from this strain was incubated with one of the two preparations of purified His6–Swi5, either full-length His6–Swi5(1–709) or His6–Swi5(539–681), which

Figure 1. Swi5 interacts with Gal11, Swi2, and Srb4 in vitro. (A) Cell extracts from DY6261 expressing Swi2-Myc, Gal11-Myc, and Srb4-Myc were incubated with *Escherichia coli* expressed His6-Swi5 derivatives, immunoprecipitated, and probed with anti-Myc antibody. Lane 1, DY6130 (Swi2-Myc) input; Lane 2, DY6145 (Gal11-Myc) input; Lane 3, DY6260 (Srb4-Myc) input; Lane 4, DY6261 (Swi2-Myc, Gal11-Myc, Srb4-Myc) input; Lane 5, His6-Swi5(1-709) immunoprecipitate; Lane 6, His6-Swi5(539-681) immunoprecipitate. (B) GST coprecipitations were performed using purified His6-Swi5(1-709) and the indicated GST fusion proteins and probed with anti-His6 antibody. Lane 1, input His6-Swi5 (20 %); Lane 2, GST-Gal11(1-441) coprecipitate; Lane 3, GST coprecipitate; and Lane 4, GST-Zap1 coprecipitate. (C) GST coprecipitations using wild-type (WT) or mutant versions of His6-Swi5. Lane 1, input His6-Swi5 (wild type); Lane 2, input His6-Swi5 (V494A); Lane 3, input His6-Swi5 (S497A); Lane 4, eluted His6-Swi5 (wild type); Lane 5, eluted His6-Swi5 (V494A); and Lane 6, eluted His6-Swi5 (S497A).

has the DNA-binding domain but lacks the Gal11 interaction region. After incubation with the yeast lysate, an anti-His6-Tag antibody was used to immunoprecipitate His6-Swi5, and anti-Myc antibody was used to detect Swi2-Myc, Srb4-Myc, and Gal11-Myc (Fig. 1A). His6-Swi5(1-709) efficiently brings down Swi2-Myc, Gal11-Myc, and Srb4-Myc from the yeast extract. In contrast, His6-Swi5(539-681), with just the DNA binding domain, fails to bind to these same proteins. These results confirm the previously described interaction between Swi5 and Swi/Snf, and also show that Swi5 interacts with Mediator.

We next asked whether the interaction of Swi5 with Gal11 was direct. A GST pull-down assay was performed with His6-Swi5(1-709) and GST-Gal11(1-441), both purified from *Escherichia coli*. After incubation, GST-Gal11(1-441) was isolated with glutathione-agarose and eluted with SDS, and the presence of His6-Swi5 in the

elution was detected by immunoblotting with anti-His6 antibody (Fig. 1B, lane 2). Comparing this signal to 20% of the input His6-Swi5 (lane 1), shows that binding of His6-Swi5 to GST-Gal11(1-441) is efficient. His6-Swi5 did not bind to two control proteins, GST or GST-Zap1 (a Zn finger transcription factor involved in zinc homeostasis; Bird et al. 2000), demonstrating specificity of the interaction. In a parallel experiment we found that His6-Pho2 did not interact with GST-Gal11(1-441) under the same binding conditions (data not shown).

We have identified single amino acid substitutions within Swi5, such as V494A and S497P, that reduce *HO* expression without affecting Swi5-Pho2 interaction (Bhoite and Stillman 1998). The V494A and S497P mutations also eliminate the interaction of His6-Swi5 with GST-Gal11(1-441) in a GST pull-down experiment (Fig. 1C), suggesting that these residues are critical for the interaction.

Effect of Mediator mutations on *HO* transcription

Genetic studies have shown that some Mediator subunits are required for general transcription in vivo and other Mediator mutations affect activation or repression at specific genes. We measured *HO* mRNA in isogenic strains where various Mediator genes, *GAL11*, *HRS1*, *MED2*, or *SIN4*, were deleted (Fig. 2A). A *gal11* mutation resulted in a fivefold drop in *HO* expression (Fig. 2A, lane 2). RNA Pol II holoenzyme preparations from *gal11* mutants are practically devoid of Hrs1, with the converse also true (Lee et al. 1999). Consistent with this finding, *HO* levels are reduced a modest but reproducible 2.5- to 3-fold in *hrs1* mutants. *med2* mutants show a similar reduction in *HO*. Combining the *gal11* mutation with either *hrs1* or *med2* did not show a further reduction in *HO* expression compared with the *gal11* single mutant. Thus, our results indicate a positive role for the Gal11 component of the Gal11-Rgr1 Mediator module in *HO* activation. *SIN4* also encodes a component of Mediator (Li et al. 1995). However, in contrast to the results with *gal11*, *hrs1*, and *med2* mutants, a *sin4* mutation does not reduce *HO* expression. Instead, a *sin4* mutation allows *HO* expression in the absence of certain activators, including Swi6, Gcn5, and Nhp6 (Yu et al. 2000).

Gal11 and Sin4 are both in Mediator, but mutations in these genes have quite different effects on *HO* expression. Intrigued by the different phenotypes of *gal11* and *sin4* mutations at *HO*, we asked if a *sin4* mutation could suppress defects in *HO* expression in a *gal11*, *med2*, or *hrs1* mutant strain. The results in Figure 2B show that a *sin4* mutation cannot suppress *gal11*, *hrs1*, or *med2* mutations.

The *SIN3* gene encodes a protein that interacts with the Rpd3 histone deacetylase, and a *sin3* mutation allows *HO* expression in the absence of certain activators. Isogenic *gal11 sin3* and *med2 sin3* double mutant strains were constructed, and *HO* mRNA measurements show that a *sin3* mutation does not suppress these Mediator mutations (Fig. 2C). Thus the change in the acetylation

Figure 2. Mutations in Mediator components affect *HO* expression. S1 nuclease protection assays with probes specific for *HO* and *CMD1* (internal control), expressed as a percentage of the wild type (WT) in lane 1. (A) Mediator mutations *gal11*, *hrs1*, and *med2* reduce *HO* expression. Strains DY150 (WT), DY5628 (*gal11*), DY6861 (*hrs1*), DY7004 (*gal11 hrs1*), DY5696 (*med2*), DY6182 (*gal11 med2*), and DY1702 (*sin4*) were used. (B) A *sin4* mutation does not suppress Mediator mutations. Strains DY150 (WT), DY1702 (*sin4*), DY5629 (*gal11*), DY5961 (*gal11 sin4*), DY6861 (*hrs1*), DY6943 (*hrs1 sin4*), DY5696 (*med2*), and DY6182 (*med2 sin4*) were used. (C) A *sin3* mutation does not suppress Mediator mutations. Strains DY150 (WT), DY984 (*sin3*), DY5629 (*gal11*), DY6256 (*gal11 sin3*), DY5696 (*med2*), and DY6184 (*med2 sin3*) were used.

state at *HO* caused by the *sin3* mutation (Krebs et al. 1999) is not sufficient to allow full *HO* expression in the absence of these Mediator components.

We have shown previously that *sin3* and *sin4* mutations differ in their ability to suppress different activators (Yu et al. 2000). These results, along with the current work on *gal11* mutants, are summarized in Table 1. The suppression analysis reveals several important features. First, *sin3* or *sin4* mutations are each able to suppress mutations in one of the DNA-binding transcription factors, Swi5 or Swi6, but not both. Second, a *gcn5* mutation eliminating the histone acetyltransferase in SAGA can be suppressed by either *sin3* or *sin4*. Finally, the Swi/Snf chromatin remodeling complex plays a critical role in *HO* activation, as both *sin3* and *sin4* fail to suppress the *swi2* defect. Interestingly, this feature is

Table 1. Suppression of *HO* activator defects by *sin* mutants

	<i>SIN+</i>	<i>sin3</i>	<i>sin4</i>
<i>SWI+</i>	+	+	+
<i>swi5</i> DNA-binding factor	–	+	–
<i>swi2</i> Swi/Snf complex	–	–	–
<i>gal11</i> Mediator complex	–	–	–
<i>gcn5</i> SAGA complex	–	+	+
<i>swi6</i> DNA-binding factor	–	–	+

Summary of *HO* expression in isogenic strains. Data are from Figure 2 and Yu et al. (2000).

also shared by a *gal11* mutation, because neither *sin3* nor *sin4* can relieve the *HO* transcription defect in a *gal11* mutant. We find this similar pattern of suppression of *gal11* and *swi2* mutants intriguing on several grounds. Although Swi6 is absolutely required for *HO* expression, a *sin4* mutation allows *HO* to be expressed in the absence of SBF (Swi4/Swi6). Moreover, this expression of *HO* is still dependent on Swi5 and requires Swi/Snf (Yu et al. 2000) as well as Mediator components such as Gal11 (Table 1). Our results suggest that Swi/Snf and Mediator are in the same genetic pathway, downstream of Swi5.

Gal11 and Srb4 binding to HO is Swi5 dependent but SBF independent

The physical interactions between Swi5 and both Mediator and SWI/SNF suggest that Swi5 would directly recruit Mediator to *HO*. To test this hypothesis, we analyzed the binding of two functionally distinct components of the Mediator complex, Gal11 and Srb4, to the *HO* promoter. *GAL11* is a non-essential gene, whereas *SRB4* is essential for viability. Expression of only some genes is affected in a *gal11* mutant, whereas a strain harboring a temperature-sensitive *srb4* mutation loses expression of most genes at the nonpermissive temperature (Thompson and Young 1995; Fukasawa et al. 2001). The Gal11 module of the yeast RNA Pol II holoenzyme is modeled as a binding target for specific activators (Park et al. 2000), whereas Srb4 is generally required and is proposed to modulate Pol II activity after activator stimulation (Lee et al. 1999; Park et al. 2000).

We performed ChIP assays to detect the association of Mediator with *HO* (Hecht et al. 1995; Cosma et al. 1999). Gal11 was tagged with Myc epitopes at the C terminus and this Gal11-Myc allele was introduced into wild-type, *swi5*, and *swi6* strains. Sheared chromatin was prepared from these cells, Gal11-Myc was immunoprecipitated, and the DNA present in the immunoprecipitated material was analyzed by PCR (Fig. 3B). Gal11-Myc efficiently binds to both the URS1 and URS2 regions of the *HO* promoter (lane 2), but this binding is eliminated in a *swi5* mutant (lane 3). Interestingly, in a *swi6* mutant, Gal11-Myc still binds to both URS1 and URS2, although the binding is slightly reduced (lane 4). Specificity of the Gal11-Myc binding to *HO* was shown by the failure to bind control fragments (the *YDL224c* promoter or the

Figure 3. Mediator binds to *HO* in vivo. (A) The positions of the URS1, URS2, and TATA regions of the *HO* promoter are shown relative to the ATG. The three promoter regions that PCR amplified in the chromatin immunoprecipitation (ChIP) are indicated. (B) Binding of Gal11-Myc to URS1 and URS2 by ChIP is shown in Lanes 1–4. Lanes 5–6 have threefold dilutions of input extract subjected to multiplex PCR. Strains DY150 (untagged control), DY6130 (Gal11-Myc), DY6197 (Gal11-Myc *swi5*), and DY6259 (Gal11-Myc *swi6*) were used. *YDL224c* and *TRA1* were negative controls. (C) Binding of Srb4-Myc to URS1 and URS2 was assessed as in part B. Strains DY150 (no tag), DY6260 (Srb4-Myc), DY6587 (Srb4-Myc *swi5*), and DY7001 (Srb4-Myc *swi6*) were used.

TRA1 ORF) and the requirement of the Myc Tag for the PCR signal (lane 1). A similar experiment was performed with Myc-tagged Srb4 strains (Fig. 3C), also showing efficient binding to URS1 and URS2 of *HO*, in a *SWI5*-dependent manner. In contrast to Gal11, however, binding of Srb4-Myc to URS2 was eliminated in a *swi6* mutant strain. The failure of both Gal11-Myc and Srb4-Myc to bind to *HO* in a *swi5* mutant suggests that Swi5 is directly required for recruitment of Mediator, because Gal11-Myc and Srb4-Myc protein levels are unaffected in *swi5* or *swi6* mutants (data not shown).

There are several important results from these ChIP experiments. First, Mediator associates with *HO* in vivo at both the URS1 region where Swi5 binds, and the URS2 region where SBF binds. Second, the dependence on Swi5 for recruitment of Mediator reveals a new role for Swi5 in *HO* activation, consistent with direct interactions observed in vitro. Lastly, both Srb4 and Gal11 bind *HO* URS1 in the absence of Swi6, suggesting that the recruitment of Mediator precedes the SBF binding.

Ordered recruitment of Gal11 and Srb4 to *HO*

Recently, Cosma et al. (1999) used ChIP to show the sequential binding of Swi5 and Swi/Snf to *HO*, and that Swi5 is required for Swi/Snf to bind. Given these observations, we examined the interdependence of Mediator and Swi/Snf binding at *HO*. ChIP experiments were performed on Gal11-Myc and Swi2-Myc tagged strains (Fig.

4A). Swi2-Myc binds both URS1 and URS2, consistent with previous observations. This Swi2-Myc binding is unaffected by a *gal11* mutation, indicating that recruitment of Mediator is not necessary for Swi/Snf binding to *HO*. In contrast, binding of Gal11-Myc to both URS1 and URS2 is eliminated in a *swi2* mutant. Our results indicate that, although Swi5 can recruit both Swi/Snf and Mediator to *HO*, stable binding of Mediator also requires Swi/Snf.

Gal11-Myc is absent from *HO* in a *swi2* mutant. It is possible that the *swi2* mutation affects expression or activity of Gal11, and thus indirectly affects Gal11-Myc binding to *HO*. Gal11-Myc binding to *CLN2* is only slightly affected by a *swi2* mutation (Fig. 4B), and we conclude that a *swi2* mutation does not globally abolish Gal11-Myc binding to promoters.

Park et al. (2000) recently showed that Gal11 is required for binding of Mediator to *GAL1*. They suggested that the Gal11 module receives signals from promoter-specific activators, which are transduced to the Srb4 module of Mediator that is associated with the C-terminal domain of Pol II. We considered such a possibility at *HO*, and asked if binding of Srb4-Myc was affected in a *gal11* mutant. The ChIP experiment in Figure 4C shows that the *gal11* mutation eliminates binding of Srb4-Myc to *HO*, and thus Gal11 is required for binding of Mediator to *HO*.

We also determined whether a *gal11* mutation affects SBF binding, a late event in the cascade of *HO* activation.

Figure 4. Swi/Snf is required for Mediator binding to *HO*. Binding of Swi2-Myc, Gal11-Myc, and Srb4-Myc proteins to URS1 and URS2 by chromatin immunoprecipitation (ChIP) is shown. The input lanes have threefold dilutions of input extract subjected to multiplex PCR. (A) Binding of Swi2-Myc and Gal11-Myc in mutants. Strains DY150 (no tag), DY6325 (Swi2-Myc), DY6128 (Swi2-Myc *gal11*), DY6130 (Gal11-Myc), and DY7065 (Gal11-Myc *swi2*) were used. PCR primers for *HO* URS1 and *HO* URS2 were used, along with negative controls *YDL224c* and *TRA1*. (B) Gal11-Myc binds to *CLN2* in a *swi2* mutant. Strains DY150 (no tag), DY6130 (Gal11-Myc), and DY7065 (Gal11-Myc *swi2*) were used. PCR primers for *CLN2*, *YDL233w*, and *SPO1* were used. (C) Srb4-Myc does not bind to *HO* in a *gal11* mutant. Strains DY150 (no tag), DY6260 (Srb4-Myc), and DY7215 (Srb4-Myc *gal11*) were used. PCR primers for *HO* URS1, *HO* URS2, *YDL224c*, and *TRA1* were used. (D) Swi4-Myc binds to *HO* in a *gal11* mutant. Strains DY150 (no tag), DY6241 (Swi4-Myc), and DY7236 (Swi4-Myc *gal11*) were used. PCR primers for *HO* URS1, *HO* URS2, *YDL224c*, and *SSB1* were used.

Our results show that binding of Swi4-Myc (in SBF) to the URS2 region of *HO* was unaffected in a *gal11* mutant (Fig. 4D). Mediator does not bind to *HO* in a *gal11* mutant (Fig. 4C), and thus we conclude that Mediator is not required for SBF binding.

Mediator binding coincides with the arrival of Swi5 at *HO*

Our results suggest a role for Swi5 in recruiting Mediator via specific interactions with Gal11. Mediator proteins are in a holoenzyme complex with RNA Pol II, and thus Swi5 recruitment of Mediator might coincide with recruitment of Pol II and transcription of *HO*. However, this is unlikely because Swi5 binds *HO* in late M/early G₁, whereas *HO* is not expressed until late G₁, at a time when most Swi5 in the nucleus has been degraded (Cosma et al. 1999).

To address this question, we examined the kinetics of Mediator binding to the *HO* promoter. Cells with an Srb4-Myc tag were synchronized by a *CDC20* arrest and release protocol. These cells have the *GAL1* promoter integrated in front of the *CDC20* cell-cycle regulatory gene, with arrest and release accomplished by withdrawing and returning galactose to cells. A high degree of synchrony through the cell cycle after the release was

shown by flow cytometry analysis (data not shown) and by analysis of cell cycle-regulated transcription of *EGT2*, *CLN2*, and *HO* by S1 protection assays (Fig. 5A). *EGT2* is activated by Swi5, and its expression peaked 25 min after release (Fig. 5C), consistent with previous observations (Kovacech et al. 1996). *CLN2* expression is controlled by SBF, and its expression peaked between 30 and 50 min (Fig. 5D), similar to the peak in *HO* expression (Fig. 5E). Timing of *HO* expression after a *CDC20* release is reproducible and consistent with previous reports (Cosma et al. 1999).

ChIP assays were performed on synchronized cultures of cells with the Srb4-Myc epitope tag. We first examined binding of Srb4-Myc to *EGT2* and *CLN2* (Fig. 5B). We find that Srb4-Myc associates with *EGT2* and *CLN2* at 15–20 and 30–50 min after the release, respectively, and thus Mediator binding parallels the mRNA levels (see quantitation in Fig. 5C,D). *PIR1* is activated by Swi5, and Srb4-Myc binding is similar to that of *EGT2*. (Mediator binding to *EGT2* slightly precedes the peak of mRNA accumulation; this could be explained if the *EGT2* mRNA is moderately unstable. The *CLN2* mRNA is unstable, and here there is a good correlation between Mediator binding and mRNA levels.)

We examined the kinetics of Srb4-Myc binding to three regions of the *HO* promoter, URS1, URS2, and

Figure 5. Srb4-Myc associates with HO in early G_1 . A log phase culture of DY7040 (*GAL-CDC20* Srb4-Myc) was arrested in metaphase by galactose depletion. After galactose additions to release from the arrest, samples were harvested at timed intervals for RNA analysis (A), chromatin immunoprecipitation (ChIP; B), and FACS analysis (data not shown). mRNA and ChIP quantitation is shown in panels C, D, and E. (A) *HO*, *EGT2*, and *CLN2* mRNA levels by S1 protection during the cell cycle. (B) Binding of Srb4-Myc to *EGT2*, *CLN2*, and *PIR1* promoters and URS1, URS2, and TATA regions of *HO* by ChIP during the cell cycle. *YDL224c*, *TRA1*, and *SPO1* were negative controls for ChIP. Lanes 9–11 have threefold dilutions of input extract subjected to multiplex PCR. (C) *EGT2* mRNA levels and Srb4-Myc binding to *EGT2* during the cell cycle. (D) *CLN2* mRNA levels and Srb4-Myc binding to *CLN2* during the cell cycle. (E) *HO* mRNA levels and Srb4-Myc binding to regions of *HO* during the cell cycle.

TATA (see map in Fig. 3A). Srb4-Myc is bound to the URS1 region of *HO* 15 min after metaphase release (Fig. 5B, lane 2; quantitation in Fig. 5E). Swi5 also binds to *HO* at this time (see following), and thus Swi5 and Mediator binding is coincident. In this experiment we also note that after maximal binding of Mediator to URS1 at 15 min, there is a decrease in the occupancy of Mediator bound at URS1. This is transient and followed closely by a second peak at 30 min, when Mediator now occupies both URS1 and URS2, preceding *HO* mRNA accumulation. This pattern of Mediator binding to URS1 and URS2 is distinct and reproducible in several experiments. It is important to note that at the 15-min time point there is essentially no binding of Mediator to URS2 or the TATA region of *HO*, whereas strong binding of Mediator to URS1 is seen.

We draw several important conclusions from this experiment. First, there is good correlation between the binding of Mediator to the URS1 region of *HO* and the arrival of transcription factor Swi5 at *EGT2*, *PIR1*, and *HO*. Second, the binding of Mediator to the *EGT2* and *CLN2* promoters is coincident with transcription at the appropriate times in the cell cycle, consistent with Mediator being associated with the RNA Pol II holoenzyme. The binding of Mediator at *HO* URS1 is clearly different, suggesting that the mechanism of transcription by the Pol II holoenzyme at *HO* may be distinct from other promoters.

Delayed arrival of Pol II to HO corresponds to HO transcription in late G₁

We have found that Mediator binds to *HO* in early G_1 , soon after Swi5 binds, but *HO* is not expressed until much later in the cell cycle after subsequent recruitment events. This observation leads to two possible scenarios. The first has Swi5 recruiting Mediator as part of the RNA Pol II holoenzyme, but the RNA polymerase II is kept in a transcriptionally inactive state until later. The second possibility has Swi5 recruiting Mediator to *HO*, but without RNA polymerase II, which only associates with the promoter subsequently. To distinguish between these possibilities, we examined the kinetics of Pol II binding to *HO* during the cell cycle. *RPB3* encodes a subunit of RNA Pol II, and we used a strain with an HA epitope-tagged *RPB3* gene (Schroeder et al. 2000). Cells with Rbp3-HA were synchronized by *CDC20* arrest and release, and samples were taken for RNA measurement (Fig. 6A) and ChIP analysis (Fig. 6B) at subsequent time points.

In the ChIP assays, we measured the association of Rbp3-HA to the URS1, URS2, and TATA regions of *HO*. For comparison, we also measured the association of Rbp3-HA to *PIR1*. *PIR1* is exclusively activated by Swi5 (Y.Yu, unpubl.; Doolin et al. 2001), and thus allows a direct comparison of Pol II binding at two Swi5-regulated genes. In an identical experiment, we also examined the kinetics of Swi5-Myc binding to *HO* and *PIR1* (Fig. 6C). At *HO*, Rbp3-HA binds transiently to the TATA region at 40–50 min after release, with kinetics very similar to

Figure 6. RNA polymerase II associates with the TATA region of *HO* in late G_1 . Log phase cultures of DY7114 (*GAL-CDC20* Rbp3-HA) and DY6693 (*GAL-CDC20* Swi5-Myc) were arrested in metaphase by galactose depletion. After galactose additions to release from the arrest, samples were harvested at timed intervals for RNA analysis (panel A), chromatin immunoprecipitation (ChIP; panels B and C), and FACS analysis (data not shown). mRNA and ChIP quantitation is shown in panels D and E. (A) *HO* and *PIR1* mRNA levels by S1 protection during the cell cycle (DY7114). (B) Binding of Rbp3-HA to *PIR1* and URS1, URS2, and TATA regions of *HO* by ChIP during the cell cycle (DY7114). *SPO1* was the negative control. Lanes 9–11 have threefold dilutions of input extract subjected to multiplex PCR. (C) Binding of Swi5-Myc to the *PIR1* promoter and the URS1 region of *HO* by ChIP during the cell cycle (DY6693). *TRA1* was the negative control. Lanes 9–11 have threefold dilutions of input extract subjected to multiplex PCR. (D) *HO* mRNA levels and Swi5-Myc and Rbp3-HA binding to the TATA region of *HO* during the cell cycle. (E) *PIR1* mRNA levels and Swi5-Myc and Rbp3-HA binding to *PIR1* during the cell cycle.

that of the mRNA accumulation (Fig. 6B, quantitation in Fig. 6E). Importantly, no binding of Rbp3-HA to the

URS1 or URS2 regions was seen at the times when Mediator is bound (Fig. 6B). At *PIR1*, Rbp3-HA binding and mRNA expression are both early in the cell cycle (Fig. 6D), at a time coincident with Swi5 binding to its target promoters (Fig. 6C). This suggests that Swi5 recruits both Mediator and RNA Pol II, possibly as a holoenzyme, to early G_1 promoters such as *PIR1*. In contrast, although Swi5 recruits Mediator to bind upstream regions of *HO*, this binding of Mediator occurs in the absence of RNA Pol II. Thus, unlike at some genes, Mediator recruitment to *HO* is not sufficient to elicit transcription, presumably because the more complex *HO* promoter is not permissive for Pol II recruitment until subsequent events transpire.

Discussion

The transcriptional regulation of *HO* is highly complex, involving the sequential recruitment of the Swi/Snf remodeling factor, the SAGA histone acetyltransferase, and finally the SBF DNA-binding factor that is believed to proximally activate transcription (Cosma et al. 1999). Here, we have identified a new factor that is recruited to *HO*, the Mediator complex. Swi5 interacts directly with the Gal11 protein, a subunit of Mediator, and a *gal11* mutation reduces *HO* expression. ChIP experiments show that Mediator binding is an early event in the ordered series of events at *HO*, binding at the same time as Swi/Snf. Importantly, although many studies have shown an association of Mediator with RNA Pol II, Mediator behaves in a unique way at *HO*, in that Swi5 recruitment of Mediator is not accompanied by RNA Pol II binding. RNA Pol II binds to *HO* only transiently in late G_1 when *HO* is actively transcribed. ChIP experiments show that, although Swi5 is required for recruitment of Mediator to *HO*, it can only do so if Swi/Snf is also recruited. Consistent with this, genetic suppression experiments suggest that Mediator and Swi/Snf may function in the same genetic pathway of *HO* promoter activation, with both factors recruited early by Swi5.

Distinct recruitment of Mediator and Pol II to *HO*

We used synchronized cells to examine binding of Mediator and Pol II to *PIR1*, *CLN2*, and *HO*. *PIR1* is exclusively activated by Swi5 in early G_1 , and *CLN2* by SBF in late G_1 . At each of these more typical promoters, binding of Mediator and Pol II coincides with binding of the promoter-specific transcription factor (Fig. 7A,B). Regulation of *HO* is complex, with both Swi5 and SBF required for activation, and our experiments show a complex pattern of Mediator binding to *HO* (Fig. 7C). At the URS1 region (~1500), Swi5 recruits both Swi/Snf and Mediator. The data suggest that both Swi5 and Swi/Snf are required for Mediator binding to URS1. Subsequently, after Swi5 is degraded, Swi/Snf binds to the proximal URS2 (~200 to ~800) region and SAGA also binds.

Mediator binds to URS2 at a time coincident with SBF

Swi5 recruits Mediator but not the holoenzyme

Figure 7. Swi5 and RNA polymerase II binding at *PIR1*, *CLN2*, and *HO*. (A) At *PIR1*, Swi5, Mediator, and RNA polymerase II all bind at the same time, which coincides with transcription in early G₁. (B) At *CLN2*, SBF, Mediator, and RNA polymerase II all bind at the same time, which coincides with transcription in late G₁. (C) At *HO*, Swi5 binds first, and recruits Swi/Snf and Mediator to URS1 in early G₁. Swi/Snf binds to URS2, and recruits SAGA. SBF and Mediator bind to URS2 at approximately the same time in late G₁, with SAGA required for SBF binding and SBF required for Mediator binding at URS2. Finally, Mediator and RNA polymerase II bind to the TATA region at the time of transcription. The model integrates data from this paper and that of Cosma et al. (1999).

binding to URS2. After this work was submitted for publication, a paper by Cosma et al. (2001) appeared showing that Mediator, TFIIB, and TFIIF bind to the TATA region of *HO* in the absence of Pol II, and Mediator binding to TATA requires SBF. At URS2, we found that a *swi6* mutation reduces binding of Gal11-Myc and eliminates Srb4-Myc binding, (Fig. 3), consistent with the idea that SBF helps recruit Mediator to the URS2 and TATA regions. The *swi6* mutation also reduces Gal11-Myc and Srb4-Myc binding to URS1. To explain this result with asynchronous cells, we note that the kinetic experiment shows two waves of Mediator binding to URS1 (Fig. 5). Thus, Swi5 may initially recruit Mediator to URS1, but later binding may depend on SBF bound at the downstream promoter element.

Whereas SBF is required for Srb4-Myc binding to URS2, Swi/Snf binds to URS2 in the absence of SBF (Cosma et al. 1999), suggesting an important difference between Swi/Snf and Mediator. Finally, at *PIR1* and *CLN2*, transcription factor binding results in binding of

both Mediator and Pol II, possibly as a holoenzyme. An interesting question arising from our studies is why Pol II fails to associate with *HO* in early G₁ when Mediator is first recruited.

Functional interactions between Swi5 and Mediator

Genetic and biochemical experiments support the interaction between Swi5 and Mediator. First, activation in vivo by a LexA-Swi5(389–513) fusion is stimulated by Gal11 overexpression. In vitro experiments show that Swi5 interacts with Mediator, and that Swi5 interacts directly with Gal11. ChIP experiments show that Swi5 recruits Mediator to *HO* and that recruitment of Srb4-Myc is lost in a *gal11* mutant, consistent with the direct interaction between Swi5 and Gal11. Missense mutations in Swi5 at residues 494 and 497 eliminate in vitro interactions with Gal11, in agreement with a crucial role for Swi5 region 398–513 in Gal11 binding. *HO* expression is comparably reduced in *gal11* mutants and in strains with Swi5(V494A) or Swi5(S497P) mutations. The residual activity in a *gal11* mutant suggests Swi5 may make additional contacts with other Mediator subunits in the absence of Gal11, or that Swi5 activation is partially Mediator independent.

Activators may require specific Mediator components for transcriptional activation in vivo, and Gcn4 and Gal4 interact with the Pol II holoenzyme in a manner that absolutely requires the Gal11 module (Myers et al. 1999; Park et al. 2000). Our finding that *E. coli* purified Swi5 associates with both the Mediator and Swi/Snf complexes parallels results with the Gcn4 activator. Natarajan et al. (1999) showed that Gcn4 can make independent interactions with Swi/Snf, SAGA, and Mediator. The requirement of Gal11 for full *HO* expression, along with the Swi5-dependent binding of Gal11 and Srb4 to the URS1 region, provides compelling evidence of a role for Swi5 in recruiting Mediator to *HO*.

Swi/Snf and Mediator at *HO*

It is interesting that the 389–513 region of Swi5 that interacts with Gal11 does not have a “classic” activation domain, in that the LexA-Swi5(389–513) fusion does not activate transcription unless Gal11 is overexpressed. Previous work has shown that the activation domains of Gcn4 and VP16 interact with the Pol II holoenzyme (Hengartner et al. 1995; Drysdale et al. 1998; Park et al. 2000). Several laboratories have recently shown that activation domains of transcription factors interact with the Swi/Snf complex (Natarajan et al. 1999; Neely et al. 1999; Yudkovsky et al. 1999). The activation domain of Swi5 is near the N terminus (D.J. Stillman, unpubl.), and thus it may be this region of Swi5 that recruits Swi/Snf while the 389–513 region recruits Mediator. Swi5 thus recruits both Swi/Snf and Mediator, and, although they function in the same genetic pathway for *HO* activation, they may act synergistically. It has been shown that physically tethering a Mediator component to a pro-

moter by fusion to a DNA-binding protein results in strong activation (Barberis et al. 1995; Farrell et al. 1996), raising the question as to why LexA-Swi5(389–513) is a weak activator. We suggest that Swi5(389–513) interacts weakly with Mediator, but that Swi5 recruitment of Swi/Snf may stimulate the Swi5 – Mediator interaction. This model is consistent with our observation that both Swi5 and Swi/Snf are required for Mediator recruitment to *HO*.

Our genetic analysis of suppression of *HO* activator mutations suggests that Swi/Snf and Mediator have related functions in *HO* activation (Table 1). Mutations in several genes, including *sin3*, *rp3*, and *sin4*, are known to suppress defects in *HO* activation (Yu et al. 2000). Unlike any of the other activator mutations, *gal11* and *swi2* mutants share the common feature of not being suppressed by either *sin3* or *sin4* mutations. Sin3 and Rpd3 are components of a histone deacetylase complex (Kadosh and Struhl 1997; Kasten et al. 1997), and mutations affect acetylation of the *HO* promoter (Krebs et al. 1999). Sin4 was originally identified as a component of the Gal11-Rgr1-Hrs1 module of Mediator (Li et al. 1995). More recently, Sin4 been shown to also be part of the SAGA complex (P. Grant and J. Workman, pers. comm.), and it may be as part of SAGA that Sin4 negatively regulates *HO*. Further work is needed to determine how a *sin4* mutation, or mutations affecting the Sin3/Rpd3 histone deacetylase, allow *HO* expression in the absence of certain activators.

What is the role of Mediator in *HO* activation?

Genetic analysis indicates that Mediator is involved in both transcriptional activation and repression, depending on the promoter (Myers and Kornberg 2000). Gal11 and Sin4 are both in Mediator, and a mutation in either *gal11* or *sin4* results in many of the same phenotypes, including reduced expression of some genes such as *CTS1* and mating-type genes, increased expression of other genes such as *GAL1*, and expression of promoters lacking UAS elements (Fassler and Winston 1989; Jiang and Stillman 1992; Chen et al. 1993; Jiang and Stillman 1995; Sakurai and Fukasawa 1997). Additionally, chloroquine gel assays show both *gal11* and *sin4* mutations affect chromatin structure of circular DNA molecules in vivo (Jiang and Stillman 1992; Nishizawa et al. 1994). It has been suggested that the effects on transcription are caused by changes in chromatin structure (Macatee et al. 1997).

Although *gal11* and *sin4* mutants share some similarities by virtue of their association with common subunits within Mediator, several experiments show that they are not functionally identical. One clear example is the opposite effects of *gal11* and *sin4* mutations on expression of *HO*. Other differences between *sin4* and *gal11* mutants include opposite effects on transcriptional regulation of *SUC2* (Vallier and Carlson 1991; Song et al. 1996), on Ty1 expression (Fassler and Winston 1989; Jiang and Stillman 1995), and on silencing of *HMR* and telomere-

linked genes (Jiang and Stillman 1995; Sussel et al. 1995). Although a DNA-binding domain fusion to Gal11 results in a strong activator, a similar fusion with Sin4 is a weak activator (Jiang and Stillman 1992; Barberis et al. 1995). A *sin4* mutation will suppress the cold sensitivity of *bur6* mutations, but a *gal11* mutation will not (Kim et al. 2000). There are also differences in growth on specific media (Chang et al. 1999), as well as differences in synthetic lethal interactions. For example, combining *gal11* with either *swi2*, *ccr4*, or *tfa1* mutations results in synthetic lethality, whereas the equivalent double mutants with *sin4* are viable (Roberts and Winston 1997; Chang et al. 1999; Sakurai and Fukasawa 2000).

Complex promoters

How does Mediator promote activation at the *HO* promoter? Purified Mediator binds to the CTD or RNA polymerase II (Myers et al. 1998), and thus the presence of Mediator at a promoter could directly influence Pol II recruitment activity or Pol II initiation by modulating CTD phosphorylation (Kim et al. 1994). Alternatively, it has been recently shown that Mediator has acetyltransferase activity (Lorch et al. 2000), and this enzymatic activity could stimulate *HO* expression. Although it is not clear exactly how Mediator acts to promote *HO* expression, the fact that Mediator is first recruited to the far upstream promoter region, long before the time of *HO* expression, is novel. We suggest that Mediator binding at URS1 brings Mediator to the *HO* promoter so that it is positioned to be quickly recruited to the proximal URS2 region by the SBF factor in late G₁, and it is from the URS2 region of the promoter that Mediator stimulates transcription by an unknown mechanism, possibly by recruiting Pol II. The recent paper by Cosma et al. (2001) showed that cell-cycle progression past START is required for RNA polymerase to bind at *HO*.

At *HO*, Swi5 recruits Swi/Snf, and this is followed by binding of SAGA and Gcn5 (Cosma et al. 1999). However, the temporal sequence of events is quite different at the IFN- β promoter (Agalioti et al. 2000). Upstream binding factors first recruit Gcn5 to the IFN- β promoter, followed by CBP and the RNA Pol II holoenzyme. Acetylation of the IFN- β chromatin template allows recruitment of the Swi/Snf complex, which allows binding of TFIID and transcriptional activation. The early recruitment of the RNA Pol II holoenzyme to IFN- β is similar to the early recruitment of Mediator to *HO*. However, at IFN- β , recruitment of RNA Pol II holoenzyme precedes Swi/Snf binding, whereas the opposite is true at *HO*, with binding of Swi/Snf being a prerequisite for the binding of Mediator.

Clearly, further work is needed to dissect the roles of Swi/Snf, Mediator, and SAGA in the activation of *HO*. Nevertheless, it is evident that *HO* uses multiple regulators at distinct stages of the transcription process. Such a multifaceted mechanism allows for fine tuning of the transcriptional activation and limits *HO* expression to a brief time within the cell cycle.

Materials and methods

Strains and plasmids

All strains listed in Table 2 are isogenic in the W303 background (Thomas and Rothstein 1989), except the two-hybrid strain DY5736 constructed from strain L40 (Vojtek et al. 1993). Plasmids are listed in Table 3. All W303 strains have *ade2*, *can1*, *his3*, *leu2*, *trp1*, and *ura3* markers; some are also *lys2*. Standard genetic methods were used for strain construction. W303 strains with disruptions in *swi5*, *swi2*, *swi6*, *sin3*, and *sin4* have been described (Yu et al. 2000), and the *med2* mutant was provided by L. Myers (Myers et al. 1998). Plasmids pJF773 and pDIS, provided by J. Fassler (University of Iowa, Iowa City) and A. Aguilera (Universidad de Seville, Spain), respectively, were used to disrupt *GAL11* and *HRS1*. Marker swap plasmids pTU10 (Cross 1997), M3926, and M3927 were used to change markers. Strains with *SWI4*-Myc (Cosma et al. 1999) and *RPB3*-HA (Schroeder et al. 2000) epitope tags were provided by K. Nasmyth and D. Bentley, respectively. Myc epitope tags were added at the chromosomal *GAL11*, *SWI2*, *SWI5*, and *SRB4* loci using PCR fragments prepared using plasmids pFA6a:13Myc:His3MX6 or pYM6, as described (Longtine et al. 1998; Knop et al. 1999). Plasmid M4154 was used to integrate a *GAL1* promoter before the *CDC20* gene.

Media and growth conditions

For most experiments, cells were grown in YEP medium containing 2% glucose at 30°C (Sherman 1991). Drop-out synthetic

Table 2. List of strains

DY150 MATa
DY984 MATa <i>sin3::ADE2</i>
DY1704 MATa <i>sin4::URA3</i>
DY5628 MATa <i>gal11::LEU2</i>
DY5629 MATa <i>gal11::LEU2</i>
DY5696 MATa <i>med2::TRP1</i>
DY5736 MATa <i>LYS2::lexA-HIS3 ura3::KanMX::lexA-lacZ</i>
DY5961 MATa <i>gal11::LEU2 sin4::TRP1</i>
DY6128 MATa <i>SWI2-Myc::KanMX gal11::LEU2</i>
DY6130 MATa <i>GAL11-Lyc::HIS3MX</i>
DY6145 MATa <i>SWI2-Myc::KanMX gal11::LEU2</i>
DY6182 MATa <i>med2::TR1 gal11::LEU2</i>
DY6184 MATa <i>med2::TRP1 sin3::ADE2</i>
DY6197 MATa <i>GAL11-Myc::HIS3MX swi5::LEU2</i>
DY6241 MATa <i>SWI4-Myc::TRP1</i>
DY6256 MATa <i>gal11::LEU2 sin3::ADE2</i>
DY6259 MATa <i>GAL11-Myc::HIS3MX swi6::TRP1</i>
DY6260 MATa <i>SRB4-Myc::TRP1(K1)</i>
DY6261 MATa <i>SWI2-Myc::KanMX GAL11-Myc::HIS3MX SRB4-Myc::TRP1(K1)</i>
DY6325 MATa <i>SWI2-Myc::KanMX</i>
DY6587 MATa <i>SRB4-Myc::TRP1(K1) swi5::hisG-URA3-hisG</i>
DY6693 MATa <i>SWI5-Myc::KanMX GALp::CDC20::ADE2 ace2::HIS3 ash1::TRP1</i>
DY6861 MATa <i>hrs1::LEU2</i>
DY6943 MATa <i>hrs1::LEU2 sin4::TRP1</i>
DY7001 MATa <i>SRB4-Myc::TRP1(K1) swi6::ADE2</i>
DY7004 MATa <i>gal11::KanMX hrs1::LEU2</i>
DY7040 MATa <i>SRB4-Myc::TRP1(K1) GALp::CDC20::ADE2</i>
DY7065 MATa <i>GAL11-Myc::HIS3MX swi2::ADE2</i>
DY7114 MATa <i>RPB3::HA::KanMX GALp::CDC20::ADE2</i>
DY7215 MATa <i>SRB4-Myc::TRP1(K1) gal11::LEU2</i>
DY7236 MATa <i>SWI4-Myc::TRP1 gal11::LEU2</i>

Table 3. List of plasmids

pJF773	<i>gal11::LEU2</i> disruptor
pDIS1	<i>hrs1::LEU2</i> disruptor
pTU10	<i>trp1::URA3</i> converter
M3926	<i>leu2::KanMX3</i> converter
M3927	<i>ura3::KanMX3</i> converter
pFA6a:13Myc:His3MX6	Myc epitope tag vector
pYM6	Myc epitope tag vector
M4154	<i>ADE2::GALp::CDC20</i> integrating plasmid
M3950	LexA bait vector (<i>URA3</i>)
M3956	LexA-Swi5(398-513) (<i>URA3</i>)
pBTM116	LexA bait vector (<i>TRP1</i>)
M3951	LexA-Swi5(398-513) (<i>TRP1</i>)
M3810	LexA-Swi5(471-513) (<i>TRP1</i>)
YEplac181	<i>LEU2</i> YEplac vector
M4047	YEplac-Gal11
M4054	YEplac-Gal11(1-441)
M3113	His6-Swi5(WT)
M2035	His6-Swi5(539-681)
M3640	His6-Swi5(V494A)
M3641	His6-Swi5(S497P)
pGEX-4T-2	GST vector
M4086	GST-Gal11(1-441)

complete media were used where appropriate to select for plasmids. For the cell-cycle experiments, strains with the *GAL1::CDC20* allele were first grown at 25°C in YEP medium containing 2% galactose and 2% raffinose to an OD₆₀₀ of 0.4, filtered rapidly, and then arrested in YEP medium containing 2% raffinose for 4 h. Cells were released from the arrest by addition of galactose to a concentration of 2%. At timed intervals samples were taken for flow cytometry, RNA analysis, and ChIP.

In vivo analysis

DY5736 was transformed with plasmid LexA-Swi5(398-513) and a library of yeast genomic DNA fused to the Gal4 activation domain (James et al. 1996). After selection on medium lacking histidine and screening for lacZ activation, sequencing of the inserts identified *PHO2* and *GAL11*. *TRP1* plasmids (pBTM116, M3951, and M3810) and *LEU2* plasmids (YEplac181, M4047, and M4054) were introduced into DY5736 to examine the ability of YEplac-Gal11 constructs to promote activation by LexA-Swi5 constructs. RNA levels were quantitated by S1 nuclease protection assays as described (Bhoite and Stillman 1998).

ChIP was performed as described (Tanaka et al. 1997). Quantitation was performed with ImageQuant software (BioRad). For each time point, the ratio of the ChIP signal (i.e., *EGT2*) to the control gene (i.e., *YDL224c*) was determined, normalized to the equivalent ratio (i.e., *EGT2/YDL224c*) for the equivalent input sample, and adjusted to a percentage of maximal observed binding. Sequence of oligos used are available on request.

In vitro interactions

His6-Swi5 proteins from plasmids M3113 and M2035 were purified after expression in *E. coli* and incubated with yeast whole cell extracts. After immunoprecipitation with anti-His antibody (Clontech), coprecipitating Myc tagged proteins were detected by immunoblotting with anti-Myc epitope antibody. GST coprecipitations were performed as described (Ausubel et

al. 1987), using purified His6-Swi5 proteins (M3113, M3640, and M3641) and GST-Gal11(1–441) (M4086), GST (pGEX-4T-2), or GST-Zap1(538–880) (a gift from M. Evans-Galea, University of Utah, Salt Lake City).

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