JOURNAL OF BACTERIOLOGY, July 1993, p. 4235–4238
0021-9193/93/134235-04$02.00/0

Vol. 175, No. 13

NOTES

The *INO1* Promoter of *Saccharomyces cerevisiae* Includes an Upstream Repressor Sequence (URS1) Common to a Diverse Set of Yeast Genes

JOHN M. LOPES,[1]* KAREN L. SCHULZE,[2] JONATHAN W. YATES,[1]
JEANNIE P. HIRSCH,[3] AND SUSAN A. HENRY[4]

Department of Molecular and Cellular Biochemistry, Stritch School of Medicine, Loyola University at Chicago, 2160 South First Avenue, Maywood, Illinois 60153[1]; Division of Neuroscience, Baylor College of Medicine, Houston, Texas 77030[2]; Department of Cell Biology, Mount Sinai Medical School, New York, New York 10029[3]; and Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, Pennsylvania 15213[4]

Received 8 April 1993/Accepted 29 April 1993

The *INO1* promoter of *Saccharomyces cerevisiae* includes a copy of an upstream repression sequence (URS1; 5′ AGCCGCCGA 3′) observed in the promoters of several unrelated yeast genes. Expression of *INO1-lacZ* and *CYC1-lacI′Z*, activated by the *INO1* UAS_{INO}, is significantly decreased by the *INO1* URS1.

The *INO1* gene of *Saccharomyces cerevisiae* encodes the enzyme inositol-1-phosphate synthase, which converts glucose-6-phosphate to inositol-1-phosphate (5, 6). This is the first committed step in the synthesis of phosphatidylinositol, a major membrane phospholipid. DNA sequence analysis of the *INO1* promoter region identified a motif previously reported by others as having a role in repression of gene expression (10). This element (5′ AGCCGCCGA 3′) was initially characterized as an upstream repression sequence (URS1) in the promoter of the *CAR1* gene of *S. cerevisiae*. Removal of this URS1 resulted in increased *CAR1* gene expression (19). Furthermore, a synthetic oligonucleotide that included the *CAR1* URS1 repressed expression of a *CYC1-lacI′Z* reporter gene (driven by an upstream activation sequence [UAS] from the *CYC1* gene). A functional URS1 element has also been observed in the promoters of the *SPO13* (3), *HOP1* (20), and *HO* (22) genes. In addition, 14 other yeast genes (*INO1* not included) were found to contain the *CAR1* URS1 in their promoters. The URS1 elements from each of these other genes were shown to also repress expression of the *CYC1-lacI′Z* system and to specifically bind a protein from yeast extracts (14).

Notably, the functions of the genes that contain the URS1 are unrelated to that of the *CAR1* gene (arginine catabolism). Similarly, there is no immediate relationship between the functions of the *INO1* and *CAR1* gene products. Control of *INO1* and *CAR1* gene expression is also unrelated. Expression of the *INO1* gene is regulated in response to the presence of inositol and choline in the growth media (8), while *CAR1* gene expression is affected by the nitrogen source (10). Control of *INO1* expression is dictated by the *INO2* (16), *INO4* (9), and *OPI1* (23) genes. This network is distinct from the regulatory circuitry controlling *CAR1* expression, which includes *GLN3* (4). Moreover, there are no obvious homologies between the reported *CAR1* (10) and *INO1* (12) *cis*-regulatory elements other than the URS1.

Experiments presented here demonstrate that the *INO1* URS1 functions in a native context. The *INO1* URS1 was also shown to repress UAS_{INO}-driven expression of the *CYC1-lacI′Z* fusion gene. The data suggest that a URS1 binding factor (URSBf) that interacts with the *INO1* URS1 is not encoded by the *INO2* or *OPI1* gene. Furthermore, URSBf is most likely a factor different from the one that binds the *CAR1* URS1.

The *INO1* promoter includes a functional URS1 element. The *CAR1* URS1 element was identified in the promoters of several genes (14), and for each of these cases it was demonstrated to function in repression of a heterologous gene (*CYC1-lacI′Z*). The only situation where the URS1 element has been shown to function in a truly native context is that of the *CAR1* gene (19). However, the URS1 has been shown to repress expression from different UASs in the promoters of the *SPO13* (3), *HOP1* (20), *HO* (18), and *CYC1* (14) genes. To determine whether the URS1 functions in regulating *INO1* expression, two yeast strains (construction was described elsewhere [12]) harboring different *INO1-lacZ* fusion genes integrated in a single copy at the *URA3* locus were assayed for β-galactosidase activity. One strain contained pJH353 (position −259; Fig. 1) which includes the URS1 element located at positions −255 to −247 while the other harbored pJH338 (position −213; Fig. 1) lacking the URS1. Each strain was grown in repressing (I+C+) and derepressing (I−C−) media and was assayed for expression of the fusion gene. The construct that included the *INO1* URS1 (pJH353) demonstrated a typical response to the two growth media (Fig. 1). As previously reported for the native *INO1* gene (8), a full-length *INO1-lacZ* fusion gene (position −543 [pJH334] [12]), and an *INO1* promoter-*CYC1-lacI′Z* fusion gene (pJH359 [12]), expression of the *INO1-lacZ* reporter gene in pJH353 was elevated when cells were grown in derepressing medium (I−C−; Fig. 1) and was barely detectable when cells were grown in medium containing inositol and choline (I+C+; Fig. 1). However, removal of the *INO1* URS1 (pJH338; Fig. 1) resulted in elevated levels

* Corresponding author.

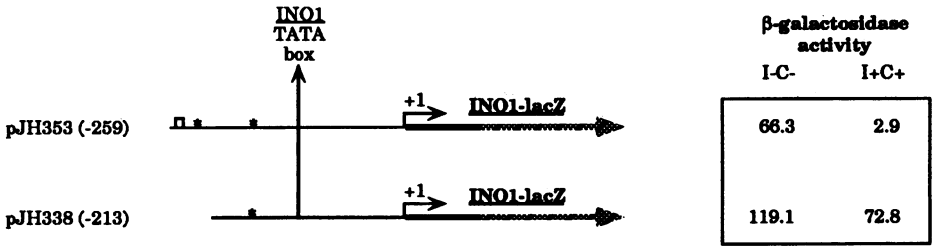
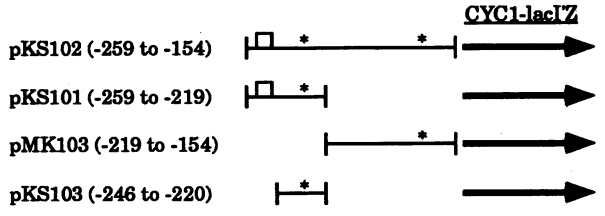


FIG. 1. Schematic representation of *INO1* promoter-*lacZ* fusions. Noted are the relative positions of the *INO1* TATA box (vertical arrow), the UAS_{INO} (asterisks), and the URS1 element (open box). Numbers in parentheses reflect the 5′ terminus of the *INO1* promoter insert relative to the start site of transcription (+1). Each construct fuses 132 amino acids from the amino terminus of the *INO1* gene product (inositol-1-phosphate synthase) to the *lacZ* gene in YIp357R (15). Expression of the *INO1-lacZ* fusion gene product in a wild-type strain is represented as β-galactosidase activity. One unit of β-galactosidase activity is equal to 1,000 × (optical density at 420 nm/min/mg of total protein). Abbreviations: I−C−, unsupplemented synthetic medium (derepressing); I+C+, synthetic medium containing 75 μM inositol and 1 mM choline (repressing).

of β-galactosidase production when cells were grown in either derepressing (I−C−) or repressing (I+C+) medium. Thus, the single copy of the *CAR1* URS1 present in the *INO1* promoter represses native expression driven by *INO1* *cis*-acting elements. More recently it has been shown that strains containing a *ume6*Δ mutant allele are defective in repression mediated by the *CAR1* URS1 (17). Consistent with this observation, expression of the native *INO1* gene and the *INO1-lacZ* fusion gene (both of which contain the URS1) is also elevated in a *ume6*Δ strain (9a).

The *INO1* URS1 reduces UAS_{INO}-directed *CYC1-lacI′Z* gene expression. To test the ability of the *INO1* URS1 to function in a heterologous system, several restriction fragments from the *INO1* promoter were inserted into the unique *Xho*I site of pJH304. This plasmid contains a *CYC1-lacI′Z* fusion gene, TATA boxes, and transcription initiation sites from the *CYC1* gene but lacks a UAS (12). Each plasmid was transformed into either a wild-type yeast strain (BRS1001 [*MAT***a** *ade2 his3 leu2 can1 trp1 ura3*]) or an *opi1* (BRS1021 [*MAT***a** *opi1 ade5 leu2 trp1 ura3*]) mutant strain and assayed for β-galactosidase activity under repressing (I+C+) and derepressing (I−C−) growth conditions. Plasmid pKS102, containing two copies of the UAS_{INO} (asterisks in Fig. 2), demonstrates a typical pattern of regulation and has previously been shown to contain sufficient information to provide full regulation to a heterologous gene. The level of derepressed expression generated by plasmids pKS101 and pMK103 is roughly additive to that observed for pKS102, although pKS101 produces nearly 1/10 as much β-galactosidase. Removal of the URS1 from pKS101 (pKS103) results in a dramatic increase (19-fold) in derepressed expression, demonstrating that the *INO1* URS1 represses UAS_{INO} activity. We have also observed that β-galactosidase expression is elevated in a *ume6*Δ strain containing pKS102 and pKS101 but not pKS103 or pMK103 (9a). Moreover, because expression of the fusion gene from pKS103 was sensitive to the *opi1* mutant allele (Fig. 2), URSBf is not likely to be encoded by the *OPI1* gene.

The *INO1* URS1 element binds a factor (URSBf) present in yeast cells. The *INO1* URS1 element was tested for the ability to form a protein-DNA complex with extracts from two yeast strains. A synthetic double-stranded oligonucleotide that included *INO1* promoter sequences from positions −259 to −238 was used as a template for electrophoretic mobility shift assays. The double-stranded DNA template, used in binding reactions, was prepared by 5′-end-labelling with T4 polynucleotide kinase (Boehringer Mannheim, Indianapolis, Ind.) and annealing two complementary oligonu-



β-galactosidase activity

	Wild type		opi1	
	I-C-	I+C+	I-C-	I+C+
pKS102 (-259 to -154)	137.2	<1.0	591.5	459.3
pKS101 (-259 to -219)	18.7	<1.0	77.8	76.4
pMK103 (-219 to -154)	113.8	<1.0	NP	NP
pKS103 (-246 to -220)	380.2	31.2	901.5	739.4

FIG. 2. Schematic representation of *INO1* regulatory fusions to a *CYC1-lacI′Z* chimera. Each plasmid was constructed by inserting relevant restriction fragments (endpoints for the insert are noted in parentheses), flanked by *Xho*I synthetic linkers, into the *Xho*I site of pJH304 (12). The relative positions of the *INO1* UAS_{INO} (asterisks) and the URS1 element (open boxes) are noted. Expression of the *INO1-CYC1-lacI′Z* fusion gene product in a wild-type and/or *opi1* (BRS1021 [*MAT***a** *opi1 ade5 leu2 trp1 ura3*]) mutant strain is represented as β-galactosidase activity. One unit of β-galactosidase activity is equal to 1,000 × (optical density at 420 nm/min/mg of total protein). Abbreviations: I−C−, unsupplemented synthetic medium (derepressing); I+C+, synthetic medium containing 75 μM inositol and 1 mM choline (repressing); NP, not performed.

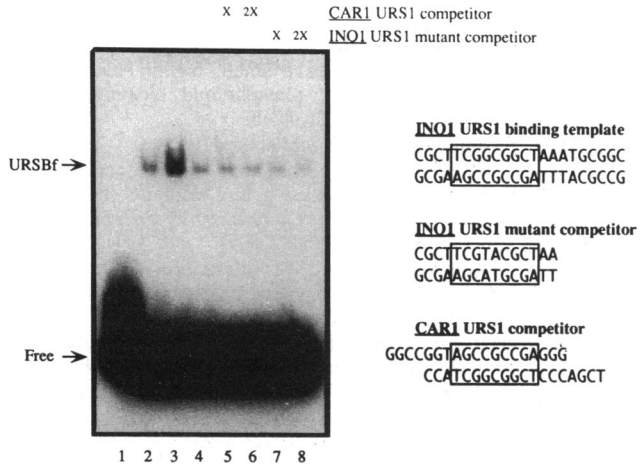


FIG. 3. Electrophoretic mobility shift assay identifying a protein-DNA complex that assembles with the URS1 element in the *INO1* promoter. A double-stranded synthetic oligonucleotide containing *INO1* promoter sequences from positions −259 to −238 served as a binding template. Extracts employed in lanes 3 to 8 were prepared from BRS1001 (wild type) and, extracts in lane 2 were prepared from BRS1017 (*ino2*). The extracts used in lanes 2 and 4 to 8 were purified from cells grown in a synthetic medium containing 75 μM inositol and 1 mM choline (I+C+), while that used in lane 3 was from cells grown in YEPD. Reaction mixtures in lanes 5 and 6 contained 100-fold (X) and 200-fold (2X) molar excess of the *CAR1* URS1 oligonucleotide. Reaction mixtures in lanes 7 and 8 contained 100- and 200-fold molar excess of the *INO1* URS1 oligonucleotide. The locations of the URS1s are denoted by the boxed areas. The URSBf protein-DNA complex and the free template are noted.

cleotides (Operon Technologies, Inc., Alameda, Calif.) as previously described (11). Extracts were prepared from a wild-type yeast strain (BRS1001) grown in either a defined complete synthetic medium (I+C+ [8]) or yeast extract-peptone-dextrose (YEPD) and a strain harboring an *ino2* mutant allele (BRS1017 [*MAT***a** *ino2 ura3 lys2*]) grown in I+C+ as previously described (11). Binding reactions (20 μl, final volume) were carried out under conditions described for the formation of protein-DNA complexes with the *CAR1* URS1 element (14), namely, 6 ng of γ-^{32}P-end-labelled DNA template, 20 μg of yeast protein [40% $(NH_4)_2SO_4$ fraction], 4 mM $MgCl_2$, 40 mM NaCl, 4 mM Tris (pH 8.0), and 50 ng of poly(dI-dC) (Boehringer-Mannheim) per ml for 15 min at room temperature. Extracts from BRS1001, grown in either YEPD or I+C+ media, formed a complex (URSBf) with the oligonucleotide template (Fig. 3, lanes 3 and 4, respectively). Two growth media were tested because a previous study employing extracts prepared from cells grown in the I+C+ medium failed to detect binding to the *INO1* URS1 element (11), while the extracts used for binding to the *CAR1* URS1 element were prepared from cells grown in YEPD (14). Thus, the medium employed to generate extracts for these experiments does not account for this discrepancy, although it is clear that the extract prepared from a YEPD-grown culture does form more of the URSBf complex (Fig. 3, lane 3). It is likely that this difference is due to the binding conditions used in the two sets of experiments. Most notably, experiments with the *CAR1* URS1 (14) used a binding buffer (same as in the experiment described in the legend to Fig. 3) including 40 mM NaCl and lacking dithiothreitol, while that used previously to show binding of proteins to the *INO1* promoter consisted of 250 mM KCl and 1 mM dithiothreitol (14). In addition, the experiment described in the legend to Fig. 3 shows that the factor interacting with the *INO1* URS1 is not dependent on a functional *INO2* gene (Fig. 3, lane 2) which encodes an activator of phospholipid biosynthetic gene expression (16).

Recently, two proteins that interact with the *CAR1* URS1 were purified and the genes that encode them were cloned (*BUF1* and *BUF2* [13]). The *BUF1* and *BUF2* genes have been shown to be essential (3a), and their products form a heterodimer that binds the *CAR1* URS1. In addition, proteins that interact with the *HO* URS1, Sdp1, have also been purified (21). Cross-linking studies have shown that Sdp1 is also a heterodimer (22). It has not yet been determined whether the Buf1p/Buf2p heterodimer is the same as the Sdp1 heterodimer. We sought to determine whether the URSBf complex is the same as the one that assembles with the *CAR1* URS1. We repeated the mobility shift assay with labelled *INO1* URS1 in the presence of a *CAR1* URS1 competitor (Fig. 3, lanes 5 and 6). The *CAR1* URS1 oligonucleotide did not compete for formation of the URSBf complex even at a 200-fold molar ratio. The *CAR1* URS1 DNA efficiently competes for the Buf1p/Buf2p binding activity with the *CAR1* URS1 at a 20-fold molar ratio (14). We also performed a competition experiment with another oligonucleotide which contains a mutant *INO1* URS1 (Fig. 3, lanes 7 and 8). The altered *INO1* URS1 was designed on the basis of mutations known to affect *CAR1* URS1 function. The mutant *INO1* URS1 DNA did not compete for the URSBf complex, while a wild-type *INO1* URS1 was an effective competitor (data not shown).

The elevated production of β-galactosidase from pJH338 in repressing media (I−C−) suggests that the single copy of the *CAR1* URS1 element within the *INO1* promoter represses native expression activated by the *INO1* UAS_{INO}, which has been localized to sequences −259 to −154 (12 [noted by asterisks in Fig. 1 and 2]). This point is further supported by the observation that pKS103 (which lacks URS1 and includes UAS_{INO}; Fig. 2) generates significantly higher β-galactosidase activity relative to pKS102 (which includes URS1 and UAS_{INO}; Fig. 2) when grown in derepressing media (I−C−). Therefore, the URS1 represses expression driven by UAS_{INO} in addition to that driven by UAS_{CYC} (14) and UAS_{CAR} (10). The effect of the URS1 on expression driven from the *INO1* promoter may address the issue of why the repressed level of *INO1* expression (I+C+) is dramatically lower than that of other phospholipid biosynthetic genes. While the repressed level of *INO1* expression (I+C+) is barely detectable (8), *CHO1* (2), *CHO2*, and *OPI3* (7) are all expressed at high levels under these same conditions. Recently, the *CHO1* promoter has been shown to also be regulated by a UAS_{INO} element (1). However, the *CHO1* promoter does not include a URS1. Thus, the presence of the URS1 in the *INO1* promoter may be responsible for its lack of expression under repressing conditions (I+C+). Consistent with this theory is the observation that removal of the URS1 from the *INO1* promoter (pJH338 in Fig. 1) results in a pattern of expression more reminiscent of the other phospholipid biosynthetic genes. Also, the *UME6* gene has been shown to be required for the repressing activity of the *CAR1* (17) and *INO1* URS1 (9a). However, we have observed that a *ume6*Δ mutant has no effect on *CHO1* or *CHO2* expression (9a).

The role of *UME6* in URS1 function is not yet fully understood. It has been proposed that *UME6* may encode a non-DNA-binding protein that interacts with proteins bound to the URS1 (13). Therefore, it is possible that the *CAR1*

URS1 and the *INO1* URS1 may assemble with different proteins. We have observed that an oligonucleotide including the *INO1* URS1 does not compete for complex formation with the *CAR1* URS1 (data not shown). Moreover, while we observe a single complex (URSBf in Fig. 3) with the *INO1* URS1, the *CAR1* URS1 forms several complexes (14, 17). The URSBf complex differs from the *CAR1* URS1 complexes in that it is a much stronger complex (data not shown). These observations are not entirely surprising, because different complexes have been observed with the URS1s of several other promoters (14).

We thank Terrance Cooper and David Stillman for invaluable discussions. We also thank Margaret Kanipes for help in constructing plasmids, Tina Gill for expert technical advice and assistance, and John Jackson for efforts in preparing the manuscript.

This work was supported by Public Health Research grant GM-16929 from the National Institutes of Health to S.A.H. and by NIH Postdoctoral fellowship GM-12099 and a Potts Foundation Award to J.M.L. K.L.S. was a participant in a Research Experience for Undergraduates program sponsored by the National Science Foundation and the Howard Hughes Medical Institute Undergraduate Biological Sciences Education Initiative.

REFERENCES

1. **Bailis, A. M., J. M. Lopes, S. D. Kohlwein, and S. A. Henry.** 1992. *Cis* and *trans* regulatory elements required for regulation of the *CHO1* gene of *Saccharomyces cerevisiae*. Nucleic Acids Res. **20:**1411–1418.
2. **Bailis, A. M., M. A. Poole, G. M. Carman, and S. A. Henry.** 1987. The membrane-associated enzyme phosphatidylserine synthase is regulated at the level of mRNA abundance. Mol. Cell. Biol. **7:**167–176.
3. **Buckingham, L. E., H.-T. Wang, R. T. Elder, R. M. McCarroll, M. R. Slater, and R. E. Esposito.** 1990. Nucleotide sequence and promoter analysis of *SPO13*, a meiosis-specific gene of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **87:**9406–9410.

3a. **Cooper, T.** Personal communication.

4. **Cooper, T. G., D. Ferguson, R. Rai, and N. Bysani.** 1990. The *GLN3* gene product is required for transcriptional activation of allantoin system gene expression in *Saccharomyces cerevisiae*. J. Bacteriol. **172:**1014–1018.
5. **Culbertson, M. R., T. F. Donahue, and S. A. Henry.** 1976. Control of inositol biosynthesis in *Saccharomyces cerevisiae*: properties of a repressible enzyme system in extracts of wild-type (*Ino*$^+$) cells. J. Bacteriol. **126:**232–242.
6. **Dean-Johnson, M., and S. A. Henry.** 1989. Biosynthesis of inositol in yeast. J. Biol. Chem. **264:**1274–1283.
7. **Gaynor, P. M., T. Gill, S. Toutenhoofd, E. F. Summers, P. McGraw, M. J. Homann, S. A. Henry, and G. M. Carman.** 1993. Regulation of phosphatidylethanolamine methyltransferase and phospholipid methyltransferase by phospholipid precursors in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta. **1090:**326–332.
8. **Hirsch, J. P., and S. A. Henry.** 1986. Expression of the *Saccharomyces cerevisiae* inositol-1-phosphate synthase (*INO1*) gene is regulated by factors that affect phospholipid synthesis. Mol. Cell. Biol. **6:**3320–3328.
9. **Hoshizaki, D. K., J. E. Hill, and S. A. Henry.** 1990. The *S. cerevisiae INO4* gene encodes a small, highly basic protein required for derepression of phospholipid biosynthetic enzymes. J. Biol. Chem. **265:**4736–4745.

9a. **Jackson, J., and J. Lopes.** Unpublished data.

10. **Kovari, L., R. Sumrada, I. Kovari, and T. G. Cooper.** 1990. Multiple positive and negative *cis*-acting elements mediate induced arginase (*CAR1*) gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:**5087–5097.
11. **Lopes, J. M., and S. A. Henry.** 1991. Interaction of *trans* and *cis* regulatory elements in the promoter of *Saccharomyces cerevisiae*. Nucleic Acids Res. **19:**3987–3994.
12. **Lopes, J. M., J. P. Hirsch, P. A. Chorgo, K. L. Schulze, and S. A. Henry.** 1991. Analysis of sequences involved in the *INO1* promoter that are involved in its regulation by phospholipid precursors. Nucleic Acids Res. **19:**1687–1693.
13. **Luche, R. M., W. C. Smart, and T. G. Cooper.** 1992. Purification of the heteromeric protein binding to the URS1 transcriptional repression site in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **89:**7412–7416.
14. **Luche, R. M., R. Sumrada, and T. G. Cooper.** 1990. A *cis*-acting element present in multiple genes serves as a repressor protein binding site for the yeast *CAR1* gene. Mol. Cell. Biol. **10:**3884–3895.
15. **Myers, A. M., A. Tzagoloff, D. M. Kinney, and C. J. Lusty.** 1986. Yeast shuttle vectors with multiple cloning sites suitable for construction of *lacZ* fusions. Gene **45:**299–310.
16. **Nikoloff, D. M., P. McGraw, and S. A. Henry.** 1992. The *INO1* gene of *Saccharomyces cerevisiae* encodes a helix-loop-helix protein that is required for activation of phospholipid biosynthesis. Nucleic Acids Res. **20:**3253.
17. **Park, H.-D., R. M. Luche, and T. G. Cooper.** 1992. The yeast *UME6* gene product is required for transcriptional repression mediated by the *CAR1 URS1* repressor binding site. Nucleic Acids Res. **20:**1909–1915.
18. **Sternberg, P. W., M. J. Stern, I. Clark, and I. Herskowitz.** 1987. Activation of the yeast *HO* gene by release from multiple negative controls. Cell **48:**567–577.
19. **Sumrada, R. A., and T. G. Cooper.** 1987. Ubiquitous upstream repression sequences control activation of the inducible arginase gene in yeast. Proc. Natl. Acad. Sci. USA **84:**3997–4001.
20. **Vershon, A. K., N. M. Hollingsworth, and A. D. Johnson.** 1992. Meiotic induction of the yeast *HOP1* gene is controlled by positive and negative regulatory sites. Mol. Cell. Biol. **12:**3706–3714.
21. **Wang, H., and D. J. Stillman.** 1990. *In vitro* regulation of a *SIN3*-dependent DNA binding activity by stimulatory and inhibitory factors. Proc. Natl. Acad. Sci. USA **87:**9761–9765.
22. **Wang, H., and D. J. Stillman.** 1993. Transcriptional repression in *Saccharomyces cerevisiae* by a *SIN3-lexA* fusion protein. Mol. Cell. Biol. **13:**1805–1814.
23. **White, M. J., J. P. Hirsch, and S. A. Henry.** 1991. The *OPI1* gene of *Saccharomyces cerevisiae*, a negative regulator of phospholipid biosynthesis, encodes a protein containing polyglutamine tracts and a leucine zipper. J. Biol. Chem. **266:**863–872.