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Functional Analysis of a Homopolymeric (dA-dT) Element That Provides Nucleosomal Access to Yeast and Mammalian Transcription Factors*

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Eukaryotic organisms ranging from yeast to humans maintain a large amount of genetic information in the highly compact folds of chromatin, which poses a large DNA accessibility barrier to rapid changes in gene expression. The ability of the yeast *Candida glabrata* to survive copper insult requires rapid transcriptional autoactivation of the *AMT1* copper-metalloregulatory transcription factor gene. The kinetics of *AMT1* autoactivation is greatly enhanced by homopolymeric (dA-dT) element (A16)-mediated nucleosomal accessibility for Amt1p to a metal response element in this promoter. Analysis of the nucleosomal positional requirements for the A16 element reveal an impaired ability of the A16 element to stimulate *AMT1* autoregulation when positioned downstream of the metal response element within the nucleosome, implicating an inherent asymmetry to the nucleosome positioned within the *AMT1* promoter. Importantly, we demonstrate that the A16 element functions to enhance nucleosomal access and hormone-stimulated transcriptional activation for the mammalian glucocorticoid receptor, in a rotational phase-dependent manner. These data provide compelling evidence that nucleosomal homopolymeric (dA-dT) elements provide enhanced DNA access to diverse classes of transcription factors and suggest that these elements may function in this manner to elicit rapid transcriptional responses in higher eukaryotic organisms.

A key component of many developmental, adaptive, and protective signaling pathways in cells is the rapid transcriptional activation of genes necessary to manifest changes in cellular homeostasis. The proximal mediators of these signaling pathways are the transcriptional activators, which must physically interact with cis-acting DNA elements within the promoters of their target genes. The organization of eukaryotic DNA into chromatin poses a unique paradox for the cell to maintain its genetic material in a compact arrangement and yet have the capacity to respond quickly to extracellular stimuli to activate gene expression. In contrast to the very high affinity that transcription factors typically have for a given target DNA sequence on free DNA, the same DNA sequence within chromatin can have >1000-fold lower affinity (1). This poses a large thermodynamic barrier to surpass in order to achieve a rapid transcriptional response for inducibly bound transcription factors. Furthermore, the magnitude of the thermodynamic barrier created by chromatin for each specific transcription factor can vary significantly and is often related to the position of the DNA-responsive element relative to the ends of the nucleosomal DNA (translational phase) and the direction the responsive element faces in relation to the histone core (rotational phase). Given that occupancy of the responsive elements by transcription factors is directly correlated with transcriptional activation, the thermodynamic barrier created by chromatin also plays an integral role in modulating the kinetics of target gene activation.

Classically, eukaryotic promoters have been categorized into two general types, preset and remodeled, based on their chromatin architecture and the need for nucleosome remodeling on the promoter to establish accessible DNA. The *Drosophila* heat shock-responsive genes *hsp26* and *hsp70* provide examples of a preset chromatin arrangement whereby the target DNA sequences recognized by the heat shock transcription factor within each of their respective promoters are maintained free of stably positioned nucleosomes. The regions of the heat shock elements within each of these promoters are hypersensitive to nuclease digestion, consistent with the lack of stably positioned nucleosomes (2).

An alternative to maintaining a preset promoter chromatin configuration is the use of stimulus-initiated nucleosome remodeling that is orchestrated by the signal-responsive transcription factors. In *Saccharomyces cerevisiae*, a signal transduction pathway is activated in response to low environmental phosphate levels, which leads to the activation of the *PHO5* gene through the Pho2p and Pho4p transcription factors. The *PHO5* gene harbors six uniquely positioned nucleosomes within its promoter, of which four are effectively remodeled during phosphate starvation (3, 4). Nucleosome remodeling on the *PHO5* promoter is dependent on the binding of Pho4p to a target DNA sequence known as UASp1, which is located between two positioned nucleosomes. Importantly, the primary Pho4p binding site is nonnucleosomal, and it is only after Pho4p-mediated nucleosome remodeling that the adjacent nucleosomal Pho4p and Pho2p binding sites become available and *PHO5* transcription is potentiated.

Another example of stimulus-initiated nucleosome remodeling is orchestrated by the activated glucocorticoid receptor (GR)[1] upon binding to a nucleosomal glucocorticoid response

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[1] The abbreviations used are: GR, glucocorticoid receptor; rGR, rat GR; bp, base pair(s); DOC, deoxycorticosterone; GPD, glyceraldehyde-3-phosphate dehydrogenase; GRE, glucocorticoid response element; MMTV-LTR, mouse mammary tumor virus-long terminal repeat.

element (GRE) located on the mouse mammary tumor virus-long terminal repeat (MMTV-LTR). In response to glucocorticoid hormone, the activated GR binds to the GREs located within a positioned nucleosome in the MMTV-LTR to facilitate the remodeling of this nucleosome, thereby providing binding site access to the NF-1 transcription factor (5–8). The affinity of the NF-1 transcription factor for DNA is severely reduced for target DNA sequences that are within a nucleosome, unlike the steroid receptors, the DNA binding of which is only modestly affected by chromatin (9–11). The stimulation of nucleosome remodeling from a nonnucleosomal site in the case of *PHO5*, and a nucleosomal site in the case of the MMTV-LTR, suggests that the exact mechanism of nucleosome remodeling used on any given promoter will be intimately associated with the nature of the transcription factor promoting the response and its ability to interact with nucleosomal DNA.

A third generally applicable mechanism for achieving accessible chromatin is through DNA structural element-facilitated nucleosome destabilization and/or the distortion of a stably positioned nucleosome to provide localized accessible DNA. This mechanism for attaining accessible chromatin was discovered by Struhl and colleagues using the *HIS3* promoter in *S. cerevisiae* (12, 13). The *S. cerevisiae HIS3* gene product functions in the histidine biosynthetic pathway and is activated by the transcription factor Gcn4p in response to amino acid starvation. The Gcn4p binding site on the *HIS3* promoter is located at −92 relative to the transcriptional start site with an imperfect 17-bp poly(dA-dT) element containing the sequence T_4CAT_{11} located 12 bp upstream of the binding site. Deletion of a 344-bp *HIS3* upstream promoter region containing this poly(dA-dT) element resulted in a severe decrease in the magnitude of Gcn4p-dependent gene activation, implicating the poly(dA-dT) element in fostering *HIS3* transcriptional activation (13). Substitution of the native imperfect poly(dA-dT) element with homopolymeric (dA-dT) elements 17, 29, or 42 bp in length restored Gcn4p-dependent transcriptional activation (12). Probing the accessibility of this region using *in vivo Hin*fI cleavage and DNA methylation studies indicated a modest poly(dA-dT)-dependent enhancement of accessibility (1.9-fold increase in *Hin*fI cleavage of the Gcn4p binding site and a 10% difference in DNA methylation by *Escherichia coli dam* methylase expressed *in vivo*). Iyer and Struhl (12) concluded that the 42-bp homopolymeric (dA-dT) element functions by improving the accessibility of the *HIS3* promoter region containing the Gcn4p binding site either by a local decrease in nucleosome occupancy and/or an altered nucleosome conformation.

Several studies have provided evidence that the region containing the Gcn4p binding site and the poly(dA-dT) element in the native *HIS3* promoter is in a region that lacks stably positioned nucleosomes. Using micrococcal nuclease to probe the chromatin structure of the wild-type *HIS3* promoter, Losa *et al.* (14) identified two strong nuclease hypersensitive sites in the region of the poly(dA-dT) element that were separated by 110 bp. They concluded that the separation between these nuclease sensitive sites was insufficient to represent the boundaries of a stably positioned nucleosome, which would be ~146 bp apart, and likely represents a nuclease sensitive region lacking stably positioned nucleosomes. Studies by Filetici *et al.* (15) further support the contention that this region of the wild-type *HIS3* promoter lacks stably positioned nucleosomes. Using radiolabeled *HIS3* gene fragments from different regions of the gene to probe a Southern blot of mononucleosomal DNA, they demonstrated that the region containing the poly(dA-dT) element and Gcn4p binding site is specifically devoid of stably positioned nucleosomes. Furthermore, in the studies by Iyer and Struhl (12), it is clear that the region of the Gcn4p binding site is ~7-fold more sensitive to *Hin*fI cleavage than an adjacent site 65 bp upstream in the absence of the poly(dA-dT) element, which is highly suggestive that the poly(dA-dT) region does not contain stably positioned nucleosomes. The 42-bp homopolymeric (dA-dT) used in the studies mentioned above may therefore be acting to further promote the unstable chromatin architecture in this region of the *his3* promoter to promote Gcn4p accessibility, or perhaps by a combination of this mechanism and altering the conformation of nucleosomes that may be transiently positioned in this region to allow nucleosomal access to Gcn4p.

Recently, we have described a distinct system that utilizes a nucleosomal homopolymeric (dA-dT) element to mediate accessibility to the copper-dependent transcription factor, Amt1p, in the yeast *Candida glabrata* (16). The Amt1p transcription factor in the yeast *C. glabrata* sits atop a hierarchy of genes that are activated by the presence of high levels of copper in the environment (17). Acting as both the sensor and transcriptional activator, Amt1p binds Cu(I) and activates its own transcription followed by the transcriptional activation of the *MTI*, *MTIIa*, and *MTIIb* metallothionein genes, which encode critical components of the copper ion detoxification pathway (18). The rapid and robust transcriptional autoactivation of the *AMT1* gene is a critical step in this signaling pathway and occurs through a single metal-responsive element (MRE) within the *AMT1* promoter (18). This MRE is located at the pseudodyad axis of symmetry of a stably positioned *AMT1* promoter nucleosome encompassing DNA from −113 to −260 relative to the transcription start site, along with a homopolymeric (dA-dT) element containing 16 contiguous adenosine residues (A16) that resides just upstream of the MRE (16). DNase I analysis and restriction enzyme access experiments with yeast chromatin suggest that the A16 element locally distorts the DNA at both ends, creating increased accessibility to neighboring DNA sequences (16). Homopolymeric (dA-dT) elements have been shown to have unique structural characteristics that make them rigid and less flexible than standard B-form DNA. In addition to having a shorter helical repeat (10.0 bp/turn as opposed to 10.5 bp/turn) and a narrow minor groove (~9 Å as compared with ~15 Å for B-form DNA), homopolymeric (dA-dT) elements have additional bifurcated hydrogen bonds that provide added structural stability (19). These characteristics of homopolymeric (dA-dT) elements, and our previous data, suggest that nucleosomal homopolymeric (dA-dT) elements can resist conforming tightly to the histone protein face of the nucleosome and create localized DNA distortions on either end of the element that provide "access windows" for transcription factors.

Analysis of the crystal structure of a reconstituted nucleosome reveals that there is an inherent asymmetry with respect to the number of histone-DNA contacts made on either side of the dyad axis of symmetry (20). Recent studies investigating triple helix formation at nucleosomal poly(dA-dT) elements experimentally identified functional differences between the ability of a triple helix to form on one side of the nucleosome dyad axis as compared with its counterpart on the other side of the dyad axis (21). In its natural context, the A16 element resides just upstream of the *AMT1* promoter MRE. Here we present the results of studies that address whether the positioning of the A16 element on the nucleosome alters the ability of the homopolymeric (dA-dT) element to stimulate *AMT1* autoregulation by placing the A16 element downstream of the MRE. Additionally, we investigate the length requirements for the nucleosomal homopolymeric (dA-dT) element to function in *AMT1* autoregulation. Importantly, we also demonstrate that the function of the A16 element is conserved in the baker's

yeast *S. cerevisiae* and does not depend on the Dat1 poly(dA-dT) binding protein. Furthermore, we demonstrate the ability of the A16 element to foster nucleosomal access to the rat glucocorticoid receptor expressed in yeast, in a rotational phase-dependent manner. Given the high frequency of occurrence of homopolymeric (dA-dT) tracts in promoter regions in yeast and mammalian genes, these elements may be of widespread use in facilitating rapid transcriptional responses to physiological and environmental stimuli.

EXPERIMENTAL PROCEDURES

Strains and Growth Conditions—The *C. glabrata* uracil auxotrophic strain (D) was used as the recipient for episomal plasmids bearing wild-type or mutant *AMT1-lacZ* reporter genes. The *AMT1* disruption strain (*amt1–1*) (17) was used for the integration of full-length wild-type or mutant *AMT1* genes in single copy at the *ura3* locus. The *S. cerevisiae* strain CY342 (*MATa ade2–101 his3–200 leu2-Δ1 lys2–801 ura3-Δ99*) was used for the glucocorticoid receptor experiments. *S. cerevisiae* strain KKY17 (CY342 + *ace1Δ225*) was used to test the ability of *AMT1* to autoregulate in baker's yeast, and *S. cerevisiae* strain KKY20 (KKY17 + *dat1Δ::kanMX2*) was used to test the role of the Dat1 protein in *AMT1* gene autoregulation. The *E. coli* strain DH5αF′ was used for the construction and maintenance of plasmids by standard techniques (22), except in the case of plasmids that were used with the Chameleon double-stranded mutagenesis kit (Stratagene), in which the manufacturer's strains were utilized.

Plasmids—The plasmids pAXHA3, pAX-S16, and pAX-A16Δ were produced in order to facilitate the construction of mutant *AMT1-lacZ* reporter plasmids. pAXHA3 contains the 635-bp *Xba*I-*Hae*III fragment of the wild-type *AMT1* gene from pBZ-12 (23) cloned into the *Xba*I and *Sma*I sites of pBluescript SK+. pAX-A16Δ is the corresponding plasmid produced from the *Xba*I-*Hae*III fragment lacking the A16 derived from pBZ12-A16Δ (16), and pAX-S16 is the corresponding plasmid derived from pRSS16 (16). The 645-bp *Xba*I-*Eco*RI fragments from pAXHA3 and pAX-S16 were used to replace the equivalent fragment in pKTP-8T (13) to produce pA16-*lacZ* and pS16-*lacZ*, respectively. The oligonucleotides 5′-CTCATCACGCCCACCTTTTTTTTTTTTTTTTTACTACTTTTAAGTCAGC-3′ and 5′-CTCATCACGCCCACCAGCATGCGGATCCTGAACTACTTTTAAGTCAGC-3′ were used in conjunction with pAX-A16Δ and the Chameleon mutagenesis kit to produce plasmids pAX-3′A16 and pAX-3′S16, respectively. The oligonucleotides 5′-GCCAAATTAGCTTATCATGATTTTTTTTTTGGATCCCAGAATGTTAGTCTCCG-3′ and 5′-GCCAAATTAGCTTATCATGATTTTTCCATGGGATCCCAGAATGTTAGTCTCCG-3′ were used with plasmid pAX-HA3 and the above mutagenesis kit to produce plasmids pAX-A10 and pAX-A5, respectively. The DNA sequence of the *Xba*I-*Eco*RI fragment from each respective plasmid was confirmed by dideoxy sequencing, and these fragments were then used to replace the equivalent fragment from plasmid pA16-*lacZ* to produce the following mutant plasmids: p3′A16-*lacZ*, p3′S16-*lacZ*, pA10-*lacZ*, and pA5-*lacZ*. Plasmids pA16/3′A16-*lacZ* and pA16/3′S16-*lacZ* were constructed via three-piece ligation by simultaneously introducing the *Xba*I-*Bsp*HI fragment of pAXHA3 and the *Bsp*HI-*Eco*RI fragment from pAX-3′A16 and pAX-3′S16, respectively, into the *Xba*I-*Eco*RI cut pA16-*lacZ* vector. Plasmid pS16/3′A16-*lacZ* was also produced by three-piece ligation using the *Xba*I-*Bsp*HI fragment of pAX-S16 and the *Bsp*HI-*Eco*RI fragment from pAX-3′A16.

The integrative plasmids pU1b-3′A16 and pU1b-3′S16 were produced by combining the *Xba*I-*Sty*I *AMT1* promoter fragment harboring their respective mutations with the remainder of the *AMT1* gene in the pU1b plasmid backbone (24). The resulting plasmids were then used to integrate, in single copy, the mutant *AMT1* genes at the *ura3* locus of the *amt1–1* strain as described previously (18). The new strains designated *3′A16::URA3* and *3′S16::URA3* were tested for copper resistance, along with strains *A16::URA3* and *S16::URA3* made previously (16). Plasmids pAX-S16GREin and pAX-S16GREout were constructed using the plasmid pAX-S16 with the Chameleon mutagenesis kit and the oligonucleotides 5′-CCTGATCATGATAAGCTCTGCTGTACAGGATGTTCTAGCTACTGGGCGTGATGAGCC-3′ and 5′-GGATCCTGATCATGACTCTGCTGTACAGGATGTTCTAGCTACGTGGTGGGC-3′, respectively. To generate pS16GREinZ and pS16GREoutZ, the *Xba*I-*Eco*RI fragments of pAX-S16GREin and pAX-S16GREout, respectively, were used to replace the corresponding fragments in pA16-*lacZ*. The plasmids pA16GREinZ and pA16GREoutZ were generated via the three-piece ligation approach described above. The plasmid p413GPD-rGR was developed to constituitively express the rat glucocorticoid receptor cDNA from the glyceraldehyde-3-phosphate dehydrogenase (GPD) promoter in *S. cerevisiae*. p413GPD-rGR contains the 2.8-kilobase *Xba*I fragment encompassing the rat glucocorticoid receptor open reading frame from CMV5rGRx subcloned into the *Xba*I site of p413-GPD (25).

Disruption of the S. cerevisiae DAT1 Gene—The oligonucleotides 5′-CTTGTGAATCTACAAACTGTCCTAAAGTATATTGGAGCAGGACATTGGGTGGAAGCTTCGTACGCTGCA-3′ and 5′-CTAGTTATTATGTGGCATATACGAATGTTTTAGTGGTATGCTGGAAATGAAGGCCACTAGTGGATCTGA3′, containing 50 bp of homology to the 5′ and 3′ ends of the *S. cerevisiae DAT1* gene, respectively, were used to amplify by polymerase chain reaction the kanMX2 cassette from the plasmid pFA-kanMX2 as described previously (26). Approximately 10 μg of the resulting polymerase chain reaction product was used to transform *S. cerevisiae* strain KKY17. The strain KKY20 was identified that exhibited G418 resistance and displayed the correct chromosomal disruption of the *DAT1* gene (*dat1Δ::kanMX2*), as ascertained by polymerase chain reaction.

Analysis of Wild-type and Mutant AMT1-lacZ Gene Expression in Vivo—The *C. glabrata* strain D was independently transformed with pA16-*lacZ*, pS16-*lacZ*, p3′A16-*lacZ*, p3′-S16-*lacZ*, pA16/3′S16-*lacZ*, pA16/3′A16-*lacZ*, pS16/3′A16-*lacZ*, pA10-*lacZ*, and pA5-*lacZ* using the protocol of Ito *et al.* (27) and plated onto SC-ura agar plates. Growth conditions, $CuSO_4$ induction of Amt1p-dependent transcription, and analysis of *AMT1-lacZ* mRNA levels by RNase protection were carried out as described previously (23). For the analysis of *AMT1* mRNA levels in *S. cerevisiae*, *ACT1* mRNA levels were used as the internal control as described elsewhere (28). RNase protection analysis was performed on at least two independent time courses of reporter gene induction for each experiment.

Analysis of Yeast Chromatin—Nuclei were isolated from 1 liter of cells grown to an absorbance at 600 nm of ~1.4 in synthetic complete medium lacking uracil, using the method of Almer and Horz (3). Micrococcal nuclease treatments were as described by Almer and Horz (3), with the concentration of micrococcal nuclease used indicated in the figure legends. After purification of the micrococcal nuclease treated DNA, the DNA was subjected to secondary digestion with *Sty*I to completion. Control samples that were used for marker lanes were left on ice without micrococcal nuclease during the initial digestion, with subsequent purification and digestion of the DNA by *Bsp*HI and *Sty*I. Digested samples were resolved on a 1% agarose gel, transferred to nitrocellulose and probed with a random prime radiolabeled 700-bp *Xba*I-*Sty*I *AMT1* fragment derived from plasmid pAXHA3.

Expression of Rat Glucocorticoid Receptor in S. cerevisiae and Induction of Glucocorticoid-responsive Reporter Gene Expression—The *S. cerevisiae* strain CY342 was cotransformed with the rat glucocorticoid receptor expression plasmid p413GPD-rGR and one of the following reporter plasmids: pA16GREinZ, pA16GREoutZ, pS16GREinZ, or pS16GREoutZ. Induction of glucocorticoid-responsive gene expression was initiated by the addition of 10 μM deoxycorticosterone (DOC) to log phase (A_{650} = 1.0–1.5) cells in SC-ura-his medium. At the time points indicated in the figure legends, cells were harvested, RNA was prepared, and steady-state *lacZ* and *ACT1* mRNA levels were analyzed by RNase protection. The levels of rGR expressed in each strain were determined by immunoblotting using a monoclonal antibody to GR and standard techniques (22).

RESULTS

Homopolymeric (dA-dT)-mediated Nucleosomal Access to Amt1p Is Affected by the Asymmetry of the Nucleosome—Previous *in vivo* footprinting analysis identified DNase I hypersensitive sites at both ends of the 16-bp homopolymeric (dA-dT) element (A16) in the *AMT1* promoter (16). Replacement of the A16 with a T16 revealed that, like the homopolymeric (dA-dT) element used in the *HIS3* promoter (12), the nucleosomal *AMT1* homopolymeric (dA-dT) element functions in an orientation-independent manner (16). Further analysis of the *AMT1* promoter revealed that these regions of hypersensitivity represent localized areas of accessible DNA within the nucleosome that may provide nucleosomal access for Cu-Amt1p to the MRE located downstream of the A16 element. To test whether the inherent asymmetry of the nucleosome would influence A16 element function, *AMT1* promoter derivatives containing either A16 or a defined nonhomopolymeric sequence (S16) positioned 3′ to the MRE were fused to the *E. coli lacZ* gene and introduced into *C. glabrata* strain D (Table I and Fig. 1*A*). The distance between the MRE and the 3′A16 was maintained the

TABLE I
Diagrammatic representation of the URA3/CEN/ARS AMT1-lacZ reporter plasmids used in this study

AMT1-lacZ reporter plasmids	Designation
A16 MRE *lacZ*	A16 (wild-type)
S16 MRE *lacZ*	S16
MRE A16 *lacZ*	3'A16
MRE S16 *lacZ*	3'S16
A16 MRE S16 *lacZ*	A16/3'S16
S16 MRE A16 *lacZ*	S16/3'A16
A10 MRE *lacZ*	A10
A5 MRE *lacZ*	A5

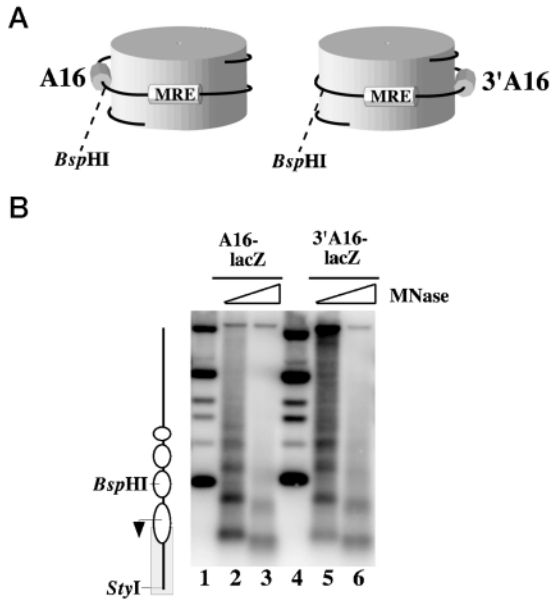

FIG. 1. **The A16 and 3′A16 elements are nucleosomal.** *A,* model for the topography of A16 and 3′A16 sequences relative to the MRE on the *AMT1* promoter nucleosome. *B,* micrococcal nuclease indirect end labeling to identify *AMT1* promoter nucleosomes on the A16 and 3′A16 promoters. *Lanes 1* and *4* contain marker genomic DNA for the A16 and 3′A16, respectively, digested with *Bsp*HI and *Sty*I. *Lanes 2* and *5* contain genomic DNA from nuclei treated with 1.56 units/ml MNase. *Lanes 3* and *6* contain genomic DNA from nuclei treated with 12.5 units/ml MNase. The genomic DNA in *lanes 2–4,* and *6* were digested after purification with *Sty*I. The *Bsp*HI site is located directly 3′ of the A16 element in the wild-type *AMT1* promoter.

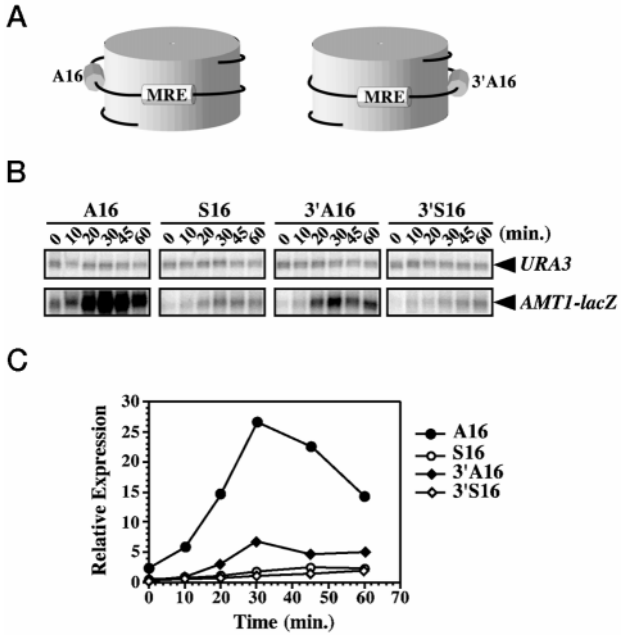

FIG. 2. **The A16 element functions asymmetrically.** RNase protection analysis of copper-induced *AMT1* gene induction for wild-type (A16), S16-, 3′A16-, and 3′S16-containing *AMT1-lacZ* reporter genes. *A,* model for the topography of A16 and 3′A16 sequences relative to the MRE on the *AMT1* promoter nucleosome. *B, C. glabrata* D strain harboring the *AMT1-lacZ* reporter plasmids described in Table I were grown to log phase in SC-ura medium and treated with 100 μM $CuSO_4$, samples were taken at 0, 10, 20, 30, 45, and 60 min after the addition of $CuSO_4$, and total RNA was extracted. Fifteen μg of RNA from each sample was analyzed by RNase protection assays. *Arrowheads* show *AMT1-lacZ* mRNA and *URA3* mRNA. *C,* quantitation of *AMT1-lacZ* mRNA expression in response to copper. All values indicated are normalized to *URA3* mRNA levels as an internal control.

same as for the wild-type *AMT1* gene. Fig. 1*B* shows the results of indirect end-labeling experiments of micrococcal nuclease treated nuclei derived from *C. glabrata* cells harboring either the wild-type or 3′A16 reporter plasmids. These results clearly show that both the wild-type *AMT1-lacZ* fusion and *3′A16-lacZ* fusion genes contain a stably positioned nucleosome over the region of the homopolymeric (dA-dT) element and the MRE. Furthermore, these results also clearly identify three additional positioned nucleosomes present on both promoters, with two nucleosomes upstream of the MRE-containing nucleosome and one nucleosome downstream downstream encompassing the TATA box and transcription initiation site. Fig. 2, *B* and *C,* shows the results and quantitation of RNase protection analysis of steady state mRNA levels expressed from wild-type and mutant *AMT1-lacZ* fusion genes during a time course of exposure to copper ions. *URA3* mRNA levels were analyzed as the internal control. The wild-type *AMT1* promoter exhibits the characteristic rapid and robust transcriptional response to copper ions, peaking at 30 min after copper addition to the cells, whereas the S16-containing promoter displays both a weaker transcriptional response and delayed kinetics of activation observed previously (16). Interestingly, the *3′A16-lacZ* promoter derivative also drives maximum levels of mRNA accumulation 30 min after copper addition, but with an overall peak magnitude that is only 25% that of the wild-type *AMT1-lacZ* fusion. The S16 and 3′S16-containing *AMT1* promoters exhibit very low, virtually indistinguishable, levels of *AMT1* gene expression in response to copper ions. Therefore, the enhanced expression rate and magnitude observed for the 3′A16 is homopolymeric (dA-dT)-dependent.

Chimeric *AMT1* promoters were constructed to determine whether the reduced activity observed for the 3′ positioned A16 element relative to the wild-type position was simply due to altered positioning of the MRE on the nucleosome, from the lack of 16 bp in the normal position of the A16 element or due to the insertion of 16 bp between the MRE and the 3′ end of the nucleosome. Reporter plasmids designated pS16/3′A16-*lacZ* and pA16/3′S16-*lacZ* (Table I) were introduced into the *C. glabrata* D strain, and the response to copper ion administration from these templates was analyzed by RNase protection. Plasmid pS16/3′A16-*lacZ* has the S16 sequence present at the wild-type position of the homopolymeric (dA-dT) element, along with the 3′-positioned A16. This plasmid was used to determine whether the lack of 16 bp at the normal A16 position created artificially low levels of mRNA expression from the 3′A16-*lacZ*

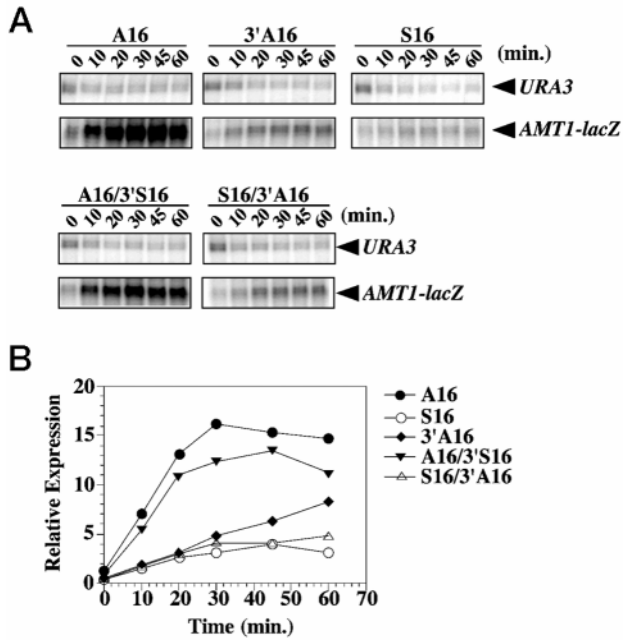

FIG. 3. **The reduced activity of the 3′A16 element is not due to repositioning of the MRE on the nucleosome.** RNase protection analysis of copper-induced *AMT1* gene induction for wild-type (A16), S16-, 3′A16-, A16/3′S16-, and S16/3′A16-containing *AMT1-lacZ* reporter genes. *A,* analysis of copper-induced *AMT1-lacZ* mRNA analysis of *C. glabrata* cells harboring the reporter plasmids indicated in Table I was performed as described in Fig. 1. *B,* quantitation of *AMT1-lacZ* mRNA expression in response to copper. All values indicated are normalized to *URA3* mRNA levels as an internal control.

reporter gene. Plasmid pA16/3′S16-*lacZ* was generated to determine whether insertion of 16 bp (S16) downstream of the MRE would affect *AMT1* autoregulation from a promoter containing a wild-type positioned A16 element. Fig. 3 shows the results of RNase protection analysis of expression from these chimeric *AMT1* reporter plasmids. Although there are small variations in the rate and extent of gene activation from experiment to experiment (compare Fig. 2 to Fig. 3), these data clearly demonstrate that the addition of 16 bp downstream of the MRE (pA16/3′S16-*lacZ*) does not significantly affect the ability of *AMT1* to autoregulate with a wild-type positioned A16 element. Additionally, placing 16 bp (S16) in the wild-type position of the A16 element, in a promoter having a 3′A16 element, does not elevate its ability to autoregulate as seen for the plasmid pS16/3′A16-*lacZ*. Taken together, these results implicate the dominant constraint of the nucleosomal core histones over A16 function in the 3′ position and suggests a functional asymmetry between the two pseudo-symmetrical halves of the nucleosome.

To ascertain whether the lower levels of copper-inducible transcription driven from the 3′A16 promoter are sufficient to foster copper resistance in *C. glabrata*, full-length *AMT1* genes were reconstituted containing either the wild-type (A16), S16, 3′A16, or 3′S16 promoter arrangements. These plasmids were integrated, in single copy, at the *ura3* locus of the *C. glabrata* strain *amt1–1*, which contains an insertionally inactivated *AMT1* gene. The resultant strains *A16::URA3*, *S16::URA3*, *3′A16::URA3,* and *3′S16::URA3* were then challenged on growth medium supplemented with a range of copper concentrations up to 1 mM. As shown in Fig. 4, all strains grow equally well on plates lacking supplemental $CuSO_4$. However, a modest challenge with 50 μM $CuSO_4$ resulted in the inability of strains *S16::URA3*, *3′A16::URA3*, and *3′S16::URA3* to grow. Furthermore, no growth differences between these strains were observed at copper concentrations lower than 50 μM (data not

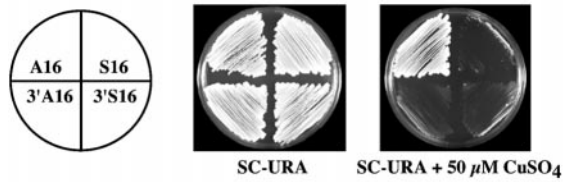

FIG. 4. **The 3′A16-containing *AMT1* gene is not competent to foster copper resistance to *C. glabrata*.** Copper resistance phenotype of *C. glabrata* strains having full-length A16 (wild-type), 3′A16-, and 3′S16-containing *AMT1* genes integrated at the *ura3* locus of the strain *amt1–1*. Strains A16::URA3, S16::URA3, 3′A16::URA3, and 3′S16::URA3 were grown on synthetic complete medium with or without the addition of 50 μM $CuSO_4$.

shown). For comparative purposes, the *A16::URA3* strain was able to grow at copper concentrations above 1 mM. These data suggest that there is a threshold level of rapid *AMT1* autoregulation that must occur to achieve sufficient levels of *C. glabrata* metallothionein gene transcription required for cell survival.

A Homopolymeric (dA-dT) Element Length Requirement for AMT1 Gene Autoactivation—Our model for A16 element-dependent nucleosomal access is based on structural perturbations of the nucleosomal DNA determined in part by the rigidity of the homopolymeric (dA-dT) element and the diameter of the nucleosome (16, 29). Given this model, we predict that there would be geometric constraints requiring a minimal length for the homopolymeric (dA-dT) to function in providing nucleosomal access to Amt1p. To test this hypothesis, the length of the homopolymeric (dA-dT) element in the *AMT1* promoter was altered from 16 bp (A16) to either 10 bp (A10) or 5 bp (A5) while maintaining a constant distance to the MRE (Table I). The results of RNase protection analysis of copper-induced *AMT1* gene expression in *C. glabrata* strains containing these reporter plasmids is shown in Figs. 5*A* and quantitated in Fig. 5*B*. Although the A5 element-containing *AMT1* gene achieves 67% the magnitude of wild-type *AMT1* gene expression, the rate of mRNA accumulation at early time points after copper addition is noticeably slower than for the wild-type gene. An *AMT1* gene with an A10 element displays similar delayed kinetics of activation to that observed for the A5 element-containing promoter but eventually achieves wild-type levels of *AMT1-lacZ* mRNA accumulation. The variation in the size of the protected fragments at the 45 min time point for the A16 and A10 samples (Fig. 5*A*) was not reproducibly observed. These data suggest that a minimal length requirement exists for maximal transcriptional activation at early time points, which is consistent with a length requirement for the homopolymeric (dA-dT) to mediate rapid nucleosomal access for Cu-Amt1p. The large stimulatory effect on *AMT1* autoregulation by the shorter homopolymeric (dA-dT) elements may indicate that localized perturbations in histone-DNA interactions, which would be predicted to increase with length of the homopolymeric (dA-dT) element, result in a more stable Amt1p-nucleosome complex that would provide a more robust transcriptional response. Although the effects of shortened (dA-dT) tracts in the natural *AMT1* gene have not been determined with respect to copper resistance *in vivo*, the slower rates of activation by promoters bearing these shorter elements, fused to the lacZ reporter, would be predicted to compromise growth in the presence of copper.

A16 Element Function Is Conserved in S. cerevisiae and Can Dispense with the Poly(dA-dT) Binding Protein Dat1p—The model for A16 element function is based on nucleosomal structural perturbations created, in large part, by inherent structural features of the A16 tract, which are independent of a poly(dA-dT) binding protein (16, 19). To date, there are no

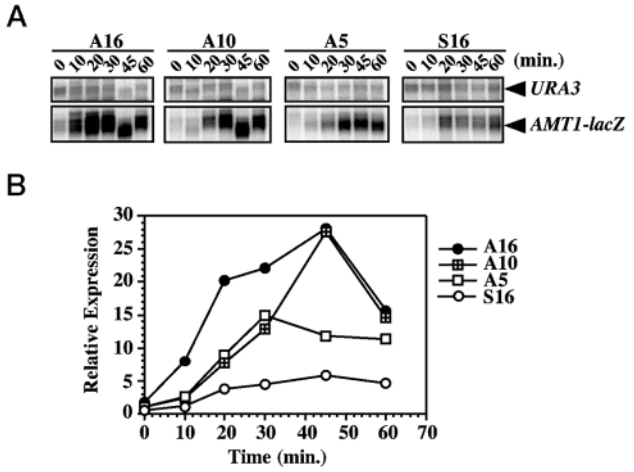

FIG. 5. **Homopolymeric (dA-dT) element function is directly related to its length.** RNase protection analysis of copper-induced *AMT1* gene induction for wild-type (A16), A10-, A5-, and S16-containing *AMT1-lacZ* reporter genes. *A,* analysis of copper-induced *AMT1-lacZ* mRNA analysis of *C. glabrata* cells harboring the reporter plasmids indicated in Table I was performed as described in Fig. 1. *B,* quantitation of *AMT1-lacZ* mRNA expression in response to copper. All values indicated are normalized to *URA3* mRNA levels as an internal control.

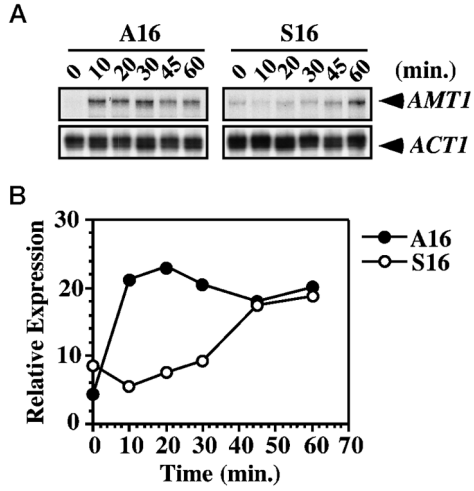

FIG. 6. **A16 element function is conserved between the opportunistic pathogenic yeast *C. glabrata* and the baker's yeast *S. cerevisiae*.** RNase protection analysis of copper-induced *AMT1* gene induction for wild-type (A16) and S16-containing *AMT1* genes in *S. cerevisiae* strain CY342. *A,* CY342 harboring plasmids pRSAMT1 or pRSS16 was grown to log phase in SC-ura media and treated with 100 μM $CuSO_4$. Analysis of copper-induced *AMT1* mRNA was performed essentially as described in Fig. 1. *B,* quantitation of *AMT1* mRNA expression in response to copper. All values indicated are normalized to *ACT1* mRNA levels as an internal control.

reports of a poly(dA-dT) binding protein in the yeast *C. glabrata,* and our previous attempts to identify an A16 element binding activity were negative (16). We therefore sought to ask whether a poly(dA-dT) binding protein might be involved in *AMT1* gene autoregulation using a surrogate genetic approach in the yeast *S. cerevisiae*. The finding that poly(dA-dT) binding activity is undetectable in protein extracts from a yeast strain harboring a *dat1*Δ allele, supports the contention that Dat1p is the predominant poly(dA-dT) binding protein in *S. cerevisiae* (30).

Dat1p has been reported to play completely opposite roles in the transcription of different genes in *S. cerevisiae*. The basal expression of the *his3* gene and a poly(dA-dT) based reporter gene was shown to be elevated in a *dat1*Δ strain, implicating Dat1p as a repressor of basal transcription (12). In contrast, Dat1p has been shown to function synergistically with the protein Reb1p to activate the basal expression of the *ILV1* gene (31). Given these two profoundly different functions on different promoters, we tested whether Dat1p would affect *AMT1* gene autoregulation through the A16 element. The entire *AMT1* promoter and structural gene, contained on a centromeric plasmid, was introduced into an *S. cerevisiae* strain harboring a deletion of the gene encoding the homologous Ace1p copper-metalloregulatory transcription factor. The ability of *AMT1* to be properly autoregulated in *S. cerevisiae* is shown in Fig. 6. In a time course of induction by copper ions, the A16 element-containing *AMT1* promoter displayed the characteristic rapid and robust activation that is observed for the regulation of the wild-type *AMT1* gene in *C. glabrata*. The S16 *AMT1* variant gene also exhibits the delayed kinetics of *AMT1* autoactivation seen in *C. glabrata*, which demonstrates that the function of the A16 element is conserved between *S. cerevisiae* and *C. glabrata*.

The *S. cerevisiae* Dat1p is a 27-kDa protein that binds to homopolymeric (dA-dT) elements, of at least 10 bp, in the DNA minor groove with very high affinity (32–34). We hypothesized that there could be an equivalent protein from *C. glabrata* that would bind to the *AMT1* promoter A16 element and modulate autoregulation in response to copper ions. We therefore investigated whether the Dat1 protein plays a role in *AMT1* gene autoregulation in *S. cerevisiae*. Wild-type (KKY17) and isogenic *dat1*Δ (KKY20) *S. cerevisiae* strains were transformed with plasmids harboring the wild-type or S16 element-containing *AMT1* genes. *AMT1* autoregulation in response to copper administration was analyzed by RNase protection, and the results are shown in Fig. 7. The results of these experiments clearly demonstrate that the homopolymeric (dA-dT) binding protein Dat1p does not play a role in regulating either basal *AMT1* gene transcription or autoactivation in response to copper in *S. cerevisiae*. These data are strong supporting evidence that the A16 element functions to provide nucleosomal access to Amt1p by virtue of its structural properties and not through the action of poly(dA-dT) binding proteins.

Homopolymeric (dA-dT) Elements Function to Provide Nucleosomal Access to Mammalian Transcription Factors—Yeast and mammalian promoters often harbor poly(dA-dT) tracts near cis-acting regulatory elements (12, 16). These observations suggest the possibility that nucleosomal access to diverse transcription factors could be facilitated by poly(dA-dT) elements. To test the ability of the *AMT1* promoter A16 element to facilitate nucleosomal access to a heterologous transcription factor, the mammalian GR was investigated as a model system. The GR has previously been shown to transcriptionally activate reporter genes in *S. cerevisiae* that contain GREs within their promoters, in a glucocorticoid-dependent manner (33). The GR is a fundamentally distinct type of transcription factor as compared with Amt1p based on several features. Amt1p is a relatively small protein of 30 kDa that binds to its responsive elements as a Cu(I)-metallated monomer, making critical DNA contacts in adjacent major and minor grooves on the same face of the DNA double helix (18, 23). GR on the other hand, binds to the GRE as a 188-kDa zinc-metallated homodimer (35–39) that interacts with two consecutive major grooves on the same face of the DNA double helix (32). To test the ability of the A16 element to confer GR access to a nucleosomal GRE, the MRE in the *AMT1* promoter was replaced by the GRE(II) sequence from the rat tyrosine aminotransferase gene promoter (40) in two predicted rotational phases. In the natural context, the *AMT1* promoter MRE has a rotational phase with the adjacent major and minor grooves of the DNA bound by Amt1p facing inward toward the histone core of the nucleosome, based on *in vitro* missing nucleoside analysis (23) and DNase I footprinting

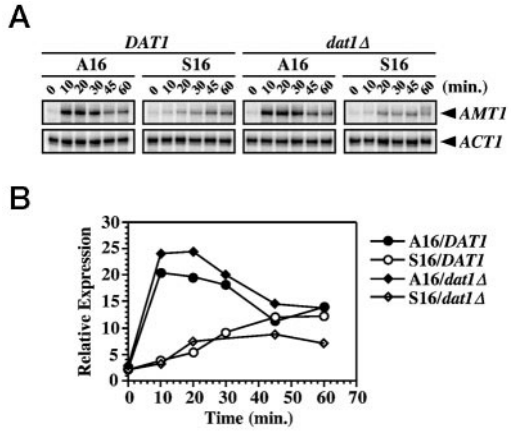

FIG. 7. **The homopolymeric (dA-dT) binding protein Dat1p is dispensable for normal *AMT1* gene autoregulation in *S. cerevisiae*.** RNase protection analysis of copper-induced *AMT1* gene induction of wild-type (A16) and S16-containing *AMT1* genes in *S. cerevisiae* strains KKY17 (*DAT1*) and KKY20 (*dat1*Δ). *A, S. cerevisiae* strains KKY17 (*DAT1*) and KKY20 (*dat1*Δ) harboring plasmids pRSAMT1 or pRSS16 were grown to log phase in SC-ura media prior to induction by copper. Copper treatment and analysis of *AMT1* and *ACT1* mRNA levels was as described in Fig. 1. *B,* quantitation of *AMT1* expression in response to copper. All values indicated are normalized to *ACT1* mRNA levels as an internal control.

studies in chromatin (16). Using the rotational phasing coordinates derived from the DNase I footprinting analysis of *AMT1* promoter chromatin, *AMT1* promoter derivatives were constructed in which the GRE is predicted to face inward (GREin) in one case and outward (GREout) in the other, as shown diagramatically in Fig. 8*A*. Both A16 element and S16-containing *AMT1* genes with either the GREin or GREout version of the GRE replacing the MRE were cotransformed into strain CY342 with the rGR expression plasmid p413GPD-rGR. DOC-induced gene expression from the *AMT1(GRE)-lacZ* reporter plasmids was analyzed by RNase protection (Fig. 8, *B* and *C*). Consistent with the inability of the GR to bind to nucleosomes *in vitro* that have the GRE rotationally phased inward toward the histone core (41, 42), little DOC-induced *AMT1* expression was observed for either the A16 or S16 element-containing promoters with a GREin arrangement (Fig. 8, *B* and *C*). In contrast, the GREout reporter plasmids exhibited robust A16 element-dependent transcriptional activation of the *AMT1(GRE)-lacZ* reporter gene that is not observed with the S16 element. Immunoblot analysis of rGR levels in each of the strains tested (Fig. 8*D*) confirmed that the differences in expression observed for the reporter genes were not due to variations in rGR expression. Additionally, indirect end-labeling experiments with yeast chromatin demonstrate that both the GREin and GREout promoters contain stably positioned nucleosomes over the region of the GRE and A16 and surrounding regions that strongly correlate with the positions of nucleosomes in the wild-type *AMT1* promoter (data not shown). Furthermore, the rotational phase dependence of rGR activation through the GRE strongly supports the presence of a nucleosome at this position that has a rotational phase similar to that which we have previously established for the MRE in the *AMT1* promoter (16). Due to the single-copy nature of the *AMT1(GRE)-lacZ* plasmids in *S. cerevisiae*, we were unable to verify the rotational phasing of the GRE by high resolution DNase I cleavage analysis. However, the A16 element dependence for GR-mediated transcriptional activation is strong evidence that homopolymeric (dA-dT)-mediated nucleosomal access is not specific to the Amt1p transcriptional activator but can function to provide nucleosomal access to a very different class of mammalian transcription factors.

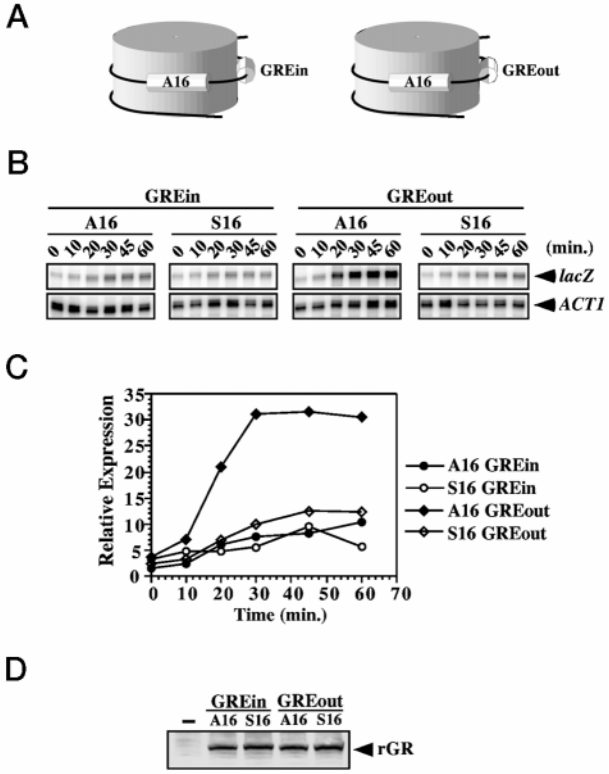

FIG. 8. **The A16 element functions to provide nucleosomal access to a mammalian transcription factor.** Glucocorticoid receptor-mediated gene activation in *S. cerevisiae* from *AMT1-lacZ* reporter genes harboring a GRE in place of the MRE. *A,* diagrammatic representation of *AMT1* promoter nucleosomes having the MRE replaced by the GRE with the GRE rotationally phased in (*GREin*) (*left*) and rotationally phased out (*GREout*) (*right*). The *white region* of the GRE represents the face of the DNA bound by the rGR. *B,* RNase protection analysis of DOC-induced *AMT1-lacZ* gene induction for wild-type (A16) and S16-containing *AMT1-lacZ* reporter genes having the MRE replaced with the GREin or GREout versions of the GRE. *S. cerevisiae* strains harboring the above *AMT1-lacZ* reporter plasmids and the rGR expression plasmid p413GPD-rGR were grown to log phase in SC-ura-his medium and treated with 10 μM DOC. Analysis of DOC-induced *AMT1-lacZ* mRNA was performed essentially as described in Fig. 1. *C,* quantitation of *AMT1-lacZ* mRNA expression in response to deoxycorticosterone. All values indicated are normalized to *ACT1* mRNA levels as an internal control. *D,* Western blot analysis of rGR expression levels for each of the strains used for the analysis of DOC-induced *AMT1-lacZ* expression. rGR levels from 30-μg samples of total protein from each strain used in *B* were analyzed using a monoclonal antibody to GR, and a 30-μg protein sample from strain CY342 not expressing the rGR (−) was used as the negative control for rGR expression.

DISCUSSION

The ability of transcription factors to interact with their target DNA sequences *in vivo* is a decisive factor in the ability of organisms to respond rapidly, robustly, and appropriately to physiological, developmental, and environmental signals. The compaction of genomic DNA into the folds of chromatin effectively reduces the affinity of DNA-binding proteins for DNA target sequences, resulting in a thermodynamic barrier that transcription factors must overcome to achieve activation of target gene expression. In many cases, the energetic cost of breaking this thermodynamic barrier is borne by nucleosome remodeling complexes, both for preset and transcription factor-mediated promoter remodeling. The finding that a DNA structural element within chromatin can function to reduce this barrier suggests that a widespread fundamental mechanism could be utilized by any eukaryotic organism to provide nucleosomal access to transcription factors. To this end, we have sought to understand the conditions under which the homopolymeric (dA-dT) element functions to facilitate transcrip-

tion factor access to its cognate target DNA sequences within chromatin.

The results of experiments with the homopolymeric (dA-dT) element positioned downstream of the *AMT1* promoter MRE provides evidence that the efficacy of A16 element-mediated nucleosomal access to Amt1p is highly dependent on the location of the homopolymeric (dA-dT) element within the nucleosome. Furthermore, the reduced transcriptional activity of the 3′A16 element is not specific to transcriptional activation by Amt1p, as this was also observed for the transactivation by the glucocorticoid receptor having a 3′-positioned A16 (data not shown). This apparent nucleosomal asymmetry is analogous to what was found by Brown and Fox (21) for triple helix formation on nucleosomes containing poly(dA-dT) elements. In this study, a fragment of DNA was reconstituted into nucleosomes that contains two stretches of 11-bp homopolymeric (dA-dT) (T11) on either side of the nucleosomal dyad axis. Triple helix formation with an 11-bp poly T oligonucleotide occurred equally well for two of the T11 elements on one side of the nucleosomal dyad, but triple helix formation was significantly hindered at the third position just 3′ to the dyad axis, and no triple helix formation was seen at the fourth site further downstream of the dyad axis (21).

The results of experiments in which the length of the homopolymeric (dA-dT) element has been altered suggest a progressive change in both the magnitude and the rate of transcription with increasing length. The studies by Iyer and Struhl (12) revealed a similar finding using a competitive growth strategy with various length homopolymeric (dA-dT) elements in the *his3* promoter. Our observation that a 5-bp homopolymeric (dA-dT) element can provide robust stimulation of copper-dependent *AMT1* transcription that is further enhanced by a 10-bp element, and yet both of these promoters do not respond as quickly as the wild-type A16 element-containing promoter, suggests the existence of a length-dependent continuum of homopolymeric (dA-dT) activity. The enhanced response rate that the A16 element provides over the A10 and A5 elements may represent a length requirement to achieve a sufficiently large perturbation of the nucleosomal structure by the rod-like structure of poly(dA-dT) element to provide adequate access to Amt1p. The robust activation of the smaller homopolymeric (dA-dT) elements may reflect their inherent ability to sufficiently disturb histone-DNA interactions to allow more stable Amt1p binding. Consistent with this hypothesis, earlier studies by Li and Wrange (43) demonstrated that the 5-bp sequence 5′-TAAAA-3′, found in its natural context directly adjacent to the GRE of the MMTV-LTR, strongly stimulated GR binding to nucleosomal GREs *in vitro*.

With the average helical pitch for homopolymeric (dA-dT) sequences being 3.2 Å/bp (23), an A16 element would be predicted to be 51.2 Å in length. Given that the DNA superhelix of the nucleosome is reported to have an average diameter of ~83 Å (18) based on the crystal structure, the A16 element would represent 61% of the length of the diameter of the nucleosome. The corresponding shorter A10 element would be 38% the length of the nucleosome diameter, and the A5 element would be 19% of the diameter. When these data are taken together with the results of Fig. 5, we can predict that the nucleosomal access window that is generated by the rod-like structure of the homopolymeric (dA-dT) element requires the length of this element to be >38% of the nucleosomal diameter for the most rapid access by Cu-Amt1p. This could vary depending on the features of the transcription factor in question, such as the size, subunit structure, nature of DNA binding, or other considerations.

Is the function of the nucleosomal *AMT1* A16 element dependent on its intrinsic structural rigidity rather than trans-acting factors? Although there are no known poly(dA-dT) binding proteins in *C. glabrata*, the Dat1 protein from *S. cerevisiae* represents the predominant poly(dA-dT) binding protein from this yeast (30). Our finding that the *AMT1* gene is properly autoregulated by copper ions when introduced into *S. cerevisiae* (Fig. 6) allowed us to test the role of Dat1p in *AMT1* gene autoregulation in this yeast. The Dat1 protein prefers to bind to homopolymeric (dA-dT) elements that are greater than 10 bp (34) and is incapable of binding to elements shorter than 10 bp *in vitro*, which is similar to the length requirement for the *AMT1* promoter homopolymeric (dA-dT) element to provide the most rapid transcriptional response to copper. Our finding that the *AMT1* gene is properly regulated in response to copper in *S. cerevisiae*, and yet does not require Dat1p, is consistent with the hypothesis of Iyer and Struhl (12) and Zhu and Thiele (16) that the homopolymeric (dA-dT) element functions largely by virtue of its intrinsic structure and is not simply a binding site for a transcription factor that recruits or stabilizes the interaction of Amt1p with the *AMT1* promoter MRE in response to copper ions. However, given the reported dominant nature of the nucleosome histone core over DNA sequence and intrinsic structure, it is possible that additional chromatin modifications are required for the *AMT1* A16 element to function in facilitating access to transcription factors to nucleosomal binding sites. Our current and future efforts address this possibility both genetically and through nucleosome reconstitution experiments.

The ability of the *AMT1* promoter A16 element to mediate nucleosomal access to the rat glucocorticoid receptor in yeast would suggest that homopolymeric (dA-dT) elements could function in higher eukaryotic organisms in this context. In contrast to the ability of the A16 element to foster interactions between Amt1p and the inwardly facing MRE in the *AMT1* promoter, the A16 element cannot provide nucleosomal access to the rGR when the GRE is positioned in a manner predicted to be rotationally phased inward. Given the large difference in size between Amt1p and rGR and the differences in the mode of DNA binding by these transcription factors, these data would suggest that a rotational phase requirement for homopolymeric (dA-dT) element function in providing nucleosomal access may exist for other transcription factors.

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