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Genetic Interactions Between Mediator and the Late G_1-Specific Transcription Factor Swi6 in *Saccharomyces cerevisiae*

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ABSTRACT

Swi6 associates with Swi4 to activate *HO* and many other late G_1-specific transcripts in budding yeast. Genetic screens for suppressors of *SWI6* mutants have been carried out. A total of 112 of these mutants have been identified and most fall into seven complementation groups. Six of these genes have been cloned and identified and they all encode subunits of the mediator complex. These mutants restore transcription to the *HO-lacZ* reporter in the absence of Swi6 and have variable effects on other Swi6 target genes. Deletions of other nonessential mediator components have been tested directly for suppression of, or genetic interaction with, *swi6*. Mutations in half of the known subunits of mediator show suppression and/or growth defects in combination with *swi6*. These phenotypes are highly variable and do not correlate with a specific module of the mediator. Mutations in tail module components *sin4* and *pgd1* showed both growth defects and suppression when combined with *swi6*, but a third tail component, *gal11*, showed neither. A truncated form of the essential Srb7 mediator subunit also suppresses *swi6* mutations and shows a defect in recruitment of the tail module components Sin4, Pgd1, and Gal11 to the mediator complex.

APPROXIMATELY 20% of yeast genes are transcribed during the cell cycle such that their mRNAs are present only during a specific phase of the mitotic cycle (Breeden 2003). The largest wave of transcription occurs in late G_1, as cells prepare to replicate their DNA and begin the cell division process. More than 300 genes are transcriptionally induced in late G_1 (Iyer *et al.* 2001; Simon *et al.* 2001) and two promoter elements (SCBs and MCBs) responsible for this regulation have been identified. The transcriptional activators of these late G_1-specific elements share a common subunit, Swi6. Swi6 does not bind DNA directly; rather, it associates with either Swi4 or Mbp1, which confer the ability to bind SCBs or MCBs, respectively. Swi4 and Mbp1 are the two founding members of a family of related DNA-binding proteins that mediate switches between mitotic, meiotic, and pseudohyphal growth (Rua *et al.* 2001; Wittenberg and Flick 2003). As the primary mitotic member of this family, Swi4 is rate limiting for the G_1-to-S transition (McInerny *et al.* 1997) and two of the critical targets of the Swi4/Swi6 complex are the late G_1 cyclins *CLN1* and *CLN2* (Ogas *et al.* 1991).

Swi4 and Swi6 were identified in screens for genes required for HO expression (Haber and Garvik 1977; Stern *et al.* 1984; Breeden and Nasmyth 1987). *HO* encodes the endonuclease involved in mating-type switching. HO transcription is restricted to late G_1 and tightly dependent upon Swi4 and Swi6 activity. Early studies showed that cells lacking Swi6 produced an intermediate constitutive level of transcripts for other Swi6 target genes (Dirick *et al.* 1992; Lowndes *et al.* 1992), suggesting that Swi6 performs a regulatory function that is both positive and negative within the cell cycle. This hypothesis is supported by the recent discovery of Whi5, which associates with Swi4/Swi6 complexes during early G_1 and dissociates when the complex is activated (Jorgensen *et al.* 2002; Costanzo *et al.* 2004; de Bruin *et al.* 2004). Activation of Swi4/Swi6 complexes requires the Cln3 cyclin and the Cdc28 cyclin-dependent kinase, which phosphorylates Whi5 and promotes its dissociation. Release of Whi5 is temporally correlated with activation of Swi4/Swi6 complexes; however, Whi5 is not the only negative regulator of Swi4/Swi6 target promoters because these transcripts retain their late G_1-specific regulation in a *whi5* mutant. Clearly, there are other negative regulatory components that have not yet been identified.

The *HO* promoter, because of its tight dependence upon Swi4 and Swi6 for activation, continues to be an important model promoter for studying the temporal order of events that are required to activate late G_1-specific transcription. The order of events that lead up to the binding and activation of *HO* by Swi4/Swi6 complexes involves several other *SWI* genes (*SWI1*, *-2*, *-3*, and *5*), which were identified first for their role in mating-type switching (Haber and Garvik 1977;

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Stern *et al.* 1984) and later as activators of *HO* transcription (Breeden and Nasmyth 1987). During M phase, Swi5 binds to a site ~1 kb upstream from the translational start site for *HO* (Stillman *et al.* 1989). Swi5 recruits the Swi/Snf chromatin-remodeling complex, which in turn enables mediator and the SAGA histone acetyltransferase to bind (Cosma *et al.* 1999; Bhoite *et al.* 2001). These large complexes modify and remodel nucleosomal structure within the 1-kb-long promoter and allow Swi4/Swi6 (or SBF) complexed with the Whi5 inhibitor to bind to downstream sites called SCBs. Once bound to SCB elements, Swi4/Swi6/Whi5 recruits mediator, but this takes place well before the complex is activated by Cln3/Cdk. Whi5 dissociates and transcription ensues (Costanzo *et al.* 2004; de Bruin *et al.* 2004), with the further recruitment of PolII, TFIIB, and TFIIH (Cosma *et al.* 2001).

The complex series of events that occur at the *HO* promoter differs from the order of events at other promoters, including other promoters whose activation is initiated by Swi5 binding (Bhoite *et al.* 2001). The most striking difference seen at the *HO* promoter, and at several other Swi4/Swi6 target promoters, is the recruitment of mediator well before transcriptional activation. This was one of the earliest examples that indicated that there is not always a direct correlation between mediator recruitment and initiation of transcription. Moreover, it is not necessarily the gene-specific activator that dictates the timing of mediator recruitment, since this can differ in the context of different promoters.

The mediator is composed of >20 proteins that transduce signals from gene-specific activators to the general transcription machinery (Lewis and Reinberg 2003). This process requires that mediator perform some stereotyped functions to direct the initiation or repression of transcription and some more individual functions tailored to its interaction with gene-specific regulators. Parsing out these functions to specific proteins within each module of the mediator is an ongoing process and few generalities have emerged. Three core modules form the head, middle, and tail of the mediator complex. A fourth, less tightly associated module composed of Srb8, -9, -10, and -11 has also been isolated as a separate complex with kinase activity (Liao *et al.* 1995; Borggrefe *et al.* 2002). The head module includes eight tightly associated proteins (Koh *et al.* 1998) and is thought to interact with the C-terminal domain (CTD) of RNA polymerase II (Lee and Kim 1998) and with two of the general transcription factors (TBP, TFIIB). The middle module with its eight subunits interacts with the CTD and TFIIE and the Srb8–11 module of the mediator (Kang *et al.* 2001). Genetics suggests that the Srb8–11 kinase module represses activator-dependent transcription (Carlson 1997). The tail module, composed of five proteins, binds to several activators *in vitro* and mutants in these subunits that are defective in activation and repression have been identified, leading to the view that this module is involved in the recognition of gene-specific regulators (Lewis and Reinberg 2003). However, *in vitro* studies suggest that this module may also be involved in activator-independent functions of the polymerase (Reeves and Hahn 2003).

Mutations in mediator components have been collected over the last two decades as suppressors of transcriptional activators (Carlson *et al.* 1984). This article identifies similar genetic interactions between the late G_1-specific transcription factor Swi6 and components of the mediator complex. Despite the absolute requirement for Swi6 for *HO* transcription, mutations in many different components of the mediator complex that activate the *HO* promoter in the absence of Swi6 have been identified. A total of 112 such mutations have been isolated and characterized in this study. These include temperature-sensitive (ts) alleles of *SIN4* and *NUT1* and a truncated allele of the essential gene *SRB7*. In addition, synthetic interactions between *swi6* and other nonessential components of the mediator have been investigated.

MATERIALS AND METHODS

Strains and growth conditions: The strains used in this study are listed in Table 1. Standard genetic methods were used for strain construction and tetrad analysis (Sherman *et al.* 1994). Cells were grown at 30°, unless otherwise specified, in either YEPD medium or synthetic complete medium supplemented with amino acids as appropriate (Sherman *et al.* 1994). In all cases, the lithium acetate protocol was used for DNA transformation (Ito *et al.* 1983).

Mutant isolation: The screens for suppressors of *SWI6* (*ssx*) mutations were carried out using BY174, BY142, BY296, and BY603. These strains carry one of three different *swi6* alleles and the *HO* promoter driving *lacZ* expression (*HO-lacZ*) integrated at the *HO* locus. In *SWI6* cells, β-galactosidase activity expressed from this reporter construct can be observed as blue colonies using the X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) filter assay as described previously (Breeden and Nasmyth 1987). *swi6* colonies remain white because Swi6 is absolutely required for *HO* transcription (Breeden and Nasmyth 1987). Suppressors of the loss of Swi6 activity that could restore *lacZ* expression in these three different *swi6* mutant backgrounds were obtained. Mutagenesis with ethyl methanesulfonate was carried out as previously described (Lycan *et al.* 1994). Survival rates were between 25 and 50%. Mutagenized cells were plated on YEPD plates and allowed to form colonies at either 30° (BY174, BY142, BY296) or 25° (BY603). Colonies grown at 25° were shifted to 37° for 4 hr before screening. Ten temperature-sensitive suppressors that expressed β-galactosidase activity only at 37° were identified.

Mutant identification: Complementation analysis in this screen is complicated by the fact that *HO* is repressed in diploid cells. To circumvent this problem, suppressors were isolated in a *MAT* deletion strain, which mates as an **a** strain, but does not produce the Mat a1 protein required for diploid-specific repression of *HO* transcription (Strathern *et al.* 1981). Suppressors generated in this strain were crossed to suppressors generated in an α-strain. Complementation between two *ssx* mutants was indicated by the inability to express

TABLE 1

***S. cerevisiae* strains used in this study**

Strain	Genotype	Source
BY131	*MATα HO-lacZ46 ura3 his3 leu2-3, -112 trp1-1 can1-100 ade2 met*	This study
BY134	*MATα HO-lacZ46 ura3 leu2 trp1-1 can1-100 ade2-1 met*	This study
BY142	*MATα HO-lacZ46 swi6-399 leu2 trp1-1 can1-100 ade2*	This study
BY150	*MATα HO-lacZ46 swi6-399 srb9-9 leu2 trp1-1 can1-100 ade2*	This study
BY155	*MATα HO-lacZ46 swi6-399 nut1-14 leu2 trp1-1 can1-100 ade2*	This study
BY165	*MATα HO-lacZ46 swi6-399 ssx6-24 leu2 trp1-1 can1-100 ade2*	This study
BY168	*MATα HO-lacZ46 swi6-399 srb10-27 leu2 trp1-1 can1-100 ade2*	This study
BY174	*mat::LEU2 HO-lacZ46 swi6-399 ura3 trp1-1 can1-100*	This study
BY175	*mat::LEU2 HO-lacZ46 swi6-399 rox3-33 ura3 trp1-1 can1-100*	This study
BY178	*mat::LEU2 HO-lacZ46 swi6-399 srb7-1 ura3 trp1-1 can1-100*	This study
BY180	*mat::LEU2 HO-lacZ46 swi6-399 ssx6-38 ura3 trp1-1 can1-100*	This study
BY183	*mat::LEU2 HO-lacZ46 swi6-399 sb10-41 ura3 trp1-1 can1-100*	This study
BY186	*mat::LEU2 HO-lacZ46 swi6-399 nut1-44 ura3 trp1-1 can1-100*	This study
BY296	*mat::LEU2 HO-lacZ46 swi6-254 his3 leu2-3, -112 trp1-1 can1-100 ade2 met*	This study
BY338	*MATα HO-lacZ46 swi6-399 srb9-9 ura3 his3 leu2 trp1-1 can1-100 ade2*	This study
BY546	*MAT***a** *HO-lacZ46 swi6::LEU2-175 ura3 his3 leu2 trp1-1 can1-100 ade2 met*	This study
BY547	*MATα HO-lacZ46 swi6::LEU2-175 ura3 his3 leu2 trp1-1 can1-100 ade2 met*	This study
BY602	*MATα HO-lacZ46 ura3 his3 leu2-1 leu2-112 trp1-1 can1-100 ade2 met*	This study
BY603	*MATα HO-lacZ46 swi6::TRP1-197 ura3 his3 leu2-1 leu2-112 trp1-1 can1-100 ade2 met*	This study
BY1078	*MATα HO-lacZ46 swi6::TRP1-197 rox3-102 ura3 his3 leu2-3, -112trp1-1 can1-100 ade2 met*	This study
BY1087	*MATα HO-lacZ46 swi6::TRP1-197 sin4-111 ura3 his3 leu2-3, -112 trp1-1 can1-100 ade2 met*	This study
BY1088	*MATα HO-lacZ46 swi6::TRP1-197 sin4-112 ura3 his3 leu2-3, -112 trp1-1 can1-100 ade2 met*	This study
BY1141	*MATα HO-lacZ46 swi6-399 SSX-14 URA his3 trp1-1*	This study
BY1152	*MATα HO-lacZ46 swi6::TRP1 sin4-112 ura3 his3 trp1-1*	This study
BY1160	*MATα HO-lacZ46 swi4::LEU2 ssx3-112 ura3 his3 leu2-3, -112 trp1-1 ade2*	This study
DY1430	*MAT***a** *HO-lacZ46 swi4::LEU2 sin4-TRP1 ura3 his3 leu2 trp1 can1-100 ade2 ade6*	D. Stillman
BY1974	*MAT***a** *gic2::LEU2 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY2125	*MAT***a** *ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY3883	*MAT***a** *srb10::HIS3MX6 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY3885	*MAT***a** *swi6::LEU2 srb10::HIS3MX6 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY3933	*MAT***a** *MED7-Flag ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY3934	*MAT***a** *MED8-Flag ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY3935	*MAT***a** *srb7-1 MED7-Flag ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY3936	*MAT***a** *srb7-1 MED8-Flag ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY4010	*MAT***a** *srb2::HIS3MX6 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY4011	*MAT***a** *srb5::HIS3MX6 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY4018	*MATα HO-lacZ46 swi6::LEU2-175 srb8::HIS3MX6 ura3 his3 leu2 trp1-1 can1-100 ade2 met*	This study
BY4019	*MATα HO-lacZ46 swi6::LEU2-175 srb11::HIS3MX6 ura3 his3 leu2 trp1-1 can1-100 ade2 met*	This study
BY4021	*MAT***a** *ROX3:HIS3MX6 ybl094c::HIS3MX6 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY4177	*MATα HO-lacZ46 swi6::LEU2-175 pGAL1-SWI6:URA3 ura3 his3 leu2 trp1-1 can1-100 ade2 met*	This study
BY4198	*MAT***a** *srb7-1 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY4199	*MAT***a** *swi6::TRP1 srb7-1 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY4200	*MATα swi6::TRP1 srb7-1 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY4201	*MAT***a** *srb7-1 srb10::HIS3MX6 ade2-1 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study
BY4203	*MAT***a** *swi6::TRP1 srb7-1 srb10::HIS3MX6 ura3 his3-11 his3-15 leu2-3 leu2-112 trp1-1 can1-100 ade2-1*	This study
BY4215	*MAT***a** *sin4::TRP1 ura3 his3 leu2-3, -112 trp1 can1-100 ade2-1*	This study
BY4401	*MAT***a** *HO-lacZ46 swi6::LEU2 sin4::TRP1 ura3 his3-1 leu2 met15*	This study
BY4402	*MAT***a** *HO-lacZ46 swi6:: LEU2 med1::KanMX4 ura3 his3-1 leu2 met15*	This study
BY4406	*MAT***a** *HO-lacZ46 swi6:: LEU2 med9::KanMX4 ura3 his3-1 leu2 met15*	This study
BY4408	*MAT***a** *HO-lacZ46 swi6:: LEU2 gal11::KanMX4 ura3 his3-1 leu2 met15*	This study
BY4410	*MATα HO-lacZ46 swi6:: LEU2 srb9::KanMX4 ura3 his3-1 leu2 met15*	This study
BY4412	*MAT***a** *HO-lacZ46 swi6:: LEU2 pgd1::KanMX4 ura3 his3-1 leu2 met15*	This study
BY4415	*MAT***a** *HO-lacZ46 swi6:: LEU2 nut1::KanMX4 ura3 his3-1 leu2 met15*	This study
BY4605	*mat::LEU2 HO-lacZ46 swi6-399 sin4-133ts ura3 his3 trp1-1 ade2*	This study
BY4608	*mat::LEU2 HO-lacZ46 swi6-399 srb9-39 ura3 his3 trp1-1 ade2*	This study
DMA688	*MAT***a** *gal11::KanMX4 ura3-0 his3-1 leu2-0 met15-0*	Winzeler *et al.* (1999)

(*continued*)

TABLE 1

(Continued)

Strain	Genotype	Source
DMA2548	*MAT***a** *pgd1::KanMX4 ura3-0 his3-1 leu2-0 met15-0*	Winzeler *et al.* (1999)
DMA3838	*MAT***a** *med9::KanMX4 ura3-0 his3-1 leu2-0 met15-0*	Winzeler *et al.* (1999)
DMA3249	*MAT***a** *med1::KanMX4 ura3-0 his3-1 leu2-0 met15-0*	Winzeler *et al.* (1999)
DMA4469	*MAT***a** *srb9::KanMX4 ura3-0 his3-1 leu2-0 met15-0*	Winzeler *et al.* (1999)
DMA4566	*MAT***a** *nut1::KanMX4 ura3-0 his3-1 leu2-0 met15-0*	Winzeler *et al.* (1999)
BY4722	*MAT*α *HO-lacZ46 swi6::LEU2-175 srb2::HIS3MX6 ura3 his3 leu2 trp1-1 can1-100 ade2 met*	This study
BY4723	*MAT*α *swi6::LEU2-175 srb5::HIS3MX6 ura3 his3 leu2 trp1-1 can1-100 ade2 met*	This study
BY4752	*MAT*α rox3-102 ade2 ura3 his3 leu2-3, -112 trp1-1 met	This study
BY4926	*MAT***a** *swi6::LEU2 ura3 his3-11 his3-15 leu2-3, -112 trp1-1 can1-100 ade2-1*	This study

β-galactosidase driven by *HO-lacZ*. The complementation analysis was performed at 30° or at 37° for ts alleles. All *swi6 ssx* mutants were also crossed against the unmutagenized *swi6 SSX* parent of the opposite mating-type strain to identify dominant suppressors. One dominant suppressor was identified among the 112 suppressors characterized.

To identify these suppressors, representative *swi6 ssx HO-lacZ* strains were transformed with the genomic DNA 2μ plasmid library (gift from F. Winston) and transformants were selected on SD plates lacking leucine. The colonies were transferred to nitrocellulose filters and assayed for β-galactosidase activity. Transformants that remained white were selected. These plasmids were isolated from the yeast strain, purified, and retransformed to confirm the dependence of the suppression phenotype on the plasmid. Plasmids that prevented suppression of *swi6* were sequenced using the Big Dye cycle sequencing method (PE-Biosystems) and the critical open reading frame was identified as indicated.

Strain constructions: *SRB8* and *SRB11* were deleted in the α-strain *swi6::LEU2 HO-lacZ* (BY547) using pFA6a-HIS3MX6 as described (Longtine *et al.* 1998). *SRB2* and *SRB5* were deleted in BY3220 *SWI6 ho* using the same method. Deletions were verified by polymerase chain reaction (PCR). No *srb2 swi6* or *srb5 swi6* double mutants could be obtained by crosses, so these crosses were performed with a *swi6* strain carrying a galactose-inducible *SWI6* gene (BY4177). The double mutants were obtained in this way, and then the plasmid was lost. The resulting strains were viable, but grew very slowly. The *ROX3* locus was followed in crosses by disrupting the adjacent YBL094C open reading frame using pFA6a-HIS3MX6 in W303 as above to create BY4021.

To get the double mutants of *swi6* and other mediator subunit genes in an *HO-lacZ* background, we crossed *sin4*, *nut1*, *pgd1*, *med1*, *med9*, *srb9*, and *gal11* deletion strains with *swi6::LEU2 HO-lacZ*. These were not isogenic crosses, so to minimize the likelihood that the phenotypes that we observed in the triple mutant were due to unknown strain variations, at least five independent segregants bearing all three mutations were analyzed from each cross. The phenotypes reported were consistent among all five isolates and thus unlikely to be caused by unknown background mutations.

RNA measurements: RNA levels were quantitated with S1 protection assays performed using oligonucleotide probes as described (Mai *et al.* 2002). The probe sequences are available upon request. mRNA levels were quantified with a PhosphorImager 400A from Molecular Dynamics (Sunnyvale, CA).

Immunoprecipitations: The C termini of Med7 and Med8 were tagged with the Flag epitope using plasmid pBS-3 Flag-KanMX as described (Gelbart *et al.* 2001) and insertion of the Flag tag into the native loci was confirmed by PCR. Cells carrying one or the other tagged locus were lysed by vortexing with glass beads five times for 60 sec in lysis buffer (20 mm HEPES pH 7.6, 1 mm EDTA, 10% glycerol, 0.25 KOAC, 0.05% NP40). One milligram of whole cell protein was diluted to 500 μl in lysis buffer and precleared with 20 μl of Protein A coupled to Dynabeads (Dynal Biotech ASA, Oslo) by rotation for 1 hr at 4°. The precleared lysates were mixed with anti-Flag M2 monoclonal antibody (Sigma, St. Louis) at 4° for 2 hr. A total of 30 μl Protein A Dynabeadads were added and incubated at 4° for 2 hr. Beads were pulled down with a magnet and proteins were eluted with 0.1 m sodium citrate pH 2.5 by rotating for 10 min at 4°. Eluate was mixed with SDS loading buffer and boiled for 5 min. Samples were separated by SDS-PAGE, transferred to PVDF membrane (Millipore, Bedford, MA), and immunoblotted with rabbit polyclonal antisera directed to Sin4, Gal11, Pgd1 (all gifts from S. Hahn), or the M2 monoclonal Flag antibody.

RESULTS

Two missense mutants and a deletion of *SWI6* were used to screen for suppressors of the requirement for Swi6 at the *HO* promoter in an *HO-lacZ* reporter construct. The results of these screens are summarized in Table 2. A total of 112 suppressors of swi6 (*ssx*) were identified. Most of the *ssx* mutations fell into seven complementation groups, but 20% of them were either single alleles of other genes or double mutants that were not further characterized. The screen for suppressors of the *swi6* deletion yielded alleles of six complementation groups, so mutations in all of these genes can bypass the requirement for Swi6 function at the *HO* promoter. Representative suppressors from each complementation group were used to clone and identify the complementing open reading frame. The identities of the suppressors are shown in Table 2 and they will be subsequently referred to by their established names. A total of 10 ts suppressors were identified in three different genes (*SIN4*, *NUT1*, and *SSX6*).

***ssx1-1* encodes a truncated allele of the essential gene *SRB7*:** The library plasmid that suppressed the *ssx1-1* phenotype contained the *SRB7* gene, which was the obvious candidate suppressor. We sequenced all the mutant's genomic DNA covered by the library plasmid and found only one mutation. The mutation was in the *SRB7* locus, causing it to be truncated after codon 89.

TABLE 2

***ssx* alleles obtained with each *swi6* mutant**

Complementation group	*swi6-399*	*swi6-254*	*swi6::TRP1*	Total	Identity of gene
SSX1	1	0	0	1	*SRB7*
SSX2	9	2	10	21	*ROX3*
SSX3	1 (1 ts)	1	17 (7 ts)	19	*SIN4*
SSX4	4	2	1 (1 ts)	7	*NUT1*
SSX5	8	6	1	15	*SRB9*
SSX6	9	1	4 (1 ts)	14	ND
SSX7	7	1	4	12	*SRB10*
Unassigned	5	4	14 (1 D)	23	
Total	44	17	51	112	

Screens were carried out with BY142 (25% survival), BY174 (25% survival), BY296 (50% survival), and BY603 (42% survival). Temperature-sensitive and dominant alleles obtained are shown in parentheses. ts, temperature sensitive; D, dominant; ND, not determined.

Since *SRB7* is an essential gene, we demonstrated linkage of *ssx1-1* to *SRB7* by deleting the adjacent gene, *GIC2*, with a *LEU2* marker in a *swi6 HO-lacZ* strain and crossing this strain to *ssx1-1 swi6 HO-lacZ*. The suppressor phenotype segregated opposite to the *gic2::LEU2* locus in 19 tetrads, indicating that a mutation linked to *GIC2* was responsible for the suppressor phenotype.

This is the first screen that has uncovered an allele of *SRB7* as a suppressor of a defect in transcriptional activation. To further characterize this mutation, the starting strain was backcrossed five times to W303 *swi6 ho* and tested for the ability to suppress the requirement for Swi6 at the native *HO* locus. The *srb7-1* mutation in *SWI6* cells increased transcription of HO by ~40% above wild-type level; in the *swi6 srb7-1 ho* strain it also shows a 40% increase above swi6 levels (Figure 1).

Truncations of *ROX3* also suppress *swi6* mutations: A library plasmid encoding *ROX3* suppressed the *ssx2-102* (BY1078) phenotype. This DNA encoded six genes, including *ROX3*. The *ROX3* locus was sequenced and a nonsense mutation terminating the protein at codon 135 was identified. Allelism between *ssx2-102* and *ROX3* was done by crossing *swi6::TRP1 ssx2-102 HO-lacZ* with *ROX3:HIS3 ho*. Among the 26 Trp+ His− segregants, one-half (13) displayed lacZ expression by the filter assay. Since *HO-lacZ* and *ho* segregated 2:2, all the segregants carried the suppressor mutation, which indicates that *SSX2* and *ROX3* are allelic.

***sin4* is allelic to *ssx3*:** Mutations in *sin4* were previously identified as suppressors of the requirement for Swi5 and Swi4 at the *HO-lacZ* locus (Nasmyth *et al.* 1987; Lycan *et al.* 1994). Moreover, diploids homozygous for *sin4* mutations sporulate inefficiently (Sternberg *et al.* 1987). We noted the same failure of *ssx3* diploids to sporulate, so we transformed a *SIN4*-containing plasmid (M1387, gift from D. Stillman) into a *swi6 ssx3-112* strain (BY1088). These transformants had no detectable *lacZ* expression, suggesting that *ssx3-112* was an allele of *SIN4*. We then performed crosses between BY1160 *swi4 ssx3* and DY1430 *swi4 sin4::TRP1* and observed the same inefficient sporulation that we observed with *ssx3* and *sin4* homozygotes. Sequencing of two *ssx3* alleles confirmed that they contained mutations in the *SIN4* locus. Interestingly, we found two mutations in both *ssx3* alleles. One of these, a glutamate-to-aspartate substitution at position 612, was shared by both mutants. We then confirmed that this change resides in the parent strain, which had a *SIN4* phenotype, and thus it appears

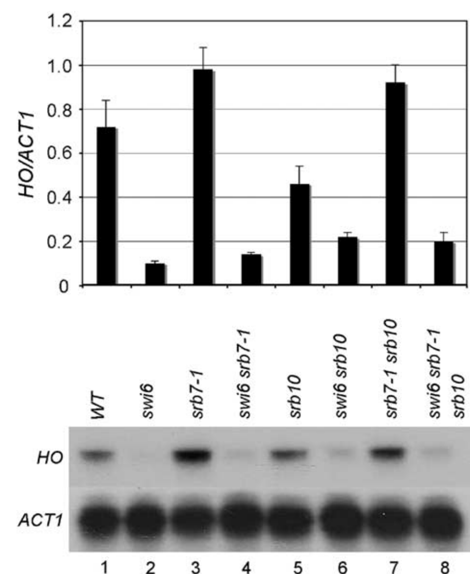

Figure 1.—*srb7-1* shows a suppression function and a synthetic interaction with *srb10* on the requirement for Swi6 at the native *HO* locus. RNA was collected from exponentially growing cells of the indicated genotypes and the level of *HO* mRNAs was measured using S1 protection. (Top) Quantification of S1 protection data, normalized to *ACT1* loading control. (Bottom) Raw data for *HO*. Strains used are BY2125, BY4926, BY4198, BY4199, BY3883, BY3885, BY4201, and BY4203.

TABLE 3

Mutations found in representative suppressors

Allele	Base substitution	Amino acid replacements
srb7-1	G265T	E89[a]
rox3-102	G405A	W135[a]
sin4-111	G383A	G128D
	G1836T	E612D
sin4-133ts	G721A	A241T
	G1836T	E612D
srb9-9	2675: deletion of T	L892YFPLSID[a]
srb10-27	768: deletion of G	M257[a]

The E612D amino acid substitution identified in both *sin4* alleles was traced back to the parent strain, which is phenotypically *SIN4*.

[a] Termination codon.

to be a neutral substitution in this assay. In addition, we identified mutations at two other sites (see Table 3), which caused a glycine-to-aspartate substitution at position 128 and an alanine-to-threonine substitution at position 241. The latter of these substitutions resulted in a *sin4* phenotype only at 37°.

***NUT1* suppresses the *ssx6* mutation, but is linked to *ssx4*:** *ssx6* resisted multiple attempts to identify this gene. Fourteen alleles of *ssx6* were isolated, but these mutants all exhibited weak suppressor activity and transformation efficiencies that were 10-fold lower than those of the parent strain. Both of these properties contributed to the difficulties in identifying the mutant gene. Individual transformations of candidate genes showed that *ssx6* could not be suppressed or complemented by *RGR1*, *SRB8*, *SRB11*, *MED1*, *MED9*, or *PGD1*. *NUT1* suppressed the *ssx6* defect, but subsequent crosses that showed that these genes were not allelic were performed between *nut1* and *ssx6*.

ssx4 is also a weak suppressor of *swi6*, which displayed poor transformation efficiency. Linkage between *ssx4* and a *nut1* deletion was demonstrated with a cross between *swi6 ssx4-14 HO-lacZ* (BY155) and *swi6::LEU2 nut1::KanMX4 HO-lacZ* (BY4415), which yielded 4:0 segregation of the suppressor phenotype.

***ssx5* and *ssx7* identify *srb9* and *srb10* as suppressors of *swi6*:** The *ssx5-9* mutation (BY338) was suppressed by a library plasmid carrying *SRB9*. Sequencing of the *SRB9* locus of BY338 showed that there was a single base deletion resulting in a reading frameshift terminating the normal coding sequence at codon 892 and replacing the terminal one-third of the protein with seven out-of-frame codons.

SRB10 was encoded on the plasmid that suppressed *ssx7-27* (BY168). *SRB10* was confirmed to be the critical locus by showing complementation with a plasmid containing only *SRB10* (pSH598, gift from Steve Hahn). Sequencing showed that *ssx7-27* harbors a single base mutation that causes termination of the protein at codon 257.

TABLE 4

Synthetic effects of *swi6* and mediator mutants grown at different temperatures

	Growth			Suppression at *HO-lacZ*
	25°	30°	37°	
swi6:	+	++	++	
Tail domain				
SWI6 sin4	+	+	–	
swi6 sin4	+/–	+/–	–	+
SWI6 pgd1	++	++	++	
swi6 pgd1	(+)	+	(+)	+
SWI6 gal11	++	++	++	
swi6 gal11	++	++	++	–
Middle domain				
SWI6 nut1	++	++	++	
swi6 nut1	++	++	++	+
SWI6 med1	+	++	++	
swi6 med1	+	+	(+)	+
SWI6 med9	++	++	++	
swi6 med9	(+)	(+)	(+)	+
SWI6 srb7	++	++	++	
swi6 srb7	++	++	(+)	+
Kinase complex				
SWI6 srb8	++	++	++	
swi6 srb8	++	++	+	+
SWI6 srb9	++	++	++	
swi6 srb9	++	++	+	+
SWI6 srb10	++	++	++	
swi6 srb10	++	++	++	+
SWI6 srb11	++	++	++	
swi6 srb11	++	++	+	+
Head domain				
SWI6 srb2	++	++	++	
swi6 srb2	+/–	+/–	+/–	ND
SWI6 srb5	+	+	+	
swi6 srb5	+/–	+/–	+/–	ND
SWI6 rox3	++	++	++	
swi6 rox3	++	++	++	+

The growth phenotypes of mediator subunit mutants in a *swi6* background grown on YEPD plates were scored from best to worst as follows: ++, +, (+), +/–, –. ND, not determined. The suppression phenotype of each double mutant is indicated as "+" if the mediator defect restores *HO-lacZ* expression on the basis of the X-Gal filter assay described in MATERIALS AND METHODS.

Defects in all components of the kinase module of mediator suppress *swi6* mutations: *srb9* and *srb10* mutants were readily isolated as suppressors of *swi6*. However, *srb8* and *srb11* were not identified. All four of these proteins are subunits of the same module. Moreover, Srb11 is the cyclin that controls the activity of Srb10. To see if *srb8* or *srb11* mutants could suppress the requirement for Swi6 at *HO-lacZ*, deletions of these two nonessential genes were generated in the *swi6 HO-lacZ* strain (BY547) and assayed for β-galactosidase activity. Both deletion mutants were capable of suppressing *swi6* (Table 4) to roughly the same extent as *srb9* and *srb10*.

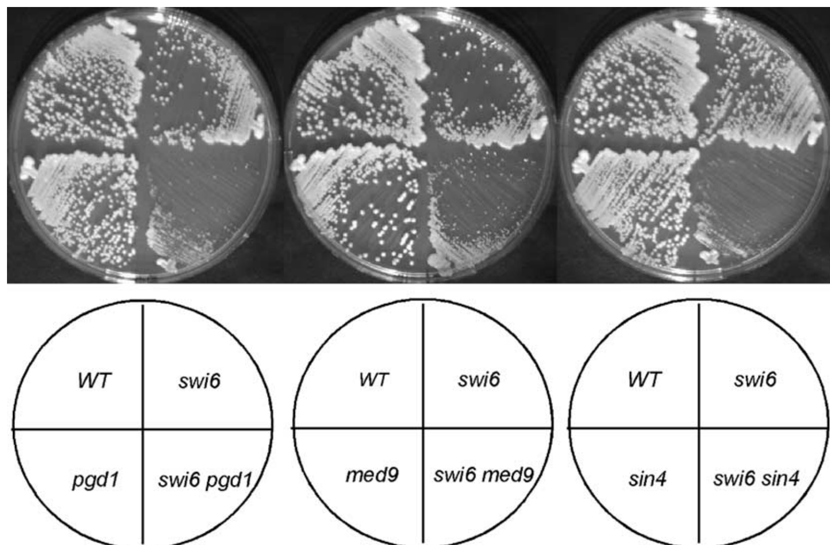

FIGURE 2.—*swi6* and mediator mutations show synthetic growth defects. Strains were streaked onto YEP-glucose plates and grown at 25° for 2 days. Strains are from left to right and counterclockwise from wild type (WT): BY134, BY546, BY4412, DMA2548, BY134, BY546, BY4406, DMA3838, BY134, BY546, BY4401, BY4215.

Mutations in half of the known subunits of mediator either act as suppressors of *swi6* or show synthetic growth defects with *swi6*: All the suppressors of *swi6* mutations that we have identified are subunits of the mediator complex and are scattered throughout all four modules that have been identified. To fully explore the suppressing capacity of mediator mutants, we combined *swi6 HO-lacZ* with deletion mutations in other subunits of the mediator complex that are known to be nonessential and screened for their ability to suppress. These results are summarized in Table 4. In addition to suppressor activity, we looked for synthetic growth defects between *swi6* and these mediator components. Many of the growth defects that we observed were more extreme at either 25° or 37° (Table 4). Mutations in all four proteins in the kinase domain behaved in a similar manner in the *swi6* background. However, the other mediator mutations that were tested showed synthetic defects in combination with *swi6* to differing extents (Figure 2). *srb2* and *srb5* showed the most extreme synthetic growth defect with *swi6*, but another head domain mutant, *rox3*, showed no such effect. All four middle domain mutations that were made could suppress the *swi6* defect in *HO-lacZ* transcription, but each showed different growth characteristics. Similarly, *SIN4* and *PGD1* mutants suppress the requirement for Swi6, but a third component of the tail module, *gal11*, does not suppress. The *swi6 pgd1* double also shows a severe growth defect at both 25° and 37°, while the *swi6 gal11* strain grows as well as *swi6* cells at all three temperatures tested.

The spectrum of suppression at other swi6 targets is highly variable: The screen for suppressors of *swi6* was originally intended to look for negative regulators that might directly interact with Swi6. However, further characterization of representative alleles of each suppressor showed that these mutants had different effects on the transcription of other Swi6-regulated genes (Figure 3). Only srb10-41 showed modest suppression of the requirement for Swi6 at the *CLN2* locus. At the *CLN1* locus all the suppressors increased mRNA levels above the *SWI6* level but the pattern was completely different from that at *HO-lacZ*. On the basis of the suppression assay at *HO:lacZ*, *srb7-1*, *rox3-33*, and *sin4-112* are the strongest of the suppressors tested, but *srb9-39*, *srb10-41*, and *ssx6-38* are all stronger suppressors at the *CLN1* locus. Interestingly, at *RNR1*, *nut1-44* increased the transcript level more than twofold, while none of the others showed any appreciable effect.

Synthetic interaction between *srb7-1* and *srb10*: Srb7 is an essential subunit of the middle module of mediator, which interacts with the kinase module (KANG *et al.* 2001). Since all four components of the kinase module were isolated as suppressors, we wondered if *srb7-1* suppressed the requirement for *swi6* by preventing association of the kinase module. If this is the case, we would expect that the phenotype of the *srb7-1 srb10* deletion would be no more extreme than the *srb10* deletion alone. To test this hypothesis, we combined *srb7-1* and *srb10*Δ in the presence or absence of *swi6* and looked at their suppression and growth characteristics. In a *SWI6* background, *srb10* had little or no effect on *ho* mRNA levels. *ho* mRNA levels were near wild type in *srb10* cells, and both *srb7* and *srb7 srb10* cells produced 140 ± 10% of the wild-type *ho* mRNA (Figure 1). In the *swi6* background, *srb7-1* and *srb10* are both weak suppressors and the *swi6 srb7-1 srb10* triple is not significantly stronger than the double mutants. This is consistent with a role for the C terminus of Srb7 in interacting with the kinase domain, since the effects of the two suppressors are not additive. However, Figure 4 shows colonies of each genotype, grown at 25° and 30°. Clearly, in both a *swi6* and a *SWI6* background, the *srb7-1 srb10*Δ combination is more deleterious than either single mutant. We conclude that Srb10 and the C terminus of Srb7 may

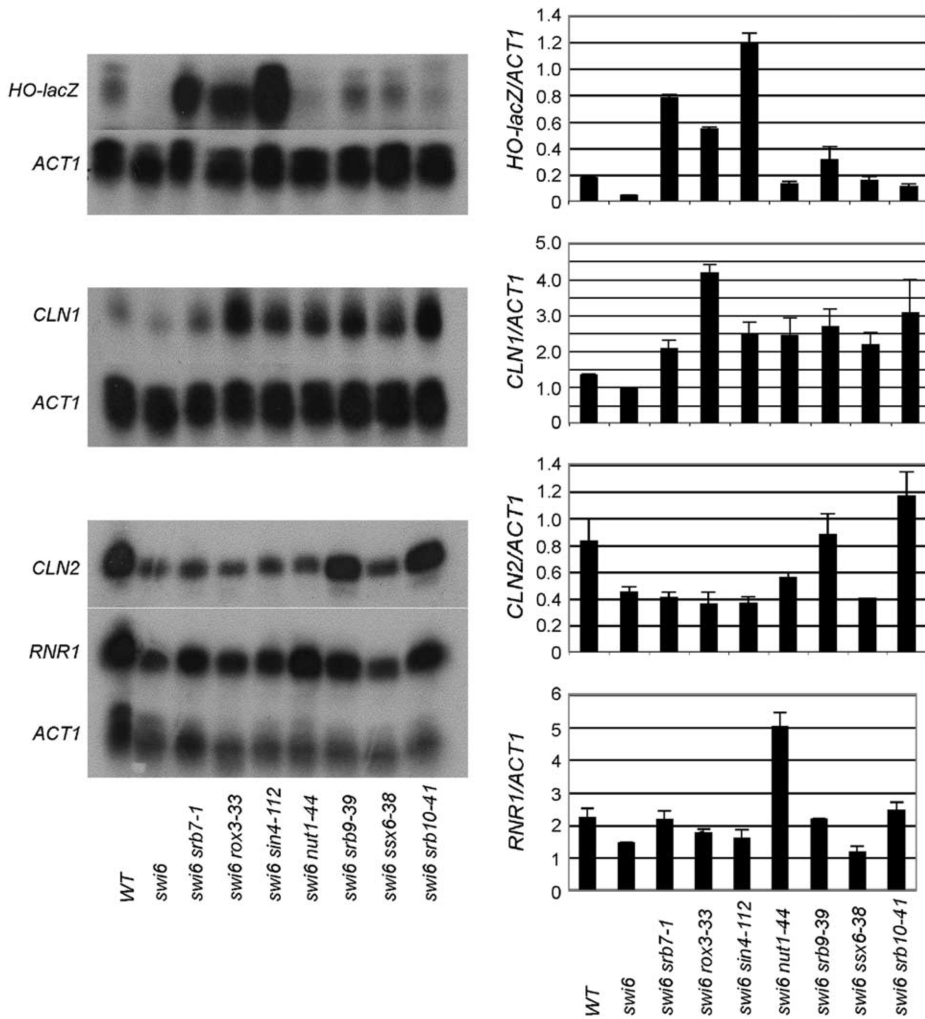

FIGURE 3.—*ssx1–ssx7* show highly variable levels of suppression *swi6* at four different Swi6 target promoters. RNA was collected from exponentially growing cells of the indicated genotypes and the levels of *HO-LacZ*, *CLN1*, *CLN2*, and RNR1 mRNAs were measured using S1 protection. (Left) Raw data for *HO-lacZ*, *CLN1*, *CLN2*, and *RNR1*. (Right) Quantification of S1 protection data, normalized to *ACT1* loading control. Strains used are BY602, BY174, BY178, BY175, BY1152, BY186, BY4608, BY180, and BY183.

function in the same pathway with respect to suppression of *swi6* mutants at the *ho* locus, but there are other critical activities that they do not share.

***srb7-1* shows reduced association with the tail module:** To see if other associations were defective in the *srb7-1* strain, we tagged Med7 and Med8, which are subunits of the middle and head domains of mediator, respectively, and looked at the association of the tail subunit components in *SRB7* and *srb7-1* cells using immunoprecipitation. These results are shown in Figure 5. It is evident that the ability of Med7 and Med8 to co-immunoprecipitate three components of the tail module (Sin4, Gal11, and Pgd1) is reduced in the absence of the C terminus of Srb7.

DISCUSSION

Swi6 plays a key role in the transition from G_1-to-S phase, due to its involvement in both transcription factor complexes that induce late G_1-specific transcription. Swi6 interacts with either Swi4 or Mbp1 in >300 genes, but none of these genes have been shown to be as dependent upon Swi6 as the *HO* gene. Although *CLN1* and *CLN2* are Swi6-regulated genes on the basis of genetics, promoter dissections, and binding studies, growing cells show less than a twofold drop in *CLN1* or *CLN2* transcript level in the absence of Swi6 (Figure 3). This difference has not been fully explored; however, several studies have indicated the existence of other regulatory elements in the *CLN1* and *CLN2* promoters (Stuart and Wittenberg 1994; Partridge *et al.* 1997).

The uniquely tight requirement for Swi6 at the *HO* promoter made it possible to identify *swi6* mutants in a genetic screen, using the *HO* promoter driving *lacZ* as the reporter (Breeden and Nasmyth 1987). This same property enabled us to identify >100 mutations that suppress the requirement for Swi6 at this promoter. Suppressors of Swi6 arose at a frequency of ~0.1% among the survivors of the mutagenesis. Interestingly, all the mutations identified were components of the mediator complex (Figure 6). Some of these mediator mutations result in a very high level of transcription (Figure 3), causing a dramatic shift from a repressed state to an activated state. Many of the same mediator components were identified as suppressors of *swi4* (Lycan *et al.* 1994; Tabtiang and Herskowitz 1998).

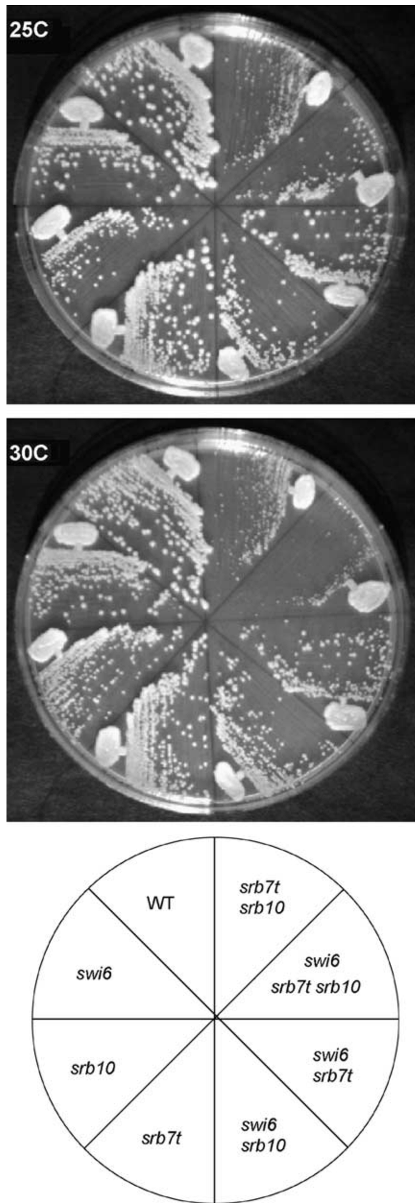

FIGURE 4.—Synthetic interactions among *swi6*, *srb7-1*, and *srb10*Δ. Strains of the genotypes indicated were grown to single colonies on YEPD plates at the temperatures indicated. Clockwise from wild type (WT): BY2125, BY4201, BY4203, BY4199, BY3885, BY4198, BY3883, BY1965.

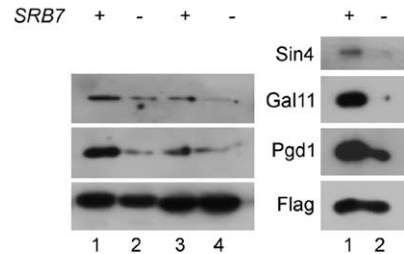

FIGURE 5.—The truncation of Srb7 causes reduced association of the tail module with the middle (Med7) and head (Med8) domains of mediator. *SRB7* (+) and *srb7-1* (−) cells carrying either a Flag-tagged Med7 (left and right, lanes 1 and 2) or a Flag-tagged Med8 (left, lanes 3 and 4) were grown at 30°, harvested, and immunoprecipitated with Anti-Flag M2 monoclonal antibodies. These precipitates were then immunoblotted with Sin4, Gal11, Pgd1, or Flag antibodies as indicated.

LYCAN *et al.* (1994) analyzed suppressors of *swi4* mutations, using the same *HO-lacZ* reporter and found mutations at roughly one-tenth the frequency of the ssx screen. Three suppressors were identified, only one of which (*sin4*) is represented in the *ssx* collection. TABTIANG and HERSKOWITZ (1998) also carried out a search for suppressors of *swi4*. These investigators used a fragment of the *HO* promoter with Swi4/Swi6-binding sites sandwiched between the *GAL1* upstream activating sequence and a *lacZ* reporter. Their search identified 14 suppressors, including several that were identified in our screen (*srb8–srb11*, *sin4*, *rox3*). This group also noted, as had previous investigators studying suppressors of Swi5, that suppression was context dependent (NASMYTH *et al.* 1987; JIANG and STILLMAN 1992). That is, *sin4* could suppress *swi4* mutations at their reporter construct, but not at the native *ho* locus. We observed a similar context dependence in our studies. Looking at three other native Swi6 target promoters, we observed a very different pattern of suppression for the seven classes of suppressor mutants that we identified.

This type of complexity has also been observed on a large scale for Gcn4-activated genes. Gcn4 is required for the full induction of >500 genes (NATARAJAN *et al.* 2001). Four native Gcn4 target genes have been

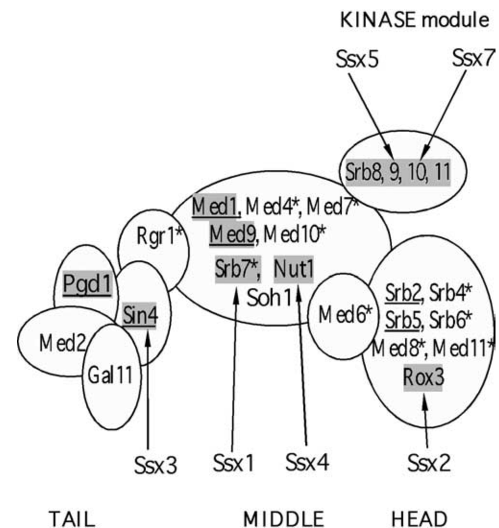

FIGURE 6.—Mutations throughout the mediator complex suppress the requirement for Swi6 for *HO-lacZ* transcription. The four mediator subdomains or modules (head, middle, tail, and kinase) are shown and the gene products that are known to be components of each module are labeled. Subunits essential for viability are indicated with asterisks. Subunits in which mutations have been found to confer suppression of *swi6* are shaded, and subunit mutants showing synthetic growth defects with *swi6* are underlined.

analyzed for their inducibility in a panel of 80 deletion mutants, each of which lacked one of the known coactivators or corepressors of transcription (Swanson *et al.* 2003). Each of the Gcn4 targets displayed a significantly different pattern of dependency, indicating that a different subset of coregulators are recruited by Gcn4 to these promoters. This complexity may be explained, in part, by the fact that Gcn4 target genes are often regulated by more than one transcription factor (Hinnebusch 1992). This appears to be a general phenomenon, because genome-wide location analysis of transcription-factor-binding sites showed that more than one-third of the promoters tested bound more than one transcription factor under the limited set of conditions tested (Lee *et al.* 2002). These and many other studies indicate that the requirements for transcription of a given promoter depend upon the constellation of transcription factors involved and the features of the surrounding chromatin.

Using the *HO-lacZ* reporter, we found that mutations in nearly half the proteins that compose the mediator could suppress the requirement for Swi6. As expected, these included all the proteins that make up the kinase domain, which is associated with repression of the transcription of many genes (Carlson *et al.* 1984; Carlson 1997; Holstege *et al.* 1998). However, we also observed suppression with mutations in every other module of the mediator, including the essential gene Srb7. This implicates all modules or the mediator as a whole in preventing transcription at *HO-lacZ* in the absence of its gene-specific activator. Unlike other promoters, mediator is recruited to the *HO* promoter well before it is activated by Cln3/Cdk, both by Swi5 and by the Swi4/Swi6 complex (Cosma *et al.* 1999, 2001; Bhoite *et al.* 2001). It may be this early localization of mediator that facilitates its role as a repressor.

Only one allele of *SRB7* was obtained from this screen and no other alleles have been identified in the dozens of such screens that have been carried out. *SRB7* is an essential gene in yeast (Hengartner *et al.* 1995; Lucau-Danila *et al.* 2000) and mice (Tudor *et al.* 1999). The *srb7-1* allele terminates the protein at position 89. Termination of Srb7 at this position eliminates 40% of the protein, the sequence of which is 40% identical to the human and Drosophila homologs (Gromoller and Lehming 2000). In spite of this, cells carrying *srb7-1* grow like wild-type cells. Only when *srb7-1* was combined with *srb10*Δ did we observe a notable drop in growth rate. We have previously shown that BY178 *swi6 srb7-1* causes the constitutive transcription of *SWI4*, which is normally transcribed in late M and early G_1 phases of the cell cycle (Breeden and Mikesell 1991). This strain also shows misregulation of *YOX1* and *YHP1*, which are cell-cycle-specific repressors of *SWI4* and other M/G_1-specific transcripts (our unpublished results). In this study we have shown that the *srb7-1* truncation reduces the amount of the three components of the tail module that are associated with mediator. This is the first role ascribed to the C terminus of Srb7. Whether this interaction between Srb7 and the tail module is mediated by a direct or indirect interaction is unknown. For example, Rgr1 is another essential component of the mediator, which has been shown to be important for the association of the tail module (Myers *et al.* 1998). It is possible that *srb7-1* disrupts association with the tail module indirectly through an effect on Rgr1.

In an effort to systematically explore the interactions between Swi6 and mediator, we combined *swi6* mutations with mutations in other nonessential subunits of the mediator. Most of these double mutants showed suppression or growth defects or both. Interestingly, with the exception of the kinase module, mutations in the individual components of each module did not behave in the same way with respect to synthetic interactions with *swi6*. Mutations in the tail module components *sin4* and *pgd1* showed both growth defects and suppression when combined with *swi6*, but a third tail component, *gal11*, showed neither. It has been suggested that *GAL11* mutants prevent association of other tail module subunits (Lee *et al.* 1999; Park *et al.* 2000). If that were the case, we would expect the *gal11* phenotypes to include all the phenotypes associated with loss of Sin4 and Pgd1. In our studies, *swi6 sin4* and *swi6 pgd1* show strong phenotypes that are undetectable in the *swi6 gal11* strain.

This study adds to the ever-growing appreciation of the complexities of transcription. With the number of proteins involved in transcription and the recognition that the unique context of each promoter influences both the sequence of events and the mechanics of the operation, it is not surprising that few generalities have emerged. Many more systematic and comprehensive studies in well-defined settings will be required before the generalities will emerge from the sea of specifics.

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