The DNA-binding Domain of the Yeast Spt10p Activator Includes a Zinc Finger That Is Homologous to Foamy Virus Integrase

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The yeast SPT10 gene encodes a putative histone acetyltransferase that binds specifically to pairs of upstream activating sequence (UAS) elements found only in the histone gene promoters. Here, we demonstrate that the DNA-binding domain of Spt10p is located between residues 283 and 396 and includes a His2-Cys2 zinc finger. The binding of Spt10p to the histone UAS is zinc-dependent and is disabled by a zinc finger mutation (C388S). The isolated DNA-binding domain binds to single histone UAS elements with high affinity. In contrast, full-length Spt10p binds with high affinity only to pairs of UAS elements with very strong positive cooperativity and is unable to bind to a single UAS element. This implies the presence of a “blocking” domain in full-length Spt10p, which forces it to search for a pair of UAS elements. Chromatin immunoprecipitation experiments indicate that, unlike wild-type Spt10p, the C388S protein does not bind to the promoter of the gene encoding histone H2A (HTA1) in vivo. The C388S mutant has a phenotype similar to that of the spt10Δ mutant: poor growth and global aberrations in gene expression. Thus, the C388S mutation disables the DNA-binding function of Spt10p in vitro and in vivo. The zinc finger of Spt10p is homologous to that of foamy virus integrase, perhaps suggesting that this integrase is also a sequence-specific DNA-binding protein.

Eukaryotic DNA is packaged into nucleosomes, which contain 147 bp of DNA wrapped around a central core histone octamer composed of two molecules each of the four core histones (H2A, H2B, H3, and H4). The core histones have “tail” domains that project out of the nucleosome core and are not necessary for the integrity of the nucleosome (1). The majority of post-translational modifications occur in the tail domains; they include acetylation, methylation, phosphorylation, ubiquitylation, and ADP-ribosylation. These post-translational modifications play important roles in gene regulation. The “histone code” hypothesis proposes that histone modifications are “marks” recognized by regulatory proteins (2).

Acetylation remains the best studied of all of the histone modifications, but new acetylation sites are still being identified (3). A recent example is the acetylation of Lys56 of histone H3 in budding yeast (4–6). This particular acetylation is unusual in that it occurs at a residue located in the nucleosome core rather than in the histone tails. It seems likely that acetylation of histone H3 Lys56 would have a major effect on nucleosome conformation because it eliminates a positive charge involved in DNA binding within the nucleosome (4), although there is as yet no evidence for this. Acetylation of histone H3 Lys56 in nucleosomes at the core histone promoters is dependent on the SPT10 gene (4), which encodes a putative histone acetyltransferase (7), suggesting that Spt10p itself is responsible for this histone H3 Lys56 acetylation. However, it has not yet been possible to demonstrate that Spt10p has histone acetyltransferase activity in vitro (4, 8). In addition, we have shown previously that targeted acetylation of histone H3 Lys79/Lys84 and of the histone H4 tail at the CLIP1 promoter is dependent on SPT10 (9). Thus, the questions of whether Spt10p is indeed a histone acetyltransferase and the identity of its target residues remain unsolved.

SPT10 was originally identified as one of a set of SPT genes. Mutations in the SPT genes suppress phenotypes associated with insertion of the yeast Ty1 transposable element into promoters (10). The SPT genes turned out to encode many important transcription proteins, including subunits of the Spt-Ada-Gcn5-acetyltransferase histone-modifying complex (11), TATA box-binding protein, and histones (12, 13). SPT10 is not an essential gene, but the null allele is associated with very slow growth and defects in gene regulation (14–16). Spt10p also activates the histone genes, which it regulates in conjunction with Spt21p (8), the Hır corepressor (17, 18), and the SWI/SNF ATP-dependent chromatin remodeling machine (4, 18).

We (9) and others (8) have proposed that Spt10p might be a coactivator recruited to promoters by activators. However, we have shown recently that Spt10p is in fact a sequence-specific DNA-binding protein that recognizes histone upstream activating sequence (UAS) elements with the consensus sequence (G/A)TTCCN6TTCNC (19). Spt10p appears to be the activator of the yeast core histone genes, which has been sought after for many years (20, 21). We found that it binds with high affinity and with extraordinary positive cooperativity to pairs of histone UAS elements. Because pairs of histone UAS elements are found only in the core histone promoters and nowhere else in the yeast genome, there are no other predicted sites for Spt10p binding. Although it remains formally possible that Spt10p is recruited to other promoters as a conventional coactivator, without employing its ability to recognize the histone UAS, it seems more likely that its effects at other genes are indirect, mediated through defects in chromatin structure arising from a deficit of histones in spt10Δ cells (19).

Here, we show that the DNA-binding domain of Spt10p is located between residues 283 and 396. It can recognize a single histone UAS element, unlike the full-length protein. This implies the presence of a...
blocking domain in Spt10p, which guarantees that the protein will bind only pairs of histone UAS elements. The binding of Spt10p to DNA is zinc-dependent, and the DNA-binding domain contains a predicted basic zinc-finger, which is necessary but not sufficient for DNA binding. We show that a mutation in this zinc finger (C388S) seriously compromises the binding of Spt10p to the histone UAS elements in vitro and in vivo. This mutation is associated with a very poor growth phenotype similar to that of the null mutant. The DNA-binding domain of Spt10p has an intriguing homology to the zinc finger domain of foamy virus integrase. We speculate that foamy virus integrase might recognize a similar DNA sequence.

EXPERIMENTAL PROCEDURES

Construction of spt10 Mutant Strains and Transforming Plasmids—All yeast strains used in this study were derived from BJ5459 (ATCC 208284): MATa ura3-52 trp1 lys2-801 leu2Δ1 his3Δ200 pep4Δ:his3 prb1Δ1.6R can1 GAL circ. BJ-spt10Δ has been described (19). BJ-SPT10-HA was constructed by transformation of BJ5459 with a SacI-HindIII digest of p355 (19); this results in the integration of DNA encoding three C-terminal hemagglutinin (HA) tags and URA3 as selection marker just downstream of the chromosomal SPT10 gene. The C388S, H355S, and H416S mutants were constructed using derivatives of p355. BJ-SPT10(C388S)-HA was constructed using p383, which was made by substituting the 731-bp BstEII-MscI fragment of wild-type SPT10 in p355 with the mutant version obtained from pGN1622 (a gift of Dr. Jef Boeke) (15). The C388S mutation was marked with a TaqI site. BJ-SPT10(H355S)-HA was constructed using p384, in which the 664-bp Swal-BstEII fragment of SPT10 in p355 was substituted with a mutant version made by PCR. The H355S mutation was marked with an AvrII site. BJ-SPT10(H416S)-HA was constructed using p402, in which the 731-bp BstEII-MscI fragment of SPT10 in p355 was substituted with a mutant version made by PCR. The H416S mutation was marked with a Clal site. Plasmid sequences were verified. The required strains were identified by Southern blot analysis; the presence of the mutation was confirmed using the marker restriction site. For the overexpression study, BJ-SPT10-HA and BJ-SPT10(C388S)-HA were transformed with plasmids based on pRS425 (a 2-μm vector carrying LEU2 as a selection marker). pRS425-SPT10-HA (p438) was made by transferring the 1679-bp HindIII-NsiI fragment carrying the SPT10-HA gene from p375 (19) to pRS425 cut with the same enzymes. pRS425-SPT10(C388S)-HA (p446) was made by substituting the 1522-bp HindIII-NdeI fragment of wild-type SPT10 in p438 with the mutant version derived from p383.

Purification of Spt10 Proteins—Baculoviral recombinant Spt10p with three HA and three FLAG tags at its C terminus was purified as described above, except that 10 mM pepstatin A was present in all buffers. Recombinant Spt10p(C388S) was prepared similarly from His6 cells, except that the multiplicity of infection was 10, and the cells were harvested 48 h post-infection. The C388S baculovirus was constructed using pU22, which is based on pFastBac1 (Invitrogen). pU22 was constructed by substituting the 742-bp BstEII-ClaI SPT10 fragment in p349 (19) with a version carrying the C388S mutation from p383. Recombinant Spt10p fragments were prepared from Escherichia coli. An expression vector for full-length SPT10 with an N-terminal His6 tag (p342) was constructed by inserting the 2293-bp BamHI fragment from pNEB-SPT10-B (9) at the BamHI site of pET-28a (+) (Novagen). A single C-terminal FLAG tag was inserted by replacing the 519-bp MscI-Xhol SPT10 fragment in p342 with a FLAG-tagged version made by PCR to obtain p348. Various Spt10p fragments were prepared using expression plasmids based on p348; the Ncol–KpnI SPT10 fragment in p348 was replaced with truncated versions made by PCR. (The Ncol site precedes the N-terminal His tag, so this was deleted; the KpnI site marks the beginning of the single C-terminal FLAG tag, which was preserved.) Expression vectors for residues 212–508 (p467), 227–433 (p466), 283–396 (p481), and 326–396 (p485) were constructed. A version of p481 carrying the C388S mutation (p490) was made using p446 as the PCR template. These plasmids were introduced into E. coli Tuner(DE3)pLysS (Novagen) for expression; cells were grown to an A600

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The Binding of Spt10p to the Histone UAS Element Is Zinc-dependent—The goal of this work was to define and characterize the DNA-binding domain of Spt10p. A search for a possible DNA-binding
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The zinc ion in a zinc finger is coordinated by the thiol groups of cysteine residues and/or the imidazole nitrogens of histidine residues. The side chain of serine differs from that of cysteine only in the substitution of oxygen for sulfur. Nevertheless, serine is a poor substitute for cysteine, and therefore, Cys-to-Ser mutations are often used to inactivate zinc fingers. To address the importance of Cys388 in the binding of Spt10p to DNA in vitro, baculoviral recombinant Spt10p carrying the C388S mutation was prepared. This turned out to be very difficult because the mutant protein was very susceptible to proteolysis, perhaps suggesting that it was partially unfolded. Nevertheless, using high concentrations of the protease inhibitor pepstatin A, it was possible to obtain preparations with some intact protein (Fig. 1E). At high concentrations, the C388S protein aggregated the DNA without measurable loss of zinc ions. This demonstrates the importance of Cys388 in the recognition of the UAS element and is consistent with a role for Cys388 in zinc binding.

The DNA-binding Domain of Spt10p Is Located between Residues 283 and 396—We analyzed the distribution of positively charged residues in the sequence of Spt10p for clues as to the possible extent of the DNA-binding domain, assuming the involvement of one of the two possible zinc fingers (Fig. 2A). This distribution revealed several clusters of basic motifs in Spt10p uncovered two possible zinc fingers (Fig. 1A). The first is $\text{HX}_2\text{H}_2\text{C}_2\text{CX}_3\text{C}$ (residues 351–388), where $X$ is any amino acid, and the second is $\text{CX}_2\text{CX}_3\text{H}_2\text{HX}_3\text{H}$ (residues 385–416). The latter possibility was first noticed by Natsoulis et al. (15), who also demonstrated that the C388S mutation has a very slow growth phenotype, similar to that of the null mutant, indicating that this residue is important in vivo (see below). However, Cys388 is common to both possible zinc fingers, and it is therefore unclear which of the two is affected by the C388S mutation. Because they share the same pair of cysteine residues, they are presumably mutually exclusive. Both zinc fingers are quite positively charged, as expected for a DNA-binding domain.

We first tested whether zinc is required for specific high affinity binding of Spt10p to the histone UAS elements using the gel shift assay. An end-labeled synthetic double-stranded 60-bp oligonucleotide corresponding to the pair of UAS elements in the HTA1 promoter (one of the two genes in yeast that encode histone H2A) (Fig. 1B) and recombinant Spt10p purified from baculovirus-infected cells were used (19).

Our Spt10p preparations contained EDTA to protect the protein from metalloproteases. Because EDTA is a potent chelator of zinc, our initial studies utilized Spt10p that had been purified further on a Mono S column in the presence of zinc to remove the EDTA, followed by a desalting column to remove zinc from the preparation. However, this Spt10p bound to the HTA1 UAS elements without measurable loss of affinity in the absence of added zinc (data not shown). This indicates that if Spt10p is a zinc-binding protein, it must bind zinc very tightly.

To test whether zinc is indeed required for the binding of Spt10p to DNA, it was therefore necessary to add EDTA to the binding reaction to strip Spt10p of zinc and then to add zinc back to determine whether it could restore DNA-binding activity. The concentrations of Spt10p and UAS DNA were fixed at 100 and 2 nM, respectively, and a range of zinc acetate concentrations (0–500 μM) was tested in the presence of 0.1 mM EDTA (Fig. 1C). Spt10p bound only very weakly to the UAS elements in the absence of zinc ions, indicating that the EDTA was effective. Addition of zinc resulted in a large increase in the binding of Spt10p to the UAS DNA, eventually reaching a plateau of ~80% DNA bound at ~50 μM zinc (Fig. 1D). The plateau corresponded to the fraction of DNA expected to be bound given the dissociation constant (19). The small amount of Spt10p binding in the absence of added zinc ions probably reflects incomplete removal of zinc from Spt10p by EDTA. We conclude that the binding of Spt10p to the histone UAS elements is dependent on zinc, consistent with the presence of a zinc finger in the DNA-binding domain.
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residues (i.e. lysine and arginine) mostly on the N-terminal side of the two candidate zinc fingers, extending into the putative histone acetyltransferase domain. Accordingly, a series of expression vectors encoding fragments of Spt10p with single C-terminal FLAG tags was constructed for expression in *E. coli*. Although it was possible to obtain sufficient quantities of soluble protein in each case, the yields were very poor, reflecting the fact that most of the protein was insoluble.

Initially, partially purified fragments of Spt10p were screened for DNA binding using the gel shift assay (Fig. 2B). The largest fragment of Spt10p tested encompassed residues 212–508, extending from within the presumptive border of the histone acetyltransferase domain (residues 113–245 as defined by homology (7)) to the border of the C-terminal deletion defined previously by others (15). In vivo, deletion of residues 509–640 is associated with transcription defects, but growth is normal (15). This fragment of Spt10p bound specifically and tightly to the histone UAS elements, yielding two complexes, presumably corresponding to the binding of Spt10p-(212–508) to each of the two UAS elements in the DNA probe. This is discussed in more detail below.

To narrow down the DNA-binding domain further, a protein corresponding to residues 227–433 was tested; although several basic residues were eliminated at each end relative to the larger protein, this protein fragment also bound tightly to the histone UAS elements, indicating that the DNA-binding domain was located within this region of Spt10p. A smaller fragment corresponding to residues 283–396 also bound to the UAS elements with high affinity. Because the C-terminal candidate zinc finger (residues 385–416) was not present in this protein, it is apparent that it is not involved in DNA binding. In contrast, the N-terminal zinc finger (residues 351–388) was entirely included in this fragment. Because it has already been shown that Cys\textsuperscript{388} is necessary for DNA binding (Fig. 1F), the C terminus of the DNA-binding domain must lie between residues 388 and 396. To narrow down the N-terminal border of the DNA-binding domain, two more proteins were prepared, corresponding to residues 326–396 (Fig. 2B) and 340–396 (data not shown). However, neither of these had any DNA-binding activity, indicating that the N terminus of the DNA-binding domain lies between residues 283 and 326. We also investigated whether the zinc finger alone is sufficient for DNA binding by testing a synthetic peptide corresponding to residues 347–393, but the peptide did not bind to DNA under any conditions tested (data not shown). We conclude that the DNA-binding domain of Spt10p is located between residues 283 and 396. Although it includes and requires the zinc finger defined by residues 351–388, this zinc finger is not sufficient for specific DNA binding.

The DNA-binding Domain Binds with High Affinity to Single Histone UAS Elements, Unlike the Full-length Protein—The wild-type and C388S versions of the DNA-binding domain of Spt10p (residues 283–396) were purified to homogeneity (Fig. 3A). Their affinities for the HTA1 oligonucleotide containing two UAS elements (as in Figs. 1 and 2) were measured using the gel shift assay (Fig. 3B). The *K* \textsubscript{D} of the wild-type DNA-binding domain was 32 ± 18 nM (n = 3), which is very similar to that of the full-length protein on the same DNA (45 ± 16 nM, n = 11). Although the DNA-binding domain carrying the C388S mutation did bind to DNA, its *K* \textsubscript{D} was only 249 ± 6 nM (n = 3) (Fig. 3B); it bound ~8-fold more weakly than the wild-type DNA-binding domain. This experiment was performed in the presence of 100 μM zinc. However, at a zinc concentration of 10 μM, the *K* \textsubscript{D} of the C388S DNA-binding domain for the UAS was 400 nM, whereas the *K* \textsubscript{D} of the wild-type DNA-binding domain was unaffected (Fig. 3C). Thus, the affinity of the C388S DNA-binding domain for the UAS was dependent on the zinc concentration, suggesting that the C388S DNA-binding domain bound zinc much more weakly compared with the wild-type DNA-binding domain, as expected. Even so, the C388S DNA-binding domain bound much more tightly to DNA compared with the full-length C388S protein, indicating that the C388S mutation had additional effects on DNA binding in the context of the full-length C388S protein (see "Discussion"). In conclusion, the C388S mutation had a strong effect on the binding of both the full-length protein and the DNA-binding domain to the histone UAS, as expected.

The two complexes formed on the HTA1 oligonucleotide reflected the presence of two UAS elements in this DNA: the faster migrating complex corresponding to binding to one of the two UAS elements and the more slowly migrating complex corresponding to binding to both UAS elements. A quantitative analysis of the binding suggested that the binding of both the wild-type and C388S DNA-binding domains to DNA was mildly negatively cooperative (data not shown). However, this analysis was complicated by the fact that their affinities for the two UAS elements were somewhat different (see below), which might account for the apparent negative cooperativity.

These observations concerning the binding of the DNA-binding domain to the HTA1 oligonucleotide were in stark contrast to those reported previously for the full-length protein (19). Full-length Spt10p bound to the pair of UAS elements with a very high degree of positive cooperativity, giving only one complex, rather than the two complexes expected for binding to two UAS elements (Fig. 3D). The cooperativity of the binding of full-length Spt10p and its isolated DNA-binding domain was compared using mutant versions of the 60-bp HTA1 oligonucleotide in which either the upstream or downstream UAS or both UAS elements had been disabled by multiple mutations (Fig. 3D). Both Spt10p and its DNA-binding domain bound strongly and with high affinity to the wild-type oligonucleotide; but Spt10p gave a single complex, and the DNA-binding domain gave two complexes, as noted above. However, Spt10p bound only extremely weakly to the oligonu-
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cleotides with only one intact UAS element ($K_D > 1 \mu M$), whereas the DNA-binding domain bound with high affinity to both oligonucleotides, giving only one complex in each case, as expected. The wild-type DNA-binding domain exhibited a mild preference for the upstream UAS ($K_D = 71 \pm 14 \text{nM, } n = 2$) over the downstream UAS ($K_D = 90 \pm 25 \text{nM, } n = 2$). These values are higher than the $K_D$ reported above for the binding of the DNA-binding domain carrying the C388S mutation to UAS elements (32 ± 18 nm) because the apparent $K_D$ is defined as the concentration of protein at which 50% of the DNA is bound, and so its value is affected by the presence of two binding sites. Neither protein bound to the oligonucleotide with both UAS elements mutated.

We also tested whether the DNA-binding domain would bind to a single UAS element (Fig. 3E). Full-length Spt10p bound extremely weakly to a 34-bp oligonucleotide containing a single UAS element from HTA1 ($K_D \gg 1 \mu M$). In contrast, the DNA-binding domain bound with high affinity to a single UAS element ($K_D = 77 \pm 5 \text{nM, } n = 2$). Thus, the DNA-binding domain did not exhibit the extreme positive cooperativity of the full-length protein. This implies that the full-length protein contains a domain that modifies the binding of the DNA-binding domain such that it binds with high affinity only if there are two UAS elements in the DNA.

Mutations in the Zinc Finger Are Associated with a Poor Growth Phenotype—We determined the consequences of mutations of the putative zinc-coordinating residues in vivo by constructing three mutants designed to distinguish between the two candidate zinc fingers: H355S, C388S, and H416S. It seemed reasonable to expect that mutations of the residues involved in coordinating the zinc ion would have very similar phenotypes, and it is already known that the C388S muta-
SPT10 (132 min), indicating that the C-terminal HA tags did not interfere with the protease mutations. To compare growth rates, we used a set of haploid strains in which the chromosomal SPT10 gene had been modified to include three HA tags at its C terminus, with the selection marker URA3 integrated just downstream. It was then possible to compare the relevant strains in the same medium (synthetic complete medium lacking uracil). The strain carrying SPT10-HA grew at the same rate as BJ5459 (132 min), indicating that the C-terminal HA tags did not interfere with SPT10 function in an obvious way. The H355S and C388S mutants grew very slowly, both having doubling times of 288 min, which is very similar to that of the spt10Δ strain (274 min). In contrast, the growth of the H416S mutant (132 min) was indistinguishable from that of the wild type. Thus, His355 and Cys388 are both important residues for SPT10 function, but His416 apparently is not. These observations are consistent with coordination of zinc by His355 and Cys388 rather than by Cys388 and His416, as suggested by the data from experiments performed in vitro described above.

However, an examination of expression levels by immunoblotting of these mutant proteins in whole cell extracts revealed that the H355S and C388S proteins were expressed at somewhat lower levels than wild-type Spt10p and the H416S protein (data not shown, see Fig. 4A for data concerning the C388S protein). This suggested that the growth phenotype might possibly reflect reduced Spt10p levels rather than the direct effect of the mutation. To address this, the C388S protein was overexpressed by transforming the C388S mutant with a 2μ-based plasmid (pRS425) carrying the spt10(C388S) gene. As a control, the wild-type protein was overexpressed in a wild-type strain. Overexpression of the wild-type and C388S proteins was confirmed by immunoblotting (Fig. 4A). Although the C388S protein was strongly overexpressed, the doubling time was not affected significantly (260 min for a single copy of the spt10(C388S)-HA gene and 268 min for multiple copies) (Table 1). Overexpression of wild-type Spt10p in wild-type cells had no effect on growth. Thus, overexpression of the C388S protein did not rescue the growth phenotype of the spt10(C388S) mutant, indicating that it was not due to reduced expression of the mutant protein. We conclude that His355 and Cys388 are essential for the function of Spt10p in vivo.

Spt10p(C388S) Does Not Bind to the Histone Gene Promoters in Vivo—Because the C388S mutation disabled the binding of Spt10p to the histone UAS elements in vitro, we expected that the mutant protein would not bind to the histone promoters in vivo. This was tested by ChIP experiments using yeast strains in which the wild-type SPT10 gene carried three HA tags at its C terminus. Chromatin fragments were prepared from untagged (wild-type) and tagged cells that had been fixed with formaldehyde, and anti-HA antibody was used for immunoprecipitation. The amount of DNA in each immunoprecipitation was determined by measuring the absorbance at 600 nm during the exponential phase of growth at 30°C and 300 rpm. For the overexpression experiments, strains were transformed with plasmids based on pRS425, a high copy vector carrying LEU2 and a 2μ plasmid origin.

### TABLE 1

| Strain name | Relevant genotype | Doubling time | Growth medium |
|-------------|-------------------|---------------|---------------|
| BJ5459      | Wild-type (ara3)  | 127 min       | SC*           |
| BJ-spt10Δ   | spt10Δ::URA3      | 274           | SC – Ura      |
| BJ-SPT10-HA | SPT10-HA::URA3    | 132           | SC – Ura      |
| BJ-SPT10(H355S)-HA | spt10(H355S)::URA3 | 288          | SC – Ura      |
| BJ-SPT10(H416S)-HA | spt10(H416S)::URA3 | 288          | SC – Ura      |
| BJ5459-pRS425 | Wild-type        | 132           | SC – Ura      |
| BJ-SPT10-HA-pRS425 | SPT10-HA (single copy) | 142        | SC – Leu      |
| BJ-SPT10-HA-pRS425-SPT10-HA | SPT10-HA (multiple copies) | 147    | SC – Leu      |
| BJ-SPT10(c388s)-HA::pRS425-SPT10(c388s)-HA | SPT10-HA (multiple copies) | 260  | SC – Leu      |
| BJ-SPT10(c388s)-HA::pRS425-SPT10(c388s)-HA | SPT10-HA (multiple copies) | 268  | SC – Leu      |

* SC, synthetic complete medium lacking either uracil or leucine as indicated.

A. Overexpression of Spt10p and Spt10p(C388S). Immunoblotting with anti-HA antibody was used to determine the relative amounts of wild-type and C388S mutant proteins. Cells expressing either a single chromosomal SPT10-HA or spt10(C388S)-HA gene and plasmid vector with no insert (pRS425) and cells overexpressing SPT10-HA or spt10(C388S)-HA from a multicopy plasmid based on pRS425 were tested. Three different isolates of each strain were analyzed. B. Spt10p carrying the C388S mutation does not bind to the HTA1 promoter in vivo. HA-tagged Spt10p was detected using anti-HA antibody in ChIP experiments. The wild-type (no tag) strain was used as a control for antibody specificity. Mock reactions lacked antibody. The relative amounts of HTA1 promoter sequence in the samples were measured by PCR and quantified using a Phosphorimager. Input DNA dilutions (40, 80, 160, and 320 ng of DNA) were used to demonstrate that equal amounts of DNA were used in the immunoprecipitation (IP) reactions and that the PCR measurements were in the linear range. The HTA1 immunoprecipitation signal for each sample was divided by the immunoprecipitation signal for the Int-V control and normalized (Norm.) to the value for the wild type (no tag), which was set equal to 1.0. Values represent the mean ± S.D. of two experiments.

FIGURE 4. The C388S mutation prevents the binding of Spt10p to the HTA1 promoter in vivo, even when overexpressed. A, overexpression of Spt10p and Spt10p(C388S). Immunoblotting with anti-HA antibody was used to determine the relative amounts of wild-type and C388S mutant proteins. Cells expressing either a single chromosomal SPT10-HA or spt10(C388S)-HA gene and plasmid vector with no insert (pRS425) and cells overexpressing SPT10-HA or spt10(C388S)-HA from a multicopy plasmid based on pRS425 were tested. Three different isolates of each strain were analyzed. B, Spt10p carrying the C388S mutation does not bind to the HTA1 promoter in vivo. HA-tagged Spt10p was detected using anti-HA antibody in ChIP experiments. The wild-type (no tag) strain was used as a control for antibody specificity. Mock reactions lacked antibody. The relative amounts of HTA1 promoter sequence in the samples were measured by PCR and quantified using a Phosphorimager. Input DNA dilutions (40, 80, 160, and 320 ng of DNA) were used to demonstrate that equal amounts of DNA were used in the immunoprecipitation (IP) reactions and that the PCR measurements were in the linear range. The HTA1 immunoprecipitation signal for each sample was divided by the immunoprecipitation signal for the Int-V control and normalized (Norm.) to the value for the wild type (no tag), which was set equal to 1.0. Values represent the mean ± S.D. of two experiments.
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Because the expression level of the C388S protein was lower than that of the wild-type protein (see above), we tested whether the C388S protein could be detected at the HTA1 promoter if it were overexpressed. Overexpression of the C388S protein had little or no effect on its binding to the HTA1 promoter (1.4-fold), which was probably not significantly different from the background level (Fig. 4B). Overexpression of wild-type Spt10p (with the HA tags) gave a very strong positive ChIP signal at the HTA1 promoter. However, after normalization to the Int-V control, the ChIP signal for overexpressed Spt10p (3-fold) was lower than that for single copy SPT10-HA cells (6-fold) apparently because there was also increased binding of Spt10p to the Int-V control. This might reflect increased nonspecific binding to chromatin by the excess Spt10p molecules. These data confirmed our conclusion that the C388S protein bound much more weakly to the HTA1 promoter compared with the wild-type protein, consistent with a large reduction in affinity for the histone UAS elements in vivo. This also accounts for the fact that the C388S mutant phenotype is very similar to the spt10Δ phenotype (see below).

The Global Effects of the spt10(C388S) Mutation on Gene Regulation Are Very Similar to Those Observed in spt10Δ Cells—Recently, we described a microarray study in which gene expression patterns in spt10Δ cells were compared with those in wild-type cells (19). We observed that ~13% of yeast genes (827 genes) were affected by >2-fold in spt10Δ cells. In that data set, we also analyzed gene expression in the spt10(C388S) mutant; these additional data are now reported here (Fig. 5 and Supplemental Table 1). The data were derived from three independently prepared RNA samples that were hybridized to Affymetrix S98 arrays at the same time, together with the triplicate RNA samples from wild-type and spt10Δ cells already described (19). Comparison of wild-type cells with spt10(C388S) cells indicated that the data were very similar to those for spt10Δ cells: ~15% of the genes (969 genes) were affected by >2-fold (Fig. 5A). As for the null mutant, a substantial majority of the genes affected in spt10(C388S) cells were up-regulated in spt10Δ cells (spt10(C388S) = 74% and spt10Δ = 77%), confirming that Spt10p repressed more genes than it activated.

The changes in gene expression occurring in spt10(C388S) and spt10Δ cells were compared directly using only data with p < 0.05 in both data sets were used. For each gene, the ratios of the expression values for spt10(C388S)/wild type and spt10Δ/wild type were calculated and plotted against one another (as base 2 logarithms for clarity of display). The R² value was calculated from the Pearson product moment correlation coefficient.

Int-V control were compared (Fig. 4B). As we (19) and others (4, 8) have reported previously, Spt10p was readily detectable at the HTA1 promoter (a 6-fold enrichment relative to Int-V). In contrast, the C388S protein was not detected at the HTA1 promoter, suggesting that this mutation did indeed prevent binding to the histone UAS in vivo, as expected from our data in vitro.

**TABLE 2**

| Gene          | spt10Δ -Fold change | p value | spt10(C388S) -Fold change | p value |
|---------------|--------------------|--------|--------------------------|--------|
| SPT10         | +61.0              | 0.00   | +1.4                     | 0.27   |
| URA3          | +16.2              | 0.00   | +15.8                    | 0.00   |
| ADH2          | +13.3              | 0.01   | +17.2                    | 0.00   |
| HUG1          | +12.5              | 0.00   | +12.7                    | 0.00   |
| THI11         | +10.3              | 0.00   | +7.7                     | 0.00   |
| PHO5          | +7.5               | 0.00   | +5.7                     | 0.01   |
| SSA3          | +7.4               | 0.04   | +6.1                     | 0.04   |
| MEP2          | −2.8               | 0.03   | −2.1                     | 0.02   |
| NPL3          | −4.5               | 0.00   | −6.0                     | 0.00   |
| HTA1*         | −1.4               | 0.26   | −1.1                     | 0.86   |
| HTB1*         | −1.6               | 0.08   | −1.8                     | 0.02   |
| HTA2*         | −1.6               | 0.12   | −1.6                     | 0.17   |
| HTB2*         | −3.9               | 0.01   | −4.8                     | 0.00   |
| HHT1/HHT2*    | −1.5               | 0.01   | −1.5                     | 0.20   |
| HHEF1/HHEF2*  | −1.2               | 0.42   | −1.0                     | 0.88   |

a Histone gene.

**FIGURE 5.** The spt10(C388S) and spt10Δ mutations have very similar effects on gene expression. **A,** the spt10(C388S) mutation has a major effect on global gene expression by Spt10p. The plot shows a comparison of gene expression levels in wild-type and spt10(C388S) cells derived from microarray data. The plot includes all open reading frames; noncoding genes were excluded. Each point represents the average of triplicate samples. The values are plotted as their base 2 logarithms for clarity of display. The black diagonal line represents no difference in expression between wild-type and spt10(C388S) cells. Points above the line represent genes expressed at higher levels in spt10(C388S) cells (i.e., repressed by Spt10p), and points below the line indicate genes expressed at lower levels in spt10(C388S) cells (i.e., activated by Spt10p). The dashed lines above and below the black diagonal line indicate the arbitrary 2-fold cutoff. The data for wild-type cells were published previously (19). All of the genes in a subset of genes that were strongly affected in spt10Δ cells and selected for further study (19) were also strongly affected in spt10(C388S) cells (indicated; see Table 2). **B,** the spt10(C388S) and spt10Δ mutations affect mostly the same genes. For this analysis, only genes with p < 0.05 in both data sets were used. For each gene, the ratios of the expression values for spt10(C388S)/wild type and spt10Δ/wild type were calculated and plotted against one another (as base 2 logarithms for clarity of display). The R² value was calculated from the Pearson product moment correlation coefficient.
The analysis above indicated that the expression data for the spt10(C388S) and spt10Δ mutants were generally similar. More specifically, the genes strongly affected in the spt10Δ mutant discussed previously (19) were similarly affected in the spt10(C388S) mutant (Fig. 5A and Table 2). These included ADH2 (an alcohol dehydrogenase-encoding gene), HUG1 (a DNA damage-inducible gene), THI11 (an enzyme involved in thiamin biosynthesis), PHOS (an inducible acid phosphatase), SSA3 (a heat shock protein), MEP2 (an ammonia transporter), and NPL3 (a nuclear shuttling protein). All of these genes were affected quantitatively very similarly (Table 2). SPT10 itself was not significantly affected in spt10(C388S) cells, but was absent in spt10Δ cells, as expected; and in both cases, LIRA3 was used to select for integrands and therefore showed a large increase in expression relative to the wild type (16-fold) (Table 2).

The effects of the spt10(C388S) and spt10Δ mutations on the histone genes were also similar (Table 2). We have argued that the major core histone genes are the only direct targets of Spt10p, acting through binding to the histone UAS elements (19). The effects on the histone genes were relatively modest, except on HTB2 (one of two genes encoding histone H2B; a 4.8-fold decrease in spt10(C388S) cells), even though all of the major histone promoters have multiple binding sites for Spt10p and even though Spt10p is detected at all of them in vivo (19). In fact, it is probable that all of these histone genes were affected much more strongly than is apparent from our data, which were obtained using non-synchronized cells, because others have detected a large reduction in the levels of all of the major core histone mRNAs in synchronized spt10Δ cells (4). We conclude that the C388S mutation confers a phenotype very similar to that of the null mutation, reflecting the fact that the C388S mutation disables the DNA-binding function of Spt10p.

**DISCUSSION**

The DNA-binding Domain of Spt10p Contains an N-terminal Basic Region and a Zinc Finger.—The goal of this study was to identify the DNA-binding domain of Spt10p, the yeast protein that activates histone gene transcription through specific binding to the histone UAS elements (19). We have shown that the DNA-binding function is located between residues 283 and 396; its N-terminal border lies between residues 283 and 326, and its C-terminal border lies between residues 388 and 396. The domain includes the H110-Cys25 zinc finger that encompasses residues 351–388. We have shown that specific binding to the histone UAS element requires zinc and that mutation of Cys388 to Ser results in a large reduction in DNA-binding affinity in vitro and prevents the binding of Spt10p to the HTA1 promoter in vivo.

It is somewhat surprising that the affinity of the C388S DNA-binding domain for the histone UAS elements in vitro was reduced by only 8-fold relative to the wild-type DNA-binding domain, although the effect was stronger (13-fold) at lower zinc concentrations. This certainly represents a very strong effect on DNA binding, but a still greater reduction in affinity might have been expected in the case of a classical zinc finger, such as those in the yeast regulatory protein Adr1 (22). However, finger 1 of the Zif268 protein provides an exception in which alanine can partially substitute for the loss of one of the histidine ligands, perhaps with the help of an exogenous water molecule (23). In addition, the zinc finger in Spt10p is of an unusual type (see below) and might be less sensitive to the loss of one of its four Cys/His ligands.

An intriguing possibility is that the putative C-terminal zinc finger, which is not required for high affinity binding, might nevertheless play a role in Spt10p function by competing with the N-terminal finger for the zinc ion. However, if this occurs, it is unlikely to be of critical importance because the H4116S mutation has no discernable phenotype.

In conclusion, the DNA-binding domain of Spt10p consists of an N-terminal basic region linked to a Cys25-His110 zinc finger. In this regard, it is similar to that of the Dro sophila GAGA protein, which consists of an N-terminal basic region linked to a Cys24-His14 zinc finger (24).

The Activity of the DNA-Binding Domain Is Modulated by the Rest of the Protein.—The DNA-binding domain of Spt10p binds to histone UAS elements with high affinity, similar to the full-length protein. However, the DNA-binding domain binds to pairs of UAS elements with little or no cooperativity, whereas the full-length protein binds with very strong positive cooperativity. This difference in cooperativity might be accounted for if Spt10p is a dimer and if the DNA-binding domain is a monomer; this is under investigation.

The full-length protein requires two UAS elements and binds only extremely weakly to a single UAS element. In contrast, the DNA-binding domain binds with high affinity to a single UAS element. Clearly, there is a domain(s) in the full-length protein that modulates the activity of the DNA-binding domain such that it requires two UAS elements for high affinity binding. This domain is able to prevent recognition of a single UAS element by Spt10p, but its blocking effect on DNA binding is overcome if two such elements are present. We are currently attempting to identify this “blocking” domain. An interesting possibility is that the putative histone acetyltransferase domain, which is situated just N-terminal to the DNA-binding domain, is involved (Fig. 6), but this remains to be determined. In addition, it has been suggested that Spt21p, which has been shown to interact with Spt10p (8), might induce a conformational change in Spt10p and so modulate its activity (8).

The proposed blocking domain might also be responsible for the very low affinity of the full-length C388S protein for the UAS element compared with the C388S DNA-binding domain. The C388S mutation
might prevent the full-length protein from countering the inhibitory effect of the blocking domain even when two UAS elements are present. The biological importance of the proposed modulatory blocking domain is likely to be in guaranteeing that Spt10p recognizes only pairs of UAS elements, resulting in extremely high specificity; the histone promoters are the only places in the yeast genome where two such sequences are found close together (19).

The Spt10p Zinc Finger Is Homologous to the Zinc Finger of Foamy Virus Integrase—A BLAST search (25) for a mammalian homolog of Spt10p was negative, but we did find a striking homology between the zinc finger region of Spt10p and the putative zinc finger domain of human/simian foamy virus integrase (Fig. 6). It extends from residues 326 to 390 of Spt10p and so spans most (and perhaps all) of the DNA-binding domain, although the most convincing homology is in the zinc finger itself. Both zinc fingers are of the type HX_3HX_25CX_2C; both are quite basic; and a good number of residues are conserved.

What might be the significance of the homology between Spt10p and integrase? We propose that they are similar because they recognize a similar DNA sequence, i.e. the integrase might recognize a DNA sequence similar to the histone UAS element. This would presumably be the result of convergent evolution. Foamy viruses are complex retroviruses similar to human immunodeficiency virus (HIV), although foamy viruses are not associated with disease (26). The HIV zinc finger is of the type HX_3HX_25CX_2C and is therefore a little shorter than the foamy virus and Spt10p zinc fingers; the HIV finger is also less basic. However, the HIV-1 integrase zinc finger can substitute for the human foamy virus finger in vitro (27). Similar zinc finger domains are found in all retroviral integrases (28). Despite much study, the precise function of the integrase finger is unclear (29). If it does indeed recognize a specific DNA sequence, as we are suggesting, the target DNA could be in the viral genome or in that of the host. Some evidence in support of the former comes from the observation of specific binding of HIV-1 integrase to the HIV-1 long terminal repeat sequence (30). If host DNA were the target, the implication is that integration of foamy virus DNA into host DNA might not be random but targeted. If so, this would be an important consideration in the possible use of foamy virus-based vectors in gene therapy (31). We are currently addressing the possibility that foamy virus integrase might be a sequence-specific DNA-binding protein.

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