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Mutations in the bZIP domain of yeast GCN4 that alter DNA-binding specificity

(protein–DNA interactions/bZIP proteins/DNA sequence recognition)

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ABSTRACT **The bZIP class of eukaryotic transcriptional regulators utilize a distinct structural motif that consists of a leucine zipper that mediates dimerization and an adjacent basic region that directly contacts DNA. Although models of the protein–DNA complex have been proposed, the basis of DNA-binding specificity is essentially unknown. By genetically selecting for derivatives of yeast GCN4 that activate transcription from promoters containing mutant binding sites, we isolate an altered-specificity mutant in which the invariant asparagine in the basic region of bZIP proteins (Asn-235) has been changed to tryptophan. Wild-type GCN4 binds the optimal site (ATGACTCAT) with much higher affinity than the mutant site (*T*TGACTCA*A*), whereas the Trp-235 protein binds these sites with similar affinity. Moreover, the Trp-235, Ala-235, and Gln-235 derivatives differ from GCN4 in their strong discrimination against *G*TGACTCA*C*. These results suggest a direct interaction between Asn-235 and the ±4 position of the DNA target site and are discussed in terms of the scissors-grip and induced-fork models of bZIP proteins.**

Mutant DNA-binding proteins with altered sequence recognition properties have been extremely useful for defining specific protein–DNA contacts mediated by the helix–turn–helix structural motif (1–8). Such specificity mutants contain single amino acid substitutions and alter DNA sequence recognition at a particular base pair, thus providing strong functional evidence for direct contacts between individual amino acids and base pairs. In general, direct interactions inferred from genetic and biochemical studies have been confirmed by high-resolution structures of the protein–DNA complexes (9–11).

The bZIP class of eukaryotic transcriptional regulators, which includes the Jun and Fos oncoproteins, C-EBP, and yeast GCN4, utilize a distinct structural motif for specific DNA binding (12). The bZIP domain is largely α-helical (13–16) and consists of a dimerization element, the leucine zipper, and an adjacent basic region that directly contacts DNA (13, 17–21). As predicted by models of the protein–DNA complex (14, 22), the leucine zipper symmetrically positions a diverging pair of α-helical basic regions to make sequence-specific contacts with the DNA target (23). However, the basis of DNA-binding specificity is essentially unknown.

Yeast GCN4 protein binds to the promoters of many amino acid biosynthetic genes and coordinately activates their transcription (24, 25). Optimal binding is observed with a 9-base-pair (bp) dyad symmetric sequence, ATGASTCAT (S = C or G), with the central 7 bp being most important (26, 27). GCN4 binds as a dimer to overlapping and nonequivalent half-sites (28), and the optimal half-site is ATGAC (29). The basic region is necessary and sufficient for DNA-binding specificity (13, 14, 21) and, as in other bZIP proteins, contains an invariant asparagine residue (Asn-235). The two models for DNA binding by bZIP proteins propose distinct roles for this asparagine residue. In the scissors-grip model (22), the invariant asparagine is proposed to break the α-helix in the basic region, thus permitting it to bend sharply and wrap around the DNA. In the induced-fork model (14), the asparagine is proposed to directly contact the target sequence.

By genetically selecting for derivatives of GCN4 that can activate transcription from promoters containing mutant binding sites, we isolate an altered-specificity mutant of yeast GCN4 in which the invariant asparagine in the basic region of bZIP proteins (Asn-235) has been changed to tryptophan. Further, we show that glutamine and alanine substitutions of Asn-235 also alter DNA-binding specificity at the same nucleotide position. These results suggest a direct interaction between Asn-235 and the ±4 position of the DNA target site, and are discussed in terms of the scissors-grip (22) and induced-fork (14) models of bZIP proteins.

MATERIALS AND METHODS

DNA Manipulations. The DNA molecules for expressing GCN4 are essentially identical to YCp88-GCN4 (30) except that the coding region has been modified by the introduction of restriction sites that do not affect the protein sequence. YCp88 is a centromeric vector with the *ura3* selectable marker that utilizes the *ded1* and SP6 promoters for protein expression *in vivo* and *in vitro*, respectively. To randomize the invariant asparagine in the basic region, a degenerate oligonucleotide containing an equimolar mixture of the four nucleotide precursors at codon 235 of GCN4, was converted to double-stranded DNA by mutually primed synthesis (31). The resulting mixture was cloned between artificially introduced *Bam*HI and *Alw*NI sites at the appropriate position of the GCN4 coding region.

Phenotypic Analysis. The *his3* promoters utilized to isolate and characterize altered-specificity mutants contain symmetrical double mutations in the GCN4 target sequences that have been described previously (29). In addition, these promoters contain Δ101, a deletion that contains an *Eco*RI linker in place of sequences between −103 and −447 and hence removes all promoter elements upstream of the GCN4 binding site. DNAs containing these promoters were introduced into the *his3* locus of yeast strain KY803 (relevant genotype *ura3-52 gcn4-Δ1*) (30) by gene replacement. For initial characterization of the mutations at position 235, GCN4 function was examined by the standard complementation assay (30), which utilizes the native *his3* promoter that contains an efficient but nonoptimal binding site (ATGACTCTT) (26). Plasmids encoding GCN4 derivatives with amino acid substitutions of Asn-235 were introduced into the resulting yeast strains, and Ura^+ transformants were assayed for growth on aminotriazole, a competitive inhibitor of the *his3* gene product. The degree of aminotriazole resistance is directly related to the level of *his3* transcription (26, 30).

DNA-Binding Specificity. The DNA-binding properties of the GCN4 derivatives were assayed in two ways. First,

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equivalent amounts of ^{35}S-labeled proteins, synthesized by transcription and translation *in vitro*, were incubated with the appropriate target sequences (final concentration, 8 nM of each DNA), and the resulting complexes were electrophoretically separated in native acrylamide gels (24). The symmetric double-mutant binding sites were obtained as 753-bp *Eco*RI–Asp-718 fragments (29), and the optimal binding site was obtained as a 435-bp *Eco*RI–*Hin*dIII fragment. Complexes with the mutant or optimal site have distinct mobilities because of differences in the sizes of DNA fragments. The conditions for the DNA-binding assay are such that the intensities of the bands representing the protein–DNA complex are roughly proportional to the binding constants (24, 26, 30). In the second method, ^{32}P-labeled oligonucleotides (65 bp) containing the various target sites flanked on each side by a primer hybridizing site were made double-stranded by the polymerase chain reaction using the identical 5′-end-labeled primer. After purification of the resulting products, 100 fmol of each DNA was incubated with the indicated *in vitro* synthesized derivatives, and the protein–DNA complexes were electrophoretically separated from the unbound DNA.

RESULTS

Isolation and Characterization of an Altered-Specificity Mutant of GCN4. As a general approach for identifying GCN4 derivatives with altered DNA-binding specificity, we utilized a set of yeast strains that differ only in the GCN4 recognition sequence upstream of the TATA element in the *his3* promoter (Fig. 1). GCN4 binding to a functional target sequence activates *his3* transcription, which permits cells to grow in the presence of aminotriazole, a competitive inhibitor of the *his3* gene product. Symmetrical double mutants representing equivalent changes in each of the adjacent half-sites (*T*TGACTCA*A*, A*C*GACTC*G*T, AT*T*ACT*A*AT, and ATG*T*-C*A*CAT) bind GCN4 poorly and are unable to support GCN4-dependent activation in yeast cells (29). To isolate potential altered-specificity mutants, collections of GCN4 proteins, generated by mutagenesis *in vitro* with degenerate oligonucleotides, are screened for their ability to activate *his3* transcription from the symmetrically mutated target sequences.

To analyze the role of the invariant asparagine (Asn-235) in DNA-binding specificity, we constructed a library of GCN4 derivatives in which this codon was randomized (Fig. 1). Initially, we used the standard complementation assay (30) to determine the effects of 12 point mutations on GCN4 function (Table 1). The Trp-235 protein behaves indistinguishably from wild-type GCN4, the Gln-235 and Ala-235 proteins display low but detectable activity (very small colonies appeared only after 1–2 weeks at low aminotriazole concentrations), and nine other derivatives are inactive. When the protein library was introduced into strains containing the four symmetrical double mutants described above, aminotriazole-resistant colonies were obtained only with *T*TGACTCA*A*. The GCN4 expression plasmids from 12 of these colonies

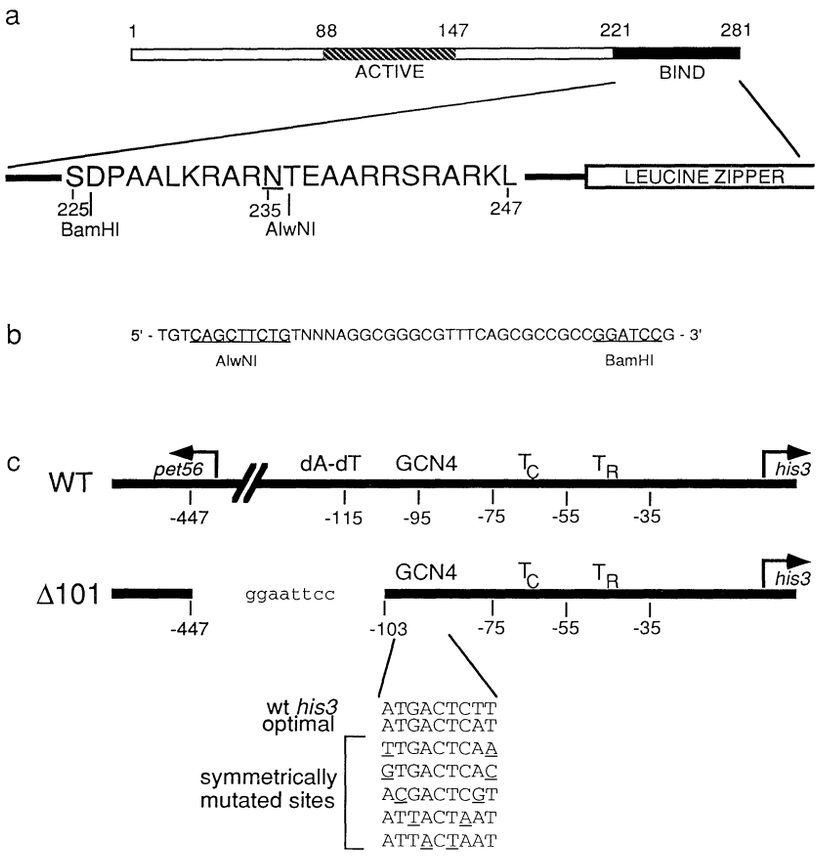

FIG. 1. Structure of promoters and proteins. (*a*) GCN4 protein (numbers indicate amino acid positions) contains an acidic transcriptional activation region and a DNA-binding domain, which consists of a leucine zipper and adjacent basic region with the indicated amino acid sequence (invariant asparagine at position 235 is underlined). (*b*) The degenerate oligonucleotide (N represents an equimolar mixture of the four nucleotide precursors) used to randomize codon 235, which corresponds to the invariant asparagine in the basic region. The double-stranded form of the oligonucleotide was cloned between the artificially introduced *Bam*HI and *Alw*NI sites at the appropriate position of the GCN4 coding region. (*c*) The *his3* promoters contain the indicated GCN4 target sequences and Δ101, a deletion that contains an *Eco*RI linker in place of sequences between −103 and −447. These promoters contain a single GCN4 binding site upstream of the T_C and T_R TATA elements but lack the poly(dA)·poly(dT) element as well as the promoter and part of the structural gene of the adjacent and divergently transcribed *pet56* gene (32).

Table 1. GCN4 function of mutant proteins tested by the standard complementation assay

Residue at position 235	Phenotype	N-cap preferences
Asparagine	+++	3.5
Tryptophan	+++	0.3
Glutamine	+	0.4
Alanine	+	0.5
Glutamic acid	−	0.4
Glycine	−	1.8
Lysine	−	0.7
Leucine	−	0.2
Proline	−	0.8
Arginine	−	0.4
Serine	−	2.3
Threonine	−	1.6
Valine	−	0.1

Plasmids encoding GCN4 derivatives with the indicated residues at position 235 were introduced into KY803 (relevant genotype, *ura3-52 gcn4-Δ1 HIS3*) and assayed for growth on aminotriazole (AT), a competitive inhibitor of the his3 gene product (30). Phenotypes are defined as +++ (normal growth on 20 mM AT), + (very poor growth on 5 mM AT and no growth at higher concentrations), and − (no growth on 5 mM AT). The N-cap preferences are taken from ref. 33 and defined as the ratio of the number of occurrences in 215 α-helices over the expected number based on the average amino acid composition.

were recovered, and all of them contained a tryptophan in place of the wild-type asparagine residue.

The wild-type (Asn-235) and Trp-235 proteins were examined for activation from sites that differ at the ±4 position (Fig. 2). As expected from the genetic selection, the Trp-235 protein stimulates transcription from the *T*TGACTCA*A*-containing promoter, whereas the Asn-235 protein does not. In contrast, when the target sequence was *G*TGACTCA*C*, the site that naturally occurs in the *his4* promoter, the Asn-235 protein activates strongly, whereas the Trp-235 protein appears inactive. Both proteins activate transcription from a promoter containing the optimal binding site, *A*TGACTCA*T*, although wild-type GCN4 is more efficient because it permits strains to grow at higher concentrations of aminotriazole (data not shown). As expected, neither protein stimulates transcription from the symmetric double mutant at the ±3 position (*AC*GACTC*GT*). These genetic results suggest that substitution of tryptophan for asparagine at residue

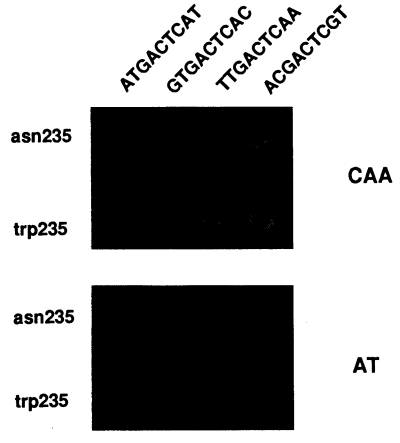

FIG. 2. Phenotypic analysis. Plasmids encoding wild-type GCN4 (Asn-235) or the Trp-235 protein were introduced into *gcn4* deletion strains containing the indicated *his3* promoters (see Fig. 1). The resulting transformants (about 10^4 cells) were plated on appropriate minimal medium containing either casamino acids (CAA) or 20 mM aminotriazole (AT), a competitive inhibitor of the *his3* gene product.

235 alters DNA sequence recognition, particularly with respect to the ±4 position of the binding site.

DNA-Binding Specificity of Mutant Proteins. To directly analyze DNA-binding specificities, we synthesized the Asn-235 and Trp-235 proteins *in vitro* and performed mobility-shift assays using the binding sites described above (Fig. 3). When the proteins were compared on individual target sequences, the Asn-235 wild-type protein binds more efficiently to the optimal (ATGACTCAT) site than the Trp-235 protein; a similar effect was observed on the native *his3* site (ATGACTC*T*T) although the absolute affinity was slightly lower. GCN4 binds the native *his4* site (*G*TGACTCA*C*) almost as strongly as the optimal site, while the Trp-235 protein does not bind detectably. In contrast, the two proteins bind *T*TGACTCA*A* with comparable affinity (a likely reason for why the Trp-235 protein, but not GCN4, can activate transcription from this site will be discussed later). To compare the relative affinities for different sites, each protein was incubated with a mutant site in the presence of an equal concentration of the optimal site, which serves as an internal standard. For GCN4, the order of binding affinity from highest to lowest is ATGACTCAT > *G*TGACTCA*C* > ATGACTC*T*T > *T*TGACTCA*A*. For the Trp-235 protein, the order is *T*TGACTCA*A* = ATGACTCAT > ATGACTC*T*T > *G*TGACTCA*C*.

We also analyzed the Gln-235 and Ala-235 proteins for their sequence preferences at the ±4 position. Surprisingly, both proteins favor the optimal site ATGACTCAT, bind weakly to *T*TGACTCA*A* and *C*TGACTCA*G*, and strongly discriminate against *G*TGACTCA*C* (Fig. 4). The binding specificities of the Gln-235 and Ala-235 proteins resemble that of the Trp-235 protein except that the Trp-235 derivative binds relatively more efficiently to *T*TGACTCA*A*. As expected from the *in vivo* complementation assay, the Gln-235 and Ala-235 proteins bind with lower affinity to any of the target sequences than the Trp-235 protein.

DISCUSSION

Evidence for a Direct Contact Between Asn-235 of GCN4 and the ±4 Position of the DNA Target Site. Specificity mutants of DNA-binding proteins provide strong genetic evidence for direct contacts between individual amino acids and base pairs (1–8), and in general such interactions have been confirmed by high-resolution structures of the protein–DNA complexes (9–11). Our biochemical experiments show that substitutions for asparagine-235 alter the DNA-binding specificity of GCN4, particularly regarding preferences at the ±4 position. In part, the Trp-235, Gln-235, and Ala-235 proteins can be interpreted as having lost some of the high-affinity interac-

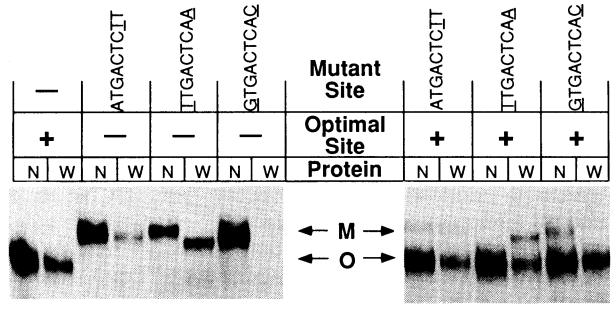

FIG. 3. DNA-binding specificity of the Trp-235 protein. Equivalent amounts of ^{35}S-labeled *in vitro* synthesized wild-type GCN4 (lanes N) or the Trp-235 derivative (lanes W) were incubated with the indicated DNA-binding sites, and the resulting complexes were electrophoretically separated in native acrylamide gels (24). Complexes with the mutant (M) or optimal (O) site have distinct mobilities because of differences in the sizes of DNA fragments.

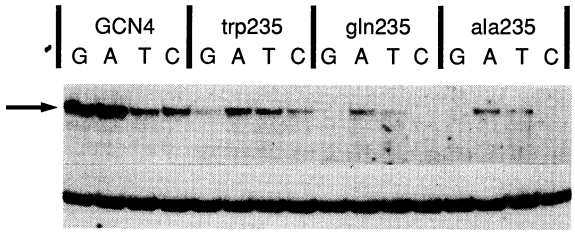

FIG. 4. DNA-binding specificities of the Gln-235 and Ala-235 proteins. ^{32}P-labeled oligonucleotides (65 bp) containing the target sites *G*TGACTCA*C*, ATGACTCAT, *T*TGACTCA*A*, and *C*TGACTCA*G* (indicated by the 5′-most nucleotide) were incubated with the indicated *in vitro* synthesized derivatives, and the protein–DNA complexes (9-hr exposure) (*Upper*) were electrophoretically separated from the unbound DNA (15-min exposure of the identical gel) (*Lower*). The arrow indicates the position of the predominant complex that contains the full-length proteins; the faint lower bands represent complexes with truncated proteins.

tions at position ±4 because all of these strongly discriminate against *G*TGACTCA*C*, a site efficiently bound by GCN4. This strongly suggests that asparagine-235 contributes to high-affinity GCN4 binding by recognizing, directly or indirectly, the ±4 position. However, Asn-235 is clearly not responsible for all specificity at position ±4, because the Trp-235, Gln-235, and Ala-235 substitutions retain some of the normal sequence preferences. On the other hand, the Trp-235 protein is not simply a "loss of specificity" mutant because it has the novel property of binding with comparable affinity to *T*TGACTCA*A* and the optimal site. Moreover, as tryptophan is the only substitution at position 235 that permits activation from *T*TGACTCA*A*, the altered specificity is not easily explained simply by the absence of Asn-235.

Although indirect conformationally transmitted effects cannot be excluded, we favor the idea of a direct contact between amino acid 235 and the position ±4. First, tryptophan, glutamine, and alanine are structurally very different and are unlikely to confer similar conformational effects, yet all three substitutions at position 235 result in a strong discrimination against *G*TGACTCA*C*. This argues for a direct interaction between Asn-235 and −4G/+4C. Second, the Asn-235 and Trp-235 proteins have distinct and sometimes opposite nucleotide preferences at the ±4 position. Third, in a chimeric protein containing the Jun basic region in place of the GCN4 basic region, the only position-235 derivative that appears to activate transcription from *T*TGACTCA*A* is the equivalent change from asparagine to tryptophan (W.T.P. and K.S., unpublished results). The homologous effects of the Trp-235 substitutions argue for direct interactions between Trp-235 and −4T/+4A, especially because the Jun and GCN4 basic regions are only 50% identical in sequence (33, 34). Fourth, in accord with a direct contact model, the relative DNA-binding affinities of the Trp-235 and Asn-235 proteins are not affected by a change in the +3 position (compare ATGACTCAT to ATGACTC*T*T; Fig. 3). Furthermore, the Trp-235 protein is unlikely to be altered significantly in its specificity for the central 7 bp because it behaves indistinguishably from GCN4 in the standard complementation assay, which requires the activation of many genes containing natural GCN4 binding sites (30, 33). In this regard, cells containing the Trp-235 protein also display wild-type levels of resistance to 5-methyltryptophan and canavanine (D.T. and K.S., unpublished results), thus indicating that this derivative can efficiently activate the tryptophan and arginine biosynthetic genes (35). Fifth, the GCN4 basic region is almost entirely α-helical and hence conformationally rigid when bound to DNA (15). Sixth, our proposed protein–DNA contact is consistent with affinity-cleavage experiments that suggest that the N termini of the GCN4 basic regions (residues 222–226) are located around residues ±5 of the binding site (36).

The above observations do not address the chemical nature of the putative interaction, nor do they preclude additional base pair contacts made by Asn-235 or interaction of other amino acid residues with position ±4. Indeed, it is likely that other amino acids account for some, and perhaps all, of the preference for the optimal −4A/+4T base pair. Thus, the hypothesis of a direct interaction between Asn-235 and position ±4 is strongly supported by the above arguments, but conclusive proof requires a high-resolution structure of the protein–DNA complex.

Comments Regarding the Scissors-Grip and Induced-Fork Models. The scissors-grip (22) and induced-fork (14) models for DNA binding by bZIP proteins differ considerably in their proposed roles for the invariant asparagine in the basic region. Our results are consistent with the general proposal of the induced-fork model, which predicts that Asn-235 is one of the "quartet" of residues that directly contact DNA. However, the initial prediction of the induced-fork model was that Asn-235 directly interacts with position ±3, whereas our observed specificity changes occur at position ±4. It seems more difficult to account for the altered binding specificity of the Trp-235 protein in the scissors-grip model, which proposes that Asn-235 serves as the N-terminal residue of an α-helix (N-cap), thus permitting the basic region to bend sharply and wrap around the DNA. In particular, the correlation between favorable N-cap formation (37) and GCN4 functional activity is extremely poor. The functional substitutions, tryptophan and glutamine, are among the most unfavorable N-cap residues, whereas nonfunctional substitutions include glycine, serine, and threonine, which are the most favorable for N-cap formation (except for asparagine). However, it is possible that disruption of the putative structural relationship between the α-helices adjoining the N-cap could indirectly alter binding specificity.

Given the general views and known structural constraints of bZIP proteins, the hypothesis that Asn-235 directly contacts position ±4 has implications for aligning the protein along the DNA. It suggests that sequence-specific contacts to the more central (and more crucial) base pairs of the GCN4 binding site would primarily involve amino acid residues more proximal to the leucine zipper (i.e., C-terminal to Asn-235). Although residues in the basic region located further from the zipper are crucial for high-affinity binding, the region of DNA covered by GCN4 extends considerably beyond the ATGAC half-site sequences that determine DNA-binding specificity (38). Perhaps the more distal basic residues contribute to overall affinity by direct but nonsequence-specific interactions to positions adjacent to the half-site determinants.

Relationship Between DNA-Binding *in Vitro* and Transcriptional Activation *in Vivo*. As mentioned above, the Trp-235 and Asn-235 proteins bind with comparable affinity to *T*TGACTCA*A* (Fig. 3), but only the Trp-235 derivative activates *his3* transcription *in vivo* (Fig. 2). This apparent discrepancy most likely reflects competition of the many genomic binding sites for the limited amount of GCN4 protein that exists *in vivo* (26). Since wild-type GCN4 strongly prefers to bind the native *his3* and *his4* sites over *T*TGACTCA*A* (Fig. 3), it is very likely that the protein will almost exclusively occupy DNA sequences in wild-type promoters subject to GCN4 control—i.e., the *T*TGACTCA*A* site is likely to be unoccupied. In contrast, *T*TGACTCA*A* competes effectively with the optimal sequence and presumably with many natural sites for binding by the Trp-235 protein. Transcriptional activation *in vivo* depends on promoter occupancy by GCN4, which in limiting situations should be affected more by competitive ability rather than by absolute binding affinity. However, these considerations in no way detract

from the biochemical experiments that clearly show that the Trp-235 protein has altered DNA-binding specificity.

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1. Youderian, P., Vershon, A., Bouvier, S., Sauer, R. T. & Susskind, M. M. (1983) *Cell* **35,** 777–783.
2. Ebright, R. H., Cossart, P., Gicquel-Sanzey, B. & Beckwith, J. (1984) *Nature (London)* **311,** 232–235.
3. Hochschild, A. & Ptashne, M. (1986) *Cell* **44,** 925–933.
4. Hochschild, A., Douhan, J. I. & Ptashne, M. (1986) *Cell* **47,** 807–816.
5. Wharton, R. P. & Ptashne, M. (1987) *Nature (London)* **326,** 888–891.
6. Lehming, N., Sartorius, J., Niemoller, M., Genenger, G., vonWilcken-Bergmann, B. & Muller-Hill, B. (1987) *EMBO J.* **6,** 3145–3153.
7. Hanes, S. D. & Brent, R. (1989) *Cell* **57,** 1275–1283.
8. Treisman, J., Gonczy, P., Vashishtha, M., Harris, E. & Desplan, C. (1989) *Cell* **59,** 553–562.
9. Jordan, S. R. & Pabo, C. O. (1988) *Science* **242,** 893–899.
10. Aggarwal, A. K., Rodgers, D. W., Drottar, M., Ptashne, M. & Harrison, S. C. (1988) *Science* **242,** 899–907.
11. Kissinger, C. R., Liu, B., Martin-Blanco, E., Kornberg, T. B. & Pabo, C. O. (1990) *Cell* **63,** 579–590.
12. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1988) *Science* **240,** 1759–1764.
13. Talanian, R. V., McKnight, C. J. & Kim, P. S. (1990) *Science* **249,** 769–771.
14. O'Neil, K. T., Hoess, R. H. & DeGrado, W. F. (1990) *Science* **249,** 774–778.
15. Weiss, M. A., Ellenberger, T., Wobbe, C. R., Lee, J. P., Harrison, S. C. & Struhl, K. (1990) *Nature (London)* **347,** 575–578.
16. Patel, L., Abate, C. & Curran, T. (1990) *Nature (London)* **347,** 572–575.
17. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. (1989) *Science* **243,** 1681–1688.
18. O'Shea, E. K., Rutkowski, R. & Kim, P. S. (1989) *Science* **243,** 538–542.
19. Kouzarides, T. & Ziff, E. (1989) *Nature (London)* **340,** 568–571.
20. Sellers, J. W. & Struhl, K. (1989) *Nature (London)* **341,** 74–76.
21. Agre, P., Johnson, P. F. & McKnight, S. L. (1989) *Science* **246,** 922–926.
22. Vinson, C. R., Sigler, P. B. & McKnight, S. L. (1989) *Science* **246,** 911–916.
23. Pu, W. T. & Struhl, K. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6901–6905.
24. Hope, I. A. & Struhl, K. (1985) *Cell* **43,** 177–188.
25. Arndt, K. & Fink, G. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 8516–8520.
26. Hill, D. E., Hope, I. A., Macke, J. P. & Struhl, K. (1986) *Science* **234,** 451–457.
27. Oliphant, A. R., Brandl, C. J. & Struhl, K. (1989) *Mol. Cell. Biol.* **9,** 2944–2949.
28. Hope, I. A. & Struhl, K. (1987) *EMBO J.* **6,** 2781–2784.
29. Sellers, J. W., Vincent, A. C. & Struhl, K. (1990) *Mol. Cell. Biol.* **10,** 5077–5086.
30. Hope, I. A. & Struhl, K. (1986) *Cell* **46,** 885–894.
31. Oliphant, A. R., Nussbaum, A. L. & Struhl, K. (1986) *Gene* **44,** 177–183.
32. Struhl, K. (1986) *Mol. Cell. Biol.* **6,** 3847–3853.
33. Struhl, K. (1987) *Cell* **50,** 841–846.
34. Vogt, P. K., Bos, T. J. & Doolittle, R. F. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 3316–3319.
35. Wolfner, M., Yep, D., Messenguy, F. & Fink, G. R. (1975) *J. Mol. Biol.* **96,** 273–290.
36. Oakley, M. G. & Dervan, P. B. (1990) *Science* **248,** 847–850.
37. Richardson, J. S. & Richardson, D. C. (1988) *Science* **240,** 1648–1652.
38. Gartenberg, M. R., Ampe, C., Steitz, T. A. & Crothers, D. M. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 6034–6038.