Improper control of expression of ATP binding cassette transporter-encoding genes is an important contributor to acquisition of multidrug resistance in human tumor cells. In this study, we have analyzed the function of the promoter region of the Saccharomyces cerevisiae YOR1 gene, which encodes an ATP binding cassette transporter protein that is required for multidrug tolerance in S. cerevisiae. Deletion analysis of a YOR1-lacZ fusion gene defines three important transcriptional regulatory elements. Two of these elements serve to positively regulate expression of YOR1, and the third element is a negative regulatory site. One positive element corresponds to a Pdr1p/Pdr3p response element, a site required for transcriptional control by the homologous zinc finger transcription factors Pdr1p and Pdr3p in other promoters. The second positive element is located between nucleotides −535 and −299 and is referred to as UASYOR1 (where UAS is upstream activation sequence). Interestingly, function of UASYOR1 is inhibited by the downstream negative regulatory site. Promoter fusions constructed between UASYOR1 and the PDR5 promoter, another gene under Pdr1p and Pdr3p control, are active, whereas analogous promoter fusions constructed with the CYC1 promoter are not. This suggests the possibility that UASYOR1 has promoter-specific sequence requirements that are satisfied by another Pdr1p/Pdr3p-regulated gene but not by a heterologous promoter.

Mammalian tumor cells can acquire the ability to detoxify several unrelated cytotoxic agents through alteration of a small number of genetic loci. This phenomenon, termed multidrug resistance, often involves the increased expression of an ATP binding cassette (ABC) transporter protein known as MDR1 (reviewed in Ref. 1). Recently, overexpression of a second ABC transporter protein was shown to confer a multidrug-resistant phenotype on cells. This membrane transporter protein was designated the multidrug resistance protein (Mrp) (2) and later shown to be responsible for production of a glutathione S-conjugate transporter activity in hepatocytes (3, 4). Control of the levels of expression of these mammalian ABC transporters is a critical determinant in the relative drug tolerance of an animal cell.

The yeast Saccharomyces cerevisiae also contains loci that can be altered to give rise to multiple or pleiotropic drug resistance (reviewed in Ref. 5). Dominant mutations in the Cys6 zinc finger transcription factors PDR1 and PDR3 result in elevated resistance to a broad array of toxic agents including cycloheximide and oligomycin (6–8). The ABC transporter protein-encoding gene PDR5 is transcriptionally activated by Pdr1p and Pdr3p and is required for Pdr1p/Pdr3p-mediated cycloheximide resistance (9, 10). Cells containing a Δpdr5 allele are cycloheximide-hypersensitive but display no oligomycin sensitivity, indicating the presence of a second Pdr1p/Pdr3p target gene required to provide oligomycin resistance. This oligomycin resistance gene, YOR1, was subsequently cloned on the basis of its ability to strongly elevate tolerance to this compound when present on a high copy plasmid (11).

DNA sequence analysis of the YOR1 gene (11) demonstrated that this locus encodes a protein with striking sequence similarity to the human cystic fibrosis transmembrane conductance regulator (12), Mrp (2), and S. cerevisiae Ycf1p (13). YOR1 is transcriptionally regulated by Pdr1p and Pdr3p and inspection of the YOR1 promoter region indicated the presence of a potential Pdr1p/Pdr3p response element (PDRE) centered 215 bp upstream of the transcription start site (11). These PDREs have been defined previously by analysis of similar elements in the promoters of PDR3 (14), PDR5 (15), and SNQ2 (16, 17). Northern blot and lacZ gene fusion analysis showed that YOR1 retained significant expression in a Δpdr1,3 strain (11). This is a marked difference from the behavior of the Pdr1p/Pdr3p-regulated PDR5 gene which is essentially inactive in a Δpdr1,pdr3 mutant background, explaining the cycloheximide-hypersensitive phenotype of this strain (10). It was anticipated that YOR1 expression would also strongly depend on PDR1/PDR3 since a Δpdr1,3 strain is extremely oligomycin-sensitive (10, 18).

To explore the nature of the differences between the co-regulated PDR5 and YOR1 promoters, we have carried out an analysis of the transcriptional control elements of YOR1. Deletion mutagenesis of a YOR1-lacZ gene fusion defined three regions important for normal regulation of YOR1 expression, two positively acting cis-elements and one negative regulatory site. One of the positive elements corresponds to the PDRE, confirming the role of this site in YOR1 transcriptional control. The second positive element is located upstream of the PDRE and is normally repressed by the action of the negative regulatory region. The activities of these other two elements appear to be independent of PDR1 and PDR3. These data are consistent with the YOR1 promoter receiving multiple regulatory inputs from PDR1/PDR3 and other as yet unknown factors.

MATERIALS AND METHODS

Yeast Strains and Media—Yeast transformations were performed using the lithium acetate procedure (19). The previously described strains used in these studies are SEY6210 (MATα; leu2-3, -112; ura3-52;
Plasmids—The wild-type YOR1-lacZ plasmid pSM109-4 has been previously described (11). Briefly, pSM109-4 corresponds to a YOR1-lacZ gene fusion containing 1066 bp upstream of the transcription start site mapped to a 25 bp of coding sequence from YOR1. This same YOR1 DNA fragment was cloned into pBlueScript KS II+ as a BamHI/EcoRI fragment to form pTH40. 5’ deletions were generated by digesting pTH40 with EcoRI, treating this DNA with the bidirectional exonuclease Bal31, forming blunt ends with Klenow enzyme and deoxyribonucleotides, and then ligating an EcoRI linker to the resulting blunt-ended fragments. The ligation reaction was then cleaved with EcoRI/BamHI and cloned into similarly digested pSEY102 (24). The 5’ end of each deletion mutant was sequenced to precisely determine the 5’ end point. An oligonucleotide (CGG GAA TTC GAA TAT GTC GAT TAC CGT GGG GGA T) was used to introduce an EcoRI linker upstream of the YOR1 ATG in a PCR reaction, resulting in the plasmid pTH70. 3’ promoter deletions were generated in the same fashion as the 5’ deletion mutants, except pTH40 was cut initially with BamHI. These 3’ deletions were cloned as EcoRI fragments into YOR1 5’ deletion mutant constructs resulting in internal promoter deletions.

A clustered point mutation was introduced into the YOR1 PDRE by PCR mutagenesis as described (15). The plasmid pTH40 was PCR-amplified using the YOR1 primer (GGG TCT CTA CTA AAT GCT TGC ACC ACG CGT TAT C) and the T3 primer. A separate PCR reaction was performed on pTH40 using the mutagenic primer (ATT TCC GCC TAT GTC GAC GGA ACC ACG CTT TAC C) and the T3 primer. The mutant base pairs are indicated by lowercase letters. PCR products from these reactions were purified, combined, and re-amplified with the T3 and T7 primers. The product from this reaction was digested with Eco47III and BglII and cloned into Eco47III/BglII cut pTH40. Resulting plasmids were sequenced to verify that the clustered point mutation had been introduced into the PDRE and that no other mutations had been introduced.

The promoter fusion plasmids were constructed using either the CYC1-lacZ vector pCBS1 (25) or the PDR5-lacZ vector pPB1112 (10). These plasmids contained versions of each promoter that replaced the normal upstream activation sequences with a unique EcoRI site. YOR1 promoter fragments were inserted as EcoRI fragments in their normal orientation relative to the heterologous promoters.

DNase I Footprint Analysis—DNase I footprint analysis was performed as described (28). The vector pOTS-Nco12 (29) expressing a Myc-epitope tagged version of the N-terminal 248 amino acids of Pdr1p was used to produce protein for these experiments. This plasmid was constructed by inserting an oligonucleotide encoding the Myc epitope into the NcoI site of a pOTS-Nco12 derivative expressing the N-terminal 248 residues of Pdr1p (provided by Laurence Lambert).

RESULTS

Primer Extension Mapping of the YOR1 Transcription Start Site—Previously, we localized the YOR1 transcription start site using an RNase protection assay (11). This assay provided an approximation of the site for transcription initiation accomplished by comparing the protected RNA species with an end-labeled set of DNA fragments. To locate precisely the YOR1 transcription start site, primer extension analysis was carried out. Total RNA was isolated from a wild-type strain transformed with the vector pRS315 or with pRS315 containing a dominant, gain-of-function allele of PDR1 designated PDR1–6. This gain-of-function PDR1 allele produced both elevated cycloheximide and oxoligycin tolerance in addition to increasing expression of PDR5 and YOR1 (11, 30).

A 25-nucleotide YOR1-specific primer was radiolabeled with 32P[γ-32P]ATP and T4 polynucleotide kinase and then annealed to the RNA samples. This oligonucleotide was also used for dye extension sequencing of a YOR1 template to generate a size standard for the primer extension products. The reverse transcription reaction products and the sequencing reactions were electrophoresed through a 6% polyacrylamide/urea gel in parallel with dye sequencing reactions performed on a YOR1 template using the same oligonucleotide primer.

DNase I footprint analysis was performed using a 25-base pair oligonucleotide primer (CCG GAA TTC GAA TAT GTC GAT TAC CGT GGG GGA T) was used to introduce an EcoRI linker upstream of the YOR1 ATG in a PCR reaction, resulting in the plasmid pTH70. 3’ promoter deletions were generated in the same fashion as the 5’ deletion mutants, except pTH40 was cut initially with BamHI. These 3’ deletions were cloned as EcoRI fragments into YOR1 5’ deletion mutant constructs resulting in internal promoter deletions.

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YOR1 mRNA levels were elevated in the presence of PDR1–6. Having accurately located the start site for YOR1 transcription, we next carried out deletion analysis of the promoter region.

5′ Deletion Mapping of the YOR1 Promoter—Low copy plasmids carrying the YOR1-lacZ fusion with progressively larger 5′ promoter deletions were transformed into a wild-type strain or an isogenic Δpdr1,3 strain. YOR1-lacZ-dependent 5′-galactosidase activities were determined for each construct (Fig. 2).

A YOR1-lacZ construct with a 5′ end point 1065 bp upstream of transcription start generated 15 units/A600 of 5′-galactosidase activity in a wild-type strain and 5 units/A600 in a Δpdr1,3 strain as described before (11). Additional 5′ truncations had no significant effect on YOR1-lacZ activity until the region between −222 and −129 was removed. The −129 5′ deletion pTH42 produced only 6 units/A600 of 5′-galactosidase activity in a wild-type strain, and this reduced activity was not further diminished in a Δpdr1,3 strain. Further reduction in the extent of YOR1 5′-noncoding sequence progressively reduced 5′-galactosidase expression.

These data suggest that the DNA region from −222 to −129 contains the major PDRE and is consistent with our previous identification of a candidate PDRE centered at position −215 (11). This candidate YOR1 PDRE is identical in sequence with the site 2 PDRE present in the PDR5 promoter, which we demonstrated to function as a positive regulatory site for PDR5 transcription (15). These data support the view that the PDRE at position −215 is required for Pdr1p/Pdr3p control of YOR1 gene expression and that this element may represent the only PDRE present in the promoter.

YOR1 Promoter Internal Deletions—Internal deletions in the YOR1 promoter were constructed to identify cis-acting elements that might have been missed in the 5′ deletion analysis. Each internal deletion mutant was assayed in both the wild-type and Δpdr1, pdr3 strains (Fig. 3).

An unexpected result from this analysis arose when the behavior of an internal deletion lacking the −115 to −50 region of the YOR1 promoter was assayed. This mutant YOR1-lacZ gene fusion produced 65 units/A600 in wild-type cells, and this elevated activity was only mildly affected by loss of PDR1 and PDR3 (reduced to 42 units/A600). A more extensive internal deletion mutation, lacking −190 to −50, also produced this same enhanced expression phenotype, with 57 units/A600 in wild-type cells and 45 units/A600 in the Δpdr1, pdr3 strain. Further elimination of YOR1 promoter sequence (−299 to −50) caused a reduction in the expression of the resulting YOR1-lacZ construct but still produced a construct that was significantly more active than the wild-type, especially in the Δpdr1/pdr3 background. The most extensive internal deletion mutant assayed (−535 to −50) essentially eliminated YOR1 expression.

The finding that deletion of the −115 to −50 region served to elevate expression of YOR1-dependent 5′-galactosidase activity was consistent with the belief that loss of this segment of YOR1 enhanced gene transcription. Since the deleted region mapped close to the YOR1 mRNA start site, one possible concern was that the increase in fusion gene expression came about from a change in mRNA form, influencing fusion gene translation rather than amount of YOR1-lacZ mRNA. To evaluate this possibility, we analyzed the 5′ ends of the YOR1-lacZ transcripts produced in these deletion mutants. Each fusion gene was transformed into a strain lacking the 5′ end of the endogenous YOR1 locus and the transcription start sites produced by each fusion gene analyzed by RNase mapping (Fig. 4).

RNase mapping confirmed that the level of YOR1-lacZ mRNA was increased in each promoter derivative that exhibited elevated 5′-galactosidase activity. Two differences were seen between the pattern of 5′ mRNA ends produced by the promoter mutants lacking the −115 to −50 region and the wild-type YOR1 promoter. First, the mutant promoters gave rise to a new transcription start site located upstream of the normal +1 start site. Second, a transcription start site (approximately +14) that is used less frequently than the +1 site in the wild-type YOR1 promoter was utilized as the major site for transcription initiation when the −115 to −50 segment was deleted. This analysis suggests that the region between −115 and −50 may play a role both in selection of the transcription start site that is utilized during YOR1 gene transcription and acts to negatively regulate YOR1 mRNA production. It should be noted that we cannot exclude that the appearance of these shorter mRNA species might be the result of degradation of a larger transcript.

These data suggest the presence of a positive regulatory site in the YOR1 upstream region between −535 and −299. This control element was designated UAS\textsubscript{YOR1}. Function of UAS\textsubscript{YOR1} is negatively regulated by an element located between −115 to −50. To define better the negative regulatory site, two internal deletion mutants were prepared that removed smaller segments of the −115 to −50 interval. Each of these mutant YOR1 promoters (−86 to −50 and −115 to −86) had the effect of elevating expression of the resulting construct by approximately 2-fold relative to the wild-type promoter in both genetic backgrounds. Neither of these internal deletion mutants was able to elevate YOR1-dependent 5′-galactosidase activity to the same extent as the derivative lacking the −115 to −50 region, suggesting the presence of multiple negative...
tracts were prepared from E. coli. The wild-typetracts were incubated with radiolabeled DNA templates pre-recognize the PDREs in the fragment of Pdr1p that contains the zinc finger DNA binding expression vector or the same vector expressing an N-terminal recombinant protein to bind to the produced Pdr1p in bacteria and assayed the ability of this sion by Pdr1p (and likely Pdr3p). To test this idea directly, we was likely to serve as a site for positive control of gene expres-sion by Pdr1p (and likely Pdr3p). To test this idea directly, we produced Pdr1p in bacteria and assayed the ability of this recombinant protein to bind to the YOR1 PDRE. Protein ex-tracts were prepared from E. coli cells carrying the empty expression vector or the same vector expressing an N-terminal fragment of Pdr1p that contains the zinc finger DNA binding domain. This Pdr1p fragment has previously been found to recognize the PDREs in the PDR5 promoter (15). Protein ex-tracts were incubated with radiolabeled DNA templates prepared from the wild-type YOR1 promoter or from a mutant YOR1 promoter that lacks the PDRE. This mutant PDRE was constructed by changing 2 base pairs that have previously been found to be essential for function of a PDRE in the PDR5 promoter (15). The results of this DNase I protection experiment are shown in Fig. 5.

Bacterially expressed Pdr1p was able to protect the YOR1 DNA region from -226 to -208 from DNase I cleavage. No protection was seen if protein was omitted or if an equal amount of protein extract prepared from cells containing the empty expression vector was used. The mutant YOR1 promoter with the 2-base pair substitution mutation in the PDRE was not bound by Pdr1p.

This finding indicates that Pdr1p can bind in vitro to the YOR1 PDRE and that this binding can be blocked by an alter-ation in the PDRE. To confirm that the PDRE is the in vitro target of Pdr1p action, we analyzed the function of the YOR1 promoter containing the 2-base pair substitution in S. cerevi-siae cells.

The PDRE Is Required for Control of YOR1 Expression by Pdr1p—To assess the contribution of the PDRE to YOR1 pro-moter function, the mutant PDRE was introduced into the context of the YOR1-lacZ fusion gene present in pSM109-4. Both the wild-type and mutant PDRE-containing YOR1-lacZ fusion genes were transformed into Δpdr1,pdr3 cells along with a low copy plasmid vector or this same vector plasmid carrying wild-type PDR1 or the dominant PDR1-6 allele. YOR1-depend-ent β-galactosidase activities were then determined for each transformant (Fig. 6).

The YOR1-lacZ fusion gene exhibited a strong increase in β-galactosidase levels in the presence of the PDR1-6 allele. The 2-base pair mutation in the PDRE completely blocked the ability of the fusion gene to respond to the PDR1-6 allele. The mutant PDRE also reduced the ability of the presence of the wild-type PDR1 gene to enhance YOR1-lacZ expression. The levels of YOR1-dependent β-galactosidase activity were similar, irrespective of the presence of the PDRE in the Δpdr1,pdr3 cells carrying the low copy vector.

These data provide a strong argument that the PDRE located at position -215 is the only site for Pdr1p (and likely Pdr3p) control of YOR1 transcription. We next evaluated the pheno-typic requirement for the PDRE in YOR1-mediated oligomycin resistance.

Normal Oligomycin Tolerance Requires the Presence of a Functional YOR1 PDRE—The 2-base pair substitution mutation in the PDRE of a YOR1-lacZ fusion gene blocked the ability of this promoter to respond to PDR1. To evaluate the consequence of loss of this sequence element on YOR1-mediated oligomycin tolerance, the mutant PDRE was introduced into the context of the wild-type YOR1 structural gene. This altered YOR1 structural gene was introduced into yor1 cells along with a low copy plasmid carrying the PDR1-6 allele or the vector plasmid alone. An equivalent wild-type YOR1 gene was transformed in a parallel fashion to provide a control for normal YOR1 function. Appropriate transformants were placed on rich medium lacking or containing oligomycin (Fig. 7).

Loss of the PDRE in the YOR1 promoter eliminated the ability of this mutant gene to normally complement the oligomycin hypersensitivity of the yor1 cells. This lack of comple-mentation could not be suppressed by the presence of the PDR1-6 allele. The wild-type YOR1 gene conferred stronger
oligomycin resistance in response to the PDR1–6 allele than in the presence of the wild-type PDR1 gene, consistent with the behavior of the YOR1-lacZ gene (Fig. 6). These data confirm that the PDRE is both required for normal Pdr1p responsive-ness of the YOR1 promoter as well as for wild-type oligomycin tolerance.

Evidence for Promoter-specific Function of the Pdr1p-independent YOR1 UAS—Along with the PDRE, the analysis described above pointed to the presence of a second positive transcriptional control element located between −299 and −535 (UASYOR1). To explore the function of this positive regulatory region, we constructed promoter fusions between various upstream fragments of YOR1 and two different UAS-deficient promoter-lacZ fusion constructs. The two promoter-lacZ constructs were the well characterized CYC1-lacZ fusion gene (22) or a similarly deleted PDR5-lacZ fusion that we have previously shown to lack its UAS (10). The YOR1 promoter fragments were placed upstream of these test promoters and introduced into Δpdr1, pdr3 cells along with a low copy plasmid carrying the wild-type or PDR1–6 alleles of PDR1 or the vector alone. YOR1 promoter fragments were inserted upstream of the heterologous promoters in the same relative orientation as in the native YOR1 promoter. β-Galactosidase activities of appropriate transformants were then determined (Table I).

The successful use of the disabled CYC1-lacZ fusion plasmid as a probe for UAS function has been well documented (22), and these were the first YOR1 promoter fusions that were analyzed. None of the YOR1 upstream fragments tested were able to significantly activate CYC1-dependent lacZ expression, even in the presence of the gain-of-function PDR1–6 allele. To ex-
This finding suggested the presence of other positive regulatory elements for transcription in the YOR1 promoter, a suggestion we have confirmed in the current study.

The second positive regulatory element for YOR1 expression (UASYOR1) lies between positions 535 and 299. The function of this element was not detected by 5′ deletion analysis as UASYOR1 activity is under negative control by an upstream repression sequence (URSYOR1) lying between −115 and −50. YOR1 appears to represent a new example of a PDR gene in terms of having this fairly complex arrangement of interacting positive and negative transcriptional control elements superimposed on the PDR-mediated control of expression. Studies of the PDR5 promoter have not indicated the presence of any regulatory sequences other than the PDREs (10, 15). Removal of the PDREs from the PDR3 promoter eliminates the ability of this gene to elevate the cycloheximide resistance of a Δpdr1,pdr3 cell (14). This comparison suggests that YOR1 is likely to integrate transcriptional control signals from factors other than Pdr1p/Pdr3p. Identification of these other factors will provide new insight into the physiological role of Yor1p.

Another unique feature presented by the YOR1 promoter is the presence of a single PDRRE in its 5′-noncoding region. All other known PDRRE-containing promoters contain at least 2 (PDR3) to 3 (PDR5) PDRREs. Interestingly, these multiple PDRRE-containing promoters also exhibit markedly more responsiveness to Pdr1p/Pdr3p than does YOR1. This observation is consistent with our previous finding that each PDRRE in the PDR5 promoter contributes approximately equally to the overall response of PDR5 to Pdr1p levels (15). These data suggest that the degree of Pdr1p/Pdr3p responsiveness of a given promoter can be largely predicted by the number of PDRREs found in the promoter of interest.

The presence of the negatively acting URSYOR1 element was detected by an increase in YOR1-lacZ expression that occurred upon deletion of the −115 to −50 region of the YOR1 promoter. We believe that this increase in expression is due to an unmasking of the UASYOR1 rather than a change in spacing of promoter elements for two reasons. First, several other YOR1 internal deletions also exhibit this enhancement of expression (Fig. 3) while each varies significantly in terms of its position relative to the transcription start site. Second, when these same internal deletion end points are used to construct chimeric promoters with PDR5, all are capable of conferring significant Pdr1p/Pdr3p-independent levels of lacZ expression (Table I). The simplest explanation of these results is that the −535/−299 UASYOR1 is activated upon removal of the URSYOR1 site. Additionally, the −115 to −50 promoter segment appears to contribute to 5′ end production in YOR1 transcription.

Other S. cerevisiae promoters have been found to have this arrangement of positive elements controlled by linked negative regulatory sites. The CAR1 gene encodes arginase and is highly repressed unless arginine is present as the nitrogen source (31). CAR1 also has a UAS element that is under control of a downstream URS. The negative regulation of this downstream URS is overcome in CAR1 when the inducer of CAR1 expression is present. Interestingly, this URS does not respond to the presence of inducer when it is placed between the UAS and TATA region of the heterologous CYC1 gene (32). The inability of this CAR1 promoter segment to function in a heterologous environment is reminiscent of the activity we have seen for the YOR1 promoter fragments placed upstream of CYC1 or PDR5. We do not yet know if the YOR1-PDR5 chimeras are active due to the presence of an additional positive site or the absence of a negatively acting element that is found in CYC1.

The behavior of the YOR1 UAS region placed upstream of CYC1-lacZ was unexpectedly complex. This UAS region was

![Diagram](image-url)
not able to stimulate CYC1-dependent β-galactosidase activity to an appreciable extent, irrespective of the genetic background examined. Even in the presence of the PDR1–6 allele, the YOR1-CYC1-lacZ fusion genes were essentially inactive. This was especially surprising considering that we previously have shown that an oligonucleotide corresponding to a PDRE present in the PDR5 promoter is capable of acting as a UAS when placed upstream of the same CYC1-lacZ test promoter (15). There are two important differences between the current study and these previous experiments. First, a different PDRE was placed upstream of the same CYC1 test vector. In these previous experiments, the TATA elements are only capable of responding to particular potential TATA utilization has been seen for the YOR1-CYC1-lacZ fusion genes were essentially inactive. This inability of the TATA element provided by each fusion plasmid. The inability of the TATA element to productively function with the TATA elements contributed by CYC1. Differential TATA utilization has been seen for the HIS3 (33) and HIS4 (34) promoters, consistent with the idea that certain TATA elements are only capable of responding to particular types of transcriptional regulatory proteins. Alternatively, the presence of some feature of the CYC1 promoter may act to inhibit the productive interaction between the YOR1 fragments and the CYC1 TATA region. Further experimentation is required to discriminate between these possible explanations for the unusual promoter specificity of the YOR1 UAS.

Recent experiments have demonstrated that YOR1 is required for resistance to the drug reovemycin A and that YOR1 transcription was inducible by this drug (35). It will be of great interest to determine which DNA sequences are involved in this observed transcriptional induction of YOR1. The work presented here provides important information about candidate regulatory elements that may mediate reovemycin A induction of YOR1 expression. Future work will determine if this drug inducibility of YOR1 acts through the PDRE or the other Pdr1p/Pdr3p-independent transcriptional control elements we have identified.

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