1964–1969 Nucleic Acids Research, 1995, Vol. 23, No. 11 

# *SIN3* works through two different promoter elements to regulate *INO1* gene expression in yeast

**Kimberly Hudak Slekar and Susan A. Henry***

Department of Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, USA

Received February 3, 1995; Revised and Accepted April 17, 1995

## ABSTRACT

**The *SIN3* global regulatory factor affects expression of many yeast genes, including the phospholipid biosynthetic gene, *INO1*. Mutations in the *SIN3* gene result in elevated levels of *INO1* expression under conditions that normally confer full repression of *INO1* transcription, indicating that *SIN3* is a negative regulator of *INO1*. In this study, the *INO1* promoter was analyzed for sequences that play a role in responding to *SIN3*-mediated repression. Two distinct promoter elements, the upstream repression sequence (URS1) and the *INO1* upstream activation sequence ($UAS_{INO}$) both were found to be involved in enabling *SIN3* to repress *INO1* expression.**

## INTRODUCTION

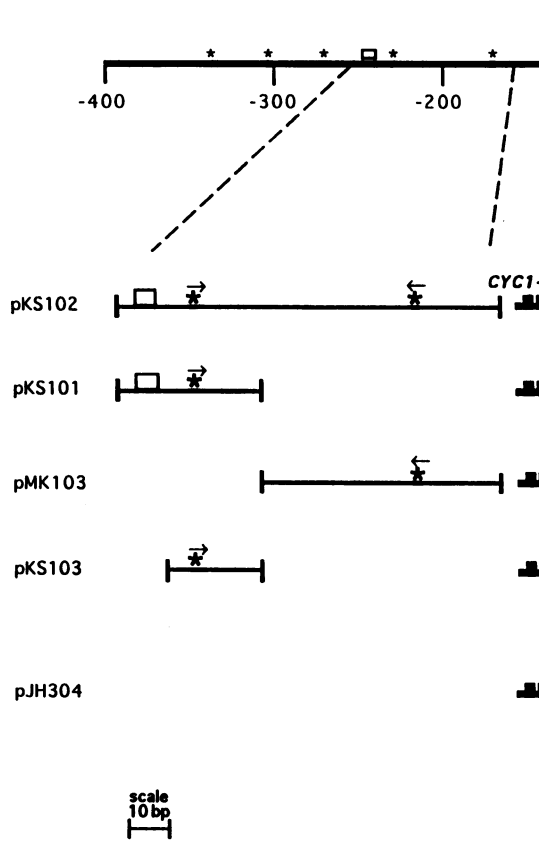
The *SIN3* gene encodes a global regulatory factor that has been shown to affect expression of numerous unrelated genes in yeast. Examples of some of the genes that are affected by a *sin3* mutation include *HO* (1,2), *TRK2* (3), *SPO11*, *SPO13* (4), *IME2* (5), *PHO5* (6), and the phospholipid biosynthetic genes *INO1*, *CHO1*, *CHO2* and *OPI3* (7). Generally, expression of these genes is moderately elevated in a *sin3* mutant background under conditions that normally allow for their repression. The *SIN3* gene encodes a 175 kDa protein containing four paired amphipathic helix (PAH) domains that lack the preceding basic regions thought to be important for DNA binding (6,8). There has been no reported evidence demonstrating direct DNA binding of the Sin3 protein. It has been proposed that the *SIN3* gene product may act to control expression of its numerous target genes through protein–protein interactions with more specific DNA binding proteins (6,9), but the precise mode of *SIN3* action remains unknown.

There is no single common element that is known to be present in the promoters of all genes that are regulated by *SIN3*. However, *sin3* mutations have been shown to affect expression of several yeast genes that contain an upstream repression sequence (URS1) (10) in their promoters, including *INO1* (11), *HO* (12), *SPO13* (13) and *IME2* (5). A functional relationship between *SIN3* and the URS1 element has been reported. Work by Bowdish and Mitchell (5) suggests a model in which the *UME6* and *SIN3* gene products work through the *IME2* URS1 element to repress *IME2* transcription in non-meiotic cells. A separate study demonstrated that a binding activity on the *HO* promoter, called Sdp1, is missing in cell extracts derived from *sin3* mutants (14). Interestingly, the binding site for Sdp1 in the *HO* promoter shows strong similarity to the URS1 sequence (9). It is not clear whether all genes that are regulated by *SIN3* contain a URS1 element in their promoters. The *CHO2*, *OPI3* and *CHO1* promoter regions, for example, contain no URS1 consensus element although they may contain URS-like sequences (D. Stillman, personal communication). In addition, it has also been reported that a *sin3* mutation has little effect on repression of the *CYC1* UAS through URS1 (15,16). Therefore, the precise relationship between *SIN3* and the URS1 element in the overall regulation of yeast genes remains unclear at this time.

Regulation of the yeast *INO1* phospholipid biosynthetic gene is affected by a mutation in the *SIN3* gene. *INO1* encodes the enzyme inositol-1-phosphate synthase, which catalyzes a key step in the synthesis of phospholipids. Expression of *INO1* is maximal in the absence of the phospholipid precursors inositol and choline (derepressing conditions). When wild-type yeast cells are grown in the presence of inositol and choline (repressing conditions), *INO1* expression is repressed at the transcriptional level (17). In *sin3* mutant strains this regulation is altered, resulting in elevated levels of *INO1* expression under repressing growth conditions (7).

The *INO1* promoter region has been extensively analyzed for sequences that may be involved in the control of *INO1* expression (11,17–19). A 10 bp element of consensus sequence 5′-CATGTGAAAT-3′, designated $UAS_{INO}$, is repeated six times in the *INO1* promoter (Fig. 1) (17,19). This element is also found upstream of the *CHO1*, *CHO2* and *OPI3* phospholipid structural genes (17,19). None of the native $UAS_{INO}$ elements located upstream of the phospholipid structural genes are perfect matches to the consensus, as they all represent 7–9 nt matches to the 10 bp consensus. The $UAS_{INO}$ element has been identified in the promoters of many other genes involved in phospholipid metabolism in addition to the ones discussed here, including genes that encode enzymes of the Kennedy pathway, the inositol transporter, the *INO2* and *INO4* regulatory genes, genes involved in fatty acid synthesis, and others (19). In addition, $UAS_{INO}$-like elements have been identified in some genes that are unrelated to phospholipid synthesis (19). There are two known examples of non-phospholipid genes that are regulated by *SIN3* and that also contain a $UAS_{INO}$ in their promoters: *PHO5* (19) and *SPO13* [J. Lopes, personal communication; (13)]. In addition

* To whom correspondence should be addressed




| | wild type | | sin3Δ | | sin3Δ WT ratio |
|---|---|---|---|---|---|
| | I-C- | I+C+ | I-C- | I+C+ | I+C+ |
| pKS102 | 638.2 ± 25.6 | 1.1 ± 0.9 | 1061 ± 174 | 37.0 ± 9.1 | 33.6 |
| pKS101 | 165.5 ± 17.0 | 1.1 ± 0.7 | 403.0 ± 96.8 | 16.8 ± 2.6 | 15.3 |
| pMK103 | 264.0 ± 23.3 | 2.3 ± 0.28 | 323.3 ± 52.5 | 7.3 ± 0.97 | 3.2 |
| pKS103 | 1431 ± 240 | 60.7 ± 7.3 | 989 ± 142 | 68.0 ± 9.0 | 1.1 |
| pJH304 | 14.5 ±3.2 | 6.6 ±1.0 | 12.7 ±1.7 | 8.4 ±1.7 | |

**Figure 1.** Analysis of heterologous reporter gene expression driven by fragments of the *INO1* promoter. Top line represents 400 bp of *INO1* sequence 5′ to start of *INO1* transcription. Asterisks show positions of $UAS_{INO}$ elements. Open box represents the URS1 element. Black box represents the *INO1* TATA box. Each vector (pKS102, pKS101, pMK103, pKS103) contains the designated portion of the *INO1* promoter fused to the *CYC1–lacI'Z* chimaera. For specific endpoints of *INO1* promoter sequences contained in the vectors, see Table 1. Arrows indicate orientation of the $UAS_{INO}$ elements, relative to the start of *INO1* transcription. Vector pJH304 contains no *INO1* sequences. Black boxes in *CYC1–lacI'Z* represent *CYC1* TATA boxes. Each plasmid was transformed into wild-type (SH338) and *sin3*Δ (SH296) strains, and β-galactosidase activity was assayed under both derepressing ($I^-C^-$) and repressing ($I^+C^+$) conditions. Numbers shown in the table are β-galactosidase activity units. Each assay was repeated four to six times using three to five independent transformants. Standard deviations are shown. In the last column, activity in the *sin3* strain under repressing conditions is divided by activity in the wild-type strain under repressing conditions.

to the $UAS_{INO}$ element, a sequence identical to the URS1 consensus element, AGCCGCCGA, has also been found in the *INO1* promoter (Fig. 1) (11). It has been demonstrated that this element plays a functional role in repression of *INO1* gene expression (11). The purpose of this work was to identify sequences in the *INO1* promoter that are involved in *SIN3*-mediated repression of *INO1*.

## MATERIALS AND METHODS

### Strains and plasmids

The congenic yeast strains used in this study were: SH296, *MAT***a** *his3 trp1 ura3 sin3::TRP1 leu2 lys2;* SH338, *MAT*α *his3 trp1 ura3.* Plasmids used in this study are listed in Table 1. Plasmid pNB404 (N. Bachhawat, personal communication) was constructed by deleting the CYC1-UAS from plasmid pNG22 (20) and then using the pNG22 CYC1 ΔUAS promoter region, including the polylinker region, to replace the CYC1 ΔUAS promoter region of pJ304 (Table 1), which does not have a polylinker region. The plasmid pNB404, therefore, contains the polylinker region from pNG22, but is based upon pJH304, as is pCON1. To construct pNB503, an oligonucleotide with the sequence:

```
ggccgtcCATGTGAAATg
     cagGTACACTTTAcagct
```

was cloned into the polylinker region of pNB404 at the *Sal*I and *Eag*I sites. The 10 bases in capital letters represent the $UAS_{INO}$ consensus sequence as discussed in the Introduction.

### Yeast media

*Vitamin-defined complete synthetic media.* Three percent glucose, 0.17% YNB Salts (recipe below), 0.0002% trace components (recipe below), 0.5% ammonium sulfate, 1% vitamin mix (21) and supplements (amino acids, uracil and adenine) as described by Culbertson and Henry (22). Where appropriate, medium was supplemented with 75 μM inositol and 1 mM choline ($I^+C^+$). Unsupplemented medium is designated $I^-C^-$. When necessary for plasmid maintenance, uracil was omitted from media. (The YNB salts, trace components and ammonium sulfate, as described in this media recipe, replace 'Yeast Nitrogen Base without Vitamins', formerly manufactured by Difco.)

**Table 1.** Plasmids

| Plasmid | Description | Source/ Reference |
|---|---|---|
| pCON1 | Consensus $UAS_{INO}$ oligo inserted into pJH304 with XhoI linkers, → orientation | J. Koipally |
| pJH304 | *CYC1* TATA-*lacI'Z* fusion vector, lacking UAS sequences | (18) |
| pKH200 | single insert of KH1+2 oligos into XhoI site of pJH304, → orientation | This work |
| pKH201 | single insert of KH3+4 oligos into XhoI site of pJH304, → orientation | This work |
| pKH202 | single insert of KH3+4 oligos into XhoI site of pJH304, ← orientation | This work |
| pKS101 | *INO1* promoter sequences -259 to -219 inserted into pJH304 with XhoI linkers | (18) |
| pKS102 | *INO1* promoter sequences -259 to -154 inserted into pJH304 with XhoI linkers | (18) |
| pKS103 | *INO1* promoter sequences -246 to -220 inserted into pJH304 with XhoI linkers | (11) |
| pNB404 | Produced from pJH304 by replacing its CYC1 promoter region with the CYC1 promoter region (ΔUAS) derived from pNG22 (20), as described in the Methods. The CYC1 promoter region from pNG22 contains a polylinker region. | N. Bachhawat |
| pNB503 | Consensus $UAS_{INO}$ oligo inserted into pNB404, → orientation in the polylinker region. | N. Bachhawat |
| pMK103 | *INO1* promoter sequences -219 to -154 inserted into pJH304 with XhoI linkers | (11) |

*Yeast nitrogen base (YNB) salts.* Recipe to make enough for 100 l media: 100 g potassium phosphate monobasic, 50 g magnesium sulfate, 10 g sodium chloride, 10 g calcium chloride.

*Trace components.* Recipe to make enough for 10 000 l media: 5 g boric acid, 0.4 g cupric sulfate, 1 g potassium iodide, 2 g ferric chloride, 4 g magnesium sulfate, 2 g sodium molybdate, 4 g zinc sulfate.

### β-galactosidase assays

β-galactosidase assays were performed as described by (18), except that aliquots were removed at 5, 10 and 15 min. β-galactosidase units are defined($OD_{420}$/min/mg total protein) × 1000. The total protein concentration (23) of each extract was determined using a BioRad assay kit as described by Lopes *et al.* (18).

### Vector construction

Vectors pKH200, pKH201 and pKH202 were constructed by inserting synthetic oligonucleotides (listed below) into the *Xho*I site of vector pJH304. The appropriate oligonucleotides for construction of each vector are listed in Table 1. Oligonucleotides used in this study were: KH1, 5′-TCGAG**CATGTGAAAA**C-3′; KH2, 5′-TCGAG**TTTTCACATG**C-3′; KH3, 5′-TCGAG**CATGTGAATT**C-3′; KH4, 5′-TCGAG**AATTCACATG**C-3′, all ordered from Operon Technologies, Inc. Verification of inserts was performed using a Sequenase kit (US Biochemicals Corp.).

## RESULTS

### Analysis of heterologous reporter gene expression driven by fragments of the *INO1* promoter

A region of the *INO1* promoter known to contain essential regulatory elements for controlling *INO1* expression extends from nucleotides −259 to −154, 5′ to the start of *INO1* transcription (18). This 105 bp fragment contains the only URS1 element found in the *INO1* promoter and two copies of the 10 bp $UAS_{INO}$ element (Fig. 1, expanded section of promoter). Both of these $UAS_{INO}$ elements contain a 'C' at the first position of the 10 bp sequence, the significance of which will be addressed in the following section. This 105 bp fragment from the *INO1* promoter was previously shown to provide full regulated expression to the heterologous reporter gene *CYC1–lacI'Z* (18). This result was confirmed by transforming plasmid pKS102 (contains sequences −259 to −154) into wild-type strain SH338, and measuring β-galactosidase production under both derepressing ($I^-C^-$) and repressing ($I^+C^+$) conditions (Fig. 1). In the wild-type strain used for these studies, the *INO1* promoter sequences present on plasmid pKS102 were capable of providing >600-fold repression in response to inositol and choline (Fig. 1). However, when the same experiment was performed in the *sin3* disruption strain SH296, expression of this construct was elevated under repressing growth conditions, relative to wild-type levels. Under repressing conditions, plasmid pKS102 supported ~34-fold more β-galactosidase production in the *sin3* mutant than it did in the wild-type strain, expressed as a ratio of *sin3*/WT (Fig. 1).

The *INO1* promoter sequences present on plasmid pKS101 were also capable of driving expression that was regulated in response to inositol and choline (Fig. 1) (18). This construct contains *INO1* promoter sequences −259 to −219, including the URS1 element and the first $UAS_{INO}$ element. The level of expression driven by this promoter fragment was ~15-fold greater in the *sin3*Δ mutant than it was in the wild-type strain, when grown under repressing conditions (Fig. 1).

When the URS1 element is removed from pKS101 to create plasmid pKS103 (−246 to −220), a much higher level of reporter gene expression was observed in the wild-type strain, relative to expression from pKS101 (Fig. 1). This is consistent with previous studies by Lopes *et al.* (11), who conclude that the *INO1* URS1 element is a functional repression sequence. Construct pKS103, which contains the first $UAS_{INO}$ but lacks URS1, supported reporter gene expression that was not appreciably different in *sin3*Δ and wild-type strains, under repressing conditions (Fig. 1).

Plasmid pMK103 contains *INO1* sequences −219 to −154, including the second $UAS_{INO}$ element. Reporter gene expression driven by these promoter sequences is regulated by inositol and choline, in a wild-type strain (18). In the *sin3*Δ mutant, this plasmid supported ~3-fold more β-galactosidase production than wild-type, under repressing conditions (Fig. 1).

Vector pJH304 contains *CYC1* TATA boxes, fused to *lacI'Z*, but lacks any *INO1* promoter sequences. There is no appreciable difference in pJH304 expression in the wild-type and *sin3* strains (Fig. 1).

### Analysis of $UAS_{INO}$ in *SIN3*-mediated control of *INO1* expression

The 10 bp $UAS_{INO}$ element alone has been shown to be sufficient to drive expression of a heterologous reporter gene when a 'C' or an 'A' residue is present at the first nucleotide position of the element (J. Koipally and J. Lopes, personal communication). This expression is regulated in response to inositol and choline. These results are illustrated in Table 2, where reporter gene expression driven by construct pCON1 was examined in wild-type strain SH338. In this experiment, an oligonucleotide consisting of a

**Table 2.** Analysis of $UAS_{INO}$ in *SIN3*–mediated control of *INO1* expression

| vector: | 10 bp oligo: | wild type | | *sin3Δ* | | *sin3Δ* WT ratio |
|---|---|---|---|---|---|---|
| | | I-C- | I+C+ | I-C- | I+C+ | I+C+ |
| pCON1 | → CATGTGAAAT | 1307 ±241 | 64.8 ±4.6 | 1372 ±367 | 144.4 ±10.8 | 2.2 |
| pKH200 | → CATGTGAAAa | 1190 ±200 | 74.2 ±11.3 | 983 ±256 | 138.2 ±20.8 | 1.9 |
| pKH201 | → CATGTGAAtT | 1132 ±169 | 85.1 ±16.6 | 1024 ±208 | 149.8 ±20.2 | 1.8 |
| pKH202 | ← CATGTGAAtT | 1383 ±64.7 | 84.0 ±2.4 | 1098 ±96.6 | 182.0 ±29.6 | 2.2 |
| pNB503 | → CATGTGAAAT | 491.2 ±30.5 | 9.4 ±0.86 | 571.1 ±86.5 | 18.5 ±1.0 | 2.0 |

Vectors harboring the indicated 10 bp oligonucleotide sequences were transformed into wild–type (SH338) and *sin3*Δ (SH296) strains, and β-galactosidase activity was assayed under both derepressing (I-C-) and repressing ($I^+C^+$) conditions. Numbers shown are β-galactosidase activity units. Each assay was repeated four to five times using three to five independent transformants. Standard deviations are shown. Arrows indicate orientation of the inserted oligonucleotide, and lower case nucleotides indicate changes from the consensus. In the last column, activity in the *sin3* strain under repressing conditions is divided by activity in the wild-type strain under repressing conditions. The relationships of the 10 bp oligonucleotides to the native $UAS_{INO}$ elements in the *INO1* promoter are described in the text.

perfect match to the $UAS_{INO}$ 10 bp consensus sequence was sufficient to support expression of the heterologous reporter gene *CYC1–lacI'Z*, and this expression was repressed 20-fold in response to inositol and choline. When this same construct was tested in *sin3*Δ mutant strain SH296, expression was ~2-fold greater than wild-type levels, under repressing conditions (Table 2).

As discussed previously, none of the native $UAS_{INO}$ elements are perfect matches to the 10 bp consensus sequence 5'-CATGTGAAAT-3'. In order to study the native $UAS_{INO}$ elements present on the –259 to –154 fragment of the *INO1* promoter, oligonucleotides that matched the two native 10 bp elements in question were placed upstream of the heterologous reporter gene *CYC1–lacI'Z* to create the vectors pKH200 and pKH201. The construct pKH200 contains sequences identical to the first native 10 bp element (corresponding to the first $UAS_{INO}$ on the pKS102 fragment, Fig. 1). This construct differs from the pCON1 construct discussed above only by the single base change of T to A at the 10th position of the inserted $UAS_{INO}$. Levels of pKH200 expression were similar to levels of pCON1 expression in the wild-type strain (Table 2). However, like the result with pCON1, expression of pKH200 was ~2-fold greater in the *sin3* mutant than it was in wild-type, under repressing conditions (Table 2).

The construct pKH201 contains sequences that are identical to the second native 10 bp element in question (corresponding to the second $UAS_{INO}$ on the pKS102 fragment, Fig. 1). Plasmid pKH201 differs from pCON1 only by the single base change of A to T at the ninth position of the inserted $UAS_{INO}$. Levels of pKH201 expression in the wild-type strain were similar to levels of pCON1 and pKH200 expression (Table 2). Under repressing conditions, pKH201 supported more β-galactosidase production in the *sin3* mutant than it did in the wild-type strain (Table 2). Construct pKH201 differs from its corresponding native $UAS_{INO}$ element in one respect: orientation relative to the start of transcription. To test the effect of orientation on expression driven by this sequence, vector pKH202 was constructed, which differs from pKH201 only by the reversed orientation of the inserted sequence. Thus, pKH202 contains its corresponding $UAS_{INO}$ element in the 'native' orientation. As predicted by other studies (J. Koipally and J. Lopes, personal communication), expression driven by the 10 bp element was not affected by orientation in a wild-type strain (Table 2, compare pKH201 with pKH202). When pKH202 was tested in the *sin3*Δ mutant strain under repressing conditions, expression levels were ~2-fold greater than wild-type (Table 2).

It has been demonstrated that 5' sequences immediately flanking the 10 bp $UAS_{INO}$ element are also critical for expression. That is, modifying the sequences that flank the 10 bp oligonucleotide caused a change the level of expression of a heterologous reporter gene driven by the $UAS_{INO}$ (N. Bachhawat, personal communication). For example, basal expression driven by plasmid pNB503 was ~7-fold lower than that of plasmid pCON1, in a wild-type strain (Table 2 and N. Bachhawat, personal communication). Both pCON1 and pNB503 contain the same 10 bp oligonucleotide, identical to the $UAS_{INO}$ consensus sequence, but the sequences that flank the element are different in the two vectors. However, expression of both constructs was affected in the same manner in a *sin3*Δ mutant background. Plasmid pNB503 supported reporter gene expression at levels

2-fold higher than wild-type levels in the *sin3* mutant, under repressing conditions (Table 2).

## DISCUSSION

To learn how the *SIN3* gene product may function to control phospholipid gene expression, experiments were carried out to define the regions of the *INO1* promoter that were critical for responding to *SIN3*-mediated repression. Expression of reporter constructs containing different fragments of the *INO1* promoter fused to *CYC1–lacI'Z* was analyzed in wild-type and *sin3*Δ strains. The contribution of both the URS1 and the $UAS_{INO}$ promoter elements to *SIN3*-mediated repression of *INO1* was examined.

In this study, reporter gene expression driven by promoter sequences on both pKS102 (contains URS1 and two 'C'-containing $UAS_{INO}$ elements) and pKS101 (contains URS1 and the first of the two $UAS_{INO}$ elements) was elevated in a *sin3*Δ mutant strain under repressing conditions, relative to wild-type (Fig. 1). Therefore, the *SIN3* gene product is required to mediate the repression conferred by these *INO1* promoter sequences. Expression of plasmid pKS103 (contains the first $UAS_{INO}$ element) was not affected by a *sin3* mutation (Fig. 1). Since the only difference between constructs pKS103 and pKS101 is the presence of the *INO1* URS1 element in pKS101, this result strongly implicates the URS1 element as being involved in *SIN3*-mediated repression of *INO1* .

A comparison of pKS101 with pKS103 expression in the *sin3* strain (Fig. 1) indicates that the *SIN3* gene product is not absolutely required for all repression from the URS1 element. If *SIN3* were absolutely required for URS1-mediated repression, then one would expect to find that pKS101 and pKS103 each supported similar levels of reporter gene expression in the *sin3* mutant strain. However, this was not the case, as removing the URS1 element resulted in a 4-fold increase in expression in the *sin3* strain under repressing conditions, and a 2.5-fold increase under derepressing conditions (Fig. 1). A comparison of pKS101 with pKS103 expression in the wild-type strain indicated that removal of the URS1 element resulted in a 55-fold increase in expression under repressing conditions, and an 8.6-fold increase in expression under derepressing conditions (Fig. 1); a much greater effect than that seen in the *sin3* mutant strain. Therefore, the URS1 element is not capable of mediating wild-type levels of repression without the *SIN3* gene product, but does remain partially functional when *SIN3* is absent.

Under repressing conditions, plasmid pMK103 supported expression that was 3-fold elevated in a *sin3* mutant strain, relative to the basal expression in wild-type (Fig. 1). Therefore, the *SIN3* gene product is required to achieve full levels of repression of the *INO1* promoter fragment present on pMK103. This 65 bp fragment contains a $UAS_{INO}$ element, suggesting that this element may also be involved in *SIN3*-mediated repression. However, expression of plasmid pKS103, which contains a $UAS_{INO}$ element, was not affected by a *sin3* mutation.

Additional experimentation proved to be successful in defining the role of the $UAS_{INO}$ element in *SIN3*-mediated repression. Reporter gene expression driven only by the $UAS_{INO}$ elements present in vectors pCON1, pKH200, pKH201, pKH202 and pNB503 was consistently elevated 2-fold in the *sin3*Δ strain, under repressing conditions (Table 2). These independent experiments, involving a study of five separate vectors, yielded results that were extremely reproducible. Therefore, the *SIN3* gene product must be required to achieve wild-type levels of repression of the $UAS_{INO}$ element. These results demonstrate that *SIN3* is capable of mediating repression of the consensus $UAS_{INO}$ element, as well as both of the native $UAS_{INO}$ elements present on the *INO1* promoter fragment between residues –259 and –154. This result is not affected by the orientation of the 10 bp element relative to the start of transcription. A puzzling result was that expression of pKS103, which contains the first $UAS_{INO}$, was not affected by a *sin3* mutation (Fig. 1). However, when the same $UAS_{INO}$ element was isolated in vector pKH201 without any native flanking DNA, expression was elevated in a *sin3* background (Table 2). Most probably, sequences flanking the $UAS_{INO}$ that are present on the 26 bp fragment in pKS103 were responsible for modifying its response to a *sin3* mutation.

In a previous study, it was demonstrated that regulation of the *CHO1, CHO2* and *OPI3* genes, which also contain $UAS_{INO}$ elements, was affected by a *sin3* mutation (7). The repression of *CHO1, CHO2* and *OPI3* appeared to be affected to a lesser degree by a *sin3* mutation than was repression of *INO1* (7). Unlike *INO1*, these three genes contain no consensus URS1 element. However, David Stillman (personal communication) recently re-evaluated the *CHO2, OPI3* and *CHO1* promoters and found URS-like elements that have some homology to the functional URS1 element as determined by Luche *et al.* (20). None of the elements detected by Stillman, however, is a perfect match for the URS1 consensus and none has been tested for functionality.

In summary, the results reported here implicate both the URS1 element and the $UAS_{INO}$ element in *SIN3*-mediated repression of *INO1*. A $UAS_{INO}$ element was responsible for ~2-fold repression by the *SIN3* gene product, while the *INO1* URS1 contributed significantly more to *SIN3*-mediated repression.

The *SIN3* gene product may act directly or indirectly (or both) at *INO1* and the promoters of co-regulated genes of phospholipid metabolism. Evidence for an indirect, as well as a direct, effect of *SIN3* on the structural genes *INO1, CHO2, OPI3* and *CHO1,* comes from a recent study demonstrating that a *sin3* mutation resulted in increased expression of the *INO2* regulatory gene (J. Jackson and J. Lopes, personal communication). The *INO2* gene has been shown to be autoregulated via a $UAS_{INO}$ element located in its own promoter (24). *INO2* encodes a protein of the basic helix–loop–helix (bHLH) class that is a positive regulatory factor required for *INO1* expression. The *INO2* gene product is also required for maximal derepression of the *CHO1, CHO2* and *OPI3* genes (19). *INO2* and several other regulatory factors that are critical for controlling *INO1* expression have been shown, like *SIN3*, to work through the $UAS_{INO}$ element. For example, *in vitro* studies have suggested a model in which Ino2p and Ino4p are both bHLH proteins, dimerize and bind to the $UAS_{INO}$ element to activate *INO1* transcription (25). Thus, any effect of *SIN3* on *INO2* expression might cause an indirect affect on *INO1* expression. Presumably, however, the effect of *SIN3* on *INO2* would also occur through the $UAS_{INO}$ located in the *INO2* promoter since *INO2* is autoregulated (24). Thus, modulation of the expression of the structural genes, *INO1, CHO1, CHO2* and *OPI3*, might occur directly through $UAS_{INO}$, as well as through modulation of regulatory gene products interacting with $UAS_{INO}$. It is interesting in this context, and perhaps significant, that two recent reports have described direct interaction of a *SIN3* mammalian homologue with regulatory proteins of the bHLH category (26,27). The ultimate goal of future experiments will be

to understand the interplay between *SIN3* and the specific regulatory factors, such as the *INO2* gene product, in controlling *INO1* gene expression.

## ACKNOWLEDGEMENTS

We would like to thank J. Lopes for providing constructs, for critical reading of the manuscript, and for many helpful discussions about this work. We also thank J. Koipally for technical assistance and helpful discussions and N. Bachhawat for providing construct pNB503. This work was supported by NIH grant GM-19629 to S. A. Henry.

## REFERENCES

1 Sternberg, P. W., Stern, M. J., Clark, I. and Herskowitz, I. (1987) *Cell,* **48**, 567–577.
2 Nasmyth, K., Stillman, D. and Kipling, D. (1987) *Cell,* **48**, 579–587.
3 Vidal, M., Buckley, A. M., Hilger, F. and Gaber, R. F. (1990) *Genetics,* **125**, 313–320.
4 Strich, R., Slater, M. R. and Esposito, R. E. (1989) *Proc. Natl. Acad. Sci. USA,* **86**, 10018–10022.
5 Bowdish, K. S. and Mitchell, A. P. (1993) *Mol. Cell. Biol.,* **13**, 2172–2181.
6 Vidal, M., Strich, R., Esposito, R. E. and Gaber, R. F. (1991) *Mol. Cell. Biol.,* **11**, 6306–6313.
7 Hudak, K. A., Lopes, J. M. and Henry, S. A. (1994) *Genetics,* **136**, 475–483.
8 Wang, H., Clark, I., Nicholson, P., Herskowitz, I. and Stillman, D. (1990) *Mol. Cell. Biol.,* **10**, 5927–5936.
9 Wang, H. and Stillman, D. J. (1993) *Mol. Cell. Biol.,* **13**, 1805–1814.
10 Sumrada, R. A. and Cooper, T. G. (1987) *Proc. Natl. Acad. Sci. USA,* **84**, 3997–4001.
11 Lopes, J. M., Schulze, K. L., Yates, J. W., Hirsch, J. P. and Henry, S. A. (1993) *J. Bacteriol.,* **175**, 4235–4238.
12 Stillman, D. J., Bankier, A. T., Seddon, A., Groenhout, E. G. and Nasmyth, K. A. (1988) *EMBO J.,* **7**, 485–494.
13 Buckingham, L. E., Wang, H.-T., Elder, R. T., McCarroll, R. M., Slater, M. R. and Esposito, R. E. (1990) *Proc. Natl. Acad. Sci. USA,* **87**, 9406–9410.
14 Wang, H. and Stillman, D. J. (1990) *Proc. Natl. Acad. Sci. USA,* **87**, 9761–9765.
15 Park, H., Luche, R. M. and Cooper, T. G. (1992) *Nucleic Acids Res.,* **20**, 1909–1915.
16 Vershon, A. K., Hollingsworth, N. M. and Johnson, A. D. (1992) *Mol. Cell. Biol.,* **12**, 3706–3714.
17 Carman, G. M. and Henry, S. A. (1989) *Annu. Rev. Biochem.,* **58**, 635–669.
18 Lopes, J. M., Hirsch, J. P., Chorgo, P. A., Schulze, K. L. and Henry, S. A. (1991) *Nucleic Acids Res.,* **19**, 1687–1693.
19 Paltauf, F., Kohlwein, S. D. and Henry, S. A. (1992) In Jones, E., Pringle, J. and Broach, J. (eds), *The Molecular and Cellular Biology of the Yeast Saccharomyces: Gene Expression.* Cold Spring Harbor Laboratory Press, Vol. 2, pp. 415–500.
20 Luche, R. M., Sumrada, R. and Cooper, T. G. (1990) *Mol. Cell. Biol.,* **10**, 3884–3895.
21 Greenberg, M. L., Reiner, B. and Henry, S. A. (1982) *Genetics,* **100**, 19–33.
22 Culbertson, M. R. and Henry, S. A. (1975) *Genetics,* **80**, 23–40.
23 Bradford, M. M. (1965) *Anal. Biochem.,* **72**, 248–254.
24 Ashburner, B. P. and Lopes, J. M. (1995) *Mol. Cell. Biol.,* **15**, 1709–1715.
25 Ambroziak, J. and Henry, S. (1994) *J. Biol. Chem.,* **269**, 15344–15349.
26 Ayer, D. E., Lawrence, Q. A. and Eisenman, R. N. (1995) *Cell,* **80**, 767–776.
27 Schreiber-Agus, N., *et al.* (1995) *Cell,* **80**, 777–786.