Characterization of a short unique sequence in the yeast *HO* gene promoter that regulates *HO* transcription in a *SIN1* dependent manner

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Received 24 January 1996

Abstract **Recently it has become clear that general chromatin proteins as well as sequence-specific DNA binding proteins are important in the control of gene expression. SIN1 in *Saccharomyces cerevisiae* is a chromatin component that regulates the transcription of a family of genes. Previously, we identified a 32 bp unique sequence (here termed XBS) in the promoter of one of those genes, *HO*, which specifically binds a protein that interacts with SIN1. We also found that this sequence can function as a weak UAS in a heterologous promoter that is dependent on the presence of SIN1. Here we report a relationship between the level of *HO* expression and the presence of the short sequence in situ in the *HO* gene. By comparing the expression of *HO* from wild type or XBS deleted *HO* promoters, we concluded that XBS serves as a weak UAS in situ in the *HO* gene, that it influences *HO* transcription via the SWI/SNF complex, and that sequences other than the XBS mediate the affect of *SIN1* on *HO* transcription. In addition, we show that a portion of the SIN1 protein that has sequence similarity to mammalian HMG1 preferentially binds the XBS.**

Key words: *SIN1*; SWI/SNF complex; *HO* transcription; HMG1; *Saccharomyces cerevisiae*

1. Introduction

The transcription of the *HO* gene in the yeast *Saccharomyces cerevisiae* is very tightly regulated by a combination of positive and negative transcriptional regulators (reviewed in [1]). A long DNA sequence upstream of the *HO* translational start site consisting of about 1400 bp has been shown to be involved in this regulation [2]. Based on deletion studies, the region has been divided into two functional regions termed URS1 and URS2 [3]. Further analysis has shown that a short 32 bp sequence (termed here XBS for X Binding Sequence) found in URS1 is capable of being bound by a protein that can interact with SIN1, a negative regulator of *HO* [4]. Subcloning of the XBS into a heterologous promoter has shown that this sequence can serve as a weak upstream activating sequence (UAS) [4].

Analysis of the amino acid sequence of SIN1 shows that it contains a domain that has sequence and structural similarity to the mammalian chromatin protein HMG1 [5]. In *sin1-2* mutants, *HO* is transcribed regardless of the presence of the *SWI1, SWI2, SWI3* positive regulators [3]. Interestingly, sin1-2 mutant protein is able to specifically remove the protein that binds the XBS in vitro [4]. SIN1 has been shown to be a DNA binding protein [5], though DNA sequence specificity has not been demonstrated. In the work reported here, we show that the XBS serves as a weak UAS in situ in the *HO* gene, and that the 'HMG1 domain' of SIN1 can preferentially bind this sequence.

2. Materials and methods

2.1. Yeast strains and plasmids

A series of yeast strains shown in Table 1 were constructed by insertion of recombinant *HO-lacZ* genes into the *URA3* gene of wild type, *sin1, swi1, swi2, or swi3* mutants. The recombinant *HO-lacZ* gene contains the entire upstream regulatory region of *HO*. The sequence TTTTCACTCTACGGATCTGTGAGAAACTGAT (wild type) was replaced with GCGGCCGC (containing a *Not*I site) in the *HO* mutant. The following procedure was used to make the replacement and to insert the *HO-lacZ* gene into the genome.

The URA3 gene was inserted into the *Hin*dIII site of pAED4 (D. Doering, Whitehead Institute) (pAED4/URA3). The *HO-lacZ* gene was excised from pLB47 (I. Herskowitz) with *Sal*I and *Pst*I, and ligated into the *Eco*RI site of pAED4/URA3 following the creation of blunt ends on both the plasmid and the insert (pAED4/URA3/HO-lacZ).

Replacement of the sequence studied in this paper was accomplished by removing an internal *Kpn*I fragment from the pAED4/URA3/HO-lacZ plasmid, subcloning it into pTZ18r (Pharmacia), performing site directed mutagenesis [6] using the oligonucleotide TCGATGTGCTGCGGCCGCTTGGGCCGA (Biotechnology General, Nes Tziona), and then returning the *Kpn*I fragment back into the pAED4/URA3/HO-lacZ plasmid.

Insertion of the wild type or mutant *HO-lacZ* genes into the yeast genome was accomplished by linearizing the plasmids with *Apa*I which cuts within the URA3 gene, and transformation of the appropriate strains (see Table 1). Constructs were confirmed by PCR and digestion with *Not*I.

2.2. Measurement of HO-lacZ expression

β-Galactosidase activity was determined as described [7].

2.3. Expression of SIN1-HMG1 in bacteria

A plasmid coding for the GST/SIN1-HMG1 fusion was synthesized by amplification of the HMG1 domain of the *SIN1* gene (corresponding to amino acids 100–162) in a PCR reaction, and subsequent subcloning into the vector pGEX-3X as described [4]. The PCR reaction amplified the putative HMG1 domain from primers GCGGATCCGGTTTAAGAGGTCTATTG and CCGAATTCAACCTGGCTTGTTAAAATG (Biotechnology General, Nes Tziona). Protein expression and purification from *E. coli* were accomplished as described [4].

2.4. Gel retardation assay

The gel retardation assay was performed as described [4] except that competing unlabeled DNA was added as described in the legend to Fig. 2.

3. Results and discussion

3.1. XBS serves as a weak UAS in situ in the HO gene

To understand the transcriptional regulation of a gene that

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Abbreviations: ABS, X-binding sequence; WT, wild type

SSDI S0014-5793(96)00159-7

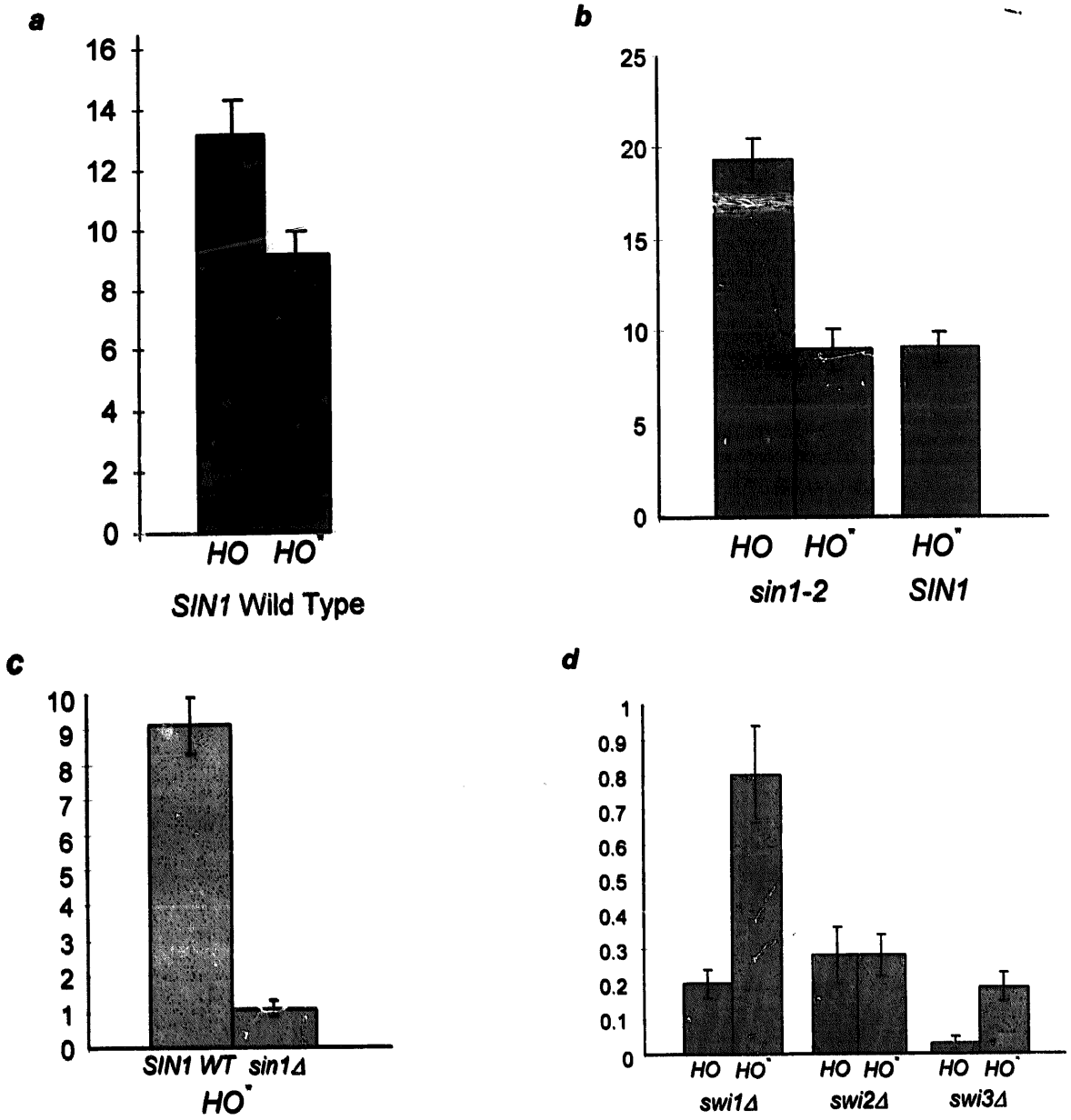

Fig. 1. Comparison of β-galctosidase levels in the strains described in Table 2. The ordinate indicates Miller units [7]. *HO*-lacZ* indicates that the *HO* promoter has been mutated.

is influenced by numerous cellular factors such as *HO*, it is important to determine the contribution of each of the *cis*-acting elements that are in the regulatory region of the gene. To determine the role that the XBS in URS1 of *HO* plays in the transcription of *HO*, we constructed plasmids containing the entire *HO* upstream regulatory region attached to a lacZ reporter gene either with or without the XBS. Plasmids not containing the XBS contained instead an unrelated DNA sequence. These plasmids were then used to transform isogenic yeast strains of differing in *SWI1,2,3* and *SIN1* such that the recombinant *HO-lacZ* genes integrated into the chromosome. Integration was verified in each case by PCR analysis (data not shown). The level of lacZ expression was used as an indicator of *HO-lacZ* transcription.

Earlier experiments had indicated that the XBS can act as a weak UAS when attached to a heterologous promoter [4]. We therefore asked whether the replacement of this sequence with an unrelated sequence in the intact *HO* promoter would affect *HO* transcription. Here we confirm that this sequence does in fact act as a weak UAS in situ in the *HO* gene, since its replacement causes a significant reduction in *HO* transcription in a wild type strain (Table 2 and Fig. 1a).

3.2. *Events at the XBS cause the overexpression of HO in sin1-2 mutants*

We have shown previously that the XBS can bind a protein that interacts with the C-terminal of SIN1 [8]. A bacterially produced mutant sin1-2 protein containing a single amino acid difference from the wild type SIN1 was able to remove this protein from the XBS [4]. We therefore were interested to

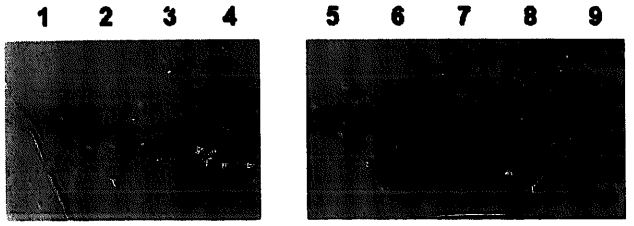

Fig. 2. Gel retardation of the XBS using purified recombinant glutathione *S*-transferase/SIN1-HMG1 fusion protein. 0.2 ng of radiolabeled XBS was used in all lanes. Lanes 1-4 contained 0, 0.2, 0.4, and 0.8 ng of unlabelled XBS as competitor, respectively. Lanes 5-9 contained 0, 0.8, 3.2, 12.8, and 51 ng of unlabeled herring sperm DNA that had been digested with *Eco*RI.

know how the 32 bp fragment would affect *HO* transcription in *sin1-2* mutants. As can be seen in Table 2 and Fig. 1b, *sin1-2* mutants with the wild type *HO* sequence overexpressed *HO* as expected, while those lacking the XBS expressed *HO* at exactly the same levels as cells wild type in *SIN1*. This experiment indicates that the overexpression of *HO* in *sin1-2* cells results from interactions that occur at the XBS.

3.3. Sequences other than the XBS mediate the effect of SIN1 on HO transcription

Earlier experiments had shown that *SIN1* affects *HO* transcription at both URS1 and URS2 [3]. We therefore asked whether SIN1 can exert its affect on *HO* transcription at DNA sequences other than the XBS studied here which is found in URS1. As shown in Table 2 and Fig. 1c, the earlier observation is suggested here as well by comparing the large reduction of *HO* transcription from a promoter lacking the XBS in mutants that are *sin1Δ* (1.1 units) when compared to *SIN1* wild type cells (9.1 units).

3.4. XBS influences HO transcription via the SWI/SNF complex

A principal antagonist to the SIN1 molecule is the SWI/SNF complex [3,9]. We sought to ask whether the components of this complex can function via the XBS. While the expression of *HO* was predictably low when *SWI1*, *SWI2*, or *SWI3* were disrupted, only in the cases of *SWI1* and *SWI3* was there a significant difference in transcription between those promoters that contained, and those that lacked the DNA sequence (Table 2 and Fig. 1d). These results indicate that SWI1 and SWI3 are required in the SWI/SNF complex to activate *HO* via the XBS, possibly by removing the proteins bound to the XBS, while SWI2 is not. The fact that *HO* transcription is not affected by the presence or absence of the XBS in a *swi2Δ* strain is particularly interesting, since SWI2 contains a DNA helicase motif [10,11] and therefore presumably is not required for displacement of proteins bound to the XBS.

Our data show that the SWI/SNF complex influences *HO* transcription through the XBS, but they also argue that the complex exerts its influence via other DNA sequences as well. While no difference is seen between the levels of *HO* transcription with or without the XBS in *swi2Δ* mutants, the overall level of *HO* transcription is greatly reduced in *swi2Δ* mutants relative to wild type presumably because the helicase is required to efficiently modify the chromatin structure leading to transcription.

3.5. The HMG1 domain of SIN1 preferentially binds the XBS

It has been reported that SIN1 binds DNA, though not in a sequence-specific manner [5]. Despite this earlier finding, we sought to determine whether SIN1 would bind the XBS preferentially over random DNA sequences. In the same experiment we tested whether the HMG1 domain of SIN1 is sufficient to bind DNA. To do this we carried out a gel mobility shift assay using the XBS as a probe. The protein used was a purified recombinant glutathione *S*-transferase/SIN1-HMG1 fusion protein. Glutathione *S*-transferase alone was unable to bind the labeled DNA at all (data not shown). To measure the affinity of the recombinant protein for the XBS relative to other DNA sequences, we added increasing amounts of unlabeled random herring sperm or dIdC DNA sequences (not shown) to the binding reaction, and asked how much unlabeled competing DNA was necessary to prevent protein binding to the probe. These experiments (Fig. 2) indicated that about 60 times more random DNA was required to bind the recombinant SIN1 molecule than the XBS. Control unlabeled double-stranded XBS competed stoichiometrically with

Table 1
Yeast strains

Strain	Genotype	Source
CY26	α *ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ1 lys2-801 ade2-101*	C. Peterson
CY110	a CY26 plus *sin1Δ::TRP1*	C. Peterson
CY57	α CY26 plus *swi2Δ::HIS3*	C. Peterson
CY58	a CY26 plus *swi1Δ::LEU2*	C. Peterson
CY72	α CY26 plus *swi3Δ::TRP1*	C. Peterson
EY18	α *trp1-Δ1 lys2-801 met sin1-2 ura3-52 his leu2*	this work
EY113	CY110 plus *ura3-52::*HO*-lacZ URA3*	this work
EY144	CY110 plus *ura3-52::*HO-*lacZ URA3*	this work
EY815	CY18 plus *ura3-52::*HO*-lacZ URA3*	this work
EY843	CY18 plus *ura3-52::*HO-*lacZ URA3*	this work
EY212	CY26 plus *ura3-52::*HO*-lacZ URA3*	this work
EY246	CY26 plus *ura3-52::*HO-*lacZ URA3*	this work
EY312	CY58 plus *ura3-52::*HO*-lacZ URA3*	this work
EY343	CY58 plus *ura3-52::*HO-*lacZ URA3*	this work
EY518	CY57 plus *ura3-52::*HO*-lacZ URA3*	this work
EY543	CY57 plus *ura3-52::*HO-*lacZ URA3*	this work
EY719	CY72 plus *ura3-52::*HO*-lacZ URA3*	this work
EY745	CY72 plus *ura3-52::*HO-*lacZ URA3*	this work

HO*-*lacZ* indicates that the *HO* promoter has been mutated as described in section 2.

Table 2
β-Galactosidase levels in Miller units [7] in the indicated strains

	HO wild type	*HO* disrupted
SIN1 wild type	13.1±1.2 (15) EY246	9.1±0.8 (15) EY212
sin1Δ	4.0±1.2 (27) EY144	1.1±0.2 (27) EY113
sin1-2	19.4±1.1 (30) EY843	9.0±1.1 (30) EY815
swi1Δ	0.28±0.08 (15) EY343	0.28±0.06 (15) EY312
swi2Δ	0.2±0.04 EY543	0.8±0.14 EY518
swi3Δ	<0.05 EY745	0.19±0.04 EY719

(number of repeat experiments indicated in parentheses)

the labeled XBS. These results clearly show that while SIN1 can bind DNA non-specifically, it preferentially binds the XBS. Furthermore SIN1 binding to DNA can occur via the HMG1 domain alone.

4. Conclusions

SIN1 is known to be a component of chromatin that transcriptionally regulates a specific family of genes (e.g. *HO*) while it has not been shown to bind DNA in a sequence-specific manner. Previously we showed that the C-terminal of SIN1 is able to contact a protein that does specifically bind a short DNA sequence (XBS) in the *HO* promoter [8]. In this paper we have further pursued the role of this DNA sequence in regulating the transcription of *HO*, and have shown that the HMG1 domain of SIN1 itself can preferentially bind this sequence. These experiments have demonstrated that the XBS is an important component of the *cis*-regulatory sequences that participate in the modulation of *HO* transcription, and that this modulation is accomplished by interactions of SIN1 and the SWI/SNF complex at this locus. Interestingly, the HMG1 domain of SIN1 shows sequence preference for binding to this sequence.

Taken together, these data suggest a model in which SIN1 is recruited to the XBS, (and probably other sequences as well) by sequence-specific DNA binding proteins that can interact both with the DNA and with SIN1. SIN1 binds the DNA via its HMG1 domain. While the DNA binding of SIN1 is not sequence-specific, SIN1 does preferentially bind this sequence, possibly stabilizing the interactions, and preventing *HO* transcription. *HO* transcription is accomplished, however, when the SWI/SNF complex interacts with the proteins bound to the XBS and other sequences, remodeling the chromatin [9,11–14] such that sequence-specific transactivating proteins are able to bind the *cis*-acting sequences. Future work will endeavor to test and improve this model.

Acknowledgements: We thank I. Herskowitz and D. Doering for plasmids, and C. Peterson and G. Herskovits for yeast strains. We also thank A. Navon for help with the in vitro mutagenesis. This work was supported by an internal university grant administered by Profs. S. Grossman and M. Kaveh to D.J.K., by the Israel Science Foundation administered by the Israel Academy of Sciences and Humanities to D.J.K. and by the Ben Gurion postdoctoral fellowship of the Israeli Ministry of Science and the Arts to S.S. It reports the results of research that was carried out by E.Y. in partial fulfillment of the requirements for his Ph.D. thesis.

References

[1] Nasmyth, K. (1993) Curr. Opin. Genet. Dev. 3, 286–294.
[2] Nasmyth, K. (1985) Cell 42, 213–223.
[3] Sternberg, P.W., Stern, M.J., Clark, I. and Herskowitz, I. (1987) Cell 48, 567–577.
[4] Katcoff, D.J., Yona, E., Hershkovits, G., Friedman, H., Cohen, Y. and Dgany, O. (1993) Nucleic Acids Res. 21, 5101–5109.
[5] Kruger, W. and Herskowitz, I. (1991) Mol. Cell. Biol. 11, 4135–4146.
[6] Ausbel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K. (1991) Current Protocols in Molecular Biology, pp. 8.0.1–8.5.7, Wiley, New York.
[7] Miller, J.H. (1972) Experiments in Molecular Genetics, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[8] Yona, E., Bangio, H., Erlich, P., Tepper, S.H. and Katcoff, D.J. (1995) Mol. Gen. Genet. 246, 774–777.
[9] Winston, F. and Carlson, M. (1992) Trends Genet. 8, 387–391.
[10] Cairns, B.R., Kim, Y.J., Sayre, M.H., Laurent, B.C. and Kornberg, R.D. (1994) Proc. Natl. Acad. Sci. USA 91, 1950–1954.
[11] Peterson, C.L., Dingwall, A. and Scott, M.P. (1994) Proc Natl Acad Sci USA 91, 2905–2908.
[12] Hirschhorn, J.N., Brown, S.A., Clark, C.D. and Winston, F. (1992) Genes Dev. 6, 2288–2298.
[13] Laurent, B.C., Yang, X. and Carlson, M. (1992) Mol. Cell Biol. 12, 1893–1902.
[14] Peterson, C.L. and Tamkun, J.W. (1995) Trends Biochem. Sci. 20, 143–146.