Determining the Requirements for Cooperative DNA Binding by Swi5p and Pho2p (Grf10p/Bas2p) at the HO Promoter*

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Robert M. Brazas, Leena T. Bhoite, Michael D. Murphy, Yaxin Yu, Yiyou Chen, Deborah W. Neklason, and David J. Stillman§

From the Division of Molecular Biology and Genetics, Department of Oncological Sciences, University of Utah Health Sciences Center, Salt Lake City, Utah 84132

SWI5 encodes a zinc finger DNA binding protein required for the transcription of the Saccharomyces cerevisiae HO gene, and PHO2 encodes a homeodomain DNA binding protein. In vitro biochemical studies using purified Swi5p and Pho2p proteins have demonstrated that Swi5p and Pho2p bind cooperatively to the HO promoter. In this report we investigate the regions of the Swi5p and Pho2p proteins required for cooperative DNA binding. The analysis of each protein gives a similar result: the zinc finger or homeodomain DNA binding domains are each sufficient for in vitro DNA binding, but additional regions of each protein are required for cooperative DNA binding. In vitro and in vivo experiments were conducted with promoters with altered spacing between the Pho2p and Swi5p binding sites. Mutations that increased the distance between the two binding sites had minimal effects on either in vitro cooperative DNA binding or in vivo upstream activating sequence activity. These observations suggest that the interaction domains of Swi5p and Pho2p are flexible and can tolerate an increase in distance between the two binding sites. The mechanism of the cooperative DNA binding by Swi5p and Pho2p is discussed.

The Saccharomyces cerevisiae SWI5 gene was identified as a positive transcriptional regulator of the yeast HO gene (1, 2), which encodes an endonuclease responsible for initiating mating type switching in yeast (3). Transcriptional regulation of HO by SWI5 is highly complex (4, 5). Swi5p binds specifically to two sites within the HO promoter (6, 7). The first, site A, is located approximately 1800 nucleotides upstream of the HO start codon, and the second, site B, is located approximately 1300 nucleotides upstream of the HO start codon. Site B functions as a SWI5-dependent upstream activation sequence in a heterologous promoter reporter plasmid (7, 8). In addition, Tebb et al. (6) have demonstrated that both site A and site B contribute to the SWI5-dependent activation of HO gene transcription. Nasmyth et al. (9) have shown that the localization of Swi5p is cell cycle regulated, and that this cell cycle regulated nuclear localization plays an important role in the transcriptional regulation of HO. The Swi5p protein is located in the cytoplasm during the G2 phase of the cell cycle, and only at the end of mitosis does Swi5p enter the nucleus, where it can activate HO transcription once START has been traversed.

We have analyzed previously the in vitro binding of Swi5p to site A of the HO promoter (8, 10). Swi5p binds to site B cooperatively with an additional protein, Pho2p. In addition, both SWI5 and PHO2 are required for full transcription of an HO-lacZ reporter in vivo (8), suggesting that the cooperative interaction of Swi5p and Pho2p at site B is required for transcription of the HO gene. PHO2 (also known as GRF10 and BAS2) was identified originally as a transcriptional activator of the HO5 gene (11), and it was subsequently shown to also regulate the transcription of the HIS4 and ADE1, 2, 5, 7, and 8 genes (12–14). Notably, Pho2p requires the presence of an additional factor, Pho4p, to activate transcription of PHOS (15), and Pho2p also requires the presence of Bas1p to activate transcription of the HIS4 and ADE genes (12, 14); however, thus far it has not been demonstrated that Pho2p binds cooperatively to the HO5, HIS4, or ADE promoters with either Pho4p or Bas1p.

There are many examples of cooperative interactions between DNA binding proteins, and in many cases cooperative DNA binding is required for transcriptional regulation (16). The cooperative DNA binding of Swi5p and Pho2p to the HO promoter is the first demonstration of cooperative DNA binding by a zinc finger DNA binding protein (Swi5p) and a homeodomain DNA binding protein (Pho2p). We therefore analyzed the in vitro DNA binding characteristics of various Swi5p and Pho2p deletion derivatives in order to define the cooperative interaction domains of each protein.

Each protein requires an additional protein sequence outside of their DNA binding domains to interact at the HO promoter. In order for Swi5p and Pho2p to bind cooperatively to the HO promoter, a region of Swi5p located amino-terminal to its zinc finger domain and a region of Pho2p located carboxyl-terminal to its homeodomain must be present. Promoter mutations have also been constructed with altered spacing between the Swi5p and Pho2p binding sites, and both in vitro and in vivo experiments indicate that there is flexibility in the interaction between Swi5p and Pho2p.

MATERIALS AND METHODS

Plasmid Constructions

The construction of expression plasmids GST-Swi5(275–709), GST-Swi5(384–709), and GST-Swi5(51–709) was described previously (10). The construction of pET-Pho2(35–528)-HIS (M2025) was also described.

1 The abbreviations used are: GST, glutathione-S-transferase; bp, base pair(s); kb, kilobase pair(s); UAS, upstream activating sequence; HIS, 6-histidine tag.

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‡ Predoctoral trainee supported by National Institutes of Health Genetics Training Grant 5 T32 GM07464. Present address: Dept. of Microbiology and Immunology, University of California, San Francisco, CA 94143.

§ To whom correspondence should be addressed. Tel.: 801-581-5429; Fax: 801-581-3607; Internet: stillman@bioscience.utah.edu.
previously (8). Plasmid pCITE-2a was obtained from Novagen.

GST-Swi5(496–709) Expression Plasmid (M1643)—The GST-Swi5(1–709) expression plasmid (M1202) (10) was digested with AFLII and EcoRI to completion, and the 950-bp SWI5 fragment was isolated and ligated into EcoRI-digested pGEX-3X (Pharmacia Biotech Inc.). The nonligated, incompatible ends were blunt-ended with a Klenow fragment and ligated into pBluescript II plasmid M1202 (Novagen). The resulting plasmid was digested partially with XhoI and BamHI, generating the following fragments: GAA GGT GGG ATC CCC GGG AAT TTT AAG TCA CAA. The bold letters represent the SWI5 sequence.

GST-Swi5(1–609) Expression Plasmid (M1536)—The GST-Swi5(1–709) plasmid M1202 was digested partially with Nski. The Nski 3’ overhangs were blunt-ended with Klenow fragment and ligated into EcoRI-digested expression plasmid (M1202) (10) was digested with EcoRI (1–709) plasmid M1202 was digested partially with Nski and HindIII, and the 320-bp fragment containing the PHO2 homeodomain and some polylinker sequences was isolated and ligated into the carboxyl terminus of GST-Swi5(1–609).

pET-Pho2(4–170)-HIS Expression Plasmid (M2067)—The 499-bp BglII-SacI fragment of PHO2 containing the homeodomain sequences was ligated into pBluescript II KS+ (Stratagene) creating M2045. Second, M2045 was digested with BamHI and HindIII, and the 320-bp fragment containing the PHO2 homeodomain and some polylinker sequences was isolated and ligated into BamHI- and HindIII-digested pET-21a (Novagen). The vector: PHO2 amino-terminal junction has the following sequence where the bold letters represent the actual PHO2 sequence: GGT CGC GGA TCC

CGACATCAATTTAAAAACCAGCATGCTAT-3'. The altered XhoI site in the vector and at the unique EcoRV site in PHO2, mixed, and ligated. The resulting plasmid expresses amino acids 1–538 of PHO2. This construct adds 12 amino acids to the amino terminus of PHO2, and 20 amino acids, including the six-histidine residue tag, to the PHO2 carboxyl terminus.

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GST-Swi5(1–595) Expression Plasmid (M2362)—The GST-Swi5(1–595) expression plasmid was constructed as follows. The GST-Swi5(1–709) plasmid M1202 was digested partially with BspHI, blunt-ended with a Klenow fragment and blunt-end-ligated. The mutation at the 5’ overhangs was performed with T4 DNA polymerase, and ligated to delete the internal fragment. The resulting plasmid expresses amino acids 1–595 of PHO2. This construct adds 12 amino acids to the amino terminus of PHO2, and 19 amino acids, including the six-histidine residue tag, to the PHO2 carboxyl terminus.

pET-Pho2(4–170)-HIS Expression Plasmid (M2067)—The 499-bp BglII-SacI fragment of PHO2 containing the homeodomain sequences was ligated into pBluescript II KS+ (Stratagene) creating M2045. Second, M2045 was digested with BamHI and HindIII, and the 320-bp fragment containing the PHO2 homeodomain and some polylinker sequences was isolated and ligated into BamHI- and HindIII-digested pET-21a (Novagen). The vector: PHO2 amino-terminal junction has the following sequence where the bold letters represent the actual PHO2 sequence: GGT CGC GGA TCC

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Fig. 1. Pho2p deletion proteins used in the DNA binding analyses and a summary of those analyses. The amino acid position of each Pho2p deletion is denoted below each deletion figure. The shaded region of Pho2p indicates the homeodomain. Each protein was tested as a Pho2p-HIS fusion protein, in which the histidine tag was fused to the carboxy-terminal residue diagrammed in the figure. The proteins whose expression in E. coli did not produce a stable product are indicated on the right. The results of the DNA binding analyses from Figs. 2 and 3 are displayed on the right.

Table: Pho2p and Swi5p Interaction Domains

| Pho2p deletion | Stable Expression in E. coli | DNA Binding | Cooperative DNA Binding with Swi5p |
|----------------|-----------------------------|-------------|-----------------------------------|
| Pho2(35-526)   | +                           | +           | +                                 |
| Pho2(71-170)   | +                           | +           | -                                 |
| Pho2(4-170)    | +                           | +           | -                                 |
| Pho2(71-526)   | +                           | +           | +                                 |
| Pho2(71-403)   | -                           | -           | -                                 |
| Pho2(71-386)   | -                           | -           | -                                 |
| Pho2(71-368)   | -                           | -           | -                                 |
| Pho2(71-247)   | -                           | -           | -                                 |

Following changes: culture volumes were 250 ml, harvested cells were resuspended in 20 ml of binding buffer before sonication, the washing step was omitted, and the entire extract was loaded onto the HiTrap (Pharmacia) nickel column.

Templates for Pho2p and Swi5p in vitro transcription/translation were linearized by cleavage with SapI and XbaI restriction enzymes, respectively. The Single Tube Protein System (Novagen) was used for in vitro protein expression. The histidine tagged fusion proteins were purified by nickel chelate chromatography on a HiTrap (Pharmacia) column as described (10), except that imidazole was omitted from the binding buffer.

Binding Reactions and Gel Retardation Assays

Unless otherwise indicated, all binding reactions (20 μl volume) used in gel retardation experiments contained the following components: 15 mM Tris (pH 8.0), 75 mM NaCl, 750 μg/ml bovine serum albumin (Sigma), 12.5 mM dithiothreitol, 7.5% glycerol, 50 μg/ml poly(dI-dC)poly(dI-dC) and approximately 1 ng (about 30,000 cpm) of labeled DNA probe (10). The protein composition of the binding reactions and the labeled DNA probe used are described in the figure legends. The gel retardation assays were performed as described previously (10). The DNA probes with altered spacing (the 1-bp mutant, wild type HO sequence, +5-bp mutant, and +10-bp mutant) were prepared as BglII-Nde restriction fragments (labeled at the BglII site) isolated from plasmids M2896, M2642, M2699, and M2761, respectively.

Other Procedures

A Pho2-HIS fusion protein was expressed in Escherichia coli from plasmid M2025, purified by nickel column chromatography, and used to immunize rabbits (HRP Inc., Denver, PA.). Western blots were incubated with the anti-Pho2p sera and visualized with the enhanced chemiluminescence kit (ECL, Amersham Corp.). Yeast strains DY3730 (HMLa ade2-1 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1), DY3734 (HMRa ade2-1 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1), DY3939 (SWI5), and DY4032 (SWI5Δ111), DY3773 (SWI5Δ157), and DY4035 (SWI5Δ43) are isogenic in the K1107 background; other markers include HO ace2::HIS3 HIS1 HMRa HMLa ade2-1 ade6 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1. Yeast strain DY2001 (MATa ace2::HIS3 ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1) is in the W303 background. The Hi lithium method (21) was used for yeast transformations. Cells were grown in synthetic complete medium (22) supplemented with adenine and the appropriate amino acids, but lacking tryptophan and/or uracil, to select for plasmids, as described in the figure legends. Quantitative determinations of β-galactosidase activity were performed in triplicate as described previously (2). Computer-generated images of autoradiographs were generated with Adobe Photoshop.

RESULTS

Pho2p, a homeodomain protein, and Swi5p, a zinc finger protein, bind cooperatively to the HO promoter (8, 10). However, the two proteins do not interact detectably in the absence of DNA (10). In order to identify specific regions of these two proteins required for cooperative DNA binding, we constructed deletion derivatives which were expressed and purified. A gel retardation assay was used to determine (i) whether each deletion protein could bind DNA on its own, and (ii) whether it could bind DNA cooperatively.

DNA Binding by Pho2p Deletions—The first four Pho2p deletion derivatives diagrammed in Fig. 1 were constructed and expressed in E. coli and purified by nickel column chromatography (23). These Pho2-HIS deletion proteins were tested for their ability to bind DNA on their own and in a cooperative fashion with Swi5p (Fig. 2). Increasing amounts of each Pho2-HIS protein were incubated with a probe in the presence or absence of added HIS-Swi5 protein. Each of the four Pho2-HIS deletion proteins bound the probe in the absence of added HIS-Swi5 protein (lanes 2–4, 8–10, 16–18, and 22–24). When increasing amounts of the Pho2(35–528)-HIS protein were used (lane 5), this increase in ternary complex formation compared to the binary complex formation was much greater than the amount of Pho2(35–528)-HIS protein used (lanes 2–4, 8–10, 16–18, and 22–24). When increasing amounts of the Pho2(35–528)-HIS protein were used (lane 5), the slower migrating ternary complex composed of HIS-Swi5 protein and Pho2(35–528)-HIS was formed as described previously (8). This ternary complex formed at the lowest concentration of Pho2(35–528)-HIS protein used (lane 5), and the amount of ternary complex formed was much greater than the amount of binary complex formed by either HIS-Swi5 or Pho2(35–528)-HIS only (compare lanes 2, 5, and 14). This increase in ternary complex formation compared to the binary complex formation demonstrates the cooperative DNA binding of these two proteins, as described previously (8, 10). In contrast, the Pho2(71–170)-HIS protein did not bind cooperatively with HIS-Swi5 to the probe. In this case, the addition of Pho2(71–170)-HIS to the binding reactions containing HIS-Swi5 led to the formation of two separate binary complexes: the HIS-Swi5DNA or
Pho2p deletion derivatives were cloned into the pCITE-2a expression vector and expressed in E. coli. The Pho2-HIS deletion derivatives were expressed as histidine tagged fusion proteins and purified by nickel column chromatography (23). In order to visualize the amount of various Pho2p deletion derivatives produced in the coupled transcription/translation reactions, parallel in vitro transcription/translation experiments were performed in the presence of [35S]methionine to label the proteins. The 35S-labeled fusion proteins were then purified on nickel columns and visualized by SDS-PAGE and autoradiography (Fig. 3A). In contrast to the E. coli expression experiments, stable proteins were produced by in vitro translation of the Pho2(71–403)-HIS, Pho2(71–386)-HIS, and Pho2(71–368)-HIS deletion derivatives.

The unlabeled, purified, in vitro translated Pho2-HIS proteins were tested for their ability to bind DNA (Fig. 3B) and to bind DNA cooperatively with full-length HIS-Swi5 using a gel retardation assay (Fig. 3C). The results are similar to those observed with the E. coli-expressed proteins. The Pho2(35–528)-HIS and Pho2(71–528)-HIS proteins are both capable of binding to DNA on their own (Fig. 3B, lanes 7 and 8), and of binding cooperatively with HIS-Swi5 (Fig. 3C, lanes 15 and 16). The Pho2(71–403)-HIS protein can bind DNA (Fig. 3B, lane 12), but is unable to interact with HIS-Swi5 (Fig. 3C, lane 20). The Pho2(71–403)-HIS, Pho2(71–386)-HIS, and Pho2(71–368)-HIS deletion derivatives were unable to bind DNA alone (Fig. 3B, lanes 9–11) or cooperatively with HIS-Swi5 (Fig. 3C, lanes 17–19). A smaller deletion derivative, Pho2(71–247)-HIS, was similarly defective in both DNA binding assays (data not shown). These results show that only the Pho2p homeodomain (Pho2(4–170)-HIS) or a Pho2p derivative containing the entire carboxyl-terminal region (Pho2(71–528)-HIS) are unable to bind DNA alone or cooperatively with Swi5p.

DNA Binding by Swi5p Deletions—The same experiments outlined above were performed using Swi5p deletion proteins. The six different Swi5p deletion derivatives diagrammed in Fig. 4 were expressed in E. coli and purified by glutathione-agarose chromatography (25). Each GST-Swi5 deletion protein

FIG. 2. The carboxyl-terminal region of E. coli produced Pho2 derivatives is required for cooperative DNA binding with Swi5p. Various Pho2-HIS deletion proteins were analyzed for their ability to bind cooperatively with the HIS-Swi5(1–709) protein to the HO promoter fragment probe using a gel retardation assay. The following amounts of HIS-Swi5 protein were added to each binding reaction: lanes 1, 14, 15, and 28, no protein; lanes 2 and 5, 0.007 μl of Pho2(35–528)-HIS; lanes 3 and 6, 0.021 μl of Pho2(35–528)-HIS; lanes 4 and 7, 0.063 μl of Pho2(35–528)-HIS; lanes 8 and 11, 0.007 μl of Pho2(71–170)-HIS; lanes 9 and 12, 0.021 μl of Pho2(71–170)-HIS; lanes 10 and 13, 0.063 μl of Pho2(71–170)-HIS; lanes 16 and 19, 0.0007 μl of Pho2(4–170)-HIS; lanes 17 and 20, 0.0021 μl of Pho2(4–170)-HIS; lanes 18 and 21, 0.0063 μl of Pho2(4–170)-HIS; lanes 22 and 25, 0.04 μl of Pho2(71–528)-HIS; lanes 23 and 26, 0.12 μl of Pho2(71–528)-HIS; and lanes 24 and 27, 0.36 μl of Pho2(71–528)-HIS. The amount of HIS-Swi5(1–709) added, where indicated, was 1.3 μl. The CS169 DNA probe was used.

Pho2(71–170)-HIS:DNA complexes (compare lanes 11–13 to lanes 8–10 and 14). These results demonstrate that the Pho2p homeodomain is sufficient for Pho2p DNA binding activity, as demonstrated previously (24), but they also demonstrate that the homeodomain is not sufficient for cooperative DNA binding with Swi5p.

Two additional Pho2p deletion derivatives were used in gel retardation experiments to test whether the carboxy-terminal or the carboxyl-terminal region of Pho2p was required for interaction with Swi5p. The first, Pho2(4–170)-HIS, contains the homeodomain plus the amino-terminal portion of the Pho2p protein. The second, Pho2(71–528)-HIS, contains the homeodomain plus the carboxyl-terminal portion of the Pho2p protein. The Pho2(4–170)-HIS protein displayed DNA binding characteristics similar to those seen for the Pho2(71–170)-HIS protein (Fig. 2, lanes 16–21). The Pho2(4–170)-HIS protein was unable to bind cooperatively with HIS-Swi5 to the HO promoter probe (compare lanes 19–21 to lanes 16–18 and 28). The Pho2(71–528)-HIS protein, however, behaved quite differently. The addition of the Pho2(71–528)-HIS protein to the binding reactions containing HIS-Swi5 led to the formation of the slower migrating ternary complex (lanes 25–27), and the ternary complex again formed at the lowest concentration of added Pho2(71–528)-HIS (lane 25). Therefore, the Pho2(71–528)-HIS protein also binds cooperatively with HIS-Swi5 to the HO promoter in vitro. These results demonstrate that the carboxyl-terminal portion of Pho2p between amino acids 170 and 528 is required for cooperative interaction with Swi5p.

We made a number of additional plasmid constructions that express Pho2p deletions with carboxyl-terminal end points between amino acids 170 and 528, in an attempt to define more precisely the region of Pho2p that interacts with Swi5p (Fig. 1). However, none of these protein products was stable when expressed in E. coli. This result suggests that the carboxyl-terminal region of Pho2p is folded into one specific domain, and that removal of part of this domain leads to the production of an unstable protein.

To overcome this problem of unstable Pho2p proteins in E. coli, we used an in vitro transcription/translation system to prepare additional Pho2p deletion proteins. Restriction fragments encoding the full-length Swi5p protein and various Pho2-HIS deletion derivatives were done into the pCITE-2a expression vector and expressed in vitro. The Pho2-HIS deletion derivatives were expressed as histidine tagged fusion proteins and purified by nickel column chromatography (23). In order to visualize the amount of various Pho2p deletion derivatives produced in the coupled transcription/translation reactions, parallel in vitro transcription/translation experiments were performed in the presence of 35S]methionine to label the proteins. The 35S-labeled fusion proteins were then purified on nickel columns and visualized by SDS-PAGE and autoradiography (Fig. 3A). In contrast to the E. coli expression experiments, stable proteins were produced by in vitro translation of the Pho2(71–403)-HIS, Pho2(71–386)-HIS, and Pho2(71–368)-HIS deletion derivatives.

The unlabeled, purified, in vitro translated Pho2-HIS proteins were tested for their ability to bind DNA (Fig. 3B) and to bind DNA cooperatively with full-length HIS-Swi5 using a gel retardation assay (Fig. 3C). The results are similar to those observed with the E. coli-expressed proteins. The Pho2(35–528)-HIS and Pho2(71–528)-HIS proteins are both capable of binding to DNA on their own (Fig. 3B, lanes 7 and 8), and of binding cooperatively with HIS-Swi5 (Fig. 3C, lanes 15 and 16). The Pho2(71–403)-HIS protein can bind DNA (Fig. 3B, lane 12), but is unable to interact with HIS-Swi5 (Fig. 3C, lane 20). The Pho2(71–403)-HIS, Pho2(71–386)-HIS, and Pho2(71–368)-HIS deletion derivatives were unable to bind DNA alone (Fig. 3B, lanes 9–11) or cooperatively with HIS-Swi5 (Fig. 3C, lanes 17–19). A smaller deletion derivative, Pho2(71–247)-HIS, was similarly defective in both DNA binding assays (data not shown). These results show that only the Pho2p homeodomain (Pho2(4–170)-HIS) or a Pho2p derivative containing the entire carboxyl-terminal region (Pho2(71–528)-HIS) are competent for DNA binding. Deletion derivatives with a truncated carboxyl-terminal region (Pho2(71–403)-HIS, Pho2(71–386)-HIS, and Pho2(71–368)-HIS) are unable to bind DNA alone or cooperatively with Swi5p.

DNA Binding by Swi5p Deletions—The same experiments outlined above were performed using Swi5p deletion proteins. The six different Swi5p deletion derivatives diagrammed in Fig. 4 were expressed in E. coli and purified by glutathione-agarose chromatography (25). Each GST-Swi5 deletion protein
Not every GST-Swi5 protein that was able to bind DNA alone was able to bind DNA cooperatively with Pho2p. When increasing amounts of GST-Swi5(1–709) were incubated with a set amount of Pho2(35–528)-HIS protein, a slower migrating ternary complex was detected (Fig. 5, compare lanes 5–7 to lanes 2–4 and 14). This ternary complex was evident at the lowest concentration of GST-Swi5(1–709) protein used, and the amount of ternary complex formed was much greater than the amount of binary complex formed by either GST-Swi5(1–709) or Pho2(35–528)-HIS alone (compare lanes 2, 5, and 14). The increase in ternary complex formation compared to binary complex formation demonstrates the cooperative DNA binding by these two proteins. The GST-Swi5(275–709) and GST-Swi5(384–709) also bound to the probe cooperatively with Pho2(35–528)-HIS (Fig. 5, lanes 8–14 and 16–21). The GST-Swi5(496–709) protein did not bind cooperatively to the HO promoter probe with Pho2(35–528)-HIS (lanes 22–27). Although one can barely detect a ternary complex in lanes 25–27, the level of the signal is not above the level of the binary complexes. This ternary complex is most easily explained as the additive probability of occupancy by independent protein-DNA interactions, although it is possible that a weak interaction occurred between Pho2(35–528)-HIS and GST-Swi5(496–709) producing this ternary complex. As seen in Fig. 6, the carboxyl-terminal deletion protein GST-Swi5(1–609), which lacks its carboxyl terminus in addition to its third zinc finger domain (see Fig. 6), was also able to bind cooperatively to the HO promoter with Pho2(35–528)-HIS (Fig. 5, compare lanes 11–13 to 8–10 and 20). The GST-Swi5(1–595) protein was unable to bind DNA on its own (Fig. 6, lanes 14–16), and it was also unable to bind to the HO promoter cooperatively with Pho2(35–528)-HIS (Fig. 6, lanes 14–19). In summary, we conclude that amino acids 1–384 and 610–709 of Swi5p are not required for cooperative interaction with Pho2p, suggesting that residues 384–609 may be sufficient for cooperativity.

Activity of Deletions in Vivo—We have shown previously the Pho2p and Swi5p binding sites from the HO promoter function together as an upstream activating sequence (UAS) when inserted into the heterologous CYC1 promoter (8). The transcriptional activity of this UAS is sharply reduced in either a pho2 or a swi5 mutant (8), and thus the HO(31)-CYC1-lacZ construct acts as an in vivo reporter dependent on cooperative interactions between Pho2p and Swi5p.

Tebb et al. (6) constructed a series of Swi5p deletions and tested their ability to activate HO transcription in vivo. They demonstrated that the DNA binding domain of Swi5p is essential for activity. The large region of Swi5p NH2-terminal to the DNA binding domain is required for activity, but it appears there is redundancy within this region. To test the ability of specific Swi5p NH2-terminal deletions to activate HO transcription in vivo, they constructed a plasmid reporter with the HO promoter together with Swi5p deletion mutants on a multi-copy plasmid. They found that the HO promoter was activated by Swi5p, but that the NH2-terminal deletions showed varying degrees of activity. The HO(31)-CYC1-lacZ reporter plasmid, and promoter activity were determined by the extent of β-galactosidase activity. The results in Fig. 7 show that the Δ30, Δ11, and Δ57 deletion derivatives activate the reporter as well or better than wild type. The Swi5pΔ141 mutant, however, which lacks amino acids 370–465, shows a significant defect in transcriptional activation of this reporter. We have constructed a plasmid reporter containing only Swi5p binding sites that is independent of PHO2; importantly, the Δ141 mutant is completely effective in activating this PHO2-independent promoter.

Comparison of the Δ57 and Δ41 mutants suggests that the region between amino acids 421 and 465 is needed for full activation of the reporter, and this region could play a role in Pho2p interaction. The in vitro analysis of NH2-terminal dele-
tion mutants suggested that amino acids 384 to 609 of Swi5p are required for cooperative DNA binding with Pho2p. Thus, the in vitro and in vivo results are consistent. However, in addition to the region 421–465, amino acids downstream of residue 465 in Swi5p may also contribute to Pho2p interaction, as the Swi5p\textsuperscript{D41} mutant still has residual activity.

We also determined whether deletion derivatives of Pho2p could complement a pho2 mutant in vivo. The PHO2 gene was cloned into a yeast CEN plasmid, and various plasmids were constructed which express COOH-terminal truncated versions of Pho2p. These PHO2 plasmids, or the vector control, were transformed into a pho2 mutant strain carrying the HO\textsuperscript{(31)}-CYC1-lacZ reporter. All of the COOH-terminal truncations were defective for activation of the Pho2p/Swi5p-dependent promoter (data not shown).

PHO2 (5\textsuperscript{GRF10}-5\textsuperscript{BAS1}) functions as an activator of basal transcription of the HIS4 gene\textsuperscript{(12)}. In addition to the basal pathway of HIS4 expression, there is an inducible pathway which requires the Gcn4p transcriptional activator. In a gcn4 mutant HIS4 expression is completely dependent upon PHO2, and thus we can use histidine prototrophy in a gcn4 mutant as an assay for Pho2p function in vivo. All of the COOH-terminal truncation mutants were unable to support growth of a gcn4 mutant in the absence of exogenous histidine (data not shown). Thus, these PHO2 truncation mutants are defective in HIS4 transcriptional activation.

Spacing Requirements for Cooperative Binding—In previous experiments we used methylation interference and missing nucleoside interference methods to identify nucleotide residues at the HO promoter that are essential for DNA binding by Swi5p and Pho2p\textsuperscript{(10)}. We can now use these data on protein-DNA contacts, along with structural studies performed on zinc finger and homeodomain DNA binding proteins, to predict which specific regions of Swi5p and Pho2p would be near each other in the protein-DNA complex. However, we must first orient each protein along the DNA.

Fig. 8 presents a summary of the DNase I protection and the methylation and missing nucleoside interference data (from Brazas and Stillman\textsuperscript{(8, 10)}) displayed on a DNA projection. Swi5p and Pho2p bind two distinct, but adjacent, regions of DNA.
DNA. Crystallographic and NMR studies of homeodomain proteins demonstrate that the homeodomain contains three α helices, including a helix-turn-helix motif which contacts DNA in the major groove (27–30). In addition, homeodomains utilize an amino-terminal arm adjacent to helix one to contact DNA in the minor groove. Since the methylation interference data places the Pho2p minor groove contacts closest to the Swi5p binding site, we have oriented Pho2p such that the amino-terminal arm points toward the Swi5p binding site. This placement sets the DNA-contacting helix three of Pho2p in the major groove, consistent with the major groove contact observed in the methylation interference experiment. The illustration below the DNA projection shows this orientation of Pho2p. We have used the data of Nakaseko et al. (26) to orient Swi5p on the HO promoter. They synthesized two different derivatives of Swi5p in E. coli. The first derivative contains all three zinc finger domains, while the second derivative contains only fingers one and two. They performed DNase I protection experiments using these proteins and an HO DNA probe. The three-finger protein protects a region which is very similar to the footprint we obtained (8). However, the two-finger protein protected only two-thirds of that region, with the 3′ portion of the footprint missing. Based on these results we can place the first zinc finger of Swi5p at the 5′ end of the Swi5p binding site and the second and third fingers toward the 3′ end of the binding site, as shown in the illustration (Fig. 8).

We used computer imaging to examine potential interactions between Swi5p and Pho2p as they bind to DNA (data not shown). We utilized the protein-DNA coordinates from the engrailed homeodomain-DNA (29) and the Zif268 zinc finger-DNA (31) co-crystals and placed them on a single computer generated B-DNA helix based on their DNA contact data. The computer model shows that the carboxyl-terminal region of Pho2p, extending from homeodomain helix 3, is on the same side of the DNA helix as the region amino-terminal to Swi5p finger one. Thus, the computer model of Swi5p and Pho2p bound to DNA, based on protein-DNA co-crystals of homologous DNA binding domains, suggests that the interacting regions of the two proteins are well positioned for cooperative protein-protein interactions.

In addition to their DNA binding domains, Swi5p and Pho2p each contain a region important for cooperative interactions. However, it is not clear for either protein whether the DNA binding domain and the interaction region are part of one rigid structure, or whether they are in distinct domains separated by a flexible linker. To address this question we tested the ability of Swi5p and Pho2p to interact when the spacing between their binding sites was altered. If the domains interact in a rigid manner, then changing the spacing between the two binding sites by 5 bp, approximately half of a helical turn, should block cooperative binding. Alternatively, if flexibility between the DNA binding and interaction domains occurs, then a spacing change should not interfere with cooperative binding.

The spacing between the Swi5p and Pho2p binding sites was altered by −5, +2, +5, and +10 bp, and the effect of these spacing changes on the activity of the HO(46)-CYC1-lacZ reporter was assayed in vivo (Fig. 9A). The greatest effect on promoter activity was seen when the spacing was changed by −5 bp. This spacing change resulted in a complete loss of promoter activity, possibly due to the binding sites being so close together that both sites cannot be occupied simultaneously due to steric hindrance. Varying effects on promoter activity were seen when the spacing between the two binding sites was increased. Increasing the spacing by 5 or 10 bp resulted in only a slight loss in promoter activity, while the 2-bp change resulted in a slightly greater decrease in promoter activity. Swi5p and Pho2p are required for activation of the −2, +5, or +10-bp promoter constructs, since promoter activity is lost in swi5 or pho2 mutants (data not shown). The results from the mutants with increased spacing suggest that the cooperative interaction domains of Swi5p and Pho2p are quite flexible and can interact over varied distances.

The in vivo experiments with promoters with various spacing changes suggested flexibility in the Swi5p and Pho2p interaction, and we wished to determine whether this flexibility could be seen in vitro. Therefore in vitro DNA binding experiments were performed on these altered binding sites to characterize the effect of spacing changes on cooperative DNA binding by Swi5p and Pho2p. Gel retardation experiments were conducted with DNA probes that were prepared from the −5-bp, wild type, and +5- and +10-bp binding sites (Fig. 9B). In these experiments, a small amount of Swi5p protein was used such that minimal Swi5p-DNA complexes could be detected in the absence of Pho2p (lanes 8, 16, 24, and 32). Addition of Pho2p to this small amount of Swi5p led to the efficient formation of the ternary Swi5p-Pho2p-DNA complex (lanes 13–15, 21–23, and 29–31) for the wild type and +5- and +10-bp probes, but not for the −5-bp probe (lanes 5–7). A different preparation of Swi5p, which contains some truncated Swi5p species, was used for this experiment. The multiple Swi5p proteins produce multiple bands in the gel shift assay, and thus it is difficult to distinguish the Swi5p/Pho2p/DNA bands resulting from cooperative binding. However, cooperative DNA binding leads to more efficient binding by proteins, which can be seen by examining the amount of the free DNA probe unbound by protein. Incubation of both Swi5p and Pho2p leads to a significant decrease in free DNA probe for the wild type and +5- and +10-bp probes (lanes 13–15, 21–23, and 29–31), indicating cooperativity in binding. A decrease in the free DNA probe is not seen with the −5-bp probe (lanes 5–7), suggesting the lack of cooperativity. However, this mutation may somewhat reduce the affinity of Swi5p (lane 8), resulting indirectly in decreased cooperative binding. Thus, both the in vivo and in vitro experiments demonstrate that the spacing between the Swi5p and Pho2p binding sites can be increased without affecting cooperative interactions.
DISCUSSION

The Swi5p zinc finger protein and the Pho2p homeodomain protein bind to the HO promoter cooperatively, but they do not interact in the absence of DNA (8, 10). In this report we have used deletion derivatives to map regions of each protein that are required for cooperative DNA binding in vitro. Swi5p requires amino acids 384–545, a region located amino-terminal to the first zinc finger domain, for cooperative DNA binding.

FIG. 7. In vivo analysis of Swi5p deletions. The amino acids missing from each Swi5p deletion mutant are indicated. The strain designated as "no Swi5p" bears a swi5::TRP1 gene deletion. Isogenic yeast strains with the indicated SWI5 allele (at the SWI5 chromosomal locus) were transformed with either the HO(31)-CYC1-lacZ reporter plasmid M1853 or the CTS1(46)-CYC1-lacZ reporter plasmid M1912. The HO(31)-CYC1-lacZ reporter is PHO2-dependent (8), and the CTS1(46)-CYC1-lacZ reporter is PHO2-independent (18). Cells were grown in the absence of uracil to maintain the plasmid, and extracts were prepared from log phase cells to measure lacZ activity. Strains DY3730 (SWI5+), DY3734 (swi5::TRP1), DY3939 (SWI5Δ30), DY4032 (SWI5Δ11), DY3773 (SWI5Δ57), and DY4035 (SWI5Δ41) were used. n.d. indicates not determined.

FIG. 8. Pho2p and Swi5p on a DNA projection. The upper part of the figure shows the sequence of the HO promoter and indicates the residues important for DNA binding. The methylation interference and hydroxyl radical interference data are from Brazas and Stillman (10). The binding interference data are displayed on a helical projection of DNA. The DNase I footprint data are from Brazas and Stillman (8). The illustration of a homeodomain and the zinc finger domains is shown below, indicating the relative placement and orientation of the two proteins. See text for discussion.
In vivo analysis of SWI5 mutants with internal deletions suggests that amino acids 421–465 are required for full activation of a Pho2p/Swi5p-dependent promoter. Moreover, the ability of Swi5p to bind DNA on its own is a necessary prerequisite for cooperative interactions with Pho2p. Pho2p requires a region located carboxyl-terminal to its homeodomain in order to interact with Swi5p at the HO promoter. Thus, for each of these proteins, the DNA binding domain is not sufficient for cooperative interactions.

The region of Pho2p defined by the initial deletion experiments required for cooperative interactions with Swi5p is quite large. In order to more precisely map the Swi5p interaction domain of Pho2p, we constructed other deletion derivatives with carboxyl-terminal end points between amino acids 170 and 528. However, these Pho2p derivatives were unstable in E. coli, suggesting that this region may not be properly folded leading to degradation of the protein. In order to circumvent the in vivo instability of these Pho2p deletions, the deletion derivatives of Pho2p were synthesized using a coupled in vitro transcription/translation system. However, these proteins were still unable to bind DNA, either on their own or in conjunction with Swi5p. This result, in conjunction with the fact that the minimal Pho2p homeodomain is stable and binds DNA, suggests strongly that the Pho2p deletion derivatives with carboxyl-terminal end points between amino acids 170 and 525 do not fold properly. Thus, the region between 170 and 528 that interacts with Swi5p may form a specific protein domain, and deletion end points within this region may fold aberrantly.

The cooperative DNA binding of Swi5p and Pho2p to the HO promoter is the first demonstration of cooperative DNA binding between a zinc finger DNA-binding protein and a homeodomain protein. However, there are a number of examples of cooperative interactions involving homeodomain proteins, and it is apparent that cooperative DNA binding by homeodomain proteins plays a critical role in gene regulation in vivo. Although most homeodomain proteins show very similar DNA binding properties in vitro, work in Drosophila melanogaster clearly demonstrates that these proteins regulate different genes in vivo (32, 33). It is believed that the cooperative interactions with other DNA-binding proteins determine the specificity of promoter recognition for homeodomain proteins in vivo.

Regions of homeodomain proteins required for cooperative interactions have been identified. The Phox1 homeodomain

Fig. 9. Increased spacing does not block cooperative binding. A, strain DY2001 was transformed with either the pYCl7 vector without a UAS insert, or the indicated HO(46)-CYC1-lacZ plasmid. Cells were grown in the absence of uracil to maintain the plasmid and extracts were prepared from log phase cells to measure lacZ activity. Plasmid pYC7 contains the lacZ gene under the control of the CYC1 promoter, but lacking a UAS. The wild-type plasmid contains an insert of 46 nucleotides from the HO promoter containing the Swi5p and Pho2p binding sites. Site-directed mutagenesis was used to create plasmids with the indicated spacing changes between the Swi5p and Pho2p binding sites. B, DNA probes containing either the −5 bp mutant, the wild type HO sequence, the +5 bp mutant, or the +10 bp mutant, were analyzed for cooperative binding by HIS-Swi5p and Pho2p(35–528)-HIS. Increasing amounts of Pho2p(35–528)-HIS was added to reactions, as indicated, at one of the following amounts: 0.35, 1.16, or 3.5 µg. The amount of HIS-Swi5p added, where indicated, was 0.25 µg. The preparations of the HIS-Swi5p and Pho2p(35–528)-HIS proteins expressed in E. coli used in this experiment were different from the other figures. The position of the free DNA probe is indicated.

Swi5p and Pho2p Interaction Domains
β-sheet that is formed by amino acid residues amino-terminal to the first finger. This additional strand is necessary for complete DNA binding activity of the Swi5p zinc finger protein (26). Similar regions amino-terminal to the zinc fingers of the Drosophila Tramtrak and yeast Adr1p proteins have also shown to be essential for their complete DNA binding activities (18, 46, 47). One interesting hypothesis is that Pho2p interacts with Swi5p through this β-strand. It is possible that other zinc finger DNA-binding proteins also contain this additional β-strand, and thus this region may represent a common motif important for interactions of zinc finger proteins with other DNA-binding proteins.

In this study we have identified regions of Swi5p and Pho2p that are required for cooperative interactions. We suggest that at least one of the two proteins, probably Pho2p, possesses a flexible linker region between its DNA binding domain and the interaction domain such that interaction with Swi5p can be maintained over varying distances. Further mutational analyses will be required to more precisely map the actual interaction surfaces of Swi5p and Pho2p.

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REFERENCES

1. Stern, M., Jensen, R., and Herskowitz, I. (1984) J. Mol. Biol. 178, 853–868
2. Breeden, L., and Nasmyth, K. (1987) Cell 48, 389–397
3. Kostrikien, R., Strathern, J. N., Klar, A. J. S., Hicks, J. B., and Hinnen, F. (1983) Cell 35, 167–174
4. Nasmyth, K. (1993) Curr. Opin. Genet. Dev. 3, 286–294
5. Herskowitz, I., Andrews, B., Kruger, W., Ogas, J., Sil, A., Coburn, C., and Peterson, C. (1992) in Transcriptional Regulation (McKnight, S. L., and Yanamandra, K. R., eds) pp. 949–974, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
6. Tebb, G., Moll, T., Dowser, C., and Nasmyth, K. (1993) Genes & Dev. 7, 517–528
7. Stillman, D. J., Bankier, A. T., Seddon, A., Groenhout, E. G., and Nasmyth, K. A. (1988) EMBO J 7, 485–494
8. Braza, R. M., and Stillman, D. J. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 11237–11241
9. Nasmyth, K., Adolph, G., Lydall, D., and Seddon, A. (1990) Cell 62, 631–647
10. Braza, R. M., and Stillman, D. J. (1993) Mol. Cell. Biol. 13, 5524–5537
11. Oshima, Y. (1983) in The Molecular Biology of the Yeast Saccharomyces cerevisiae Metabolism and Gene Expression (Strathern, J. N., Jones, E. W., and Broach, J. R., eds) pp. 159–180, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
12. Ahmad, K. T., Style, C., and Fink, G. R. (1987) Science 237, 874–880
13. Braus, G., Mosch, H. U., Vogel, K., Hinnen, A., and Hutter, R. (1989) EMBO J 8, 939–945
14. Daigain-Forier, B., and Fink, G. R. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 6746–6750
15. Vogel, K., Horz, W., and Hinnen, A. (1989) Mol. Cell. Biol. 9, 2050–2057
16. Macknight, S. L., and Yanamandra, K. R. (eds) (1993) Transcriptional Regulation, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
17. Guarente, L., Lalonde, B., Gifford, P., and Alani, E. (1984) Cell 36, 503–511
18. Thukral, S., Eise, A., and Young, E. T. (1991) Mol. Cell. Biol. 11, 1566–1577
19. Chang, Y. C., and Timberlake, W. E. (1993) Genetics 133, 29–38
20. Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. E., Sadowsky, J. G., Smith, J. A., and Struhl, K. (1987) Current Protocols in Molecular Biology, pp. 8.1.1–8.1.6, Wiley & Sons, New York
21. Jiang, Y. W., and Stillman, D. J. (1995) Genetics 140, 103–114
22. Rine, J., Dyer, M. D., Winston, F., and Hayer-Hartline, W. (1991) Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
23. Smith, M. C., Furman, T. C., Ingolia, T. D., and Pidgeon, C. (1988) J. Biol. Chem. 263, 7212–7215
24. Tice-Baldwin, K., Fink, G. R., and Arndt, K. T. (1989) Science 246, 931–935
25. Smith, D. B., and Johnson, K. S. (1988) Genes & Dev. 13, 24–40
26. Nakatani, Y., Neuhaus, D., Klug, A., and Rhodes, D. (1992) J. Mol. Biol. 228, 619–636
27. Phillips, C. L., Veronos, A. K., Johnson, A. D., and Dahlquist, F. W. (1991) Genes & Dev. 5, 764–772
28. Wolberger, C., Veronos, A. K., Liu, B., Johnson, A. D., and Pabo, C. O. (1991) Cell 67, 517–528
29. Kissinger, C. R., Liu, B., Martin-Blanco, E., Kornberg, T. B., and Pabo, C. O. (1990) Cell 63, 579–590
30. Ottling, G., Qian, Y., Müller, M., Affholter, M., Gehring, W., and Wüthrich, K. (1988) EMBO J 7, 4305–4309
31. Pavletich, N. P., and Tschajov, O. (1991) Science 252, 809–817
32. Hayashi, S., and Scott, M. P. (1990) Cell 63, 883–894
33. Laughon, A. (1991) Biochemistry 30, 11357–11372
34. Grueneberg, D. A., Natesan, S., Alexandre, C., and Gilman, M. Z. (1992) Science 257, 1089–1095
35. Xue, D., Tu, Y., and Chalfie, M. (1993) Science 261, 1324–1328
36. Galang, C. K., and Hauser, C. A. (1993) Mol. Cell. Biol. 13, 4609–4617
37. Mendel, D. B., Hansen, L. P., Graves, M. K., Conley, P. B., and Crabtree, G. R. (1993) Genes & Dev. 7, 1042–1056
38. Johnson, A. (1992) in Transcriptional Regulation (McKnight, S. L., and Yamamoto, K. R. (eds) pp. 975-1006, Cold Spring Harbor, NY
39. Mak, A., and Johnson, A. D. (1993) Genes & Dev. 7, 1862–1870
40. Vershon, A. K., and Johnson, A. D. (1993) Cell 72, 105–112
41. Treisman, R., Marais, R., and Wynne, J. (1992) EMBO J. 11, 4631–4640
42. Thompson, C. C., Brown, T. A., and McKnight, S. L. (1991) Science 253, 762–768
43. Neuhaus, D., Nakaseko, Y., Schwabe, J. W. R., and Klug, A. (1992) J. Mol. Biol. 228, 637–651
44. Lee, M. S., Gippert, G. P., Soman, K. V., Case, D. A., and Wright, P. E. (1989) Science 245, 635–637
45. Klevit, R. E., Herrick, J. R., and Horvath, S. J. (1990) Proteins 7, 215–226
46. Camier, S., Kacherovsky, N., and Young, E. T. (1992) Mol. Cell. Biol. 12, 5758–5767
47. Fairall, L., Harrison, S. D., Travers, A. A., and Rhodes, D. (1992) J. Mol. Biol. 226, 349–366
Determining the Requirements for Cooperative DNA Binding by Swi5p and Pho2p (Grf10p/Bas2p) at the HO Promoter
Robert M. Brazas, Leena T. Bhoite, Michael D. Murphy, Yaxin Yu, Yiyou Chen, Deborah W. Neklason and David J. Stillman

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