The Coiled Coil Dimerization Element of the Yeast Transcriptional Activator Hap1, a Gal4 Family Member, Is Dispensable for DNA Binding but Differentially Affects Transcriptional Activation*

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The heme activator protein Hap1 is a member of the yeast Gal4 family, which consists of transcription factors with a conserved Zn₂Cys₆ cluster that recognizes a CGG triplet. Many members of the Gal4 family contain a coiled coil dimerization element and bind symmetrically to DNA as homodimers. However, Hap1 possesses two unique properties. First, Hap1 binds asymmetrically to a direct repeat of two CGG triplets. Second, Hap1 binds to two classes of DNA elements, UAS₁/CYC1 and UAS/CYC7, and permits differential transcriptional activation at these sites. Here we determined the residues of the Hap1 dimerization domain critical for DNA binding and differential transcriptional activation. We found that the Hap1 dimerization domain is composed of functionally redundant elements that can substitute each other in DNA binding and transcriptional activation. Remarkably, deletion of the coiled coil dimerization element did not severely diminish DNA binding and transcriptional activation at UAS₁/CYC1 but completely abolished transcriptional activation at UAS/CYC7. Furthermore, Ala substitutions in the dimerization element selectively diminished transcriptional activation at UAS/CYC7. These results strongly suggest that the coiled coil dimerization element is responsible for differential transcriptional activation at UAS₁/CYC1 and UAS/CYC7 and for making contacts with a putative coactivator or part of the transcription machinery.

Many of these Zn₂Cys₆ cluster proteins, such as Gal4, Ppr1, and Put3, bind to symmetrical DNA sites containing an inverted repeat of two CGG triplets separated by spacers of variable length (2–5, 8). Hap1 is unique in this family because the Hap1 homodimer binds to asymmetric sites containing a direct repeat of two CGG triplets (consensus sequence, CG-GnnnTAnCGG; CGG triplets in UAS/CYC7), separated by a six-nucleotide spacer (11, 12). However, this asymmetry in Hap1 DNA binding is not dictated by asymmetric dimerization but by an asymmetric interaction between the residues within the zinc cluster of one Hap1 subunit and the residues immediately N-terminal to the Zn₂Cys₆ cluster of the other Hap1 subunit (7, 12). The x-ray crystal structures of Gal4-, Ppr1-, Put3-, and Hap1-DNA complexes (2, 3, 7, 8) show that the coiled coil dimerization elements of these proteins form similar coiled coils and permit symmetrical dimerization (2, 3, 7, 8, 13).

Hap1 is the key regulator that mediates the effects of oxygen and heme on the expression of genes required for respiration and for controlling oxidative damage in Saccharomyces cerevisiae (14, 15). In response to heme, Hap1 binds to the upstream activation sequences (UASs) of numerous genes, such as those encoding cytochrome c-iso-1 (CYC1) (16), iso-2 (CYC7) (17, 18), cytochrome c1 (CYTI) (19, 20), catalase (CTTT) (20, 21), and flavohemoglobin (YHB1) (22, 23), and activates transcription of these genes. Hap1 contains several functional domains and modules (see Fig. 1): the Zn₂Cys₆ binuclear cluster, the dimerization domain, the activation domain, seven heme-responsive motifs (HRM₁–6 and HRM7), and three repression modules RPM₁–3 (10–12, 24). The RPMs permit Hap1 repression whereas the HRMs, particularly HRM7, allow heme binding and heme activation of Hap1 (24). Although the previously defined dimerization domain spans residues 105–244, the minimal dimerization element was mapped between residues 105–135 (7, 10). This minimal dimerization element is sufficient to allow dimerization and DNA binding, as shown by electrophoretic mobility shift assays and x-ray crystal structural analysis (7, 12, 15).

Further, Hap1 binds to two different classes of DNA elements, exemplified by the UAS₁ of the CYC1 promoter (UAS₁/CYC1, CGGCGTTTACGGACGAC) and the UAS of the CYC7 promoter (UAS/CYC7, CGCTATTACGGATATA) (25). The major difference between UAS₁/CYC1 and UAS/CYC7 is that UAS₁/CYC1 contains two CGG triplets, whereas UAS/CYC7 contains two CGC triplets (26). In addition, the TA sequence in the spacer of the UAS/CYC7 site is repeated (26). Although Hap1 binds to these different sites with equal affinity, previous evidence strongly suggests that Hap1 activates transcription at these sites by different mechanisms (27–29). Many Hap1 positive control mutants specific for one of these two binding sites.

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1 The abbreviations used are: HRM, heme-responsive motif; RPM, repression module.
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RESULTS

Western Blotting—For Western blotting, whole cell extracts were first separated on 7% SDS-polyacrylamide gels and then transferred to polyvinylidene difluoride or nitrocellulose membranes. Hap1 was visualized by using a purified antibody against GST-Hap1 (residues 1–171) and a chemiluminescence Western blotting kit (Roche Molecular Biochemicals), as described previously (30).

β-Galactosidase Assays—To determine the β-galactosidase levels from reporter genes in cells containing Hap1, DD1–DD4, M1, and M2, SD5-HAP1 expression plasmids expressing wild-type Hap1 and mutants from the GAL1-10 promoter were transformed into the strain L51 bearing the UAS/CYC1-lacZ or UAS/CYC7-lacZ reporter. Cells were grown in 2% raffinose to OD 0.3 and then induced with 2% galactose for about 6 h. Cells were harvested and resuspended in three packed cell volumes of buffer (20 mM Tris, 10 mM MgCl₂, 1 mM dithiothreitol, 0.3 M NaCl, 1 mM phenylmethylsulfonfluoride, 1 μM/ml pepstatin, 1 μg/ml leupeptin). Cells were then permeabilized by agitation with four packed cell volumes of glass beads, and extracts were collected as described (25). This method consistently yielded extracts with protein concentrations of approximately 10 mg/ml.

DNA binding reactions were carried out in 20 μl volume with 5% glycerol, 4 mM Tris, pH 8, 40 mM NaCl, 4 mM MgCl₂, 2 ng/ml heme, 10 mM dithiothreitol, 3 μg of salmon sperm DNA, 10 μM ZnOAc₂, 300 μg/ml bovine serum albumin as described (26). Approximately 0.03 pmol of labeled UAS1/CYC1 or UAS/CYC7 and 20 μg of protein extracts were used in each reaction. The reaction mixtures were incubated at room temperature for 1 h and then loaded onto 4% polyacrylamide gels in 30 mM Tris borate/EDTA for gel electrophoresis at 4 °C. The radioactive bands were visualized and quantified by using a PhosphorImager™ (Molecular Dynamics). Oligonucleotide probes for UAS1/CYC1, UAS/CYC7 (27), and the corresponding mutant sites were synthesized. The top strand sequences are as follows: UAS1/CYC1, GTGGCCGGGGTTTATACGATGA; mutant UAS1/CYC1, GTGGCCGGGGTTTATATACGATGA; UAS/CYC7, CCTTCGCTATTATCGGCAGTACGA; mutant UAS/CYC7, CCTTCGCTATTATCGATGACTAGC.

FIG. 1. Hap1 domain structure and the primary amino acid sequence of the dimerization domain. Shown here are the six-cysteine zinc cluster (Zn, residues 55–95), the dimerization domain (DD, 105–244), repression module RPM1 (residues 245–278), RPM2 (residues 1061–1185), RPM3 (residues 203–244), heme-responsive motifs HRM1–6 (residues 279–444), HRM7 (residues 1191–1198), and the activation domain (residues 1309–1483). The amino acid sequence of the dimerization domain is shown, and the deleted regions and mutated regions in DD1 (residues 203–244), DD2 (residues 172–202), DD3 (residues 136–171), DD4 (residues 105–135), M1 (RERV to ALAA), and M2 (VKS to AAA) are indicated. The hydrophobic residues in the 4-3 hydrophobic repeat, coiled coil dimerization element are shown in bold type. The dimerization element encompassing residues 103–134 forms an α-helical coiled coil as shown by X-ray crystal structural analysis (7) and is marked by a heavy line. The region encompassing residues 171–195 is predicted to form an α-helix and is marked by a light line.
rately to generate mutants DD1–DD4, and the activities of these mutants at UAS/CYC7 and UAS1/CYC1 were measured. Combined deletion of any two segments, such as those in DD1 and DD2, resulted in a completely labile and inactive protein and thus is not shown here. As shown in Fig. 2, deletion of residues 203–244 (DD1), 172–202 (DD2), or 136–171 (DD3) did not significantly affect transcriptional activation at both UAS/CYC7 and UAS1/CYC1; the mutants had slightly lower activity than wild-type Hap1 but were still highly active at these sites. Western blotting analysis showed that DD1–DD3 were expressed at about the same level as wild-type Hap1 (Fig. 3). Further, we determined DNA binding by wild-type Hap1 and mutants (Fig. 4). As expected, DD2, like wild-type Hap1, bound specifically to both UAS/CYC7 and UAS1/CYC1 with high affinity. DD1 and DD3 behaved identically to DD2 and are therefore not shown. These results show that the three elements containing residues 203–244 (DD1), 172–202 (DD2), and 136–171 (DD3) are functionally redundant. Deletion of any one of the three elements had little effect on Hap1 DNA binding and transcriptional activation.

The Coiled Coil Dimerization Element Containing Residues 105–135 Has a Strong Impact on Transcriptional Activation at UAS/CYC7 but Not UAS1/CYC1—Strikingly, contrary to the deletion of residues 203–244 (DD1), 172–202 (DD2), or 136–171 (DD3), deletion of residues 105–135 (DD4) of the coiled coil dimerization element caused a complete loss of transcriptional activation at UAS/CYC7. However, mutant DD4 still retained a moderate level (about 40% of that of wild-type Hap1) of activity at UAS1/CYC1. Western blotting analysis showed that mutant DD4 was expressed about 3-fold lower than wild-type Hap1 (Fig. 3 and legend, note that about 3-fold more extracts containing DD4 were loaded). The lower expression level of DD4 explains the approximately 2-fold reduction of its activity at UAS1/CYC1 (Fig. 2) but does not explain the complete lack of activity at UAS/CYC7.

The Coiled Coil Dimerization Element Containing Residues 105–135 Is Not Required for DNA Binding at Both UAS/CYC7 and UAS1/CYC1—The lack of DD4 transcriptional activity at UAS/CYC7 may be attributable to two possible causes: lack of DNA binding or lack of transcriptional activation at the site. To distinguish between these possibilities, we carried out electrophoretic mobility shift assays. As shown in Fig. 4, DD4 bound to both UAS/CYC7 and UAS1/CYC1 sites with about 3-fold lower affinity (taking into account that the DD4 protein level was about 3-fold lower as well; see Figs. 3 and 4) than wild-type Hap1 or DD2, at two different concentrations. Thus, deletion of the coiled coil dimerization element has only a minor effect on Hap1 DNA binding, showing that the dimerization element is dispensable for DNA binding. Interestingly, DD4 binding to UAS/CYC7 was even slightly stronger than binding to UAS1/CYC1 (Fig. 4). Further, the DNA binding reactions were carried out in the presence of an excessive amount (3 μg) of salmon sperm (competitor) DNA, suggesting that DD4 binding to UAS1/CYC1 and UAS/CYC7 is sequence-specific. These results suggest that the complete and selective loss of DD4 activity at UAS/CYC7 was not attributable to a selective defect in DNA binding at UAS/CYC7.

Furthermore, we do not think that the low activity of DD4 at UAS/CYC7 is caused by the higher sensitivity of UAS/CYC7 to Hap1 levels for the following reasons. First, DD4, like wild-type Hap1 and DD2, actually binds to UAS/CYC7 with higher affinity than to UAS1/CYC1 (Fig. 4). Second, many Hap1 mutations (27) affect DNA binding and transcriptional activation at both UAS1/CYC1 and UAS/CYC7 in the same manner. For example, the mutant K86I (with a mutation of Lys86 to Ile) was transcriptionally inactive at both UAS1/CYC1 and UAS/CYC7 (Fig. 2). The mutant protein level was about the same as that of wild-type Hap1 (Fig. 3), whereas its DNA binding affinity at both UAS1/CYC1 and UAS/CYC7 was about 2-fold less than
wild-type Hap1 (Fig. 5). These results strongly suggest that the mutant protein level does not dictate the transcriptional activity of a particular mutant at either UAS1/CYC1 or UAS/CYC7. If Hap1 transcriptional activity was dictated by protein levels, then mutant K86I would be highly active at both UAS1/CYC1 and UAS/CYC7. Thus, the selective loss of DD4 transcriptional activity at UAS/CYC7 very likely reflects a unique property of mutant DD4 at UAS/CYC7. These results together suggest that the coiled coil dimerization element encompassing residues 105–135 has a strong and selective effect on transcriptional activation at UAS/CYC7 but not DNA binding nor transcriptional activation at UAS1/CYC1.

The Hap1 Mutant Protein Lacking the Coiled Coil Dimerization Element Binds Specifically to DNA Sites Containing a Direct Repeat of Two CGG/CGC Triplets—Because DD4 does not contain the coiled coil dimerization element, perhaps it binds to UAS/CYC7 as a monomer and is thus defective in transcriptional activation. However, data shown in Fig. 4 argue against this possibility. Both DD4-UAS1/CYC1 and DD4-UAS/CYC7 complexes (Fig. 4, lanes 3–6) migrated at the same positions, and the mobility of these complexes was very similar to that of the wild-type Hap1-DNA complex (Fig. 4, lanes 1 and 2). If DD4 binds to UAS1/CYC1 or UAS/CYC7 as a monomer, the mobility of the DD4-DNA complex should be much higher than that of the wild-type Hap1-DNA complex, as shown previously (12). Further, previous experiments (12) have clearly shown that DNA binding by Hap1 monomers requires only a single CGG/CGC triplet, apparently because a Hap1 monomer can make contacts with only one CGG/CGC triplet, as shown by the crystal structure of the Hap1-DNA complex (7). Therefore, to ascertain whether DD4 binds to UAS1/CYC1 and UAS/CYC7 as a dimer, we synthesized two mutated UAS1/CYC1 and UAS/CYC7 sites. The mutated UAS1/CYC1 site contains one CGG triplet; the second CGG in UAS1/CYC1 (CGGGGTTTACGG) is changed to TAT (see “Materials and Methods”). The mutated UAS/CYC7 site contains one CGC triplet; the second CGC in UAS/CYC7 (CGCTATTATCGC) is changed to ATA (see “Materials and Methods”). If DD4 binds to UAS1/CYC1 and UAS/CYC7 as a monomer, it should bind to the wild-type and mutated sites with equal affinity. However, if DD4 binds to UAS1/CYC1 and UAS/CYC7 as a dimer, it should not bind or should bind with much lower affinity to the mutated sites.

As expected, DD4 bound specifically to the wild-type UAS1/CYC1 (Fig. 6, lane 4) and UAS/CYC7 sites (Fig. 7, lane 4). The binding of DD4 to these sites was confirmed by supershift of the DD4-DNA complexes by an anti-Hap1 antibody (10) (Fig. 6, lane 3, and Fig. 7, lane 3). However, DD4 did not bind to the mutated UAS1/CYC1 (Fig. 6, lanes 1 and 2) and the mutated UAS/CYC7 (Fig. 7, lanes 1 and 2) sites, whether or not the anti-Hap1 antibody was present. These results show that DD4 binds specifically to DNA sites containing a direct repeat of two CGG/CGC triplets but not to DNA sites containing only one CGG/CGC triplet, suggesting that DD4 binds to DNA as a dimer.

To further verify these results, we carried out competition experiments. We examined the effect of the unlabeled wild-type and mutated UAS1/CYC1 site on DD4 binding to the radiolabeled wild-type UAS1/CYC1 site and the effect of unlabeled wild-type and mutated UAS/CYC7 site on DD4 binding to the radiolabeled wild-type UAS/CYC7 site. The cold wild-type UAS1/CYC1 site completely out-competed DD4 binding to the radiolabeled DNA (Fig. 6, lanes 6 and 7), even when the amount of the cold wild-type UAS1/CYC1 site was moderate (Ref. 11; about 100-fold of the radiolabeled site) (Fig. 6, lane 6).
Compared with the wild-type site, a moderate amount of the mutated UAS1/CYC1 site did not compete with DD4 DNA binding (Fig. 6, compare lane 8 with lane 5), although an excessive amount (Ref. 11; about 1000-fold of the radiolabeled site) of the mutated site out-competed DD4 binding (Fig. 6, lane 9). This result is not totally unexpected because competition of Hap1 DNA binding by mutated sites has been observed previously when the amount of mutated site was excessive (11). Clearly, DD4 cannot bind to the mutated UAS1/CYC1 site with an affinity that is high enough to allow the formation of a stable DD4-DNA complex that is detectable by electrophoretic mobility shift assays (Fig. 6, lanes 1 and 2). Nevertheless, the competition experiments (Fig. 6, lane 9) suggest that the mutated UAS1/CYC1 site can still interact with DD4 when its amount was high enough.

Similarly, the cold wild-type UAS/CYC7 site competed efficiently with DD4 DNA binding to the radiolabeled site (Fig. 7, lanes 6 and 7), whereas the mutated UAS/CYC7 site did not...
significantly compete with DD4 DNA binding even when its amount was excessive (Fig. 7, lanes 8 and 9). The mutated UAS1/CYC1 site competed much better than the mutated UAS/CYC7 site (compare lane 9 in Fig. 6 with lane 9 in Fig. 7) when their amounts were excessive, very likely because additional bases adjacent to the second CGC triplet are changed in the mutated UAS/CYC7 site but not in the mutated UAS1/CYC1 site (see “Materials and Methods”). Together, these data shown in Figs. 6 and 7 strongly suggest that DD4 binds to both UAS1/CYC1 and UAS/CYC7 as a dimer in a sequence-specific manner.

Ala Substitutions in the Coiled Dimerization Element Suggest That the Dimerization Element Makes Selective Contacts at UAS/CYC7.—To investigate further the molecular interactions mediating differential transcriptional activation, we examined the structure of the dimerization element in the Hap1-DNA complex (7). We decided to explore the effects of Ala substitutions in the dimerization element on transcriptional activation at UAS1/CYC1 and UAS/CYC7. We generated two mutants, M1 and M2, with residues in the middle of the coiled coil dimerization element (Fig. 1) substituted by Ala. M1 contains mutations of residues RERV to AAAL (L was introduced to generate a HaeII site) on one face of the coiled coil; M2 contains mutations of residues VKSL to AAAA on the other face of the coiled coil. Because Ala substitutions usually do not significantly alter protein structure (32), we expect that the coiled coil formed by the dimerization element should not be disrupted by these mutations. However, these mutations should disrupt interactions provided by the side chains of the original amino acid residues in the dimerization element. In other words, if residues RERV or VKSL interact with a putative coactivator, then the mutations should disrupt the interaction between Hap1 and the coactivator. Further, residues RERV and VKSL are on opposite faces of the coiled coil. Thus, mutations of these two sets of residues may have differential effects on transcriptional activation.

To determine whether RERV and VKSL are important for transcriptional activation, we detected the activities of M1 and M2 at UAS1/CYC1 and UAS/CYC7. Both M1 and M2 showed a very high (even higher than wild-type Hap1, for unknown reasons) level of activity at UAS1/CYC1. The activity of M2 is about half of that of M1 (Fig. 2), which is consistent with the fact that M2 protein level is about half of that of M1 (Fig. 3). Interestingly, at UAS/CYC7, both M1 and M2 exhibited significantly lower activity at UAS/CYC7 (Fig. 2). In particular, compared with wild-type Hap1, the activity of mutant M2 was reduced by 37-fold, whereas the activity of M1 was reduced by 5-fold at UAS/CYC7 (Fig. 2). However, M2 activity is generally lower than M1 (Fig. 2, UAS1/CYC1) even at UAS1/CYC1, probably because of its lower protein level. Thus, the effects of mutations in M1 and M2 on transcriptional activation at UAS/CYC7 should be assessed in comparison with their activities at UAS1/CYC1. Compared with their activities at UAS1/CYC1, M1 activity is reduced 8-fold, whereas M2 is reduced 32-fold, but wild-type Hap1 activity is higher at UAS/CYC7. These results show that residues VKSL on one face of the coiled coil have a stronger impact on transcriptional activation at UAS/CYC7 than residues RERV on the opposite face, suggesting that the face containing residues VKSL preferentially makes contacts critical for transcriptional activation at UAS/CYC7.

Further, we examined DNA binding by mutants M1 and M2 at UAS/CYC7 and UAS1/CYC1 (Fig. 8). For unknown reasons, the background bands, which resulted from DNA binding by non-Hap1 proteins in extracts containing M1 and M2 (Fig. 8), were much more intense than those containing wild-type Hap1 or DD4 (Fig. 4). We therefore used an anti-Hap1 antibody (10) to verify the position of the Hap1-DNA complex. As shown in Fig. 8, M1-DNA and M2-DNA complexes formed at UAS/CYC7 (Fig. 8, lanes 6 and 12) were supershifted, whereas those formed at UAS1/CYC1 were mostly disrupted (Fig. 8, lanes 5 and 11). In both cases, the data prove that the disrupted or supershifted complexes were Hap1-DNA complexes. Furthermore, like DD4, M1 and M2 bound specifically to DNA sites containing a direct repeat of two CGG/CGC triplets (Fig. 8 and data not shown). Clearly, M1 and M2 binding at UAS/CYC7 was stronger than their binding at UAS1/CYC1, suggesting that the selective and significant reduction of M1 and M2 activity at UAS/CYC7 was not attributable to their reduced DNA binding affinity at the site.

**DISCUSSION**

In this report, we examined the roles of amino acid residues in the previously defined Hap1 dimerization domain in DNA binding and transcriptional activation at UAS1/CYC1 and UAS/CYC7. Three themes emerge from our analyses. First, strikingly, the coiled coil dimerization element, which is found in many Gal4 family members (2, 10), is dispensable for Hap1 DNA binding, although its deletion slightly reduces Hap1 DNA binding activity (Figs. 3 and 4). Sequence-specific DNA binding by the Hap1 deletion mutant lacking the coiled coil dimerization element still requires a complete Hap1 site containing a direct repeat of two CGG/CGC triplets, suggesting that the mutant binds to DNA as a dimer (Figs. 6 and 7). Second, the coiled coil dimerization element is critical for differential tran-
scriptional activation at UAS1/CYC1 and UAS/CYC7. Deletion or mutations of the residues in the dimerization element caused a loss of transcriptional activity at UAS/CYC7 but not at UAS1/CYC1 (Fig. 2). All three mutants, DD4, M1, and M2 bound to UAS/CYC7 with higher affinity than to UAS1/CYC1 (Figs. 4 and 8) but lost the ability to activate transcription at UAS/CYC7, not at UAS1/CYC1 (Fig. 2). Third, the dimerization domain contains highly redundant elements in DNA binding and transcriptional activation. Remarkably, deletions of any one of the three elements containing 30–40 amino acid residues outside the coiled coil in the dimerization domain (Fig. 1) had very little effect on DNA binding or transcriptional activation (Figs. 2–4).

X-ray crystal structures of several Gal4 family members (2, 3, 7, 8) show that the coiled coil dimerization element plays an essential role in the formation of homodimers by these proteins. In the Hap1-DNA complex, residues 103–135 provide the majority of the hydrophobic interactions that allow Hap1-Hap1 dimerization (7). Therefore, it is quite intriguing that deletion of this element in Hap1 did not result in a complete loss of DNA binding activity (Fig. 4). In addition, DD4 required the full Hap1-binding site for optimum binding. Like Hap1, DD4 binding to sites with changes in the conserved nucleotides of the Hap1-binding site, CGGnnnTAnCGG (11), was greatly reduced (Figs. 6 and 7). These results suggest that Hap1 exhibits remarkable flexibility in allowing one part of Hap1 to compensate functionally another part when deleted. In addition, sequence analysis of the dimerization domain showed that residues 171–195 have a considerable potential to form an α-helix. Perhaps this putative α-helix compensates for the function of the dimerization element when deleted. Still, this α-helix is separated from the ZnCys6 cluster by 40 amino acid residues in DD4 (Fig. 1), again illustrating the remarkable flexibility of the Hap1 protein.

The DNA-binding domains of many transcriptional activators have been shown to affect transcriptional activation (33–39). Positive control mutants that affect transcriptional activation but not DNA binding have been identified in numerous proteins, including A repressor (33, 34), FIS (35), glucocorticoid receptor (36), Oct-1 (37), MyoD (38), and MEF2C (39), but the mechanisms by which the DNA-binding domains affect transcriptional activation remain unclear. In the case of Hap1, when positive control mutations are modeled onto the Hap1-DNA complex, these mutations scatter over the protein surface, and no obvious protein-protein interaction surface that may be responsible for interacting with a putative coactivator or part of the transcription machinery can be discerned (40). Thus, previous data cannot suggest a clear mechanism by which the Hap1 DNA-binding domain may affect transcriptional activation. Interestingly, our data reveal that the coiled coil dimerization element is critical for transcriptional activation at UAS/CYC7 but not at UAS/CYC1. This result strongly suggests that the coiled coil dimerization element is responsible for interactions with the putative coactivator or part of the transcription machinery (27–29). Further, data from mutants M1 and M2 suggest that one face of the coiled coil has a stronger effect on transcriptional activation than the other face (Fig. 2), again supporting the idea that the dimerization element makes contacts with another protein or protein complex critical for transcriptional activation at UAS/CYC7.

X-ray crystal structures of the Hap1-, Gal4-, Ppr1-, and Put3-DNA complexes (2, 3, 7, 8) show that the residues in the coiled coil dimerization element are largely exposed and thus may easily interact with other proteins nearby. Perhaps the residues in the dimerization element, not the residues bracketing the ZnCys6 cluster, which may be imbedded in the Hap1-DNA complex (7), are more likely to interact with a putative coactivator or part of the general transcription machinery. The effect of positive mutations bracketing the ZnCys6 cluster (27) may be mediated by their interactions with the dimerization element. Perhaps the effects of positive control mutants in the DNA-binding domains of other transcriptional activators might also be attributable to the interactions mediated by their dimerization domains (35–39).

REFERENCES

1. Johnston, M. (1987) Microbiol. Rev. 51, 458–476
2. Marmorstein, R., Carey, M., Phashne, M., and Harrison, S. C. (1992) Nature 356, 408–414
3. Marmorston, R., and Harrison, S. C. (1994) Genes Dev. 8, 2504–2512
4. Reece, R. J., and Phashne, M. (1993) Science 261, 909–911
5. Siddiqui, A. H., and Brandizzi, M. C. (1989) Mol. Cell. Biol. 9, 4706–4712
6. Gardner, K. H., Anderson, S. F., and Coleman, J. E. (1995) Nat. Struct. Biol. 2, 898–905
7. King, D. A., Zhang, L., Guarente, L., and Marmorston, R. (1999) Nat. Struct. Biol. 6, 71–75
8. Swainathan, K., Flynn, P., Reece, R. J., and Marmorston, R. (1997) Nat. Struct. Biol. 4, 753–759
9. Hellauer, K., Rochon, M. H., and Turcotte, R. (1996) Mol. Cell. Biol. 16, 6096–6102
10. Zhang, L., Bermingham, M. O., Turcotte, B., and Guarente, L. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2851–2855
11. Zhang, L., and Guarente, L. (1994) Genes Dev. 8, 2110–2119
12. Zhang, L., and Guarente, L. (1998) EMBO J. 17, 4676–4681
13. Zitomer, R. S., Carriero, P., and Deckert, J. (1997) Kidney Int. 51, 507–513
14. Cresuet, F., Verdiere, J., Gaisne, M., and Slonimski, P. P. (1988) J. Biol. Chem. 263, 263–276
15. Pfeifer, K., Kim, K. S., Kogan, S., and Guarente, L. (1989) Cell 56, 291–301
16. Guarente, L., Lalonde, B., Gifford, P., and Alani, E. (1984) Cell 36, 903–911
17. Lowry, C. V., and Zitomer, R. S. (1988) Mol. Cell. Biol. 8, 4651–4658
18. Prezant, T., Pfeifer, K., and Guarente, L. (1987) Mol. Cell. Biol. 7, 3252–3259
19. Schneider, J. C., and Guarente, L. (1991) Mol. Cell. Biol. 11, 4934–4942
20. Winkler, H., Adam, G., Mattes, E., Schanz, M., Hartig, A., and Ruiss, H. (1988) EMBO J. 7, 1799–1804
21. Lodhi, T., and Guirard, B. (1991) Mol. Cell. Biol. 11, 3762–3772
22. Buisson, N., and Labbe-Beis, R. (1998) J. Biol. Chem. 273, 9527–9533
23. Zhan, X. J., Raitt, D., Burke, P. V., Clewell, A. S., Kwaest, K. E., and Peyton, R. O. (1996) J. Biol. Chem. 271, 25313–25318
24. Hach, A., Hor, T., and Zhang, A. (1995) Cell. Biol. 19, 4324–4333
25. Pfeifer, K., Prezant, T., and Guarente, L. (1987) Cell 49, 19–27
26. Zhang, L., and Guarente, L. (1994) J. Biol. Chem. 269, 14443–14447
27. Turcotte, R. (1992) Gene Dev. 6, 2001–2009
28. Kim, K. S., and Guarente, L. (1989) Nature 342, 200–203
29. Kim, K. S., Pfeifer, K., Powell, L., and Guarente, L. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4524–4528
30. Zhang, L., Hach, A., and Wang, C. (1998) Mol. Cell. Biol. 18, 3819–3828
31. Zhang, L., and Guarente, L. (1994) Genetics 136, 813–817
32. Wells, J. A. (1991) Methods Enzymol. 202, 390–411
33. Guarente, L., New, J. S., Hochschuld, A., and Phashne, M. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 2368–2373
34. Hochschuld, A., Irwin, N., and Phashne, M. (1983) Cell 32, 319–325
35. Gosink, K. K., Gnaal, T., Bokal, A. J. T., and Gourse, R. L. (1996) J. Bacteriol. 178, 5182–5187
36. Schena, M., Freedman, L., and Yamamoto, K. R. (1989) Genes Dev. 3, 1590–1603
37. Stern, S., and Herr, W. (1991) Genes Dev. 5, 2555–2566
38. Bengal, E., Flores, O., Ranganjan, P. N., Chen, A., Weintraub, H., and Verma, I. M. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 6221–6225
39. Molkentin, J. D., Black, B. L., Martin, J. F., and Olson, E. N. (1996) Mol. Cell. Biol. 16, 2627–2636
40. King, D. A., Zhang, L., Guarente, L., and Marmorston, R. (1999) Nat. Struct. Biol. 6, 22–27