THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 274, No. 30, Issue of July 23, pp. 21029–21036, 1999

Printed in U.S.A.

Distinct Regions of the Swi5 and Ace2 Transcription Factors Are Required for Specific Gene Activation*

(Received for publication, April 8, 1999)

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Swi5 and Ace2 are cell cycle-regulated transcription factors that activate expression of early G_1-specific genes in *Saccharomyces cerevisiae*. Swi5 and Ace2 have zinc finger DNA-binding domains that are highly conserved, and the two proteins bind to the same DNA sequences *in vitro*. Despite this similarity in DNA binding, Swi5 and Ace2 activate different genes *in vivo*, with Swi5 activating the *HO* gene and Ace2 activating *CTS1* expression. In this report we have used chimeric fusions between Swi5 and Ace2 to determine what regions of these proteins are necessary for promoter-specific activation of *HO* and *CTS1*. We have identified specific regions of Swi5 and Ace2 that are required for activation of HO and CTS1, respectively. The Swi5 protein binds *HO* promoter DNA cooperatively with the Pho2 homeodomain protein, and the *HO* specificity region of Swi5 identified in the chimeric analysis coincides with the region of Swi5 previously identified that interacts with Pho2 *in vitro*. Swi5 and Ace2 also activate expression of a number of other genes expressed in G_1 phase of the cell cycle, including *ASH1*, *CDC6*, *EGT2*, *PCL2*, *PCL9*, *RME1*, and *SIC1*. Analysis of the Swi5/Ace2 chimeras shows that distinct regions of Swi5 and Ace2 contribute to the transcriptional activation of some of these other G_1-regulated genes.

How do transcription factors of the same DNA-binding class activate specific target genes? This problem, called promoter-specific activation, can be explained by several simple mechanisms involving differential DNA binding, subcellular localization of related transcription factors, or the timing of expression of those factors. The zinc finger DNA-binding transcription factors Swi5 and Ace2 bind to the same sequences *in vitro* with approximately the same affinity, yet *in vivo* they activate distinct genes (1, 2). Swi5 activates transcription of the *HO* gene, encoding the site-specific endonuclease required for mating type switching (3). Ace2 activates *CTS1*, which encodes chitinase, required to degrade the cell wall between mother and daughter cells to complete cytokinesis (2). Both *CTS1* and *HO* are expressed in late G_1 of the cell cycle, while *SWI5* and *ACE2* genes are transcribed in late G_2 (2, 4). When these factors are translated during G_2, both proteins are retained in the cytoplasm. At the end of mitosis, the transcription factors enter the nucleus and are subsequently degraded after activating their gene targets (2, 5). Although the timing of entry into the nucleus for both proteins has not been determined simultaneously, it is clear that the times of nuclear entry are very close. In addition, the nuclear localization sequence for Swi5 has been mapped, and this region is conserved between Swi5 and Ace2, making it unlikely that there are large differences in their nuclear localization patterns (2, 5).

The Swi5 and Ace2 proteins show similarity at the amino acid level, as shown in Fig. 1. The two proteins are almost identical in the zinc finger DNA-binding domain region, with 83% identity, rising to 94% with conservative amino acid substitutions. The amino acids predicted to make nucleotide-specific contacts with DNA, based on the Zif268 zinc finger protein/DNA co-crystal (6), are highly conserved between Ace2 and Swi5, leading to the prediction that these two proteins would recognize the same DNA sequences (2). *In vitro* DNA-binding experiments with both truncated proteins expressed in *Escherichia coli* and full-length proteins produced in rabbit reticulocyte lysates demonstrated that Swi5 and Ace2 do in fact bind to *HO* and *CTS1* promoter sequences with similar affinity (1, 7). However, the N-terminal regions of Swi5 and Ace2 (520 and 570 amino acids, respectively, are poorly conserved, with 18% identity through this region. There are, however, three small blocks of similarity, ranging from 20 to 27 amino acids each, in this large N-terminal region (Fig. 1).

In addition to *HO* and *CTS1*, Swi5 and Ace2 also activate transcription of the *ASH1*, *CDC6*, *EGT2*, *RME1*, *SIC1*, *PCL2*, and *PCL9* genes (8–15). These genes encode a diverse set of proteins (Table I), but a common feature is that all of these genes are expressed in early G_1 phase of the cell cycle. The Swi5 and Ace2 proteins are present in the nucleus for a limited time in early G_1 and are thus G_1-specific transcription factors. The role of Swi5 and Ace2 in the activation of these G_1 genes is complex. For some of these target genes, either Swi5 or Ace2 is competent for activation, while for other genes Swi5 is the primary activator with only a minor role for Ace2. We describe these genes as being "jointly" regulated by Swi5 and Ace2, to distinguish their regulation from that of *HO* and *CTS1*, where only one of the zinc finger proteins is an effective transcriptional activator. Therefore, promoter-specific activation by Swi5 and Ace2 cannot be explained by a simple mechanism, inasmuch as, at some target genes, Swi5 and Ace2 appear equivalent for transcriptional activation.

We expect that specific domains within the Swi5 and Ace2 proteins are involved in promoter-specific activation of *HO* and *CTS1*. Both positive and negative factors determine the ability

* This work was supported by National Institutes of Health Grants GM39067 and GM48624 and by NCI Grant 5 P30 CA42014 (for oligonucleotide synthesis and DNA sequencing performed at the Huntsman Cancer Institute DNA/Peptide and DNA Sequencing Facilities, respectively). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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TABLE I
Genes jointly regulated by Swi5 and Ace2

Gene	Function
ASH1	Daughter specific repressor of *HO* expression
CDC6	Initiator of DNA replication
EGT2	Required for cell separation
PCL2	Partner for Pho85 cyclin-dependent kinase
PCL9	Partner for Pho85 cyclin-dependent kinase
RME1	Repressor of meiosis; activator of *CLN2* transcription
SIC1	Inhibitor of Cdc28 cyclin-dependent kinase

of Swi5 and Ace2 to activate *HO* and *CTS1* (1, 16–18), and presumably these transcription factors interact with Swi5 and Ace2. For example, Swi5 binds to the *HO* promoter cooperatively with another factor, the Pho2 homeodomain protein (17). Ace2 does not interact with Pho2 (1), and thus the interaction of Swi5 with Pho2 may contribute to the specific activation of *HO*. Similarly, we have identified negative regulators that prevent Swi5 from activating *CTS1* expression (1). In this paper, we describe the identification of regions for both the Swi5 and Ace2 proteins that are responsible for promoter-specific activation of *HO* and *CTS1*, respectively. In addition, we show that particular regions of Swi5 and Ace2 contribute to the activation of some of the jointly regulated genes. These results are a first step toward understanding the mechanism of promoter-specific activation by the Swi5 and Ace2 transcription factors.

EXPERIMENTAL PROCEDURES

Plasmids—Plasmids used in this study are listed in Table II. Recombinant PCR[1] was used to generate all chimeric fragments, which were then cloned into YCplac33. The end points of the fusion are indicated in Table II by the amino acid end points of the fusions. Primer and template sequences will be provided upon request. The YIp plasmids M3319–M3325 were generated by replacing a *Pvu*I fragment in YIplac211 with a *Pvu*I fragment containing the chimera from the appropriate YCp plasmid. The *CTS1* (46)-*lacZ* reporter plasmid M2864 has been described previously (1). The *HIS4-lacZ* plasmid M2296 was constructed by moving a 5-kilobase pair *Sal*I fragment with *HIS4-lacZ* from plasmid pLL54 (19) into a Bluescript derivative containing *TRP1*; this plasmid was the gift of Yi Wei Jiang.

Yeast Strains—Saccharomyces cerevisiae strains are listed in Table III. Strains DY150, DY411, DY1142, DY1143, and DY1148 have been described previously (2). The *CTS1-lacZ* reporter integrated at the *LEU2* locus in DY1897 has been described (1). Strains DY4497–DY4503 are derived from DY1143 by integrating plasmids M3319–M3325, respectively, at the *URA3* locus after digestion with *Stu*I. C-terminal 13 Myc epitope tags were added as described by Longtine *et al.* (20) using PCR products generated using plasmid pFA6a:13MycKanMX6 as template. Oligonucleotides F665 (5′-AATGGAACGGGGATTATGGTTTCGCCAATGAAAACTAATCAAAGGCGGATCCCCGGGTTAATTAA-3′) and F666 (5′-TGTTACCCACATTCTCCACTCTTCCACAGAAAAAATTCCTAAAGTGAATTCGAGCTCGTTTAAAC-3′) were used to tag the C terminus of Swi5, and oligonucleotides F667 (5′-GAGCAAAACTCGAACCGCACCCTTTCAAACGAAACTGATGCTCTCCGGATCCCCGGGTTAATTAA-3′) and F668 (5′- GGCCCTTAAGACTACAGTGTACGTAAATCGTAAAGAAATAAATGTGAATTCGAGCTCGTTTAAAC-3′) were used to tag the C terminus of Ace2.

Quantitation of RNA Levels—RNA measurements were made by S1 protection assays as described (21) using RNA isolated from 200 ml of YEPD cultures harvested at an OD_{660} = 0.5. All samples were sonicated for 5 s before the OD_{660} was measured to compensate for OD differences between clumpy and nonclumpy strains. The following oligonucleotides were used: *ASH1* (5′-GCCTTGGGACGCACAGGTGAGGATGATCTTGATCTCATCTTGCCACTTTTCGTCGGTGTGAGAAAG-3′), *CDC6* (5′-CATTCAGATCTTGGAAGGAATCGAAAATCTTCTGGAAAATGGAGGACGGCTCTCCCAACGGCGCGT-3′), *CMD1* (5′-GGGCAAAGGCTTCTTTGAATTCAGCAATTTGTTCTTCGGTGGAGGC-3′), *CTS1* (5′-CGCAAAATCAATGTCTGCATTTTCCAACAAGTCACCAACAGAAGCATCCGGGTATGGACATTGTGGTGCGATGAGG-3′), *EGT2* (5′- CCGCTACTCAATGATGCAGTAACCGCAACTTCAGGGATAGCGGATGATTGCGAAGTATAAGAAGAAAGCGGGTCCG-3′), *HO* (5′-GCCCTGTGTGACATTTATGACGCGGGCAGCGGAGCCATCTGCGCACATAACGTAAGAGTTAGCCCACCGC-3′), *PCL2* (5′-GATAAATTCGATCTTCTAGATTCCAACGTGGAGAGGGTCGAAGTGGAAGCTAAAGATGGGCGCTCA-3′), *PCL9* (5′-GGATGTCAGTGAAGGAACCGATATAGCAGAAGAAGTTCTAGAATGCGAAACCGGTCTTCTGCAGCC-3′), *RME1* (5′-GGGACACTTACACGGTCTTTCAAGGTTGAATTCGTCTAAGTGCGCGGCAAACTCAACTAACGTTGCGAACCCCCTG-3′), and *SIC1* (5′-CGACCCAATGGTTCCTGCTCTTCCCTTACTGTTCCATTATCATGACTTTCAAATTGGAATAGTGTCCTCTGACAGT-3′). Quantitative analysis was performed using ImageQuant software and a Molecular Dynamics PhosphorImager. Radioactivity in each lane was measured, the background level was subtracted, and the value was normalized by dividing by the value for the *CMD1* internal control.

Quantitative determinations of β-galactosidase activity were performed as described previously (22). Each value is the average of four independent transformants. Western blots were performed with 9E10 anti-Myc monoclonal antibody and ECL Western kit (Amersham Pharmacia Biotech).

RESULTS

Identification of HO and CTS1 Specificity Regions of Swi5 and Ace2—In order to identify what regions of Swi5 and Ace2 contribute to promoter-specific activation of *HO* and *CTS1*, we generated chimeric fusions between Swi5 and Ace2. In a previous report we described Swi5/Ace2 chimeras that were created by exchanging restriction fragments (2). In particular, the Swi5-(1–538)/Ace2-(591–770) chimera was unable to activate either *HO* or *CTS1* (2). Due to the insertion of an oligonucleotide linker with a restriction site, this particular chimera has a two-amino acid insertion in a region highly conserved between Swi5 and Ace2. An NMR structural analysis of the DNA-binding domain of Swi5 has recently shown that these two extra nucleotides are part of an α-helical region that contributes to the stability of the first zinc finger of Swi5 (23). Thus, the failure of this Swi5-(1–538)/Ace2-(591–770) chimera to activate either *HO* or *CTS1* can be attributed to perturbation of the protein structure.

We therefore made more precise fusions using recombinant PCR. We were concerned that the fusion junction between two proteins would affect protein secondary structure, and decided to use the regions of homology shown in Fig. 1 as locations for fusions. This method of generating the chimeras proved much more successful in that all the fusions were active at least one of the transcriptional reporters tested. In addition, the chimeras activated transcription to the degree of either wild type Swi5 or Ace2, indicating that not only are the proteins expressed, but perhaps more importantly, that they are functional.

Chimeric constructs were cloned into YCp vectors. The chimeras were expressed from either the *SWI5* or *ACE2* promoters, depending on what protein fragment is N-terminal. Using the regions of similarity as a guide, Swi5 and Ace2 were broken into six parts, designated A, B, C, D, E, and F. Region E contains the DNA-binding domain, and region F contains the nuclear localization and cytoplasmic retention sequences. This nomenclature will be used throughout this paper to indicate what protein domains a chimeric fusion contains. For example, $ABCD^{Swi5}EF^{Ace2}$ contains regions A, B, C, and D from Swi5 and regions E and F from Ace2.

The chimeric fusions between Swi5 and Ace2 were analyzed for their ability to activate *HO-lacZ* or *CTS1-lacZ* reporter. YCp plasmids with the chimeric fusions were transformed into yeast strains that lacked endogenous Ace2 and Swi5, and that contained an integrated *HO-lacZ* or a *CTS1-lacZ* reporter. Promoter activity was quantitated by measuring β-galactosidase levels, and are shown as a percentage of either the level of activation that Swi5 gives at *HO-lacZ* or that Ace2 gives at *CTS1-lacZ* (Fig. 2).

The first set of chimeras show that the DNA-binding domain

[1] The abbreviation used is: PCR, polymerase chain reaction.

TABLE II
Plasmids

YCpac33	YCp plasmid with URA3 marker
M2292	YCplac33 with SWI5 in YCplac33
M2297	YCplac33 with ACE2 in YCplac33
M2452	YCplac33 with ABCD^{Swi5}EF^{Ace2} expressed from *SWI5* promoter; Swi5-(1–521)-Ace2-(576–770)
M2474	YCplac33 with ABCD^{Ace2}EF^{Swi5} expressed from *ACE2* promoter; Ace2-(1–575)-Swi5-(522–709)
M2552	YCplac33 with ABC^{Ace2}DEF^{Swi5} expressed from *ACE2* promoter; Ace2-(1–469)-Swi5-(394–709)
M2253	YCplac33 with ABC^{Swi5}DEF^{Ace2} expressed from *SWI5* promoter; Swi5-(1–393)-Ace2-(470–770)
M2849	YCplac33 with AB^{Ace2}CDEF^{Swi5} expressed from *ACE2* promoter; Ace2-(1–301)-Swi5-(260–709)
M2850	YCplac33 with AB^{Swi5}CDEF^{Ace2} expressed from *SWI5* promoter; Swi5-(1–259)-Ace2-(302–770)
M2866	YCplac33 with AB^{Swi5}C^{Ace2}DEF^{Swi5} expressed from *SWI5* promoter; Swi5-(1–259)-Ace2-(302–469)-Swi5(394–709)
YIplac211	YIp plasmid with *URA3* marker
M3319	YIplac211 with ABCD^{Swi5}EF^{Ace2} expressed from *SWI5* promoter
M3320	YIplac211 with ABCD^{Ace2}EF^{Swi5} expressed from *ACE2* promoter
M3321	YIplac211 with ABC^{Ace2}DEF^{Swi5} expressed from *ACE2* promoter
M3322	YIplac211 with ABC^{Swi5}DEF^{Ace2} expressed from *SWI5* promoter
M3323	YIplac211 with AB^{Ace2}CDEF^{Swi5} expressed from *ACE2* promoter
M3324	YIplac211 with AB^{Swi5}CDEF^{Ace2} expressed from *SWI5* promoter
M3325	YIplac211 with AB^{Swi5}C^{Ace2}DEF^{Swi5} expressed from *SWI5* promoter
M2864	CTS1 promoter (−560 to −515) inserted into CYC1(TATA)-lacZ reporter in YEp plasmid with *TRP1* marker
M2296	HIS4-lacZ reporter in YRp plasmid with *TRP1* marker

TABLE III
Yeast strains

All strains are in the W303 strain background (29) except DY1148, which is a K1107 strain (30).

DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY411	*MAT***a** *swi5*::*hisG ade2 can1 his3 leu2 trp1 ura3*
DY1142	*MATα ace2*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY1143	*MAT***a** *swi5*::*hisG ace2*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY1148	*MAT***a** *HO-lacZ swi5*::*TRP1 ace2*::*HIS3 ade2 ade6 can1 his3 leu2 met trp1 ura3*
DY1897	*MAT***a** *LEU2*::*CTS1-lacZ swi5*::*hisG ace2*::*HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY4497	*MAT***a** *swi5*::*hisG ace2*::*HIS3 URA3*::ABCD^{Swi5}EF^{Ace2} *ade2 can1 his3 leu2 trp1*
DY4498	*MAT***a** *swi5*::*hisG ace2*::*HIS3 URA3*::ABCD^{Ace2}EF^{Swi5} *ade2 can1 his3 leu2 trp1*
DY4499	*MAT***a** *swi5*::*hisG ace2*::*HIS3 URA3*::ABC^{Ace2}DEF^{Swi5} *ade2 can1 his3 leu2 trp1*
DY4500	*MAT***a** *swi5*::*hisG ace2*::*HIS3 URA3*::ABC^{Swi5}DEF^{Ace2} *ade2 can1 his3 leu2 trp1*
DY4501	*MAT***a** *swi5*::*hisG ace2*::*HIS3 URA3*::AB^{Ace2}CDEF^{Swi5} *ade2 can1 his3 leu2 trp1*
DY4502	*MAT***a** *swi5*::*hisG ace2*::*HIS3 URA3*::AB^{Swi5}CDEF^{Ace2} *ade2 can1 his3 leu2 trp1*
DY4503	*MAT***a** *swi5*::*hisG ace2*::*HIS3 URA3*::AB^{Swi5}C^{Ace2}DEF^{Swi5} *ade2 can1 his3 leu2 trp1*

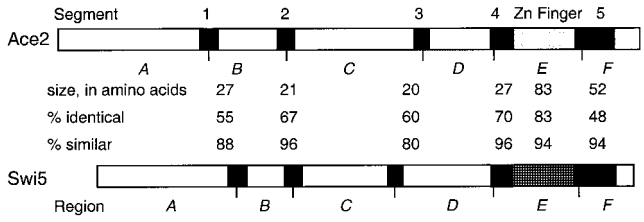


FIG. 1. **The Swi5 and Ace2 transcription factors are similar at the amino acid level.** In addition to the zinc finger region, there are five segments of similarity between Swi5 and Ace2. The regions A–D, between the similar segments, are not conserved.

does not determine promoter specificity at *HO* and *CTS1*. Chimeras ABCD^{Ace2}EF^{Swi5} and ABCD^{Swi5}EF^{Ace2} (Fig. 2, *lines 3* and *4*) were constructed with regions E and F, containing the DNA-binding domain of one transcriptional activator, fused to regions A–D of the other. The results show clearly that the N-terminal part of the proteins, regions A–D, confer promoter-specific activation. Swi5 and Ace2 proteins bind *in vitro* to *HO* or *CTS1* promoter fragments with similar affinities (1), and thus it was not unexpected that the DNA-binding domains of Swi5 and Ace2 are not the critical determinants of promoter-specific activation. Region F, which controls the regulated nuclear entry of Swi5, is highly conserved between Swi5 and Ace2. Since Swi5 and Ace2 enter the nucleus at similar times (2, 24), this region was not expected to be critical for promoter specificity.

What regions within A–D are important for promoter-specific activation? To address this question, additional chimeric fusion proteins were generated. Chimera ABC^{Ace2}DEF^{Swi5} (Fig. 2, *line 5*) is able to activate *HO-lacZ*, while ABCD^{Ace2}-EF^{Swi5} (Fig. 2, *line 4*) cannot. These results suggest that region D of Swi5 (amino acids 394–521) is necessary for activation of the *HO-lacZ* reporter, and we designate this region the *HO* specificity region. This region of Swi5 has been also shown to be necessary for interaction of Swi5 with the Pho2 homeodomain protein (25, 26).

The next set of chimeras suggest that region C of Ace2 is required for specific activation of *CTS1*. Chimera ABC^{Ace2}DEF^{Swi5} (Fig. 2, *line 5*) can activate *CTS1-lacZ*, while AB^{Ace2}CDEF^{Swi5} (Fig. 2, *line 7*) cannot, and the difference between these two constructs is the presence of region C of Ace2. An additional chimera was constructed to test whether region C of Ace2 is sufficient for *CTS1* activation in the context of the Swi5 protein. The AB^{Swi5}C^{Ace2}DEF^{Swi5} chimera can activate *HO-lacZ*, due to the presence of region D from Swi5, and it can also activate *CTS1-lacZ* (Fig. 3). We have designated region C of Ace2 (amino acids 303–469) as the *CTS1* specificity region. A fusion protein consisting of the lexA DNA-binding domain fused to region C of Ace2 is a strong activator of a transcriptional reporter containing lexA binding sites in the *CYC1* promoter (data not shown). This result demonstrates that region C contains a transcriptional activation domain capable of activating transcription from the *CYC1* TATA element.

The ABC^{Swi5}DEF^{Ace2} chimera is not able to activate either *HO-lacZ* or *CTS1-lacZ* (Fig. 2, *line 6*), raising the concern that this protein is not functional or stable *in vivo*. We used two other reporter constructs that can be activated by either Swi5 or Ace2 to determine whether ABC^{Swi5}DEF^{Ace2} is functional. The *CTS1* (46)-*lacZ* reporter, which contains the Ace2 binding sites but lacks the negative regulatory site that blocks activation by Swi5, is inactive in a *swi5 ace2* double mutant (1), but

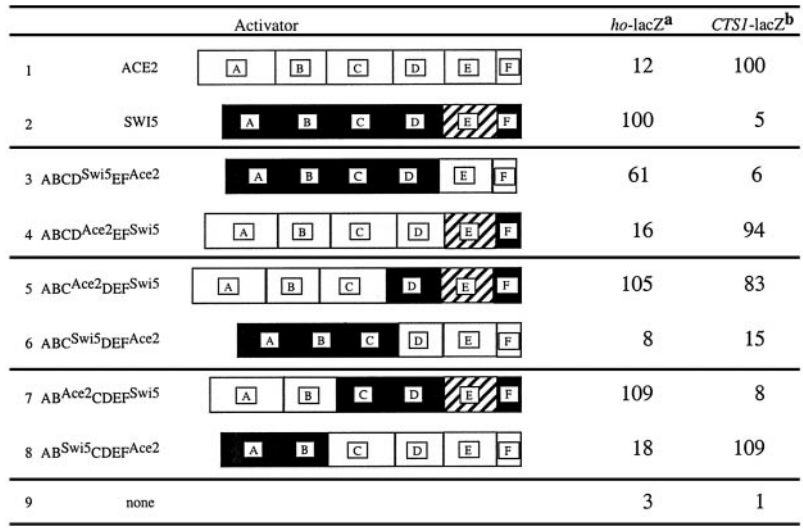


FIG. 2. **Activation of the *HO-lacZ* and *CTS1-lacZ* reporters by the Swi5/Ace2 chimeric proteins.** YCp plasmids expressing chimeric fusions between Swi5 and Ace2 were transformed into yeast strains and grown in medium lacking uracil, and extracts were prepared for β-galactosidase assays. Four independent transformants were assayed; standard deviations were less than 20%. The chimera diagrams, which are not to scale, have regions A–F labeled with Swi5 sequences in *black* and Ace2 sequences in *gray*. The DNA-binding domain, region E, is shown as a *stippled block*. *Footnote a*, expression of the integrated *HO-lacZ* reporter in DY1148 (*ace2 swi5*) is given as a percentage normalized to the Swi5 control (*line 2*); *footnote b*, expression of the integrated *CTS1-lacZ* reporter in DY1897 (*ace2 swi5*) is given as a percentage normalized to the Ace2 control (*line 1*).

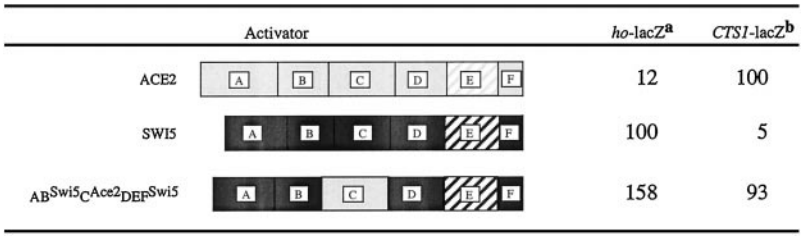


FIG. 3. **Region C from Ace2 is sufficient to confer *CTS1-lacZ* activation upon Swi5.** YCp plasmids expressing chimeric fusions between Swi5 and Ace2 were transformed into yeast strains and grown in medium lacking uracil, and extracts were prepared for β-galactosidase assays. Four independent transformants were assayed, and standard deviations were less than 20%. Chimera diagrams are as in Fig. 2. *Footnote a*, expression of the integrated *HO-lacZ* reporter in DY1148 (*ace2 swi5*) is given as a percentage normalized to the Swi5 control (*line 2*); *footnote b*, expression of the integrated *CTS1-lacZ* reporter in DY1897 (*ace2 swi5*) is given as a percentage normalized to the Ace2 control (*line 1*).

can be activated by either Swi5 or Ace2 (Fig. 4). The *HIS4-lacZ* reporter responds similarly to either Swi5 or Ace2 (Fig. 4; Ref. 7). The ABC^{Swi5}DEF^{Ace2} chimera is able to activate both *CTS1* (46)-*lacZ* and *HIS4-lacZ* equivalently to full-length Swi5, indicating that this chimeric fusion is functional *in vivo*. To directly determine levels of the chimeric proteins *in vivo*, we generated strains with a Myc epitope fused in frame at the C terminus of Ace2, Swi5, and each of the chimeras. Western analysis shows that the fusion proteins are expressed at similar levels as the wild type Ace2 and Swi5 proteins (Fig. 5). Although the ABC^{Swi5}DEF^{Ace2} chimera (*lane 7*) is expressed at slightly lower levels than other chimeras, it is expressed at a level similar to that of native Swi5 (*lane 2*). We conclude that the reason the ABC^{Swi5}DEF^{Ace2} chimera is unable to activate *HO-lacZ* and *CTS1-lacZ* is because it lacks a promoter specificity domain from either Swi5 or Ace2.

We used an S1 nuclease protection assay of mRNA levels to measure the ability of the various chimeras to activate transcription of the native *HO* and *CTS1* genes in strains generated by integrating the various chimeric fusions at the *URA3* locus of a *swi5 ace2* double mutant strain. The rationale for this experiment is twofold. First, we have observed differences in regulatory properties of the integrated *HO-lacZ* reporter compared with the native *HO* locus (18, 27). Second, we have also noted subtle differences in regulation of the jointly regulated genes (*i.e. SIC1*) when YCp plasmids with *SWI5* and *ACE2* were used compared with integrated genes (data not shown). We attribute this difference to the slightly higher copy number of YCp plasmids compared with single copy integrants. The pattern of activation by the different chimeras at the endogenous *HO* and *CTS1* genes was similar to that seen for the *lacZ* reporters (Fig. 6).

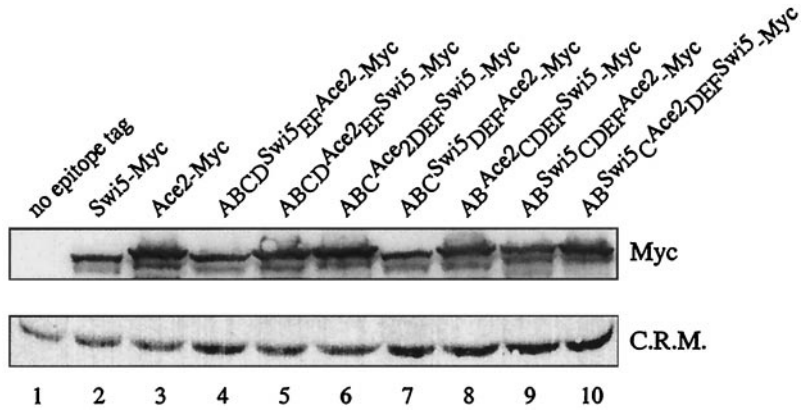
G_1 Genes Activated by Swi5 and Ace2—There are a number of genes expressed in early G_1 whose expression has been reported to be activated by Swi5 or Ace2 (Table I). RNA was isolated from four isogenic strains differing at the *SWI5* and *ACE2* loci and used for S1 nuclease protection analysis using probes specific for each of these seven genes. The experiment was performed three times, and the average values are presented in Table IV. All of these genes show a significant reduction in expression in the *swi5 ace2* double mutant. However, none of them show a reduction as strong as that seen for *HO* and *CTS1* in either the *swi5* or *ace2* single mutants. For convenience, we have designated these as jointly regulated genes. These genes show varying dependence on Swi5 and Ace2 for transcriptional activation. For example, either Swi5 or Ace2 can activate *ASH1*, *EGT2*, *RME1*, or *PCL2* as expression is only modestly reduced in the *swi5* and *ace2* single mutants, but is significantly reduced in the *swi5 ace2* double mutant. Reduction in mRNA levels in the *swi5 ace2* double mutant is more substantial for *EGT2* and *RME1* than for *ASH1* or *PCL2*. In contrast, Swi5 is the major activator of *SIC1* and *PCL9* expression, but we note that expression of *SIC1* and *PCL9* is further reduced in the *swi5 ace2* double mutant. Swi5 also plays the major role in activation of *CDC6*, as an *ace2* mutation has little effect. Although *CDC6* expression is not decreased by an *ace2* mutation, we discuss the regulatory properties of *CDC6* with the jointly regulated genes because its expression is only partially reduced by a *swi5* mutation. It has been shown that the Swi4/Swi6 transcription factors, along with Swi5, activate *CDC6* (9), and thus there is a high residual level of *CDC6* expression in the *swi5 ace2* double mutant.

Activation of Jointly Regulated Genes by Swi5-Ace2 Chimeric Fusions—We examined the ability of the chimeric fusions between Swi5 and Ace2 shown in Fig. 2 to activate transcription of the jointly regulated genes (*ASH1*, *CDC6*, *EGT2*, *PCL2*,

Activator	*CTS1*(46)-lacZ[a]	*HIS4*-lacZ[b]	*ho*-lacZ[c]	*CTS1*-lacZ[d]
Ace2	100	85	12	100
Swi5	53	100	100	5
NONE	1	2	3	1
$ABC^{Ace2}DEF^{Swi5}$	178	100	105	83
$ABC^{Swi5}DEF^{Ace2}$	37	149	8	15

FIG. 4. **Chimera $ABC^{Swi5}DEF^{Ace2}$ is functional for transcriptional activation.** Yeast strains were transformed with YCp plasmids expressing chimeric fusions between Swi5 and Ace2 and, for *a* and *b*, also transformed with *lacZ* reporter plasmids. Transformants were grown in medium lacking uracil or uracil and tryptophan to select for plasmids, and extracts were prepared for β-galactosidase assays. Four independent transformants were assayed, and standard deviations were less than 30%. *Footnote a*, expression of the *CTS1*(46)-*lacZ* reporter on plasmid M2894 in strain DY1143 (*ace2 swi5*) is given as a percentage normalized to the Ace2 control (*line 1*); *footnote b*, expression of the *HIS4-lacZ* reporter on plasmid M2296 in strain DY1143 (*ace2 swi5*) is given as a percentage normalized to the Swi5 control (*line 2*); *footnote c*, *HO-lacZ* expression (from Fig. 2) is given as a percentage normalized to the Swi5 control (*line 2*); *footnote d*, *CTS1-lacZ* expression (from Fig. 2) is given as a percentage normalized to the Ace2 control (*line 1*).

FIG. 5. **Chimeras are expressed at wild type levels.** Western blotting was used to determine protein levels of strains with Swi5, Ace2, or various chimeras containing a C-terminal Myc epitope tag. Electrophoretic mobilities of the different proteins are consistent with their predicted molecular masses. The cross-reacting material (*C.R.M.*) serves as a loading control. *Lane 1*, DY150 with no epitope tag; *lanes 2–10*, epitope tags were added to wild type Swi5 in strain DY1142, wild type Ace2 in strain DY150, and various chimeras in strains DY4497 through DY4503.



PCL9, *RME1*, and *SIC1*). RNA was prepared from *swi5 ace2* strains containing integrated versions of the chimeric fusions, and mRNA levels were determined by S1 nuclease protection with probes specific for each of the jointly regulated genes. This experiment was performed three times, and mRNA levels were quantitated by PhosphorImager analysis. Our results indicate that, although the regions identified as required for *HO* and *CTS1* activation are in fact necessary for discrimination between those genes, they also contribute to activation of the jointly regulated genes.

Analysis of activation of *EGT2*, *PCL2*, and *ASH1* by the chimeras did not identify any particular region of either Swi5 or Ace2 as critical for their activation (data not shown). This means that almost all of the chimeric fusions analyzed were capable of activating expression of these genes. This suggests that either protein can contribute to gene activation equally, and that no one region of Swi5 or Ace2 is required for expression of these genes. This is consistent with a model in which promoter-specific activation is distinct from activation at promoters where Swi5 and Ace2 are equivalent. In contrast, the C-terminal regions of Swi5 and Ace2, containing the DNA-binding domains and nuclear localization sequences, are involved in the activation of several of the jointly regulated genes.

SIC1 and PCL9—SIC1 and *PCL9* show very similar profiles of activation by the different chimeras (Fig. 7). These genes are primarily activated by Swi5, and the pattern seen for chimera activation is very similar to that seen for *HO* (Figs. 2 and 6), in that all chimeras with region D of Swi5 can activate *SIC1* and *PCL9*. However the $ABCD^{Ace2}EF^{Swi5}$ chimera (Fig. 7, *line 6*), which does not activate *HO* expression, does drive transcription of *SIC1* and *PCL9*. This chimera contains region EF from Swi5, and indeed, all chimeras with region EF from Swi5 can activate *SIC1* and *PCL9*. Thus, either region D or region EF of Swi5 is required for activation of these genes, indicating that there are multiple regions within Swi5 involved in activation of *SIC1* and *PCL9*.

Chimeras $ABC^{Ace2}DEF^{Swi5}$ (Fig. 7, *line 7*) and $AB^{Swi5}C^{Ace2}$-DEF^{Swi5} (Fig. 7, *line 11*) activate *SIC1* and *PCL9* well beyond the wild type expression level. Along with region DEF of Swi5, which is required for *SIC1* and *PCL9* expression, these chimeras also contain region C from Ace2, the *CTS1* specificity region. Region C of Ace2 does not effectively activate these genes without region D and region EF of Swi5 (*i.e.* in wild type Ace2, *line 2*). As described above, region C from Ace2 contains an activation domain when targeted to a heterologous promoter, and it is possible that it is this generalized activation domain in region C that further stimulates the activity of these two chimeras.

RME1—The *RME1* gene shows an equal dependence on both Swi5 and Ace2, as *RME1* expression is reduced to similar extents in the *swi5* and *ace2* single mutants but strongly reduced in the double mutant (Fig. 8*A*). These characteristics distinguish it from the other jointly regulated genes. The pattern of chimera activation of *RME1* is similar to that seen for *HO*, *SIC1*, and *PCL9*, as chimeras with region D of Swi5 can activate *RME1* expression well (*lines 5*, *7*, *9*, and *11*). There are some subtle differences, however. First, $ABCD^{Swi5}EF^{Ace2}$ (*line 5*) activates *RME1* much more effectively than full-length Swi5, surpassing the amount of activation seen in the wild type strain. This suggests that region EF of Ace2 contributes some activation potential at *RME1*. An alternative explanation is that region ABCD of Ace2 contains an inhibitory activity that ABCD of Swi5 lacks. The reciprocal chimera, $ABCD^{Ace2}EF^{Swi5}$ (*line 6*), activates similar to the level seen for full-length Ace2. These results differ from that seen at *SIC1* and *PCL9*, where both the $ABCD^{Ace2}EF^{Swi5}$ and $ABCD^{Swi5}EF^{Ace2}$ (*lines 5* and *6*)

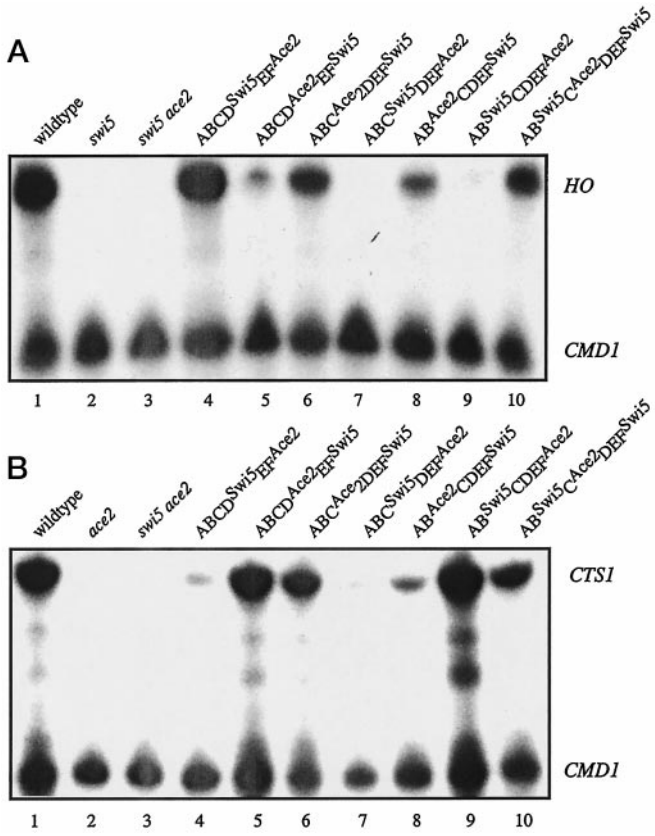


FIG. 6. **Activation of *HO* and *CTS1* by the Swi5/Ace2 chimeras.** S1 nuclease protection assays were performed using probes specific for *HO* (*A*) and *CTS1* (*B*), along with *CMD1* as an internal control. RNAs were prepared from the following strains: (*A*) DY150, DY411, DY1143, DY4497, DY4498, DY4499, DY4500, DY4501, DY4502, and DY4503; (*B*) DY150, DY1142, DY1143, DY4497, DY4498, DY4499, DY4500, DY4501, DY4502, and DY4503.

TABLE IV

Relative contributions of Swi5 and Ace2 in activating early G_1 genes

S1 nuclease protection assays were performed using probes specific for each of the genes indicated, along with a *CMD1* internal control in each nuclease protection experiment. The experiment was performed three times for all probes with three independent RNA preparations. RNA levels were quantitated by PhosphorImager analysis, normalized by dividing by the value for the *CMD1* control, and values are given as a percentage of wild type expression. Standard deviations were all less than 40%; most were less than 20%. RNAs were prepared from the following strains: DY150, DY411, DY1142, and DY1143.

Gene	Wild type	*swi5*	*ace2*	*ace2 swi5*
HO	100	1	60	1
CTS1	100	87	3	1
ASH1	100	88	92	33
CDC6	100	45	227	50
EGT2	100	80	92	1
PCL2	100	104	103	57
PCL9	100	36	101	18
RME1	100	75	52	11
SIC1	100	61	99	26

chimeras activate expression to wild type levels. Thus activation of *RME1* indicates a difference between the EF regions of Swi5 and Ace2; the EF region of Ace2 stimulates transcriptional activation better than the analogous region from Swi5.

CDC6—This gene shows a sharp dependence upon Swi5 for activation, as *CDC6* expression is reduced to similar extents in *swi5* single and *swi5 ace2* double mutants (Fig. 8*B*). Expression of *CDC6* is also activated by the Swi4p/Swi6p complex and Mcm1 (9, 28) and is probably responsible for the CDC6 expression remaining in the *swi5* mutant. *CDC6* is also the only gene we have examined that shows a reproducible increase in activation in an *ace2* strain (Table IV, line 3). The basis for this increase is unclear but it is probably indirect.

The pattern of *CDC6* activation by the chimeras is also unique. The activating chimeras (*lines 6*, *7*, *9*, and *11*) all contain region EF of Swi5. For example, the $ABCD^{Swi5}EF^{Ace2}$ chimera (*line 5*) is unable to activate *CDC6*, while the $ABCD^{Ace2}EF^{Swi5}$ chimera (*line 6*) activates *CDC6* effectively. This result is similar to that seen at *SIC1* and *PCL9*, where region EF of Swi5, containing the DNA-binding domain and nuclear localization sequences for this protein, also contributes to trans-activation. Region D of Swi5 is not a significant determinant of *CDC6* expression, unlike at *SIC1*, *PCL9*, and *HO*. Unlike the situation at *CDC6*, region EF has no role in promoter specificity at *HO* or *CTS1*.

DISCUSSION

The mechanisms that determine specific transcriptional activation of genes are complex and often involve multiple factors. Domains within the transcription factor itself contribute to promoter-specific activation by determining interactions with specific proteins. We have examined transcriptional activation by the Swi5 and Ace2 transcription factors as a model for promoter-specific activation in *S. cerevisiae* and have identified domains of these proteins that are required for transcriptional activation of particular genes.

The Swi5 and Ace2 transcription factors have highly conserved zinc finger DNA-binding domains, and they bind to same DNA sequences *in vitro* (1). Nonetheless, these factors activate different genes, with Swi5 activating *HO* expression and Ace2 activating *CTS1* (2). The Swi5 and Ace2 transcription factors are cell cycle-regulated, being present in the nucleus for a brief period in G_1 of the cell cycle. Along with activating transcription of *HO* and *CTS1* in G_1, it has been shown that Swi5 and Ace2 contribute to the activation of a set of genes expressed in G_1, including *ASH1*, *CDC6*, *EGT2*, *RME1*, *SIC1*, *PCL2*, and *PCL9* (8–15). We have used the term "jointly" regulated when referring to these genes, as none of them shows a strong requirement for only Swi5 or Ace2 as observed for *HO* and *CTS1*. In order to determine what domains of Swi5 and Ace2 contribute to the transcriptional activation of these various G_1 genes, we have constructed plasmids that express protein chimeras between Swi5 and Ace2. The analysis of transcriptional activation of *HO*, *CTS1*, and the jointly regulated genes by these fusion proteins allows us to identify specific parts of the proteins required for promoter-specific activation.

Region D of Swi5 Is Necessary for Activation of HO—*HO* was activated by any protein chimera containing region D (amino acids 394–521) of Swi5, but not by chimeras lacking this region (Figs. 2 and 6). Specific activation of *HO* requires region D of Swi5, and this *HO* specificity region of Swi5 interacts *in vitro* with the Pho2 homeodomain protein. *In vitro* experiments with truncated derivatives of Swi5 demonstrated that amino acids 384–496 are required for cooperative DNA binding with Pho2 (25). A genetic screen has been performed to identify single amino acid substitutions within this region that affect Swi5 activation of *HO* (26). Fifteen mutations between residues 482 and 505 of Swi5 were recovered, and many of these substitutions also affect the ability of Swi5 to cooperatively bind DNA with Pho2, without effecting HO expression (26). The genetic analysis of these Swi5 point mutants suggests that this region of Swi5 has two functions, activation of *HO* and interaction with Pho2, and is consistent with a role for region D in specific activation of *HO*.

Region C of Ace2 Is Necessary for Activation of CTS1—Analysis of the ability of the protein chimeras to activate *CTS1* shows that region C (amino acids 303–469) of Ace2 is required

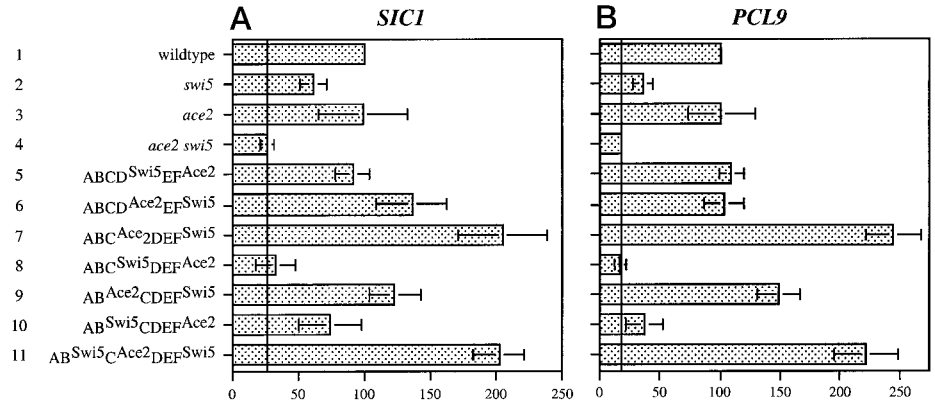


FIG. 7. **Activation of *SIC1* and *PCL9* by the Swi5/Ace2 chimeras.** S1 nuclease protection assays were performed using probes specific for *SIC1* (*A*) and *PCL9* (*B*), along with *CMD1* as an internal control, and RNA levels quantitated by PhosphorImager analysis were normalized by dividing by the value for the *CMD1* control. The experiment was performed three times with three independent RNA preparations. Values are given as a percentage of the wild type strain (*line 1*), with the standard deviations indicated. The *vertical lines* drawn across the *graph* represent the amount of activation seen in the *ace2 swi5* double mutant strain (*line 4*). RNAs were prepared from the following strains: DY150, DY411, DY1142, DY1143, DY4497, DY4498, DY4499, DY4500, DY4501, DY4502, and DY4503.

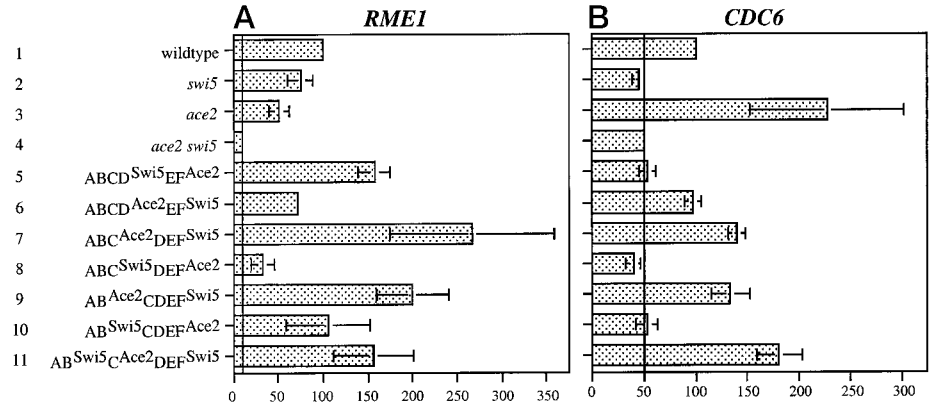


FIG. 8. **Activation of *RME1* and *CDC6* by the Swi5/Ace2 chimeras.** S1 nuclease protection assays were performed using probes specific for *RME1* (*A*) and *CDC6* (*B*), along with *CMD1* as an internal control, and RNA levels quantitated by PhosphorImager analysis were normalized by dividing by the value for the *CMD1* control. The experiment was performed three times with three independent RNA preparations. Values are given as a percentage of the wild type strain (*line 1*), with the standard deviations indicated. The *vertical lines* drawn across the *graph* represent the amount of activation seen in the *ace2 swi5* double mutant strain (*line 4*). RNAs were prepared from the following strains: DY150, DY411, DY1142, DY1143, DY4497, DY4498, DY4499, DY4500, DY4501, DY4502, and DY4503.

(Figs. 2 and 6). Additionally, the $AB^{Swi5}C^{Ace2}DEF^{Swi5}$ chimera shows that this *CTS1* specificity region of Ace2 is sufficient for *CTS1*-specific activation in the context of the Swi5 protein. $AB^{Swi5}C^{Ace2}DEF^{Swi5}$ can activate both *HO* and CTS1, as it contains region C of Ace2 and region D of Swi5 (Fig. 3). Region C of the Ace2 protein contains a strong activation domain, as a lexA-Ace2(region C) fusion is capable of activating transcription of a reporter containing lexA sites in the promoter (data not shown). Further experiments will be needed to determine whether the general activation property of region C is necessary for promoter-specific activation of *CTS1*, or whether the two functions are separable.

Role of Region EF in Promoter-specific Activation—The protein chimeras demonstrated that the DNA-binding domains of Swi5 and Ace2, present within region E, do not play a major role in discrimination between *HO* and *CTS1* (Figs. 2 and 6). However, region EF, which includes the DNA-binding domain, is important for activation of some of the jointly regulated genes. The $ABCD^{Swi5}EF^{Ace2}$ chimera activates *RME1* better than native Swi5 (Fig. 8), demonstrating that region EF of Ace2 contributes to activation. Analysis of the activation of *SIC1* and *PCL9* by the chimeras shows that either region D or region EF of Swi5 is required (Fig. 7). The fact that *SIC1* and *PCL9* are effectively activated by the $ABCD^{Ace2}EF^{Swi5}$ chimera, but poorly by native Ace2, demonstrates a role for region EF of Swi5. Finally, region EF of Swi5 is the only region required for activation of *CDC6* (Fig. 8). *CDC6* is regulated by Swi5, and not by Ace2, for at least part of its expression, making it most similar to *HO*. However, unlike at *HO*, region D of Swi5 is not critical for activation of *CDC6*; instead, region EF is important for activation of *CDC6*. This difference may eventually provide insight into promoter-specific activation by particular regions of Swi5.

What are the functional domains in regions E and F? Region E contains the DNA-binding domains of Swi5 and Ace2, and region F contains sequences that control cytoplasmic retention in G_2 and nuclear localization in M and G_1. Differences in Swi5 and Ace2 DNA binding at these promoters may play a role in the strength of activation at *SIC1*, *PCL9*, *RME1*, and *CDC6*. Interestingly, *in vitro* DNA-binding experiments conducted with a *SIC1* promoter probe show differences in binding by Swi5 and Ace2 (12). Additionally, region F can be divided into two parts. The first segment of 50 amino acids contains the nuclear localization signals and is conserved between Swi5 and Ace2 (48% identical, 94% similar), while the second segment of 30 amino acids is not conserved between Swi5 and Ace2. Further work will be needed to establish whether it is the DNA-binding domain in region E, the nuclear localization sequences in region F, or some other function contained within these regions that affects activation of *RME1*, *SIC1*, *PCL9*, and *CDC6*.

Acknowledgments—We thank Yi Wei Jiang for the *HIS4-lacZ* reporter plasmid M2296 and Warren Voth for comments on the manuscript.

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