Cooperative binding of the yeast Spt10p activator to the histone upstream activating sequences is mediated through an N-terminal dimerization domain

Geetu Mendiratta, Peter R. Eriksson and David J. Clark*

Laboratory of Molecular Growth Regulation, National Institute of Child Health and Human Development, National Institutes of Health, Building 6A Room 2A14, 6 Center Drive, Bethesda, MD 20892, USA

ABSTRACT

The yeast Spt10p activator is a putative histone acetyltransferase (HAT) possessing a sequence-specific DNA-binding domain (DBD) which binds to the upstream activation sequences (UAS elements) in the histone gene promoters. Spt10p binds to a pair of histone UAS elements with extreme positive cooperativity. The molecular basis of this cooperativity was addressed. Spt10p (640 residues) is an elongated dimer, but the isolated DBD (residues 283–396) is a monomer and binds non-cooperatively to DNA. A Spt10p fragment comprising the N-terminal domain (NTD), HAT domain and DBD (residues 1–396) binds cooperatively and is a dimer, whereas an overlapping Spt10p fragment comprising the DBD and C-terminal domains (residues 283–640) binds non-cooperatively and is a monomer. These observations imply that cooperative binding requires dimerization. The isolated NTD (residues 1–98) is a dimer and is responsible for dimerization. We propose that cooperativity involves a conformational change in the Spt10p dimer which facilitates the simultaneous recognition of two UAS elements. *In vivo*, deletion of the NTD results in poor growth, but does not prevent the binding at the *HTA1* promoter, suggesting that dimerization is biologically important. Residues 1–396 are sufficient for normal growth, indicating that the critical functions of Spt10p reside in the N-terminal domains.

INTRODUCTION

The *SPT10* gene of *Saccharomyces cerevisiae* was initially isolated using a genetic screen designed to search for factors affecting gene expression (1–3). This screen identified several genes encoding important transcription factors, including TBP (=*SPT15*), subunits of the SAGA histone acetyltransferase (HAT) complex (4) and two of the core histones (*SPT11* = *HTA1* and *SPT12* = *HTB1*). This was one of the earliest indications that gene regulation is closely tied to chromatin structure (5,6). *SPT10* was also discovered independently (7), using a genetic screen designed to identify genes which, when mutated, would allow the activation of a promoter lacking an UAS element (i.e. a basal promoter). *SPT10* and *SPT21* both encode positive regulators of the histone genes (8,9). *SPT1* is identical to *HIR2*, which encodes a subunit of the Hir co-repressor, also known to act on the histone genes (10,11). The connection between the histones and the *spt* mutations (6) was recently strengthened further by work from our own laboratory, demonstrating that *SPT10* encodes a sequence-specific DNA-binding protein that recognizes the histone UAS element ([G/A]TTCCN$_6$TTCNC) (12), through which it activates transcription of the major core histone genes. The DNA-binding domain (DBD) of Spt10p contains an unusual zinc finger (His$_2$-Cys$_2$) that is homologous to the zinc finger domain of retroviral integrases (13).

Spt10p is an unusual transcriptional activator because it appears to lack a classical activation domain. Instead, it has a putative HAT domain similar to that of Gcn5p (14). However, attempts to demonstrate the HAT activity of Spt10p have been unsuccessful so far. The absence of HAT activity in recombinant Spt10 protein might be explained by a requirement for other yeast proteins [as is the case for the Sas2p HAT activity (15)], or it could reflect a missing cofactor, or it might indicate that the actual substrates of Spt10p are not histones but non-histone proteins. However, it is clear that the HAT domain is critical for the function of Spt10p, because point mutations predicted to inactivate the HAT activity result in a weak growth phenotype similar to that of the null mutant (8). We have shown that acetylation of the H3 and H4 tails at the induced *CUP1* promoter is dependent on *SPT10* (16), but this is likely to be an indirect effect (12). There is also strong correlative evidence indicating that Spt10p might acetylate lysine-56 of histone H3 at the

---

histone promoters (17). In addition, the HAT domain itself has activating function *in vivo* (8). Thus, Spt10p appears to be an unusual type of activator, with a sequence-specific DBD fused to a putative HAT domain rather than the usual activation domain, which recruits a HAT as co-activator. The only similar protein known is the mammalian transcription factor ATF-2, which has a DBD and HAT activity (18). It seems possible that there might be many more such proteins awaiting recognition.

The histone genes are strongly expressed in S-phase to provide histones for nucleosome assembly on newly replicated DNA. The cell cycle dependence of histone gene regulation is directed by two elements: multiple positively acting UAS sequences which bind Spt10p and a negative regulatory element (11,19,20). The repressive activity of the Hir complex is mediated through the negative element (11,21). The Hir complex has been purified recently (22,23). Although the histone UAS element confers cell cycle-dependent expression on a reporter gene (11,19), Spt10p is present at three of the four core histone loci outside S-phase (8). This leads to the intriguing question of how the activating function of Spt10p is regulated during the cell cycle.

A remarkable property of Spt10p is the extreme positive cooperativity it displays with respect to the histone UAS elements: it requires two histone UAS elements for high-affinity binding (12). This cooperativity is likely to be important *in vivo* because Spt10p can only bind where there are two UAS elements; a search of the yeast genome indicates that two such elements are found only in the core histone promoters and nowhere else, suggesting that these genes are the sole targets of the Spt10 activator (12). Here, we have addressed the molecular basis of the positive cooperativity displayed by Spt10p. We demonstrate that Spt10p is a dimer and that cooperative binding correlates with dimer formation, which is mediated through its N-terminal domain (NTD). Genetic analysis indicates that the critical biological functions of Spt10p are confined to the N-terminal part of the molecule, encompassing the NTD, HAT domain and DBD.

MATERIALS AND METHODS

Purification of Spt10 proteins

Expression plasmids for Spt10p fragments were based on p348 (13) and encoded proteins with N-terminal met-gly fused to the Spt10p sequence with a single FLAG tag at the C-terminus: p487 = residues 2–396, p488 = 283–640, p492 = 80–396, p499 = 283–508, p406 = 80–640 and p505 = 2–98. The latter (p505) also encoded an N-terminal His$_6$ tag. Spt10 proteins were expressed in *Escherichia coli* BL21 Tuner (DE3) pLysS (Novagen) grown to an A_{600} of 0.5–0.6 in Luria broth supplemented with 40 µg kanamycin/ml, 30 µg/ml chloramphenicol, 1 mM zinc acetate and induced with isopropyl thiogalactoside (IPTG). For p487, p488 and p499, cells were induced with 0.05 mM IPTG for 4 h at room temperature. For p406, cells were induced with 2 mM IPTG for 2 h at 30°C; the same conditions were used for p505, except that 1 mM IPTG was used. Cell pellets were resuspended in 20 ml Buffer S per litre of culture [50 mM HEPES–KOH, pH 7.5, 0.2% Triton X-100, 0.1 mM zinc acetate, 10 mM 2-mercaptoethanol, 10 µM pepstatin A and

protease inhibitors lacking EDTA (Roche)] containing either 0.5 M NaCl (p487 and p488) or 0.25 M NaCl (p406, p499 and p505). The cells were broken by sonication on ice, the debris was removed by spinning in a Sorvall SA600 rotor (16 500 r.p.m. for 30 min at 4°C) and the supernatant was syringe-filtered (0.45 µm) before separation by ion exchange. For p487, p488, p406 and p499, the extract was diluted to 0.2–0.25 M NaCl with Buffer S and applied to a 5 ml HiTrap Mono-S column (GE Healthcare), washed with 25 ml of the same buffer and eluted using either a 0.25–1.5 M NaCl gradient (20 ml) for p487 and p488 or a 0.25–0.78 M NaCl gradient (25 ml) for p406 and p499. Fractions containing Spt10 proteins were pooled and either dialysed into Buffer S or diluted with Buffer S to adjust the salt concentration to 0.4–0.5 M. For p505, the extract was diluted to 0.1 M NaCl using Buffer S with Tris–HCl (pH 8.0) instead of HEPES, applied to a 5 ml HiTrap Mono-Q column (GE Healthcare) and eluted with a 0.1–1.0 M NaCl gradient. Spt10 proteins were purified to homogeneity by immunoprecipitation using anti-FLAG antibody as described previously (13), or using cross-linked anti-FLAG antibody agarose (Sigma).

Construction of *spt10* mutants and transforming plasmids

Yeast strains were constructed using the protease-deficient strain BJ5459 (ATCC 208284: MAT**a** *ura3-52 trp1 lys2-801 leu2Δ1 his3Δ200 pep4Δ::HIS3 prb1Δ1.6R can1 GAL cir$^+$*). BJ-spt10Δ (12) and BJ-SPT10-HA (13) have been described. BJ-SPT10ΔC-HA, BJ-SPT10ΔN-HA and BJ-SPT10(1–396)-HA were constructed by transformation with a SacI–HindIII digest of the appropriate integration plasmid, based on p355 (12): For the ΔC mutant [deletion from the EcoRI site at S508 (3)], integration plasmid p414 was constructed by replacing the 1001 bp BstEII–BsrGI fragment in p355 with a 606 bp version made by ligating the 438 bp BstEII–EcoRI fragment from p355 to an EcoRI–BsrGI PCR fragment (corresponding to S508 fused to the 3-HA sequence and stop codon). For the ΔN mutant, p509 was constructed by replacing the wild-type 1170 bp AvaI–BstEII fragment with a 977 bp mutated version made by PCR. For BJ-SPT10(1–396)-HA, p507 was constructed by replacing the wild-type 2376 bp HindIII–BsrGI fragment in p355 with a 1650 bp version made by ligating a HindIII–KpnI PCR fragment (including the *SPT10* promoter and residues 1–396 followed by a KpnI site) to a 197 bp KpnI–BsrGI fragment encoding three HA tags preceded by a KpnI site. Strains were verified by Southern blot analysis. Overexpression plasmids were derivatives of p438, which is pRS425 (2-µm origin, *LEU2*) carrying *SPT10-HA* (13). For overexpression of residues 1–396 or 1–508, pRS-SPT10 (1–396)-HA (p514) and pRS-SPT10ΔC-HA (p515) were constructed by replacing the PstI–BamHI *SPT10* fragment in p438 with that from p507 or p414, respectively. For overexpression of residues 80–640, pRS-SPT10ΔN-HA (p516) was constructed by replacing the HindIII–NdeI *SPT10* fragment in p438 with that from p509.

Gel-shift assays

Spt10p was mixed with 2 nM DNA in 15 µl of 20 mM Tris–HCl (pH 8.0), 0.15 M KCl, 5 mM MgCl$_2$, 0.1 mM

zinc acetate, 10% glycerol, 0.1 mg/ml BSA, 5 mM 2-mercaptoethanol, 0.1 mg/ml poly(dI–dC)–poly(dI–dC) (Amersham) and incubated at room temperature for 30 min (12). Samples were analysed in 5% (19:1) polyacrylamide gels containing 20 mM Tris–acetate, 1 mM Na-EDTA, pH 8.3 and 5% glycerol (12). Binding constants were estimated by curve fitting to obtain the Spt10p concentration required to bind 50% of the DNA and are quoted with standard error from n independent determinations.

Gel filtration and sedimentation analysis

About 2 µg FLAG-tagged Spt10 protein was mixed with protein standards (Amersham; 90 µg each) in 150 µl of 20 mM HEPES–NaOH, pH 7.6, 150 mM KCl, 5 mM $MgCl_2$, 0.1 mM zinc acetate, 5 mM 2-mercaptoethanol, 10 µM pepstatin A and a protease inhibitor cocktail (Roche). Samples were loaded on to a 5.5 ml 5–20% linear sucrose gradient containing the same buffer and spun in a Beckman SW55 rotor at 40 000 r.p.m. for 16 h at 4°C. The protein standards were located by analysis of the fractions in protein gels; Spt10 proteins were located by immunoblotting using anti-FLAG antibody (Sigma M2). The sedimentation coefficient ($s_{20,w}$) of the Spt10 protein was determined using a plot of $s_{20,w}$ for the standards against the peak fraction for each protein. Samples for gel filtration were fractionated on a calibrated Superdex-200 10/300 GL column (Amersham), using blue dextran 2000 to measure the void volume. The Stokes radius (R_s) of each Spt10 protein was obtained from a plot of the Stokes radii of the standards (known spherical proteins; data provided by Amersham) against K_{av} for each protein, obtained using the equation: $K_{av} = (V_e - V_0)/(V_t - V_e)$, where V_e is the elution volume, V_0 is the void volume and V_t is the total volume of the column. The standards used were as follows: ribonuclease A [$s_{20,w}$ = 2.1 S (24); R_s = 16.4 Å], chymotrypsinogen [$s_{20,w}$ = 2.6 S (25); R_s = 20.9 Å], ovalbumin [$s_{20,w}$ = 3.7 S (26); R_s = 30.5 Å], BSA [$s_{20,w}$ = 5.1 S (26); R_s = 35.5 Å], aldolase [$s_{20,w}$ = 7.4 S (27); R_s = 48.1 Å], catalase [$s_{20,w}$ = 11.3 S (26); R_s = 52.2 Å] and ferritin (R_s = 61.0 Å). The measured values of $s_{20,w}$ and R_s for each Spt10 protein were used to calculate the molecular weight (M) using the following equation:

$$M = \frac{(6\pi\eta \cdot R_s \cdot s_{20,w} \cdot N_A)}{(1 - \bar{v}\rho)},$$

where η is the viscosity of water at 20°C, N_A is Avogadro's number, v is the partial specific volume (PSV) of the protein

[calculated as described in (28); Table 1] and ρ is the solvent density. The frictional ratio (f/f_o) was calculated using the following equation:

$$\frac{f}{f_o} = \frac{R_s}{(3M \cdot \bar{v}/4\pi N_A)^{1/3}},$$

where f is the frictional coefficient and f_o is the frictional coefficient of a perfect sphere of the same molecular weight.

RESULTS

Spt10p binds with high affinity to a pair of UAS elements (K_D = 45 nM) but binds only very weakly to a single UAS element (K_D > 1 µM) (12,13). Our goal in this study was to gain some understanding of the molecular basis of this extreme positive cooperativity exhibited by Spt10p with respect to the histone UAS elements.

Mapping the domains of Spt10p required for cooperative binding

To identify the portion of Spt10p required for cooperative binding, some Spt10 protein fragments were prepared, all of which included the DBD, which is located near the centre of the molecule (Figure 1A). The Spt10 proteins were generally relatively difficult to purify from *E.coli* because they were insoluble when prepared from a standard BL21 strain. Accordingly, we resorted to a low-level expression *E.coli* system to obtain small amounts of soluble, active protein (Figure 1B). The full-length protein was purified in larger amounts using a baculovirus system (12).

We have shown previously that full-length Spt10p binds with high affinity only if two UAS elements are present (12), whereas the isolated DBD binds non-cooperatively to a single UAS with high affinity (13). These observations were confirmed using a set of *HTA1* probes carrying either two UAS elements (wild type), or with one of the two UAS elements mutated, or with both elements mutated (Figure 2A). Full-length Spt10p bound with high affinity only to the probe with two intact UAS elements, giving one complex (i.e. positively cooperative binding); it bound only very weakly to the probes having only one intact UAS and did not bind at all to the probe with both UAS elements mutated (Figure 2B). The DBD, on the other hand, bound tightly to the probe with two UAS elements, giving two complexes (Figure 2C),

Table 1. Determination of the oligomeric status of various Spt10 protein fragments using gel filtration and sucrose density gradient measurements

	PSV v (ml/g)	Average $s_{20,w}$ (S)	R_s (Å)	Measured MW	Sequence MW	Measured/sequence	Frictional ratio (f/f_o)
Spt10p (1–640)	0.715	6.4 ± 0.2	65.1 ± 0.0	167 000	79 830	2.1 = dimer	1.8
DBD (283–396)	0.720	2.0 ± 0.9	14.8 ± 0.0	12 000	14 659	0.8 = monomer	1.0
NTD–HAT–DBD (1–396)	0.727	5.3 ± 0.1	33.7 ± 0.0	74 000	46 579	1.6 = dimer	1.2
DBD-C1-C2 (283–640)	0.711	2.4 ± 0.5	42.3 ± 1.4	42 000	42 165	1.0 = monomer	2.0
DBD-C1 (283–508)	0.719	2.6 ± 0.2	33.7 ± 0.0	35 000	26 909	1.3 = monomer	1.6
NTD (1–98)	0.712	3.0 ± 0.6	18.0 ± 4.5	21 000	13 525	1.6 = dimer	1.0

MW = molecular weight. $s_{20,w}$ is the sedimentation coefficient measured in Svedbergs. R_S is the Stokes radius and PSV is the partial specific volume. All measurements represent the average of at least two independent experiments with standard error. The calculated sequence molecular weight includes the epitope tags. Note that some of the Spt10 proteins were found in a sharp peak within a single fraction in the gel filtration experiments, leading to a standard error close to zero. Thus, molecular weights determined by this method are not as accurate as those determined using other methods, but they are sufficiently precise to distinguish between monomer and dimer.
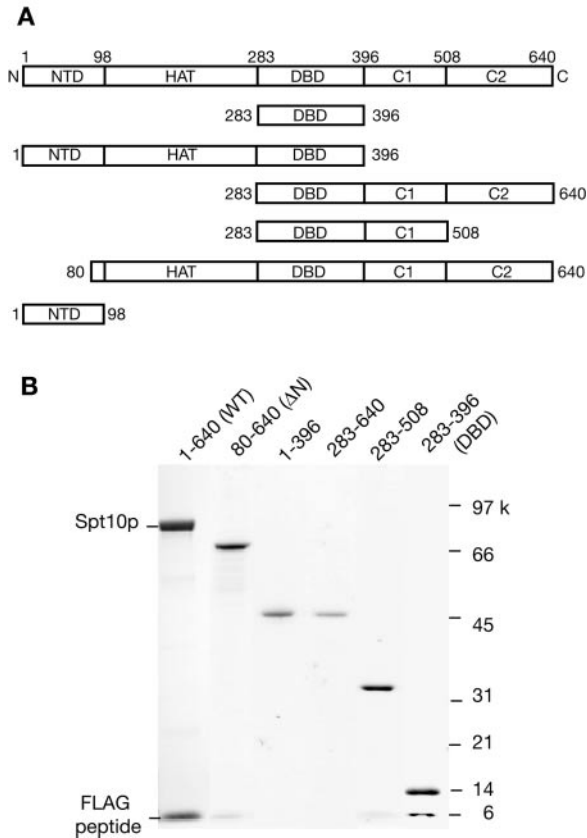
A

B

Figure 1. Purified protein fragments of Spt10p. (**A**) Diagram showing the Spt10 protein fragments purified. NTD = N-terminal domain; HAT = putative histone acetyltransferase domain; DBD = DNA-binding domain; C-terminal domains, C1 and C2, were defined arbitrarily with reference to a previous study (see text). (**B**) Analysis of purified Spt10 proteins in an SDS–polyacrylamide gel stained with Coomassie blue. WT = wild-type protein. Full-length Spt10p has three HA epitope tags followed by three FLAG tags at its C-terminus. All of the Spt10 protein fragments have a single FLAG tag at their C-termini which was used to purify the proteins by immunoprecipitation and elution with a synthetic FLAG peptide.
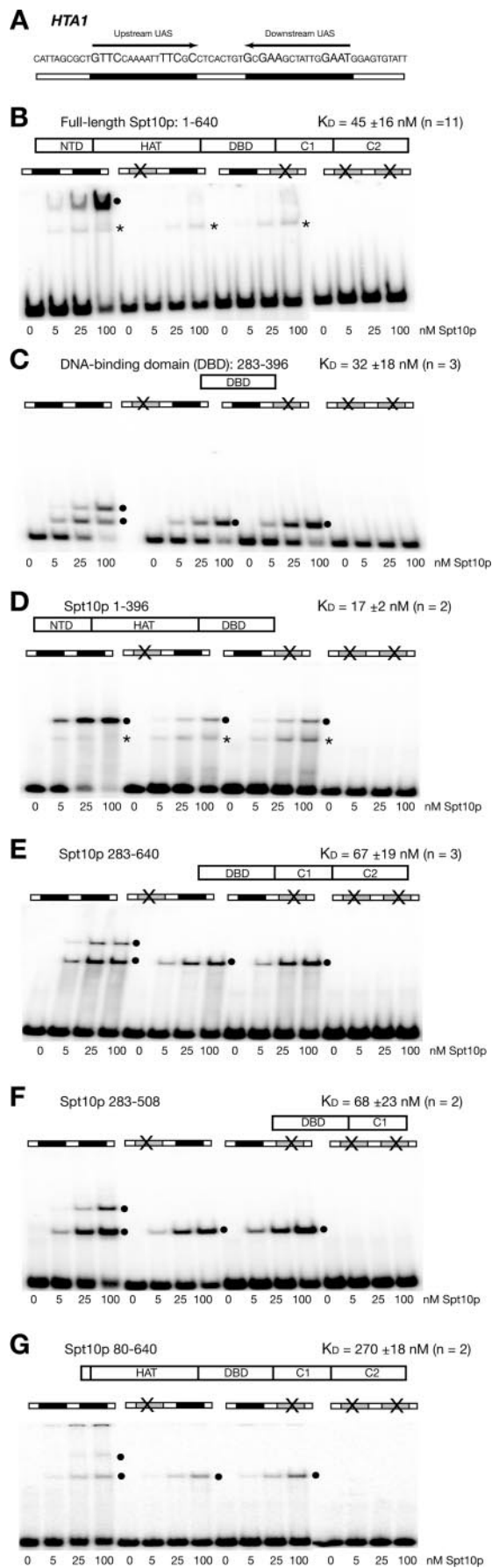
corresponding to one or two DBD proteins bound (non-cooperative binding). The DBD gave a single high-affinity complex with the probes containing a single intact UAS and did not bind at all to the probe with no intact UAS elements. These observations indicated that the full-length protein possesses a domain external to the DBD that is responsible for cooperative binding. This domain prevents the DBD in full-length Spt10p from binding to a single UAS.

The first step toward identifying the domain responsible for cooperative binding was to compare the binding of an N-terminal fragment (residues 1–396, corresponding to the NTD, the HAT domain and the DBD) with that of an overlapping C-terminal fragment (residues 283–640, corresponding to the DBD, C1 and C2 domains) to the histone UAS elements (Figure 2D and E). These overlapping protein fragments (Figure 1A) both contain the central DBD (residues 283–396). The N-terminal fragment of Spt10p behaved like the full-length protein, forming a single high-affinity complex with two UAS elements; it bound significantly more tightly than the full-length protein ($K_D = 17 \pm 2$ nM; $n = 2$). The N-terminal fragment bound very poorly to the oligonucleotides with only one intact UAS element

($K_D > 0.5$ µM). In contrast, the C-terminal fragment of Spt10p behaved similarly to the isolated DBD: it bound with high affinity to the *HTA1* probe with two UAS elements ($K_D = 67 \pm 19$ nM; $n = 3$) and to the probes with only a single intact UAS. A similar result was obtained with a truncated protein lacking the domain at the C-terminus (residues 283–508, corresponding to the DBD and the C1 domain); this protein also bound non-cooperatively to the histone UAS elements with high affinity (Figure 2F; $K_D = 68 \pm 23$ nM; $n = 2$). Thus, removal of the C2 domain had no effect on DNA binding. However, the C1 domain had a mildly inhibitory effect on binding by the DBD, which had $K_D = 32 \pm 18$ nM (13). Domains C1 and C2 were defined arbitrarily, because nothing is known of the domain structure or of the functions of this portion of Spt10p, except that a yeast strain with a deletion of the C-terminus (residues 509–640 = C2) has been described (3) (and see below). These observations indicated that cooperative binding is a property of the N-terminal domains of Spt10p, involving either the NTD and/or the HAT domain.

To determine the roles of the NTD and the HAT domain in cooperative binding, we attempted to prepare a protein corresponding to the HAT–DBD portion of Spt10p (residues 80–396). Although we were able to prepare small quantities of pure protein, it was very sensitive to proteases, despite the presence of a cocktail of protease inhibitors. The protease responsible was presumably a contaminant present in tiny amounts; it cleaved the purified protein between the HAT domain and DBD at a moderate rate, yielding functional DBD protein (data not shown). The appearance of multiple bands in the gel-shift experiment suggested that this protein bound non-cooperatively, but the degradation occurring during the binding reaction also gave rise to complexes corresponding to the DBD only, making the experiment difficult to interpret. Even performing the binding reactions on ice and for shorter times failed to prevent proteolysis (data not shown).

In an alternative approach, we prepared a Spt10 protein lacking only the NTD (Spt10ΔNp; residues 80–640). This protein was much more stable than the HAT–DBD protein, perhaps suggesting that the C-terminal domains of Spt10p have a protective effect on the protease-sensitive site between the HAT domain and DBD, or perhaps because the slightly different purification properties of the larger protein resulted in separation from the protease. Spt10ΔNp bound non-cooperatively to the histone UAS elements, yielding two complexes with the wild-type probe and single complexes with the probes having only one intact UAS (Figure 2G). However, the binding was very weak, with an apparent K_D of 270 ± 18 nM ($n = 2$). Analysis of Spt10ΔNp by gel filtration and sedimentation (see below) revealed that nearly all of the protein was aggregated, possibly the result of incorrect folding. Thus, it is likely that the low affinity observed for this protein reflected aggregation, which might result in a much lower concentration of active protein and an apparently low affinity. However, because Spt10ΔNp bound with similar affinity to the probe with two UAS elements and to the probes with a single intact UAS (after taking into account the number of binding sites; Figure 2G), it may be concluded that the NTD is required for cooperative binding of Spt10p to pairs of UAS elements.

Figure 2. Cooperative binding by Spt10p requires the NTD. Test for cooperative binding by various Spt10 protein fragments (described in Figure 1). Gel-shift assays using the wild-type *HTA1* probe with two UAS elements and versions in which either the upstream UAS or the downstream UAS or both were mutated [the mutations are described in Figure 7B of Ref. (12)]. (**A**) Sequence of the probe. The pair of UAS elements in the *HTA1* promoter (one of two genes encoding H2A) are inverted with respect to one another, as indicated by the arrows. (**B**) Gel-shift assay with full-length Spt10p. Intact UAS elements are indicated by black boxes; mutated elements are indicated by crosses through grey boxes. Spt10–DNA complexes are indicated by black dots. The asterisk indicates a complex probably formed by a degradation product of Spt10p. The apparent K_D for the binding of the Spt10 protein to the probe with two wild-type UAS elements is given with standard error and number of independent determinations (*n*). (**C**) The DNA-binding domain (DBD). (**D**) The N-terminal fragment of Spt10p (residues 1–396). (**E**) The C-terminal fragment of Spt10p (residues 283–640). (**F**) Truncated C-terminal fragment of Spt10p (residues 283–508). (**G**) Spt10p lacking its NTD (residues 80–640).

Binding of Spt10p to a single UAS element is blocked if the NTD is present

We proposed previously that Spt10p possesses a blocking domain which prevents the DBD from recognizing a single UAS element. This was based on the observation that Spt10p does not bind to a short oligonucleotide containing a single UAS element, whereas the isolated DBD binds tightly (13). The interactions of the Spt10 protein fragments with an oligonucleotide containing only the downstream UAS from the *HTA1* promoter were analyzed in gel-shift experiments (Figure 3). As shown previously (13), Spt10p bound only very weakly to this probe, whereas the DBD bound with relatively high affinity ($K_D = 77 \pm 5$ nM). Similar to full-length Spt10p, the N-terminal fragment of Spt10p (=NTD–HAT–DBD) bound extremely weakly. In contrast, the DBD-C1-C2 and DBD-C1 proteins bound relatively tightly with K_D values of 99 ± 4 and 100 ± 13 nM, respectively [these K_D values were higher than those reported above (Figure 2E and F) due to the different numbers of UAS elements in the probes]. As noted above, the C1 domain appeared to have a slight inhibitory effect on DNA binding. The binding of Spt10ΔNp was very weak, with a K_D of 740 ± 29 nM (Figure 3) but, even so, it bound more tightly to a single UAS than did the wild-type full-length protein. As discussed above, we attributed the weak binding of Spt10ΔNp to a reduced concentration of active protein as a result of aggregation. These data are consistent with those described above for longer probes and indicate that the blocking function of Spt10p requires the NTD.

Cooperative binding correlates with dimerization of Spt10p

It seemed reasonable to propose that the requirement of Spt10p for two UAS elements might be related to its oligomeric state. Accordingly, we determined the oligomeric states of the Spt10 proteins by subjecting them to gel filtration to measure the Stokes' radius (R_s) and to sedimentation in sucrose density gradients to measure the sedimentation coefficient ($s_{20,w}$), using proteins of known Stokes radii and $s_{20,w}$ for calibration. Fractions were analysed in protein gels by staining to ascertain the locations of the standard proteins and by immunoblot to detect Spt10 proteins (all of the proteins were FLAG-tagged; anti-HA antibody was used for full-length Spt10p, which had three C-terminal HA tags in

addition to the FLAG tags). The immunoblots are shown in Figure 4. The Stokes radius and $s_{20,w}$ for each Spt10 protein were derived from standard plots (see Materials and Methods) and were used to calculate the molecular weight (Table 1).

Full-length Spt10p gave rise to two peaks in both the sedimentation and gel filtration analyses (Figure 4). The major peak in the sucrose gradient corresponded to $s_{20,w} = 6.4$ S and the major component eluting from the gel filtration column had $R_s = 65.1$ Å. Using these values, the molecular
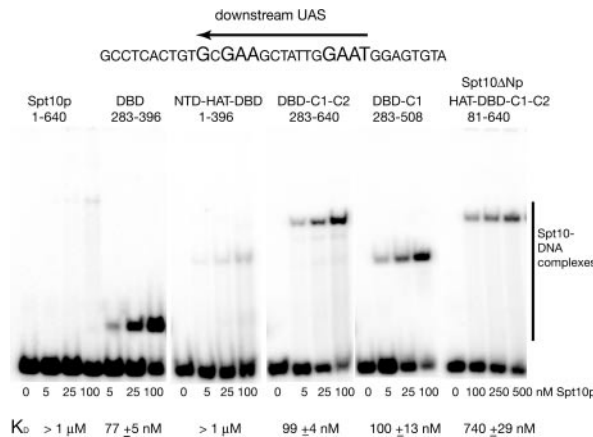

Figure 3. The NTD of Spt10p inhibits binding to a single UAS element. The downstream UAS from *HTA1* was used as a probe in gel-shift assays with the Spt10 proteins described in Figure 1. Values for the dissociation constant (K_D) are given below with standard error derived from at least two independent experiments [the data for the DBD are derived from Ref. (13)].

weight of Spt10p was determined to be 167 000 (Table 1). This corresponds to a Spt10p dimer, since the sequence molecular weight is 79 830. The less prominent peak in the sucrose gradient had $s_{20,w}$ of ~12 S (it was just outside the range of the standards) and a Stokes radius of ~75 Å, but this peak eluted partly in the void volume. Thus, it was difficult to be certain of the oligomeric state of this fraction of Spt10p, but the calculated molecular weight using these values corresponds to a tetramer. Attempts to clarify this issue using a Sephacryl S-300 HR gel filtration column (which is designed for larger proteins) failed, due to a strong interaction of Spt10p with the column (data not shown). We conclude that Spt10p exists primarily as a dimer and that Spt10p dimers interact reversibly to form a tetramer or higher oligomer.

Unlike Spt10p, the DBD was found to be a monomer and so were the C-terminal fragments of Spt10p (DBD-C1-C2 and DBD-C1; Table 1). In contrast, the N-terminal fragment of Spt10p was found to be a dimer, similar to the full-length protein, indicating that the NTD or the HAT domain is responsible for dimer formation. To determine which of these two domains is involved in dimer formation, we attempted to measure the molecular weight of Spt10ΔNp, but this was aggregated (as mentioned above) and no useful data could be obtained. Similarly, the isolated HAT domain (residues 80–277) also formed aggregates (data not shown). However, the isolated NTD (residues 1–98) was well-behaved and was found to be a dimer. Thus, our observations indicated that Spt10p is a dimer that is stabilized through interactions between the NTDs. Furthermore, cooperative binding is clearly correlated with dimer formation,
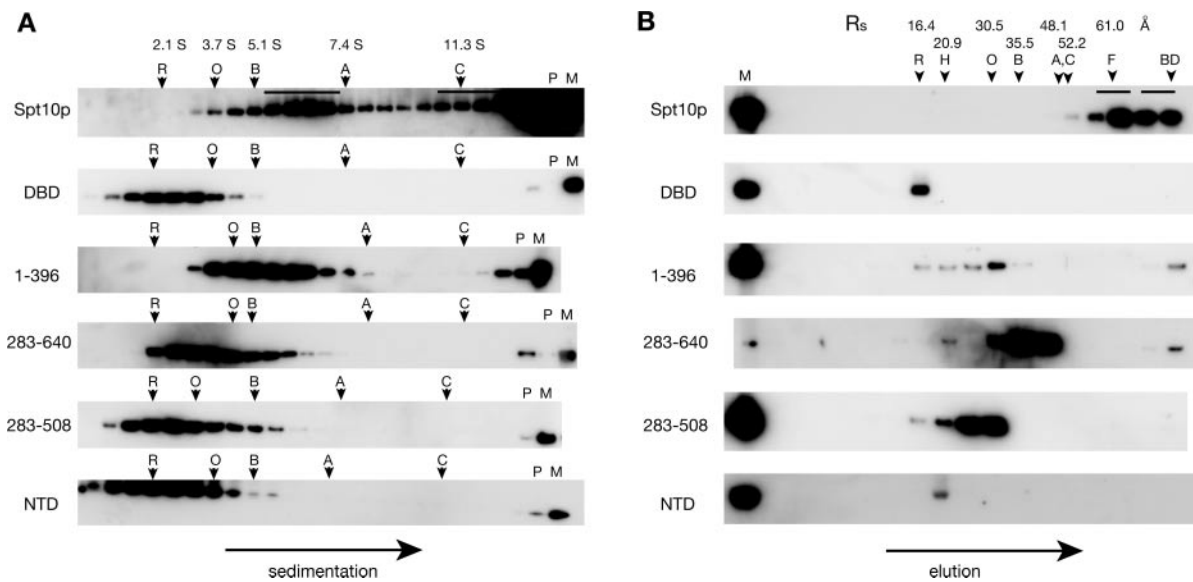

Figure 4. Determination of the oligomeric states of the Spt10 proteins. (**A**) Estimation of the sedimentation coefficient, $s_{20,w}$, of Spt10 proteins in sucrose density gradients. The various Spt10 protein fragments were sedimented in sucrose gradients in the presence of a set of standard proteins of known $s_{20,w}$. The fractions were analysed in protein gels: standards were located by staining with Coomassie blue; Spt10 proteins were located by immunoblotting using anti-FLAG antibody (or anti-HA antibody in the case of full-length Spt10p). Immunoblots are shown (P = pellet; M = Spt10 protein as a marker). The arrowheads mark the locations of the standard proteins, together with their sedimentation coefficients, $s_{20,w}$, in Svedbergs: ribonuclease (R), ovalbumin (O), BSA (B), aldolase (A) and catalase (C). The positions of the standards in the gradient varied somewhat, probably reflecting variation in the preparation and fractionation of the gradients. In the case of full-length Spt10p, two peaks were observed, indicated by black bars (the Spt10p marker was overloaded). (**B**) Estimation of the Stokes radii, R_s, of various Spt10 proteins by gel filtration using a Superdex-200 column calibrated with standard proteins of known Stokes radii, R_s (the values are shown at top). Proteins were detected as described in (A). Standards were the same as in (A), but also included chymotrypsinogen (H) and ferritin (F). Blue dextran (BD) was used to determine the void volume of the column. In the case of full-length Spt10p, two peaks were observed, indicated by black bars. See Table 1 for a summary of these data.

because only dimeric proteins containing the DBD exhibited cooperativity.

The NTD is required for wild-type growth *in vivo*

Although *SPT10* is not an essential gene, the null mutant grows very poorly (3,12) (Figure 5A). To determine which portions of Spt10p are required for wild-type growth, a set of strains expressing various truncated forms of Spt10p was constructed using BJ5459 as the parent strain. In these strains, the wild-type chromosomal *SPT10* gene was replaced with the truncated, tagged version integrated at the *SPT10* locus, with the selection marker *URA3* downstream. All of the integrated genes included three HA tags at the C-terminus of the encoded Spt10 protein to facilitate detection by immunoblotting and for chromatin immunoprecipitation (ChIP). The presence of the epitope tags had no effect on growth relative to wild type [Figure 5A; compare wild type (i.e. *SPT10*) with *SPT10-HA*]. Deletion of residues 509–640 had no effect on growth (*spt10-ΔC*), confirming this result described previously by others (3). It has also been reported that deletion of residues 477–640 had no growth phenotype (7). However, an even larger C-terminal deletion (residues 397–640) did not affect growth (Figure 5A). Thus, the N-terminal fragment of Spt10p (residues 1–396) was sufficient for normal growth. It should be noted that yeast cells expressing residues 1–396 were significantly enlarged relative to wild-type cells, pointing to a more subtle phenotype (data not shown).

The question of whether the NTD has a critical role in Spt10p function was addressed using a *spt10-ΔN* strain, which expresses residues 80–640. Deletion of the NTD had a strong effect on growth, although this strain did not grow as poorly as the *spt10Δ* mutant (Figure 5A). A trivial explanation for the effect of deleting the NTD on growth was that Spt10ΔNp was poorly expressed. Indeed, it was found that Spt10ΔNp was poorly expressed (Figure 5C), although this was also true for Spt10(1–396)p, which showed no growth phenotype. If the poor growth phenotype of the *spt10-ΔN* strain was due entirely to poor expression, then overexpression of Spt10ΔNp should rescue the phenotype. A set of strains overexpressing the various Spt10p truncations was constructed by transforming the strains used in Figure 5A with high copy plasmids expressing the same truncated form of Spt10p. The presence of the plasmid resulted in overexpression of the proteins, as expected (Figure 5C). Overexpression of full-length Spt10p with three HA tags had no effect on growth (Figure 5B); neither did overexpression of the proteins truncated at the C-terminus (Spt10p 1–508 and 1–396). Most importantly, overexpression of Spt10ΔNp did not rescue the growth phenotype, indicating that the NTD does indeed have a critical role in the function of Spt10p.

The effect of deleting the NTD on the binding of Spt10p to the histone UAS elements *in vivo* was determined by performing ChIP experiments using an anti-HA antibody, as described previously (13). The overexpression strains were used for ChIP because overexpressed Spt10ΔNp was present at similar levels to overexpressed wild-type protein, which was not the case for the single chromosomal loci (Figure 5C). The ChIP signal for Spt10ΔNp at the *HTA1* promoter was ~5-fold lower than that for wild-type Spt10p (Figure 5D), indicating that Spt10ΔNp was present at the *HTA1* promoter
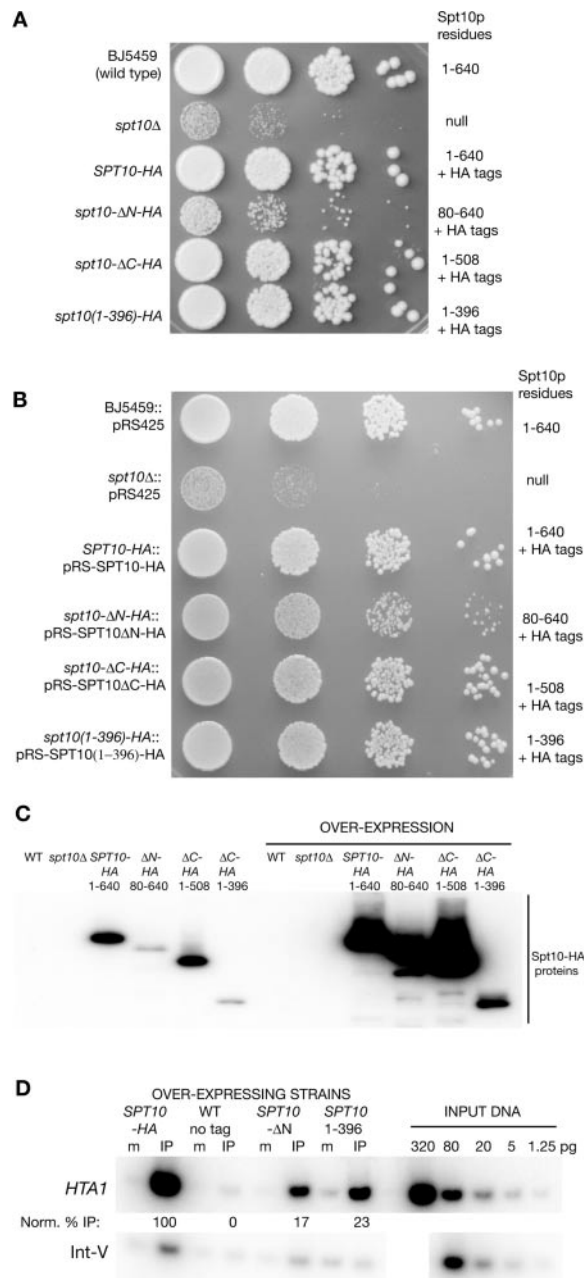

Figure 5. The NTD, HAT domain and DBD of Spt10p are sufficient for wild-type growth. (**A**) Growth tests for yeast strains expressing various portions of Spt10p. These strains were derived from wild-type strain BJ5459. Spot dilution series (10-fold) were performed on a YPD plate. In this assay, approximately equal numbers of cells are spotted on a plate; serial dilutions are used to facilitate comparison of relative colony size as a measure of growth. (**B**) Growth tests for yeast strains overexpressing various portions of Spt10p. The strains shown in (A) were transformed with the appropriate derivative of pRS425 (a high copy plasmid carrying *LEU2*). Spot dilution series (10-fold) were performed on a plate containing synthetic medium lacking leucine. (**C**) Determination of the expression levels of the various portions of Spt10p. Immunoblotting using anti-HA antibody. Equal amounts of protein were loaded in each lane. The same exposure of a single blot is shown. (**D**) Chromatin immunoprecipitation (ChIP) for HA-tagged Spt10 proteins at the *HTA1* promoter in overexpressing cells. An intergenic region from chromosome V was used as a control (Int-V) (13). A series of input DNA dilutions was used for each sample, but only one series is shown. IP, immunoprecipitate; m, mock (no antibody); norm. % IP, the fraction of input DNA present in the IP expressed as a percentage of that present in the *SPT10-HA* wild-type control.

in vivo, although it did not bind as well as wild-type protein. Spt10(1–396)p gave a ChIP signal similar to that for Spt10ΔNp (Figure 5D), even though it was expressed at much lower levels (Figure 5C). These data are consistent with our observations *in vitro*: the relatively high concentration of Spt10ΔNp in overexpressing cells would be expected to off-set a lower affinity, resulting in some binding to the histone UAS elements. Conversely, Spt10(1–396)p had a high affinity for a pair of histone UAS elements, but was present at a much lower concentration in the cell. Thus, Spt10ΔNp and Spt10(1–396)p were bound at the *HTA1* promoter at similar levels *in vivo*, but only the NTD deletion gave rise to a severe growth phenotype, suggesting that the NTD is required for a step subsequent to simple DNA binding.

DISCUSSION

The current study was based on our previous observation that intact Spt10p binds with high affinity only if two histone UAS elements are present, whereas the isolated DBD binds tightly to a single UAS element (13). This implies that another domain of Spt10p interferes with the binding of Spt10p to a single UAS element, forcing it to search for two such elements. The goal of this study was to identify the domain(s) of Spt10p responsible for this cooperative binding and to gain some insight into the molecular basis of this recognition. We have demonstrated that cooperative binding requires the NTD: in the absence of the NTD, Spt10p binds non-cooperatively. The NTD contains very few positively charged amino acid residues and is therefore unlikely to bind to DNA. Instead, it is much more likely to exert its influence on the binding of the DBD to DNA through effects on Spt10p structure, probably via dimer formation. This proposal is sup-ported by our demonstration that both Spt10p and the NTD are dimers and that the DBD is a monomer. Spt10p also forms a tetramer, or possibly a larger oligomer, which tends to undergo reversible dissociation into dimers. The formation of a Spt10p tetramer would be interesting given that there are two pairs of UAS elements in the promoters of each divergently transcribed pair of histone genes (12). In this case, a Spt10p dimer bound to a pair of UAS elements could interact with another Spt10p dimer bound at a neigh-bouring pair of elements. However, it should be noted that none of the Spt10 protein fragments exhibited a tendency to form tetramers.

Spt10p is an elongated dimer

Ideally, the molecular weight measurements would have been done using the analytical ultracentrifuge or by light scatter-ing, but these methods require greater quantities of stable protein than we were able to prepare. Therefore, we obtained approximate molecular weights by combining data from gel filtration and density gradient centrifugation [e.g. see (27)]. Both of these transport methods separate proteins based on their molecular weight and their shape, described by the frictional coefficient (f), but in different ways. In a gel filtration experiment, a protein with an elongated shape will appear to have a higher molecular weight than its actual value, because compact proteins explore more of the gel volume than extended proteins and are therefore retarded.

In sedimentation, the more compact a protein is, the more rapidly it sediments. Thus, a protein with an elongated shape will appear to have a *lower* molecular weight than the actual value (the opposite to gel filtration). The molecular weight of a protein is derived by solving an equation relating the Stokes radius to $s_{20,w}$ (see Materials and Methods). Although this method is significantly less accurate than the sedimentation equilibrium method, it gives additional infor-mation because it also provides an approximate measure of the degree of compaction, given by the value of f.

The frictional ratio of a molecule (f/f_o) is a comparison of the observed frictional coefficient of a molecule (f) with the frictional coefficient expected if the molecule were perfectly spherical (f_o). Thus, a frictional ratio of 1.0 would indicate that the molecule is essentially spherical. However, the calcu-lation of f_o does not account for hydration of the molecule; therefore, frictional ratios less than ~1.2 are usually consid-ered to indicate a globular (spherical) protein (29). Values >1.2 are indicative of elongated proteins and values of 2 or more correspond to very extended proteins (29). The frictional ratio calculated for full-length Spt10p is 1.8 (Table 1), which suggests that the Spt10p dimer has a highly extended conformation (29). In contrast the DBD monomer has $f/f_o = 1.0$, indicative of a globular protein. The NTD dimer ($f/f_o = 1.0$) and the NTD–HAT–DBD dimer ($f/f_o = 1.2$) are also globular proteins. In contrast, both C-terminal fragments of Spt10p (DBD-C1-C2 and DBD-C1) are highly elongated ($f/f_o = 2.0$ and 1.6, respectively). The implication is that the C2 domain might be extremely extended. These data imply that the extended conformation of the Spt10p dimer is due primarily to the C-terminal domains, C1 and C2.

A working model for cooperative binding by Spt10p

It is important to note that simple dimer formation by Spt10p is not sufficient to account for the requirement for two UAS elements, because the dimer would still be expected to bind a single UAS element with reasonably high affinity, even if only one of the two DBDs is bound specifically. A speculat-ive model to explain the requirement for two UAS elements is presented in Figure 6A. We propose that the two DBDs in the Spt10p dimer are oriented such that neither can recognize a complete UAS element due to steric hindrance from the rest of the Spt10p dimer: each DBD can recognize only part of an UAS element. If there is only one UAS element in the DNA concerned, this interaction is weak, resulting in the observed low affinity. However, if there are two UAS elements, it is argued that the interaction of both DBDs with part of an UAS element is sufficient to trigger a conformational change in the Spt10p dimer, allowing both DBDs to interact fully with both elements. This hypothetical model might help to explain our puzzling observation that the binding of Spt10p to two UAS elements is not significantly stronger than that of the DBD [45 ± 16 nM and 32 ± 18 nM, respectively; (13)]. A possible explanation for this is that the additional free energy gained by the interaction of Spt10p with a second UAS element might drive a conformational change in Spt10p, rather than contribute to increased affinity. Attempts to obtain evidence for a conformational change in Spt10p as a result of binding to DNA using proteases as probes were thwarted by the presence of a contaminating protease which became
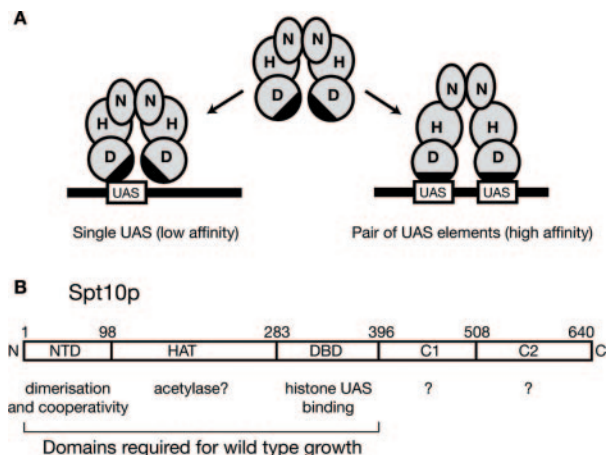
Figure 6. Working model for the cooperative binding of Spt10p to a pair of histone UAS elements. (**A**) Working model to account for the cooperative binding of the Spt10p dimer to a pair of histone UAS elements. The NTD–HAT–DBD portion of Spt10p (residues 1–396) is sufficient for wild-type growth; it is a relatively compact dimer stabilized through interactions between its NTDs (N). The C1 and C2 domains have been omitted for clarity; they appear to be relatively extended. It is proposed that the DBDs (D) in the Spt10p dimer are oriented such that the DNA-binding site (black crescent) cannot interact fully with a single UAS element. However, if two such elements are present, the combined interactions of each DBD with part of an UAS are sufficient to trigger a conformational change in Spt10p, resulting in high-affinity binding. Other models invoking different types of conformational change are also possible. (**B**) The domain structure of Spt10p. Summary of the functions of Spt10p and how they relate to its structure.

active when the protease inhibitors were removed from the Spt10p preparation.

In some respects, though not all, Spt10p appears to behave similarly to the erythroid transcription factor GATA-1, which contains two zinc fingers. The isolated C-terminal zinc finger has a high affinity for a single GATA sequence, whereas the isolated N-terminal finger exhibits only extremely weak binding to a single GATA sequence (30). However, GATA-1 protein fragments containing both fingers bind much more tightly to DNA containing two GATA sequences than to DNA with a single GATA site. To account for these observations, it has been proposed that the two zinc fingers interact with one another within the GATA-1 protein. It was suggested that this interaction is disrupted if there are two GATA sites present in the DNA, because the zinc finger–DNA interactions are together much stronger than the interaction between the fingers (30,31). Based on this analogy, it is possible that the two DBDs [each of which contains a single zinc finger (13)] might interact weakly within the Spt10p dimer and that this interaction is disrupted only in the presence of DNA containing two UAS elements. This postulated interaction between DBDs would presumably be very weak, because the isolated DBD is a monomer; however, such an interaction would be strengthened within the Spt10p dimer due to the local concentration effect.

The NTD has a critical function *in vivo*

Yeast lacking the NTD grow poorly, suggesting that the NTD is important for growth. An issue here is the question of whether Spt10ΔNp aggregated *in vivo*, as observed *in vitro*. This is difficult to rule out definitively, but aggregation in

E.coli probably reflects inclusion body formation, which has not been reported in yeast. In addition, we found that Spt10ΔNp remains in the supernatant when a yeast extract is prepared, suggesting that it is not present as large aggregates. Furthermore, Spt10ΔNp could be detected at the *HTA1* promoter *in vivo*. We have shown that Spt10(1–396)p is a dimer and it is probable that Spt10ΔNp is a monomer because it lacks the NTD dimerization domain. Although both proteins bind to the *HTA1* promoter *in vivo*, Spt10ΔNp cannot rescue the growth phenotype. The implication is that the interaction between two Spt10p monomers to form the dimer, mediated by the NTD, resulting in cooperative binding, is critical for gene activation by Spt10p *in vivo*. It should be noted that the NTD might possess other functions in addition to dimerization. Another histone gene regulator, Spt21p, is required for maximal binding of Spt10p to histone promoters in S-phase *in vivo* (8) and since there is evidence that Spt10p interacts directly with Spt10p *in vitro* (8), Spt21p might affect dimerization.

It has already been shown that point mutations in the DBD (3,13), or in the putative acetyl coenzyme A binding site in the HAT domain [(8); P. R. Eriksson, unpublished data] are sufficient to cause very poor growth, similar to that of the null strain, indicating that both the DBD and HAT domain are essential for wild-type growth. Thus, the vital functions of the Spt10p activator are confined to the N-terminal portion of the molecule, corresponding to the NTD, HAT domain and DBD (residues 1–396), which is sufficient for wild-type growth (Figure 6B). The same region of Spt10p is required for cooperative binding to pairs of histone UAS elements.

In conclusion, dimer formation by Spt10p, mediated by the NTD, appears to result in a Spt10p conformation designed to ensure that both DBDs in the dimer must bind to a histone UAS element before high-affinity binding is possible. Thus, cooperative binding results in increased specificity of Spt10p for the histone promoters. A search of the yeast genome has revealed that pairs of sequences similar to the histone UAS element occur only in the major core histone promoters and nowhere else in the genome (12). Thus, the requirement for two histone UAS elements should guarantee that Spt10p binds at the histone promoters and nowhere else.

REFERENCES

1. Fassler,J.S. and Winston,F. (1988) Isolation and analysis of a novel class of suppressor of Ty insertion mutations in *Saccharomyces cerevisiae*. *Genetics*, **118**, 203–212.
2. Natsoulis,G., Dollard,F., Winston,F. and Boeke,J.D. (1991) The products of the *SPT10* and *SPT21* genes of *Saccharomyces cerevisiae*

increase the amplitude of transcriptional regulation at a large number of unlinked loci. *New Biol.*, **3**, 1249–1259.

3. Natsoulis,G., Winston,F. and Boeke,J.D. (1994) The *SPT10* and *SPT21* genes of *Saccharomyces cerevisiae*. *Genetics*, **136**, 93–105.

4. Grant,P.A., Sterner,D.E., Duggan,L.J., Workman,J.L. and Berger,S.L. (1998) The SAGA unfolds: convergence of transcription regulators in chromatin-modifying complexes. *Trends Cell Biol.*, **8**, 193–197.

5. Winston,F. and Sudarsanam,P. (1998) The SAGA of Spt proteins and transcriptional analysis in yeast: past, present and future. *Cold Spring Harb. Symp.*, **63**, 553–561.

6. Yamaguchi,Y., Narita,T., Inukai,N., Wada,T. and Handa,H. (2001) *SPT* genes: key players in the regulation of transcription, chromatin structure and other cellular processes. *J. Biochem.*, **129**, 185–191.

7. Yamashita,I. (1993) Isolation and characterisation of the *SUD1* gene, which encodes a global repressor of core promoter activity in *Saccharomyces cerevisiae*. *Mol. Gen. Genet.*, **241**, 616–626.

8. Hess,D., Liu,B., Roan,N.R., Sternglanz,R. and Winston,F. (2004) Spt10-dependent transcriptional activation in *Saccharomyces cerevisiae* requires both the Spt10 histone acetyltransferase domain and Spt21. *Mol. Cell. Biol.*, **24**, 135–143.

9. Hess,D. and Winston,F. (2005) Evidence that Spt10 and Spt21 of *S.cerevisiae* play distinct roles *in vivo* and functionally interact with MCB-binding factor, SCB-binding factor and Snf1. *Genetics*, **170**, 87–94.

10. Sherwood,P.W. and Osley,M.A. (1991) Histone regulatory (*Hir*) mutations suppress δ insertion alleles in *Saccharomyces cerevisiae*. *Genetics*, **128**, 729–738.

11. Osley,M.A. (1991) The regulation of histone synthesis in the cell cycle. *Ann. Rev. Biochem.*, **60**, 827–861.

12. Eriksson,P.R., Mendiratta,G., McLaughlin,N.B., Wolfsberg,T.G., Mariño-Ramírez,L., Pompa,T.A., Jainerin,M., Landsman,D., Shen,C.-H. and Clark,D.J. (2005) Global regulation by the yeast Spt10 protein is mediated through chromatin structure and the histone upstream activating sequence elements. *Mol. Cell. Biol.*, **25**, 9127–9137.

13. Mendiratta,G., Eriksson,P.R., Shen,C.-H. and Clark,D.J. (2006) The DNA-binding domain of the yeast Spt10p activator includes a zinc finger that is homologous to foamy virus integrase. *J. Biol. Chem.*, **281**, 7040–7048.

14. Neuwald,A.F. and Landsman,D. (1997) *GCN5*-related histone *N*-acetyltransferases belong to a diverse superfamily that includes the yeast *SPT10* protein. *Trends Biochem. Sci.*, **22**, 154–155.

15. Sutton,A., Shia,W.J., Band,D., Kaufman,P.D., Osada,S., Workman,J.L. and Sternglanz,R. (2003) Sas4 and Sas5 are required for the histone acetyltransferase activity of Sas2 in the SAS complex. *J. Biol. Chem.*, **278**, 16887–16892.

16. Shen,C.-H., Leblanc,B.P., Neal,C., Akhavan,R. and Clark,D.J. (2002) Targeted histone acetylation at the yeast *CUP1* promoter requires the transcriptional activator, the TATA boxes and the putative histone acetylase encoded by *SPT10*. *Mol. Cell. Biol.*, **22**, 6406–6416.

17. Xu,F., Zhang,K. and Grunstein,M. (2005) Acetylation in histone H3 globular domain regulates gene expression in yeast. *Cell*, **121**, 375–385.

18. Kawasaki,H., Schiltz,L., Chiu,R., Itakura,K., Taira,K., Nakatani,Y. and Yokoyama,K.K. (2000) ATF-2 has intrinsic histone acetyltransferase activity which is modulated by phosphorylation. *Nature*, **405**, 195–200.

19. Osley,M.A., Gould,J., Kim,S., Kane,M. and Hereford,L. (1986) Identification of sequences in a yeast histone promoter involved in periodic transcription. *Cell*, **45**, 537–544.

20. Freeman,K.B., Karns,L.R., Lutz,K.A. and Smith,M.M. (1992) Histone H3 transcription in *Saccharomyces cerevisiae* is controlled by multiple cell cycle activation sites and a constitutive negative regulatory element. *Mol. Cell. Biol.*, **12**, 5455–5463.

21. Dimova,D., Nackerdien,Z., Furgeson,S., Eguchi,S. and Osley,M.A. (1999) A role for transcriptional repressors in targeting the yeast SWI/SNF complex. *Mol. Cell*, **4**, 75–83.

22. Green,E.M., Antczak,A.J., Bailey,A.O., Franco,A.A., Wu,K.J., Yates,J.R. and Kaufman,P.D. (2005) Replication-independent histone deposition by the HIR complex and Asf1. *Curr. Biol.*, **15**, 2044–2049.

23. Prochasson,P., Florens,L., Swanson,S.K., Washburn,M.P. and Workman,J.L. (2005) The HIR corepressor complex binds to nucleosomes generating a distinct protein/DNA complex resistant to remodeling by SWI/SNF. *Genes Dev.*, **19**, 2534–2539.

24. Carrasco,B., Garcia de la Torre,J., Byron,O., King,D., Walters,C., Jones,S. and Harding,S.E. (1999) Novel size-independent modeling of the dilute solution conformation of the immunoglobulin IgG Fab' domain using SOLPRO and ELLIPS. *Biophys. J.*, **77**, 2902–2910.

25. Orsini,G. and Goldberg,M.E. (1978) The renaturation of reduced chymotrypsinogen A in guanidine HCl. Refolding versus renaturation. *J. Biol. Chem.*, **253**, 3453–3458.

26. Maass,D.R. and Atkinson,P.H. (1990) Rotavirus proteins VP7, NS28, and VP4 form oligomeric structures. *J. Virol.*, **64**, 2632–2641.

27. Bresnick,E.H. and Felsenfeld,G. (1994) The leucine zipper is necessary for stabilising a dimer of the helix–loop–helix transcription factor USF but not for maintenance of an elongated conformation. *J. Biol. Chem.*, **269**, 21110–21116.

28. Harpaz,Y., Gerstein,M. and Chothia,C. (1994) Volume changes on protein folding. *Structure*, **2**, 641–649.

29. Tinoco,I., Sauer,K. and Wang,J.C. (1985) *Physical Chemistry*, *2nd edn*. Prentice-Hall, NJ.

30. Trainor,C.D., Ghirlando,R. and Simpson,M.A. (2000) GATA zinc finger interactions modulate DNA binding and transactivation. *J. Biol. Chem.*, **275**, 28157–28166.

31. Ghirlando,R. and Trainor,C.D. (2003) Determinants of GATA-1 binding to DNA. The role of non-finger residues. *J. Biol. Chem.*, **278**, 45620–45628.