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

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Keith A. Koch‡ and Dennis J. Thiele§

From the Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109-0606

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) upon binding to a nucleosomal glucocorticoid response

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§ A Burroughs Wellcome Toxicology Scholar. To whom correspondence should be addressed: Dept. of Biological Chemistry, University of Michigan Medical School, 1301 Catherine Rd., Ann Arbor, MI 48109-0606. Tel.: 734-763-5717; Fax: 734-763-4581; E-mail: dthiele@umich.edu.

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

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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 CAT 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 HinII cleavage and DNA methylation studies indicated a modest poly(dA-dT)-dependent enhancement of accessibility (1.9-fold increase in HinII 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 vivo affects the accessibility of the region containing the poly(dA-dT) element that were separated by 110 bp. They concluded that the separation between these nucleosome sensitive sites was insufficient to represent the boundaries of a stably positioned nucleosome, which would be −146 bp apart, and likely represents a nucleosome 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 HinII 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) element 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 following by the transcriptional activation of the MTI, MTIa, and MTIb 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 auxotroph
strain (D) was used as the recipient for episomal plasmids bearing
wild-type or mutant AMT1-lacZ reporter genes. The AMT1 disruption
strain (mat1−1) (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 ade2–1 his3–200 leu2–1 asd1–201 ura3–1) was used to test the ability of AMT1
to autoregulate in baker’s yeast, and the S. cerevisiae strain KKY20
(KKY17 + dat1A::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 synthetic oligonucleotide techniques
(22), except in the case of plasmids that were used with the Chameleon
double-stranded mutagenesis kit (Stratagene), in which the manufac-
turer’s strains were utilized.

Plasmids—The plasmids pXAH3, pAX-S16, and pAX-A16Δ were
produced in order to facilitate the construction of mutant AMT1-lacZ
reporter plasmids. pXAH3 contains the 635-bp XbaI-HoeIII fragment of
the wild-type AMT1 gene from pBZ12 (12) cloned into the XbaI and
SmaI sites of pBluescript SK+, pAX-A16Δ is the corresponding plasmid
produced from the XbaI-HoeIII fragment lacking the A16 derived from
pBZ12-A16Δ (16), and pAX-S16 is the corresponding plasmid derived
from pRSS16 (16). The 645-bp XbaI-EcoRI fragments from pXAH3 and
pAX-S16 were used to replace the equivalent fragment in pKPT-ST
(13) to generate pAX-S16-lacZ and pAX-A16Δ-lacZ, respectively. The oligonu-
cleotides 5′–CTTATCATGATTTTTTTTTTTTGATCCCAGAATGTTAGTCTCC-
GACTCTGCTGTACAGGATGTTCTAGCTACGTGGTGGGC–3′ and 5′–
GCCAAAATCTATCTATAGGATGGTATGCTGGAAATGAAGGCCACTA-
TGGGTGGAAGCTTCGTACGCTGCA–3′ were used with plasmid pXAH3 and
the Chameleon double-stranded mutagenesis kit (Stratagene), in which the manufac-
turer’s strains were utilized.

Analysis of Yeast Chromatin—Nuclei were isolated from 1 liter of
cells grown to an absorbance of 600 nm of ~1.4 in synthetic complete
medium lacking uracil, using the method of Almer and Horz (3). Micro-
coccal 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 StyI to
completion. Control samples that were used for marker lanes were left
on ice without micrococcal nuclease during the initial digestion, with
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
AmT1 Is Affected by the Asymmetry of the Nucleosome—Previ-
ous in vivo footprinting analysis identified DNase I hypersensi-
sitivity sites at both ends of the 16-bp homopolymeric (dA-dT)
fragment of pX-A16 and the BspHI-EcoRI fragment from pAX-3CA.

The integrative plasmids pULb3′-A16 and pULb3′-S16 were pro-
duced by combining the XbaI-StyI AMT1 promoter fragment harboring
their respective mutations with the remainder of the AMT1 gene in
the pUL1 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 (15). The new strains design-
ated 3′A16::URA3 and 3′S16::URA3 were tested for copper resistance,
along with strains 16′::URA3 and S16′::URA3 made previously (16). Plasmids pX-S16GREin and pX-S16GREout were constructed using the plasmid pAX-S16 with the Chameleon mutagenesis kit and the oligonucleotides 5′–CCTGATCATGATAGGCTGCTAGCTACGTGGTGGGC–3′ and 5′–
CCTGATCATGATAGGCTGCTAGCTACGTGGTGGCC–3′, respectively.
To generate pX-S16GREin and pX-S16GREout, the XbaI-EcoRI fragments of
pX-S16GREin and pX-S16GREout, respectively, were used to replace the corresponding fragments in pX-A16-lacZ. The plas-
mids pX-S16GREin and pX-S16GREout were generated via the three-
piece ligation approach described above. The plasmid p413GPD-rGR
was developed to constitutively express the rat glucocorticoid receptor
cDNA from the glyceraldehyde-3-phosphate dehydrogenase (GPD) pro-
motor in S. cerevisiae. p413GPD-rGR contains the 2.8-kilobase XbaI
fragment encompassing the rat glucocorticoid receptor open reading
frame from CMV5rGrs subcloned into the XbaI site of p413-GPD (25).

Disruption of the S. cerevisiae DAT1 Gene—The oligonucleotides 5′-
CCTGATCATGATAGGCTGCTAGCTACGTGGTGGGC–3′ and 5′–
CCTGATCATGATAGGCTGCTAGCTACGTGGTGGCC–3′, respectively. The plasmid
pA16/3 was used as described previously (26). Approximately 10 μg of the re-
sulting polymerase chain reaction product was used to transform
the S. cerevisiae strain KKY17. The strain KKY20 was identified that exhib-
ted G418 resistance and displayed the correct chromosomal disruption
of the DAT1 gene (dat1A::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, pS16-A16-lacZ, pA16Δ/316-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, CuSO4 induction of Amt1p-dependent transcription, and
analysis of AMT1-lacZ mRNA levels by RNase protection were carried
out as described previously (25). For the analysis of AMT1 mRNA levels
with copper, we used Propionate as the sole carbon source as described
elsewhere (28). RNase protection analysis was performed on at least two
independent time courses of reporter gene induction for each
experiment.

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Transcription Factors

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same as for the wild-type AMT1 gene. Fig. 1B 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 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 CuSO₄, samples were taken at 0, 10, 20, 30, 45, and 60 min after the addition of CuSO₄, 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 levels as an internal control. Next, 15 μg of RNA from each sample was analyzed by RNase protection assays. Arrowheads show AMT1-lacZ mRNA and URA3 mRNA levels as an 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

![Table I](Image)

**Table I**

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

| AMT1-lacZ reporter plasmids | Designation |
|-----------------------------|-------------|
| A16                         | A16 (wild-type) |
| S16                         | S16 |
| A16/S16                     | 3'A16 |
| A16/S16/A16                 | 3'S16 |
| A16/S16/S16                 | A16/S16 |
| A10                         | A10 |
| A5                          | 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. A16, 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 BspHI and StyI. 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. Genomic DNA in lanes 2–4, and 6 were digested after purification with StyI. The BspHI site is located directly 3’ of the A16 element in the wild-type AMT1 promoter.
porter genes. A, 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.

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.

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-CU. The resultant 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 CuSO4. 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 Amtp. 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. 5A and quantitated in Fig. 5B. Although the A5 element-containing AMT1 gene achieved 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. 5A) 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-Amp. 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 Amp1-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
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_ 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_ 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. 5. 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_ (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 homodimer, 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 analysis of copper-induced _AMT1_ mRNA expression in response to copper. All values indicated are normalized to _URA3_ 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 diagrammatically in Fig. 8A. 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 assay (Fig. 8B 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. 8B 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. 8D) confirmed that the differences in expression observed for the reporter genes were not due to variations in rGR expression. Additionally, indirect in vitro 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) element functions to provide nucleosomal access to a very different class of mammalian transcription factors.
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/pb (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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