A homeo domain protein lacking specific side chains of helix 3 can still bind DNA and direct transcriptional repression

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A series of mutations in the homeo domain of the yeast $\alpha 2$ protein were constructed to test, both in vivo and in vitro, predictions based on the $\alpha 2$ -DNA cocrystal structure described by Wolberger et al. (1991). The effects of the mutations were observed in three different contexts using authentic target DNA sequences: $\alpha 2$ binding alone to specific DNA, $\alpha 2$ binding cooperatively with MCM1 to specific DNA, and $\alpha 2$ binding cooperatively with $a 1$ to specific DNA. As expected, changes in the amino acid residues that contact DNA in the X-ray structure severely compromised the ability of $\alpha 2$ to bind DNA alone and to bind DNA cooperatively with MCM1. In contrast, many of these same mutations, including a triple change that altered all the "recognition" residues of helix 3, had little or no effect on the cooperative binding of $\alpha 2$ and $a 1$ to specific DNA, as determined both in vivo and in vitro. These results show that the ability of a homeo domain protein to correctly select and repress target genes does not necessarily depend on the residues commonly implicated in sequence-specific DNA binding.

[Key Words: Homeo domain proteins; DNA-binding specificity; combinatorial control]

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The yeast $\alpha 2$ protein is a member of the homeo domain family of DNA-binding proteins; this highly conserved sequence motif was first recognized in several genes that control development in *Drosophila* (Laughon and Scott 1984; McGinnis et al. 1984a,b; Shepherd et al. 1984). Homeo domain proteins have now been found in nearly all eukaryotic organisms, ranging from yeast to humans, and several hundred members of this DNA-binding family are known (for review, see Scott et al. 1989; Hayashi and Scott 1990; Wuthrich and Gehring 1992). The structures of five different homeo domains, including that of $\alpha 2$, have been determined by nuclear magnetic resonance (NMR) spectroscopy or X-ray crystallography (Qian et al. 1989; Kissinger et al. 1990; Otting et al. 1990; Phillips et al. 1991; Wolberger et al. 1991; Billeter et al. 1993; Ceska et al. 1993; Leiting et al. 1993; Klemm et al. 1994). Despite limited sequence identity among these homeo domains, they adopt nearly the same conformation. The mechanism of DNA binding also appears to be remarkably similar in that most of the DNA backbone and some of the base-specific contacts are identical in the structures of the homeo domains bound to DNA.

The X-ray and NMR structures provide a clear picture of how homeo domain proteins bind to DNA on their own. We wished to test whether predictions from these

structures hold when a homeo domain binds DNA cooperatively with other proteins. The yeast $\alpha 2$ protein provides a number of advantages for this analysis. (1) The target genes and the "other" proteins are known. In α cells, $\alpha 2$ binds DNA cooperatively with MCM1 to turn off transcription of the *a*-specific genes. The 32-bp DNA sequence recognized jointly by a dimer of $\alpha 2$ and a dimer of MCM1 is called the *a*-specific gene (*asg*) operator (Johnson and Herskowitz 1985; Keleher et al. 1988; Passmore et al. 1989; Ammerer 1990; Smith and Johnson 1992). In *a*/ α cells, $\alpha 2$ performs a second function: It binds cooperatively (as a heterodimer) with the homeo domain protein *a 1* to a second type of operator, the *hsg* operator, to turn off the haploid-specific genes (Strathern et al. 1981; Miller et al. 1985; Goutte and Johnson 1988, 1993, 1994; Dranginis 1990). (2) $\alpha 2$, *a 1*, and MCM1 have all been purified, and their DNA-binding properties have been characterized extensively (see above references). (3) The structure of the $\alpha 2$ homeo domain complexed with a biologically relevant DNA sequence (a variant of the *asg* operator) has been determined (Wolberger et al. 1991). (4) A series of *asg* operator- and *hsg* operator-containing reporter constructs have been described which, when combined with the relative ease of yeast genetics, provide a simple, quantitative way to test the effect of $\alpha 2$ mutations in vivo (Goutte and Johnson 1988; Keleher et al. 1988; Mak and Johnson 1993).

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Based on the X-ray and NMR structures, we constructed a series of point mutations predicted to damage the DNA-binding affinity of $\alpha 2$ and tested the effects of the mutations in vitro and in vivo. Although most of the changes that we examined destroyed the ability of $\alpha 2$ to work alone and in combination with MCM1, to our surprise, most of the mutant $\alpha 2$ s still functioned in combination with $\alpha 1$. We conclude that many of the specific side chains of $\alpha 2$ that contact DNA in the $\alpha 2$ /DNA X-ray structure are not required for DNA binding of $\alpha 2$ in conjunction with $\alpha 1$.

Results

Point mutations in the homeo domain of $\alpha 2$ reduce the level of $\alpha 2$ /MCM1 repression in vivo

As described in Materials and methods, we developed a scheme to construct site-specific mutations in the $\alpha 2$ gene, to introduce these mutations into yeast for in vivo repression analysis, and to introduce the mutations into bacterial expression vectors to provide a source of pure protein for DNA-binding experiments.

The $\alpha 2$ protein is 210 residues in length and contains two stable domains (Sauer et al. 1988). The homeo domain is part of the carboxy-terminal domain, and NMR and crystallography experiments have shown that this region forms three α -helices that pack into a tight bundle (Phillips et al. 1991; Wolberger et al. 1991). A sketch of the $\alpha 2$ homeo domain bound to its DNA site is shown in Figure 1. The first helix (residues 138–150 of the intact protein) and the second helix (residues 159–169) pack against each other in a roughly antiparallel fashion, and both of these pack against the third helix, which lies across them. The third helix (residues 173–189), frequently termed the "recognition helix," lies in the major groove of the DNA and makes three base-specific contacts and a series of sugar-phosphate backbone contacts. An extended region at the beginning of the $\alpha 2$ homeo domain (the amino-terminal arm), which lies in the minor groove of the DNA, appears to make at least one base-specific contact. Immediately following the carboxyl terminus of the homeo domain is a 20-amino-acid tail that does not contact DNA but is required for interaction with $\alpha 1$ (Strathern et al. 1988; Mak and Johnson 1993).

A list of the point mutations that we constructed in the $\alpha 2$ homeo domain and their effects on $\alpha 2$ /MCM1-mediated repression are shown in Figure 2. For these experiments, the mutant $\alpha 2$ proteins were expressed from the $\alpha 2$ promoter carried on a *CEN* plasmid, and their activities were monitored using an asg operator reporter construct that was integrated into the yeast genome. In most cases, the results of mutations in the homeo domain agree well with predictions made from the crystal structure. For example, Arg-173, Gln-175, Lys-186, and Lys-188 all make contacts with the DNA phosphate backbone, and point mutations in any of these residues have a large deleterious effect on the level of $\alpha 2$ /MCM1-mediated repression.

Figure 1. A sketch summarizing the base-specific contacts observed in the crystal structure of $\alpha 2$ complexed with specific DNA. The diagram is based on the crystal structure derived from Wolberger et al. (1991). Base-specific contacts in the major groove (Ser-181, Asn-182, and Arg-185) and minor groove (Arg-135) of the DNA are also shown. The DNA shown is the half-site from the asg operator.

Amino acid substitutions in residues that make major groove base-specific contacts in the X-ray crystal structure, Ser-181, Asn-182, and Arg-185, also have large damaging effects on the level of repression. This result confirms that all of these residues play major roles in the recognition of the DNA. The Ser-181-Gln mutation is noteworthy in that this position has been studied extensively in other homeo domain proteins, and, for some cases, it has been proposed to be a major determinant of DNA-binding specificity (Hanes and Brent 1989; Treisman et al. 1989; Schier and Gehring 1993). In the $\alpha 2$ X-ray structure, this side chain has been modeled to make an extended van der Waals contact with the third position in the major groove of the operator (Wolberger et al. 1991). The Gln substitution, which inserts a larger, bulkier side chain in place of Ser-181, does not have as large deleterious effect on the level of repression as might be expected if this residue provides a critical determinant of DNA-binding specificity. In the engrailed homeo domain cocrystal structure, the Gln residue at the homologous position contacts the second position of the operator (Kissinger et al. 1990). A substitution of this residue from Gln to Ala had only a small effect on its DNA-binding activity, suggesting that the Gln at this position may only play a small role in determining the engrailed DNA-binding affinity and specificity (Ades and Sauer 1994). One explanation for the partial repression by the $\alpha 2$ Ser-181-Gln mutant is that the Gln side chain

Figure 2. In vivo assays for transcriptional repression by $\alpha 2$ /MCM1 and $\alpha 1/\alpha 2$. (A) Assay for α -specific gene repression by $\alpha 2$ mutants. Plasmid pAV115, a *CEN LEU2* plasmid, containing the entire *MAT α* locus with a specific site-directed mutation in the $\alpha 2$ gene ($\alpha 2^*$ here), was transformed into a *mat Δ* strain containing an integrated copy of a derivative of pSJ3, an *asg* reporter, at the *URA3* locus on chromosome V. $\alpha 2$ /MCM1-mediated repression was monitored by measuring the β -galactosidase produced from a *CYC1-lacZ* promoter that contains an $\alpha 2$ /MCM1 operator site. The MCM1 protein was expressed from its endogenous gene on chromosome XIII. Nonrepressed conditions [tester strain transformed with pAV114, a blank control vector] produced an average 150 ± 10 units of β -galactosidase activity. Full repression of the reporter promoter (tester strain transformed with pAV115 containing a wild-type copy of $\alpha 2$), gave an average of 6 ± 1 units of β -galactosidase activity, giving ~ 22 -fold repression of the reporter promoter. (B) Assay for haploid-specific gene repression of $\alpha 2$ mutants. $\alpha 1/\alpha 2$ -mediated repression by the $\alpha 2$ mutants was measured by a similar strategy using a *MAT $\alpha 1$* tester strain that contains an integrated copy of the *CYC1-lacZ* promoter containing an *hsg* operator. The $\alpha 1$ protein was expressed from its endogenous gene at the *MAT* locus on chromosome III. The presence of wild-type $\alpha 2$ in this tester strain repressed *lacZ* expression from 30 units (pAV114) to 3 units (pAV115 wild-type $\alpha 2$), a 10-fold repression of the promoter construct. (C) The effects of site-directed mutations in $\alpha 2$ on $\alpha 2$ /MCM1- and $\alpha 1/\alpha 2$ -mediated repression. The percent activity of each mutant relative to wild-type levels is shown. The calculations are based on the average of at least three independent transformants of each mutant.

may be repositioned so that it contacts the second position of the operator instead of the third base pair.

The NMR and X-ray homeo domain structures show that the amino-terminal region of the homeo domain wraps partially around the DNA to make contacts in the minor groove (Kissinger et al. 1990; Otting et al. 1990; Wolberger et al. 1991; Billeter et al. 1993; Klemm et al. 1994). In the engrailed structure, Arg side chains at the third and fifth positions of the homeo domain contact thymines at two adjacent base pairs. Residues at the third and fifth positions of other homeo domains are mostly Arg, suggesting that these contacts with the DNA are conserved and are important in binding affinity and specificity. The $\alpha 2$ homeo domain contains two Arg residues at the fourth (Arg-132) and seventh (Arg-135) positions instead of the third and fifth positions as in other homeo domain proteins. In the $\alpha 2$ cocrystal structure, residues Arg-132–Thr-137 lie in the minor groove, but only the Arg-135 side chain is well defined and is modeled to contact two adjacent base pairs (Wolberger et al. 1991). The large deleterious effect of the Arg135–Ala mutation indicates that Arg-135 is important for repression and DNA binding. The other arginine in the amino-terminal arm, Arg-132, appears to have only a minor role. The lack of a second additional DNA contact made by the amino-terminal arm may suggest why the DNA-binding affinity of $\alpha 2$ is intrinsically relatively low and why it requires accessory proteins to bind DNA with high affinity and specificity.

Mutations in side chains that lie close to the DNA but

that do not appear to make a direct contact, such as Lys-177 and Asn-178, reduce the level of repression 5- to 10-fold, suggesting that these side chains may also play a role in binding when $\alpha 2$ is bound in complex with MCM1. In the $\alpha 2$ cocrystal structure, Asn-178 lies in the major groove, but it does not appear to make any base-specific or phosphate-backbone contacts. One substitution at this position, Asn-178–Ile, introduces a larger side chain, and it is likely that it interferes with DNA binding. The other mutant, Asn-178–Ala, is harder to

reconcile with the crystal structure because the substitution effectively removes the side chain. It is possible that Asn-178 is involved in contacting the DNA through a water-mediated hydrogen bond (Gehring et al. 1994) and that a substitution of this side chain with alanine would remove this contact (C. Wolberger, pers. comm.).

Mutations in other solvent-exposed residues that are distal from the DNA, such as residues Asn-164 and Asn-167, which lie on the back side of the homeo domain in helix 2, have little or no effect on the level of repression and serve as convenient controls. Altogether, these results show that $\alpha 2$ side chains that make contacts with the DNA in the cocrystal structure are required for efficient repression with MCM1 *in vivo*.

*Point mutations in the homeo domain of $\alpha 2$ do not reduce the level of $\alpha 1/\alpha 2$ repression *in vivo**

The effect of the $\alpha 2$ mutations on $\alpha 1/\alpha 2$ -mediated repression was tested in a similar way using an integrated hsg-operator reporter, with $\alpha 1$ being supplied from a chromosomal copy of *MATa* (Fig. 2B). We found that most of the $\alpha 2$ mutants that completely destroy $\alpha 2$ /MCM1-mediated repression (above) have little or no effect on $\alpha 1/\alpha 2$ -mediated repression. Even amino acid substitutions with large damaging effects on $\alpha 2$ /MCM1-mediated repression and predicted by the crystal structure to affect key protein–DNA contacts (e.g., Lys-188–Ile or Arg-185–Met) function at nearly wild-type levels with $\alpha 1$.

As a control for this experiment, we constructed a point mutation in $\alpha 2$ (Leu-196–Ser) that was shown previously to abolish $\alpha 1/\alpha 2$ -mediated repression of haploid-specific genes but not $\alpha 2$ /MCM1-mediated repression of the *a*-specific genes (Strathern et al. 1988). Residue Leu-196 lies in the carboxy-terminal tail region adjacent to the homeo domain, and deletion analysis of the $\alpha 2$ protein has established that this tail region is important for cooperative DNA binding between $\alpha 1$ and $\alpha 2$ (Mak and Johnson 1993). In accordance with the previous analysis of this mutant, it retains the ability to repress transcription with MCM1 at roughly wild-type levels but fails to repress transcription with the $\alpha 1$ protein (Fig. 2). This result serves as a control and indicates that the observed repression of the $\alpha 1/\alpha 2$ operator site by the $\alpha 2$ mutants that we constructed is not the result of a failure of our assays to detect a loss of repression.

One possible interpretation of these results is that the levels of $\alpha 1$ and $\alpha 2$ proteins in the cell are sufficiently high to enable them to bind to the $\alpha 1/\alpha 2$ site, even though the protein–DNA interactions have been substantially weakened. Mutations in a single side chain that contacts the DNA might therefore have a small but undetectable effect on the level of repression. To test this model, a triple mutant—Ser-181–Ala/Asn-182–Ala/Arg-185–Ala, referred to as H3-3A—that removes all three side chains that make base-specific contacts in the major groove of the DNA was constructed. As expected, this mutant was completely defective in repressing transcription with MCM1; however, it still showed signifi-

cant levels of repression with $\alpha 1$ (Fig. 2C). We conclude from these results that when in a complex with $\alpha 1$, these residues are not crucial for repression in the cell.

Integration of $\alpha 2$ mutants into the chromosomal MAT locus

In the experiments described above, the $\alpha 2$ protein was expressed from its endogenous promoter on a low copy *CEN* plasmid. To rule out complications resulting from plasmid loss or overexpression, we constructed stable chromosomal copies of the $\alpha 2$ mutations at the *MAT* locus (see Materials and methods). α Cells carrying the mutant $\alpha 2$ (*mata2* cells) were tested for repression of an *asg* reporter, and *MATa*/*mata2* cells were tested for repression of an *hsg* reporter. The results are shown in Figure 3, and, in general, agree with those utilizing *CEN* plasmids. A couple of the mutants, such as Ser-181–Ile, and Arg-185–Met, show a larger effect on $\alpha 1/\alpha 2$ -mediated repression than in the experiments utilizing *CEN* plasmids, and the amino-terminal arm mutant (Arg-135–Ala) also appears to partially affect $\alpha 1/\alpha 2$ -mediated repression. However, the most striking result is still exemplified by the phenotype of the triple mutant H3-3A: complete disruption of $\alpha 2$ /MCM1-mediated repression but wild-type levels of $\alpha 1/\alpha 2$ -mediated repression.

*Mutations in the $\alpha 2$ homeo domain destroy repression of endogenous *a*-specific genes but not endogenous haploid-specific genes*

Although it seemed unlikely, it was formally possible that the reporters used in the experiments described above misrepresent the behavior of authentic *a*-specific and haploid-specific genes. To rule out this possibility, we tested the effects of the mutations on the repression of endogenous *a*- and haploid-specific genes using several bioassays. In general, these tests agree well with those utilizing the reporter constructs; a brief description of them follows (see also Materials and methods).

α Strains, each bearing a different mutant $\alpha 2$ integrated at the *MAT* locus (i.e., *mata2* strains), were assayed for their mating type (by testing their ability to mate with either *MATa* or *MATa* tester strains) and for their production of *a*- and α -mating factor (by the formation of halos on appropriate tester strains that are growth sensitive to mating factor) (Sprague 1991). In α cells, a strong mutation in the homeo domain, such as the triple mutant H3-3A, exhibits the same phenotype as a deletion of the $\alpha 2$ gene (Table 1). This mutant fails to repress *a*-specific genes, such as *STE6* and *BAR1*, which allows the production of *a*-factor and the degradation of α -factor, respectively. Thus, a *mata2* strain carrying the H3-3A allele exhibits an *a* halo, whereas a wild-type α strain exhibits an α halo. This strain also fails to mate with either *a* or α cells, a phenotype characteristic of a *mata2* deletion strain, because both *a*-specific and α -specific genes are expressed (Strathern et al. 1981).

To test the effects of the $\alpha 2$ mutations on $\alpha 1/\alpha 2$ -mediated repression of haploid-specific genes, we examined

Figure 3. $\alpha 2$ /MCM1- and $a1/\alpha 2$ -mediated repression by strains bearing integrated $\alpha 2$ mutations. (A) Assay for a -specific gene repression of integrated $\alpha 2$ mutants. The mutant $\alpha 2$ genes expressed from their endogenous promoter were integrated at the MAT locus on chromosome III. The asg reporter construct is carried on plasmid pAV71. The expression from the reporter construct in a wild-type $\alpha 2$ strain is 1.2 ± 0.1 β -galactosidase units. The repression ratio was calculated by comparing the β -galactosidase values (155 ± 8 units) from the same strain transformed with a $CYC1-lacZ$ plasmid without an asg operator (pAV73). (B) Assay for haploid-specific gene repression of integrated $\alpha 2$ mutants. $a1/\alpha 2$ -mediated repression is measured by the repression ratios of a diploid $MATa/mata\alpha 2$ strain that is transformed with a $CYC1-lacZ$ reporter plasmid containing an hsg operator site (pYJ103). In a wild-type $MATa/MAT\alpha$ background, this reporter produces 1.0 ± 0.2 β -galactosidase units. The repression ratio was calculated by comparing β -galactosidase values (83 ± 2 units) from the same strains transformed with a reporter plasmid without an hsg operator (pAV73). (C) Repression ratios for the integrated $\alpha 2$ mutations in combination with MCM1 or with $a1$. The level of repression by each mutant was calculated as a percent activity of wild-type $\alpha 2$ repression.

$MATa/mata\alpha 2$ strains for their production of a - or α -factor and for their sporulation efficiency (Table 2). In these strains the $\alpha 2$ mutations that we constructed show two types of halo phenotypes that agree well with the results from the reporter constructs. One set of mutants, H3-3A, Ser-181-Gln, and Lys-188-Ile, produce small a halos on the tester strain. The presence of an a halo shows that these mutants fail to repress transcription of the a -specific genes (see above). The small halos indicate that only a low level of the a -factor is being expressed. The most plausible explanation for the decrease in mating-factor expression is that these $\alpha 2$ mutants are working in combination with the $a1$ protein to repress transcription of the haploid-specific gene $STE12$, which is required for full expression of the a -specific genes (Fields and Herskowitz 1985). This result agrees well with the results of the hsg reporters that show full repression in strains carrying these $\alpha 2$ mutations. The other set of mutants, exemplified by Ser-181-Ile, Arg-185-Met and Arg-135-Ala, produce a large a halo on the tester strains. All of these mutants show slightly reduced levels of repression of the hsg reporter; therefore, the most likely explanation for the larger halos is that these strains not only fail to repress a -specific genes but also allow sufficient expression of $STE12$ for a -specific gene activation.

We also examined the ability of the $MATa/mata\alpha 2$ mutant strains to sporulate. We found that strains containing the wild-type $\alpha 2$, H3-3A, Ser-181-Gln, or Lys-188-Ile mutants are able to sporulate efficiently, showing that $a1/\alpha 2$ -mediated repression is functional in all of these strains. However, strains that show a slight reduction in their ability to repress the hsg reporter fail to sporulate. The most likely explanation for this phenotype is that these mutants fail to completely repress the haploid-specific $RME1$ gene, a repressor of the meiotic pathway (Mitchell and Herskowitz 1986).

In conclusion, the tests for mating, mating pheromone production, and sporulation are in excellent agreement with the assays utilizing reporter constructs and provide an independent confirmation of the results.

$\alpha 2$ homeo domain mutants fail to bind DNA on their own in vitro but still bind cooperatively with $a1$

To examine directly the effects of the $\alpha 2$ point substitutions on DNA binding, the mutant proteins were expressed in *Escherichia coli*, purified to $>90\%$ homogeneity, and assayed by electrophoretic mobility shift assays (Keleher et al. 1988; Sauer et al. 1988; Goutte and Johnson 1993). Wild-type intact $\alpha 2$ protein was compared with the same concentration of H3-3A, Arg-135-Ala, Lys-188-Ile, and Leu-196-Ser mutant proteins in binding to the asg operator on their own and cooperatively with MCM1 (Fig. 4A,B). The DNA-binding affinity of the H3-3A mutant protein appears to be at least 100-fold lower than wild-type protein, a result expected because the mutant protein lacks all of the base-specific contacts that the homeo domain makes in the major groove of the DNA. Single amino acid substitutions in these three positions also significantly reduce the level

Table 1. Mating-type and mating-factor production in $\alpha 2$ mutant strains

Strain	Genotype	Repression of asg reporter (%)	Halo ^a		Mating ^b	
			a	α	with MAT α strain	with MATa strain
246.1.1	MAT α	100	–	+	–	+
EG123	MATa	0	+	–	+	–
23 α 182	Δ mat α 2	0	+	–	–	–
YJ08	wild type	100	–	+	–	+
YJ05	R135A	0	+	–	–	–
YJ03	S181I	1	+	–	–	–
YJ07	S181Q	2	+	–	–	–
YJ02	R185M	0	+	–	–	–
YJ06	K188I	2	+	–	–	–
YJ01	H3-3A	0	+	–	–	–
YJ04	L196S	100	–	+	–	+

^a(+) The strain forms a halo on the appropriate pheromone-sensitive tester strain.

^b(+) 90–100% of wild-type mating efficiency; (–) less than a 0.1% efficiency.

of binding to the site on their own (data not shown). The affinity of Arg-135–Ala appears to be reduced by ~50-fold; this mutation removes the base-specific contact that the amino-terminal arm of the homeo domain makes in the minor groove of the DNA. The affinity of Lys-188–Ile, a mutant in a side chain that makes phosphate–backbone contact in the cocrystal structure, is also reduced by ~50-fold. These results indicate that the reduced level of repression in vivo is the result of the failure of $\alpha 2$ to bind to the operator site with wild-type affinity.

The wild-type and mutant $\alpha 2$ proteins were also tested for cooperative binding with **a1** to the hsg operator (Fig. 4C). The binding of the H3-3A, Arg-135–Ala, and Lys-188–Ile mutant proteins are not detectably reduced from that of the wild-type protein. As a control, Leu-196–Ser is reduced at least 200-fold in binding to the hsg operator with **a1**. Thus, the results obtained in vitro are consistent with our in vivo observations and confirm that the “base-specific” contacts between $\alpha 2$ and DNA are not crucial for the ability of $\alpha 2$ to act in combination with **a1**.

Discussion

The homeo domain protein $\alpha 2$, a transcriptional repressor in yeast, acts in combination with two additional proteins: With MCM1, $\alpha 2$ turns off the **a**-specific genes; with **a1**, $\alpha 2$ turns off the haploid-specific genes. The former combination occurs in α and **a**/ α cells, and the latter occurs in the **a**/ α cell only.

The X-ray structure of the $\alpha 2$ homeo domain complexed with a variant of the **a**-specific gene operator has been described (Wolberger et al. 1991), and we constructed a series of point mutations in the $\alpha 2$ homeo domain designed to impair base-specific and phosphate–backbone contacts without destabilizing the homeo domain structure. The analysis of these mutations leads to a surprising observation: Although nearly all of the mutations impaired the specific DNA binding of the $\alpha 2$ homeo domain on its own and impaired the combined action of $\alpha 2$ and MCM1, many of these same mutations had little or no effect on the cooperative binding of $\alpha 2$ and **a1** to DNA. Moreover, many of these mutant $\alpha 2$ s worked as efficiently in vivo as did wild-type $\alpha 2$ in re-

Table 2. Mating-factor production and sporulation efficiency in diploid $\alpha 2$ mutant strains

Strain	Genotype	Repression of hsg reporter (%)	Halo ^a		Sporulation efficiency (%)
			a	α	
EG123 × 246.1.1	a / α	100	–	+	38
EG123 × 23 α 182	a / Δ mat α 2	3	+	–	<0.1
YJ18	a /WT	100	–	–	39
YJ15	a /R135A	35	+	–	<0.1
YJ13	a /S181I	20	+	–	<0.1
YJ17	a /S181Q	100	+ / –	–	11
YJ12	a /R185M	45	+	–	<0.1
YJ16	a /K188I	100	+ / –	–	16
YJ11	a /H3-3A	100	+ / –	–	20
YJ14	a /L196S	2	–	+	<0.1

^a(+) That particular strain forms a halo on the appropriate pheromone-sensitive tester strain; (+ / –) A halo is formed on the tester strain, but it is significantly smaller than a normal halo.

Figure 4. Mutations in the $\alpha 2$ homeo domain have reduced binding affinity to the asg operator with and without MCM1 but have nearly wild-type binding affinity to the hsg operator in combination with **a1**. (A) Mobility retardation assay of the asg operator produced by decreasing concentrations of wild-type $\alpha 2$ (lanes 2–6), L196S (lanes 7–11), K188I (lanes 12–16), H3-3A (lanes 17–21), and R135A (lanes 22–26) mutant proteins in the absence of MCM1. The concentrations of $\alpha 2$ proteins are varied by fivefold dilutions from 4×10^{-7} M (lanes 2,7,12,17,22) to 6.4×10^{-10} M (lanes 6,11,16,21,26). Free DNA is shown in lane 1. (B) Mobility retardation assay to the asg operator produced by decreasing concentrations of wild-type $\alpha 2$ (lanes 3–7), L196S (lanes 8–12), K188I (lanes 13–17), H3-3A (lanes 18–22), and R135A (lanes 23–27) mutant proteins in the presence of a constant amount of MCM1 are shown. The concentrations of $\alpha 2$ proteins are varied by fivefold dilutions from 1.6×10^{-8} M (lanes 3,8,13,18,23) to 2.5×10^{-11} M (lanes 7,12,17,22,27). Note that lane 4 in A has the same concentration as lane 3 in B. Lane 2 shows the shift produced by MCM1 in the absence of $\alpha 2$, and lane 1 shows the free DNA. (C) Mobility retardation assay to the hsg operator produced by decreasing concentrations of wild-type (lanes 2–6), L196S (lanes 7–11), K188I (lanes 12–16), H3-3A (lanes 17–21), and R135A (lanes 22–26) $\alpha 2$ protein in the presence of a constant amount of **a1**. The concentrations of $\alpha 2$ proteins are varied by fivefold dilutions from 4×10^{-7} M (lanes 2,7,12,17,22) to 6.4×10^{-10} M (lanes 6,11,16,21,26). The shifted band at the highest concentration of L196S (lane 7) is slightly higher than a normal **a1**/ $\alpha 2$ shift and migrates at a position observed by a dimer of $\alpha 2$ binding alone to the site.

pressing transcription of the haploid genes in combination with **a1**. Thus, when taken in combination with MCM1, the homeo domain mutations had the predicted effect; when taken with **a1**, the mutations were largely silent. How can this "split phenotype" be explained? We consider four possibilities.

In theory, the simplest explanation would be that the $\alpha 2$ homeo domain does not contact DNA in the **a1**/ $\alpha 2$ -DNA complex. However, an extensive set of protein-DNA cross-linking experiments and chemical protection experiments has shown that the $\alpha 2$ homeo domain does contact DNA when bound with **a1** (Goutte and Johnson 1993, 1994). Moreover, genetic and biochemical experiments have shown that the homeo domain of $\alpha 2$ is required for the DNA-binding activity of **a1**/ $\alpha 2$ (Porter and Smith 1986; Hall and Johnson 1987; Goutte and Johnson 1988).

A second possible explanation for the differential effects of the mutations is that $\alpha 2$ may contact DNA in

two distinct ways: one in the $\alpha 2$ /DNA and $\alpha 2$ /MCM1/DNA complexes and the other in the **a1**/ $\alpha 2$ /DNA complex. According to this explanation, the point mutations would compromise the former but not the latter mode of DNA binding. Although it is difficult to rule out small differences in orientation, several lines of experiments suggest that $\alpha 2$ contacts DNA in roughly the same way in the three different complexes. First, chemical protection experiments show that the homeo domain of $\alpha 2$ makes a similar set of DNA contacts whether or not it is complexed with **a1** (Goutte and Johnson 1994). Second, if the orientation of the $\alpha 2$ homeo domain was dramatically shifted in the **a1**/ $\alpha 2$ complex, one would expect other surface-exposed residues of the homeo domain to contact DNA. However, we have shown that mutations of residues on the surface of helix 2 or the beginning of helix 3 had no effect on **a1**/ $\alpha 2$ activity (Fig. 2C). Thus, it seems unlikely that $\alpha 2$ has two drastically different ways of contacting DNA.

A third possibility is based on a model where $\alpha 1$ and $\alpha 2$ make equal contributions to the DNA-binding affinity and specificity of the heterodimer. According to this model, only half of the heterodimer-DNA contacts would be compromised by the $\alpha 2$ mutations, because $\alpha 1$ is still wild type. In contrast, because $\alpha 2$ binds as a dimer with MCM1, the $\alpha 2$ mutations would be expected to have a much more deleterious effect (roughly the square of the heterodimer affinity decrease). Although this argument must be true in principle, we feel that it cannot adequately explain the wild-type phenotype of the $\alpha 2$ mutants taken in combination with $\alpha 1$. The binding of a dimeric protein (whether from prokaryotes or eukaryotes) to DNA is typically disrupted by the elimination of one set of monomer-DNA contacts. This applies to the binding of an $\alpha 2$ dimer with MCM1: The removal of one set of specific $\alpha 2$ -DNA contacts (by mutation of one-half of the operator) severely disrupts $\alpha 2$ /MCM1 repression in vivo (Smith and Johnson 1994). Thus, it seems unlikely that an $\alpha 1/\alpha 2$ heterodimer could accommodate a loss of half (or nearly half) of its specific DNA contacts and still function in the cell.

A fourth possibility, and the one we favor, is that $\alpha 1$ provides the majority of the DNA-binding specificity for the heterodimer and that the constraints on $\alpha 2$ -DNA recognition are therefore loosened. Two additional observations are consistent with this idea: (1) When the known hsg operators are compared, the $\alpha 1$ halves of the site are conserved much more than are the $\alpha 2$ halves (Miller et al. 1985; Goutte and Johnson 1994). (2) Under a specific set of biochemical conditions, the specific/nonspecific DNA-binding ratio is ~ 30 for the $\alpha 2$ dimer but $\sim 30,000$ -fold for the $\alpha 1/\alpha 2$ heterodimer, again suggesting that $\alpha 1$ provides a large fraction of the specificity. (Because under the same conditions $\alpha 1$ does not bind DNA alone, we were unable to directly determine the $\alpha 1$ specificity ratio.) Therefore, we propose that in the $\alpha 1/\alpha 2$ DNA complex, $\alpha 1$ is providing the majority of the specific DNA contacts and the $\alpha 2$ /DNA interface can tolerate changes in the residues that contact DNA without significantly compromising the ability of the heterodimer to recognize specific DNA in the cell. According to this model, the main role of $\alpha 2$ in the heterodimer would be to provide protein-protein contacts that convert $\alpha 1$ from a weak to a tight binding form.

Regardless of their precise molecular explanation, the results in this paper show that in one context (acting with MCM1) the $\alpha 2$ homeo domain is extremely sensitive to mutations that change residues responsible for sequence-specific binding. In another context (with $\alpha 1$), the $\alpha 2$ homeo domain appears buffered, that is, relatively insensitive to these same changes. Other studies of homeo domain proteins have also revealed, in some in vivo contexts, a relative insensitivity to mutations in the DNA-binding residues. Porter and Smith (1986) analyzed in vivo a series of point mutations in the $\alpha 2$ homeo domain. These studies were carried out before structural information for homeo domains was available, and, viewed in retrospect, many of these changes probably destabilized the homeo domain structure and thereby

inactivated both $\alpha 2$ /MCM1 and $\alpha 1/\alpha 2$ repression in vivo. However, several mutations (Asn-178-His, Lys-186-Asn, and Lys-188-Ile) showed a selective reduction in $\alpha 2$ /MCM1 repression, a result that anticipated some of the results presented in this paper. Another of their mutations, Asn-182-Asp, compromised $\alpha 2$ /MCM1 function as well as $\alpha 1/\alpha 2$ function in vivo, whereas a change of Asn-182 to Ala (Fig. 1C) inactivated only $\alpha 2$ /MCM1 function. In the crystal structure, the Asn-182 side chain contacts another side chain in helix 3 as well as the DNA, and there are several possible reasons for the different phenotypes of these two mutations. Because the Asn-182-Asp protein is deficient in all functions of $\alpha 2$, it is possible that it is expressed poorly or is less stable than the wild type. A second possibility is that the change from Asn to Asp introduces an unfavorable negative charge into the DNA-protein interface that simply prevents the close approach of DNA and protein.

Experiments with *Drosophila* homeo domains have also revealed instances where mutations in DNA-binding residues do not necessarily eliminate biological functions. Schier and Gehring (1993) showed that a *ftz* to *bcd* altered specificity mutant, created by a Gln-50-Lys change in *ftz*, provides efficient rescue of parasegments 8 and 14 but poorer rescue of other structures. Other mutations that compromise the strength of the *ftz*-DNA interaction show similar effects (Furukubo-Tokunaga et al. 1992).

These observations have several implications for the general understanding of homeo domain proteins. First, the study of homeo domain-DNA interactions in isolation, although of undeniable importance, may not necessarily reflect how these proteins act in vivo. For example, there appears to be little correlation between the behavior of many of the mutant $\alpha 2$ proteins in binding DNA alone when compared with binding DNA with $\alpha 1$. In particular, the alteration of residues that, as seen to the X-ray structure, make specific contacts with DNA (Arg-135, Ser-181, Asn-182, and Arg-185) drastically reduces specific DNA binding by $\alpha 2$ alone but has little or no effect on the cooperative binding of $\alpha 2$ and $\alpha 1$ to DNA.

A second implication of these observations relates to the way homeo domain proteins may have evolved to participate in more than one regulatory circuit. It would seem that the specific DNA-binding residues of $\alpha 2$ are tailored for binding to *asg* operator with MCM1. On the basis of homologies in other fungi, it has been proposed that the $\alpha 1/\alpha 2$ circuit is of more ancient origin than the $\alpha 2$ /MCM1 circuit (Johnson 1992). These ideas suggest a model where the $\alpha 1/\alpha 2$ circuit could be maintained while allowing the $\alpha 2$ homeo domain to undergo changes in DNA-binding specificity. That is, features of the $\alpha 1/\alpha 2$ circuit "free up" the DNA-binding residues of the $\alpha 2$ homeo domain for testing out and perhaps adopting new circuits without compromising the existing circuit. As many homeo domain proteins from other organisms are known to participate in multiple circuits, perhaps situations where homeo domains are buffered against mutational changes will be common.

Materials and methods

Plasmids

Mutations in the $\alpha 2$ gene were constructed by site-directed oligonucleotide mutagenesis using the *dut*⁻, *ung*⁻ system (Smith 1985) in plasmid pAV99, a P_{tac} - $\alpha 2$ vector with a single-stranded bacteriophage f1 origin of replication (Mak and Johnson 1993). Oligonucleotides, used in the mutagenesis or construction of DNA-binding sites, were synthesized at the Biotechnology Resource Center at University of California San Francisco (UCSF) or on an ABI 392 synthesizer at the Waksman Institute, Rutgers University. Isolates of pAV115, a *CEN LEU2* yeast plasmid with a 4.3-kb *HindIII* fragment that contains the entire *MAT α* locus and the specific site-directed mutations in the $\alpha 2$ gene, were constructed by cloning the 0.6-kb *BglIII*-*BamHI* fragment from pAV99 containing the different mutations in the $\alpha 2$ gene. The construction of plasmids pAV99, pAV114 (a version of pAV115 without the *MAT α* fragment), and pAV115 was described in Mak and Johnson (1993). Plasmids pAV71 and pAV73, reporter vectors with and without the *STE6* $\alpha 2$ /MCM1 DNA-binding site controlling expression of a *CYC1-lacZ* fusion, have been described elsewhere (Vershon et al. 1992). Plasmid pYJ103, which was used as a reporter plasmid for $\alpha 1/\alpha 2$ -mediated repression, was constructed by inserting an oligonucleotide that contains a consensus $\alpha 1/\alpha 2$ binding site (TCATGTAATTAAT-TACATCA) into the *SalI* site in the *CYC1-lacZ* fusion promoter of pAV73.

Yeast strains

Most of the strains used in this study are congenic with strain EG123 (*trp1 leu2 ura3 his4*) and only vary at the *MAT* locus (Siliciano and Tatchell 1984). Wild-type *MAT α* and *MAT α* strains are EG123 and 246.1.1, and the wild-type *MAT α /MAT α* diploid used in these experiments is a product of a mating between these two strains. *mat Δ* and *mat $\alpha 2$* cells are strains KT23 $\alpha 8$ and 23 $\alpha 182$ (Tatchell et al. 1981) and were used as controls in the *CYC1-lacZ* repression and biological assays. The control *MAT α /mat $\alpha 2$* strain is from a mating of strains EG123 $\times$ 23 $\alpha 182$. The construction of reporter strains containing integrated copies of *CYC1-lacZ* fusions with $\alpha 2$ /MCM1 or $\alpha 1/\alpha 2$ DNA-binding sites by K. Komachi has been outlined in Mak and Johnson (1993).

Strain YV50 was constructed by integration at the *MAT* locus of a 4.3-kb *HindIII* *MAT $\alpha 1$* , *mat $\alpha 2$::URA3* fragment in which the entire $\alpha 2$ gene has been replaced with *URA3*. This strain was used to integrate the $\alpha 2$ mutants into the *MAT* locus by the following procedure: Isolates of pAV115, containing different site-directed mutations in the $\alpha 2$ gene, were digested with *HindIII*, and the linear 4.3-kb *MAT α* fragment was transformed into strain YV50 by the lithium acetate procedure (Ito et al. 1983). The transformed cells were grown on YEPD plates overnight at 30°C and then replated on SD + 5-fluoro-uracil acid (5-FOA) plates to select for a *Ura*⁻ phenotype (Sikorski and Boeke 1991). Resistant colonies were rescreened on SD + 5-FOA and SD-*Ura* plates to confirm the loss of *URA3*. Positive colonies were assayed by Southern blot for the presence of a 0.8-kb *BamHI*-*BglIII* fragment, which is characteristic for the mutant $\alpha 2$ gene from pAV115. The following strains were constructed by this procedure: YJ08 (a reconstruction with wild-type $\alpha 2$), YJ01 (H3-3A), YJ02 (Arg-185-Met), YJ03 (Ser-181-Ile), YJ04 (Leu-196-Ser), YJ05 (Arg-135-Ala), YJ06 (Lys-188-Ile), and YJ07 (Ser-181-Gln).

Because strains containing integrated copies of the $\alpha 2$ mutants will not mate properly with *MAT α* cells, diploid strains containing the $\alpha 2$ mutants were constructed by transforming

these strains with a wild-type *MAT α URA3* 2 μ plasmid and mating the transformants with a *MAT α* strain containing a *TRP1 ARS* plasmid. Diploids were selected on SD-*Ura*-*Trp* plates—the plasmids were lost by growing the cells nonselectively on YEPD—and then screened for colonies that were unable to grow in the absence of uracil and tryptophan. The presence of the $\alpha 2$ mutation in the resulting strains was verified by Southern blot for the presence of the $\alpha 2$ *BamHI*-*BglIII* fragment and the *MAT $\alpha 1$* allele. Strains YJ18 (wild-type *MAT α /MAT α*), YJ11 (*MAT α /mat $\alpha 2$* H3-3A), YJ12 (*MAT α /mat $\alpha 2$* Arg-185-Met), YJ13 (*MAT α /mat $\alpha 2$* Ser-181-Ile), YJ14 (*MAT α /mat $\alpha 2$* Leu-196-Ser), YJ15 (*MAT α /mat $\alpha 2$* Arg-135-Ala), YJ16 (*MAT α /mat $\alpha 2$* Lys-188-Ile), and YJ17 (*MAT α /mat $\alpha 2$* Ser-181-Gln) were constructed by the above procedure.

Yeast media used in these experiments are described in Sherman et al. (1979). Halo, mating type, and sporulation tests were performed using the same procedures and strains as described in Mak and Johnson (1993).

Protein purification

Full-length wild-type $\alpha 2$ (residues 1–210) was purified from strain SY903.1 containing plasmid pDS-1, a P_{tac} - $\alpha 2$ expression vector, by a modified version of the procedure in Sauer et al. (1988) as described in Mak and Johnson (1993). Mutant $\alpha 2$ proteins were purified by the same procedure from transformants of strain SY903.1 with pAV100 containing mutations in the $\alpha 2$ gene. Plasmid pAV100, a P_{tac} - $\alpha 2$ vector, was constructed by removing a 1.4-kb *BamHI* fragment from pAV99 that is 200 bp past the termination codon of the $\alpha 2$ gene. We have observed that removal of this fragment allows greater expression of the $\alpha 2$ protein. Purified mutant and wild-type $\alpha 2$ proteins were dialyzed into S + 500 buffer (50 mM Tris-HCl at pH 8.0, 1 mM EDTA, 10 mM β -mercaptoethanol, 500 mM NaCl). The concentrations of the purified proteins were determined by measuring the absorbance at 280 nm and using the theoretical extinction coefficient of OD 0.60 per mg/ml for full-length $\alpha 2$. The relative purities and concentrations of the different proteins were verified by Coomassie staining of SDS gels of the samples. $\alpha 1$ Protein was purified from bacteria transformed with a P_{T7} - $\alpha 1$ expression vector and was a gift from C. Goutte (UCSF, CA). The MCM1_{1–97} protein used in the DNA-binding experiments was a fragment of the intact protein (residues 1–97) that contains the DNA-binding domain and was purified from bacteria that contain pAV154 as described in Vershon and Johnson (1993).

DNA-binding experiments

The labeled wild-type $\alpha 2$ /MCM1 (from the *STE6* promoter) and $\alpha 1/\alpha 2$ (from the *MF $\alpha 1$* promoter) sites used in the gel mobility shift experiments were generated by isolating the 86- and 73-bp fragments from an *EcoRI* and *HindIII* restriction digest of pCK1 and pCG25, respectively (Goutte and Johnson 1988; Keleher et al. 1988), and filling the 5' overhangs with ³²P using Klenow polymerase. Mobility retardation assays were performed in 20 mM Tris (pH 8.0), 1 mM EDTA, 5 mM MgCl₂, 10 mM BSA (fraction VI), 5% glycerol, 0.1% NP-40, and 10 μ g/ml of sheared salmon sperm DNA. Protein dilutions were made in 50 mM Tris (pH 8.0), 1 mM EDTA, 500 mM NaCl, 10 mM 2-mercaptoethanol, and 10 mg/ml of BSA. Five microliters of $\alpha 2$ and 5 μ l of MCM1, $\alpha 1$, or protein dilution buffer were added to 40 μ l of end-labeled operator fragment diluted in assay buffer, so that the final NaCl concentration was 100 mM. Reactions were incubated at room temperature for at least 1.5 hr and one-half of the reaction was loaded onto 6% polyacrylamide gel and electrophoresed at 200

V for 2 hr. Gels were dried and scanned on a Molecular Dynamics PhosphorImager.

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References

- Ades, S.E. and R.T. Sauer. 1994. Differential DNA-binding specificity of the engrailed homeodomain: The role of residue 50. *Biochemistry* **33**: 9187–9194.
- Ammerer, G. 1990. Identification, purification, and cloning of a polypeptide (PRTF/GRM) that binds to mating-specific promoter elements in yeast. *Genes & Dev.* **4**: 299–312.
- Billeter, M., Y.Q. Qian, G. Otting, M. Muller, W. Gehring, and K. Wuthrich. 1993. Determination of the nuclear magnetic resonance solution structure of an *Antennapedia* homeodomain-DNA complex. *J. Mol. Biol.* **234**: 1084–1097.
- Ceska, T.A., M. Lamers, P. Monaci, A. Nicosia, R. Courtese, and D. Suck. 1993. The X-ray structure of an atypical homeodomain present in the rat liver transcription factor LFB1/HNF1 and implications for DNA binding. *EMBO J.* **12**: 1805–1810.
- Dranginis, A.M. 1990. Binding of yeast $\alpha 1$ and $\alpha 2$ as a heterodimer to the operator DNA of a haploid-specific gene. *Nature* **347**: 682–685.
- Fields, S. and I. Herskowitz. 1985. The yeast *STE12* product is required for expression of two sets of cell-type-specific genes. *Cell* **42**: 923–930.
- Furukubo-Tokunaga, K., M. Muller, M. Affolter, L. Pick, U. Kloter, and W.J. Gehring. 1992. In vivo analysis of the helix-turn-helix motif of the *fushi tarazu* homeo domain of *Drosophila melanogaster*. *Genes & Dev.* **6**: 1082–1096.
- Gehring, W.J., Y.Q. Qian, M. Billeter, K. Furukubo-Tokunaga, A.F. Schier, D. Resendez-Perez, M. Affolter, G. Otting, and K. Wuthrich. 1994. Homeodomain-DNA recognition. *Cell* **78**: 211–223.
- Goutte, C. and A.D. Johnson. 1988. $\alpha 1$ protein alters the DNA binding specificity of $\alpha 2$ repressor. *Cell* **52**: 875–882.
- . 1993. Yeast $\alpha 1$ and $\alpha 2$ homeodomain proteins form a DNA-binding activity with properties distinct from those of either protein. *J. Mol. Biol.* **233**: 359–371.
- . 1994. Recognition of a DNA operator by a dimer composed of two different homeodomain proteins. *EMBO J.* **13**: 1434–1442.
- Hall, M.N. and A.D. Johnson. 1987. homeodomain of the yeast repressor $\alpha 2$ is a sequence-specific DNA-binding domain but is not sufficient for repression. *Science* **237**: 1007–1012.
- Hanes, S.D. and R. Brent. 1989. DNA specificity of the Bicoid activator protein is determined by homeodomain recognition helix residue 9. *Cell* **57**: 1275–1283.
- Hayashi, S. and M.P. Scott. 1990. What determines the specificity of action of *Drosophila* homeodomain proteins? *Cell* **63**: 883–894.
- Ito, H., Y. Kukada, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali ions. *J. Bacteriol.* **153**: 163–168.
- Johnson, A.D. 1992. A combinatorial regulatory circuit in budding yeast. In *Transcriptional regulation* (ed. K. Yamamoto and S. McKnight), pp. 975–1006. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Johnson, A.D. and I. Herskowitz. 1985. A repressor (MAT $\alpha 2$ product) and operator control expression of a set of cell type specific genes in yeast. *Cell* **42**: 237–247.
- Keleher, C.A., C. Goutte, and A.D. Johnson. 1988. The yeast cell-type repressor $\alpha 2$ acts cooperatively with a non-cell-type-specific protein. *Cell* **53**: 927–936.
- Kissinger, C.R., B. Liu, E. Martin-Blanco, T.B. Kornberg, and C.O. Pabo. 1990. Crystal structure of an engrailed homeodomain-DNA complex at 2.8 Å resolution: A framework for understanding homeodomain-DNA interactions. *Cell* **63**: 579–590.
- Klemm, J.D., M.A. Rould, R. Aurora, W. Herr, and C.O. Pabo. 1994. Crystal structure of the Oct-1 POU domain bound to an octamer site: DNA recognition with tethered DNA-binding modules. *Cell* **77**: 21–32.
- Laughon, A. and M.P. Scott. 1984. The sequence of a *Drosophila* segmentation gene: Protein structure homology with DNA binding proteins. *Nature* **310**: 25–30.
- Leiting, B., R. De Francesco, L. Tomei, R. Courtese, G. Otting, and K. Wuthrich. 1993. The three-dimensional NMR-solution structure of the polypeptide fragment 195–286 of the LBF/HNF1 transcription factor from rat liver comprises a non-classical homeo domain. *EMBO J.* **12**: 1797–1803.
- Mak, A. and A.D. Johnson. 1993. The carboxy-terminal tail of the homeo domain protein $\alpha 2$ is required for function with a second homeo domain protein. *Genes & Dev.* **7**: 1862–1870.
- McGinnis, W., M.S. Levine, E. Hafen, A. Kuroiwa, and W.J. Gehring. 1984a. A conserved DNA sequence in homeotic genes of the *Drosophila Antennapedia* and *bithorax* complexes. *Nature* **308**: 428–433.
- McGinnis, W., R.L. Garber, J. Wirz, A. Kuroiwa, and W.J. Gehring. 1984b. A homologous protein-coding sequence in *Drosophila* homeotic genes and its conservation in other metazoans. *Cell* **37**: 403–408.
- Miller, A.M., V.L. MacKay, and K.A. Nasmyth. 1985. Identification and comparison of two sequence elements that confer cell-type specific transcription in yeast. *Nature* **314**: 598–603.
- Mitchell, A.P. and I. Herskowitz. 1986. Activation of meiosis and sporulation by repression of the *RME1* product in yeast. *Nature* **319**: 738–742.
- Otting, G., Y.Q. Qian, M. Billeter, M. Mueller, M. Affolter, W.J. Gehring, and K. Wuthrich. 1990. Protein-DNA contacts in the structure of a homeodomain-DNA complex determined by nuclear magnetic resonance spectroscopy in solution. *EMBO J.* **9**: 3085–3092.
- Passmore, S., R. Elble, and B.-K. Tye. 1989. A protein involved in minichromosome maintenance in yeast binds a transcriptional enhancer conserved in eukaryotes. *Genes & Dev.* **3**: 921–935.
- Phillips, C.L., A.K. Vershon, A.D. Johnson, and F.W. Dahlquist. 1991. Secondary structure of the homeodomain of yeast $\alpha 2$ repressor determined by NMR spectroscopy. *Genes & Dev.* **5**: 764–772.
- Porter, S.D. and M. Smith. 1986. homeo domain homology in yeast MAT $\alpha 2$ is essential for repressor activity. *Nature* **320**: 766–768.
- Qian, Y.Q., M. Billeter, G. Otting, M. Muller, W.J. Gehring, and K. Wuthrich. 1989. The structure of the *Antennapedia* homeodomain determined by NMR spectroscopy in solution:

- Comparison with prokaryotic repressors. *Cell* **59**: 573–580.
- Sauer, R.T., D.L. Smith, and A.D. Johnson. 1988. Flexibility of the yeast $\alpha 2$ repressor enables it to occupy the ends of its operator, leaving the center free. *Genes & Dev.* **2**: 807–816.
- Schier, A.F. and W.J. Gehring. 1993. Functional specificity of the homeodomain protein fushi tarazu: The role of DNA-binding specificity *in vivo*. *Proc. Natl. Acad. Sci.* **90**: 1450–1454.
- Scott, M.P., J.W. Tamkun, and G.W. Hartzell. 1989. The structure and function of the homeodomain. *Biochem. Biophys. Acta* **989**: 25–48.
- Shepherd, J.C.W., W. McGinnis, A.E. Carrasco, E.M. De Robertis, and W.J. Gehring. 1984. Fly and frog homeo-domains show homologies with yeast mating type regulatory proteins. *Nature* **310**: 70–71.
- Sherman, F., G.R. Fink, and C.W. Lawrence. 1979. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Sikorski, R.S. and J.D. Boeke. 1991. *In vitro* mutagenesis and plasmid shuffling: From cloned gene to mutant yeast. *Methods Enzymol.* **194**: 302–318.
- Siliciano, P.G. and K. Tatchell. 1984. Transcription and regulatory signals at the mating type locus in yeast. *Cell* **37**: 969–978.
- Smith, D.L. and A.D. Johnson. 1992. A molecular mechanism for combinatorial control in yeast: MCM1 protein sets the spacing and orientation of the homeodomain of an $\alpha 2$ dimer. *Cell* **68**: 133–142.
- . 1994. Operator-constitutive mutations in a DNA sequence recognized by a yeast homeodomain. *EMBO J.* **13**: 2378–2387.
- Smith, M. 1985. *In vitro* mutagenesis. *Annu. Rev. Genet.* **19**: 423–462.
- Sprague, G.F. 1991. Assay of yeast mating reaction. *Methods Enzymol.* **147**: 357–372.
- Strathern, J., J. Hicks, and I. Herskowitz. 1981. Control of cell type in yeast by the mating type locus: The $\alpha 1$ - $\alpha 2$ hypothesis. *J. Mol. Biol.* **147**: 357–372.
- Strathern, J., B. Shafer, J. Hicks, and C. McGill. 1988. α / α -Specific repression by MAT $\alpha 2$. *Genetics* **120**: 75–81.
- Tatchell, K., K.A. Nasmyth, and B.D. Hall. 1981. *In vitro* mutation analysis of the mating-type locus in yeast. *Cell* **27**: 25–35.
- Treisman, J., P. Gonczy, M. Vashishtha, E. Harris, and C. Desplan. 1989. A single amino acid can determine the DNA binding specificity of homeodomain proteins. *Cell* **59**: 553–562.
- Vershon, A.K. and A.D. Johnson. 1993. A short, disordered protein region mediates interactions between the homeodomain of the yeast $\alpha 2$ protein and the MCM1 protein. *Cell* **72**: 105–112.
- Vershon, A.K., N.M. Hollingsworth, and A.D. Johnson. 1992. Meiotic induction of yeast HOP1 gene is controlled by positive and negative regulatory sites. *Mol. Cell. Biol.* **12**: 3706–3714.
- Wolberger, C., A.K. Vershon, B. Liu, A.D. Johnson, and C.O. Pabo. 1991. Crystal structure of a MAT $\alpha 2$ homeodomain-operator complex suggests a general model for homeodomain-DNA interactions. *Cell* **67**: 517–528.
- Wuthrich, K. and W. Gehring. 1992. Transcriptional regulation by homeodomain proteins: Structural, functional, and genetic aspects. In *Transcriptional regulation* (ed. K. Yamamoto and S. McKnight), pp. 535–566. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

A homeo domain protein lacking specific side chains of helix 3 can still bind DNA and direct transcriptional repression.

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