Swi5 and Ace2 are cell cycle-regulated transcription factors that activate expression of early G1-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 homodomain 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 G1 phase of the cell cycle, including ASH1, CDC6, EG12, 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 G1-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 G1 of the cell cycle, while SWI5 and ACE2 genes are transcribed in late G2 (2, 4). When these factors are translated during G2, 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 Zf268 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, EG12, 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 G1 phase of the cell cycle. The Swi5 and Ace2 proteins are present in the nucleus for a limited time in early G1 and are thus G1-specific transcription factors. The role of Swi5 and Ace2 in the activation of these G1 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...
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 PCR1 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 YEp plasmids M3319–M3325 were generated by replacing a PvuI fragment in YIp lac211 with a plasmid carrying the chimeras from the appropriate YCp plasmid. The CTS1 (46-lacZ reporter plasmid M2684 has been described previously (1). The HIS4-lacZ plasmid M2296 was constructed by moving a 5-kilobase pair SauI fragment with HIS4-lacZ reporter from pLL54 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 StuI. C-terminal 13 Myc epitope tags were added as described by Longtine et al. (20) using PCR products generated using plasmid pF6a:13MycKanMX6 as template. Oligonucleotides F665 (5′-TGTTACCCACATTCTCCACTCTTCCACAGAAAAATT-3′) and F666 (5′-GTGGTACCCACATTCTCCACTCTTCCACAGAAAAATT-3′) were used to tag the C terminus of Swi5, and oligonucleotides F667 (5′-GGACAAAACCTGCAAACGCCCACTTGCCTCCTCCGTGACCGCGGGTTAATTAA-3′) and F668 (5′-GGACAAAACCTGCAAACGCCCACTTGCCTCCTCCGTGACCGCGGGTTAATTAA-3′) were used to tag the C terminus of Ace2.

Quantitation of RNA Levels—Quantitative determinations of galactosidase activity were 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 the value for the CMD1 internal control.

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 two separate reports in this issue. 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 upon 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. Depending on what protein fragment is N-terminal. Using 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.

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Promoter-specific Activation Domains of Swi5 and Ace2

TABLE II

| Plasmids                        |
|---------------------------------|
| YCpac33                         | YCp plasmid with URA3 marker |
| M2292                           | YCplasmid with SWI5 in YCpac33 |
| M2297                           | YCplasmid with ACE2 in YCpac33 |
| M2452                           | YCplasmid with ABCD^{Swi5}EF^{Ace2} expressed from SWI5 promoter; Swi5-(1–521)-Ace2-(576–770) |
| M2474                           | YCplasmid with ABCD^{Ace2}EF^{Swi5} expressed from ACE2 promoter; Ace2-(1–575)-Swi5-(522–709) |
| M2552                           | YCplasmid with ABCC^{Ace2}DEF^{Swi5} expressed from ACE2 promoter; Ace2-(1–469)-Swi5-(394–709) |
| M2253                           | YCplasmid with ABC^{Swi5}DEF^{Ace2} expressed from SWI5 promoter; Swi5-(1–439)-Ace2-(470–770) |
| M2249                           | YCplasmid with ABC^{Ace2}DEF^{Swi5} expressed from ACE2 promoter; Ace2-(1–301)-Swi5-(260–709) |
| M2250                           | YCplasmid with ABC^{Ace2}DEF^{Swi5} expressed from ACE2 promoter; Ace2-(1–301)-Swi5-(260–709) |
| M2266                           | YCplasmid with ABC^{Swi5}DEF^{Ace2} expressed from SWI5 promoter; Swi5-(1–529)-Ace2-(302–469)-Swi5(394–709) |
| YPlac211                        | Ylp plasmid with URA3 marker |
| M3319                           | YPlac211 with ABCD^{Swi5}EF^{Ace2} expressed from SWI5 promoter |
| M3320                           | YPlac211 with ABCD^{Ace2}EF^{Swi5} expressed from ACE2 promoter |
| M3321                           | YPlac211 with ABCD^{Ace2}EF^{Swi5} expressed from ACE2 promoter |
| M3322                           | YPlac211 with ABCD^{Ace2}EF^{Swi5} expressed from ACE2 promoter |
| M3323                           | YPlac211 with ABCD^{Ace2}EF^{Swi5} expressed from ACE2 promoter |
| M3324                           | YPlac211 with ABCD^{Ace2}EF^{Swi5} expressed from ACE2 promoter |
| M3325                           | YPlac211 with ABCD^{Ace2}EF^{Swi5} expressed from ACE2 promoter |
| M3326                           | 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 |

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

TABLE III

| Yeast strains |
|---------------|
| DY150         | MAT\(\alpha\) ade2 can1 his3 leu2 trp1 ura3 |
| DY141         | MAT\(\alpha\) swi5::hisG ade2 can1 his3 leu2 trp1 ura3 |
| DY1142        | MAT\(\alpha\) ace2::HIS3 ade2 can1 his3 leu2 trp1 ura3 |
| DY1143        | MAT\(\alpha\) swi5::hisG ade2::HIS3 ade2 can1 his3 leu2 trp1 ura3 |
| DY1148        | MAT\(\alpha\) HO-lacZ swi5::TRP1 ade2 can1 his3 leu2 met trp1 ura3 |
| DY1887        | MAT\(\alpha\) LEU2::CTS1-lacZ swi5::HIS3 ade2 can1 his3 leu2 trp1 ura3 |
| DY4497        | MAT\(\alpha\) swi5::hisG ade2::HIS3 URA3::ABCD^{Swi5}EF^{Ace2} adc2 can1 his3 leu2 trp1 |
| DY4498        | MAT\(\alpha\) swi5::hisG ade2::HIS3 URA3::ABCD^{Swi5}EF^{Ace2} adc2 can1 his3 leu2 trp1 |
| DY4499        | MAT\(\alpha\) swi5::hisG ade2::HIS3 URA3::ABCD^{Swi5}EF^{Ace2} adc2 can1 his3 leu2 trp1 |
| DY4500        | MAT\(\alpha\) swi5::hisG ade2::HIS3 URA3::ABCD^{Swi5}EF^{Ace2} adc2 can1 his3 leu2 trp1 |
| DY4501        | MAT\(\alpha\) swi5::hisG ade2::HIS3 URA3::ABCD^{Swi5}EF^{Ace2} adc2 can1 his3 leu2 trp1 |
| DY4502        | MAT\(\alpha\) swi5::hisG ade2::HIS3 URA3::ABCD^{Swi5}EF^{Ace2} adc2 can1 his3 leu2 trp1 |
| DY4503        | MAT\(\alpha\) swi5::hisG ade2::HIS3 URA3::ABCD^{Swi5}EF^{Ace2} adc2 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^{Swi5}EF^{Ace2} and ABCD^{Ace2}EF^{Swi5} (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}CD^{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 AB^{Swi5}C^{Ace2}DEF^{Swi5} 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
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 ABCSwi5DEFAce2 chimera is able to activate both CTS1 (46)-lacZ and HIS4-lacZ equivalently to full-length Swi5, indicating that this chimeric protein 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 Swi5, 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 ABCSwi5DEFAce2 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 ABCSwi5DEFAce2 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 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).

**G1 Genes Activated by Swi5 and Ace2**—There are a number of genes expressed in early G1 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, EG12, 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 EG12 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 Swi5/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, EG12, PCL2,
**Fig. 4.** Chimeras ABC\^Swi5\textsubscript{EF}Ace2\textsubscript{D} are 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 \(\beta\)-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 1).

**Fig. 5.** Chimeras are expressed at wild type levels. Western blotting was used to determine protein levels of strains expressing chimeric fusions 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\textsuperscript{Ace2EF}\textsuperscript{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\textsuperscript{Ace2DEF}\textsuperscript{Swi5} (Fig. 7, line 7) and AB\textsuperscript{Ace2EF}\textsuperscript{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, lane 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. 8A). 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\textsuperscript{Ace2EF}\textsuperscript{Swi5} (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\textsuperscript{Ace2EF}\textsuperscript{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\textsuperscript{Ace2EF}\textsuperscript{Swi5} and ABCD\textsuperscript{Swi5EF}\textsuperscript{Ace2} (lines 5 and 6)
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<sup>Swi5</sup>-EF<sup>Ace2</sup> chimera (line 5) is unable to activate CDC6, while the ABCD<sup>Ace2</sup>-EF<sup>Swi5</sup> 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 G1 of the cell cycle. Along with activating transcription of HO and CTS1 in G1, it has been shown that Swi5 and Ace2 contribute to the activation of a set of genes expressed in G1, 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 G1 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 chimers lacking this region (Figs. 2 and 6). Specific activation of HO requires region D of Swi5, and this HO specificity region of Swi5 interacts 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 affecting 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.
Promoter-specific Activation Domains of Swi5 and Ace2

Figs. 7 and 8. 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.

Figs. 2 and 6. Additionally, the AB_Swi5CAce2DEF_Swi5 chimera shows that this CTS1 specificity region of Ace2 is sufficient for CTS1-specific activation in the context of the Swi5 protein. AB_Swi5CAce2DEF_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_Swi5E_Ace2 chimera activates RME1 better than native Swi5 (Fig. 5), 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_E_Ace2 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 G2 and nuclear localization in M and G1. 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.

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