Structure and expression of the PHO80 gene of *Saccharomyces cerevisiae*

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ABSTRACT

In yeast, the repression of acid phosphatase under high phosphate growth conditions requires the trans-acting factor PHO80. We have determined the DNA sequence of the PHO80 gene and found that it encodes a protein of 293 amino acids. The expression of the PHO80 gene, as measured by Northern analysis and level of a PHO80-LacZ fusion protein is independent of the level of phosphate in the growth medium. Disruption of the PHO80 gene is a non-lethal event and causes a derepressed phenotype, with acid phosphatase levels which are 3-4 fold higher than the level found in derepressed wild type cells. Furthermore, over-expression of the PHO80 gene causes a reduction in the level of acid phosphatase produced under derepressed growth conditions. Finally, we have cloned, localized and sequenced a temperature-sensitive allele of PHO80 and found the phenotype to be due to T to C transition causing a substitution of a Ser for a Leu at amino acid 163 in the protein product.

INTRODUCTION

The transcriptional regulation and subsequent expression of genes requires the interaction of positively-acting DNA binding factors with promoter elements, termed upstream activator sequences in yeast. These sequences normally are located several hundred bases upstream of the actual sites of initiation of transcription. In several cases, the binding sites and the molecules involved are known (1,2). However, in fewer systems there is also a negative control element involved in the transcriptional regulation (3,4). At the present time, little is known about the mechanism of action of these negative factors.

The transcriptional regulation of the phosphate-repressible acid phosphatase gene of *Saccharomyces cerevisiae* is controlled by a complex but genetically well-defined gene control system, involving both negative and positive trans-acting factors. A structural gene for acid phosphatase, PHO5, has been isolated (5) and the cis-acting elements which are responsible for the transcriptional response to the level of inorganic phosphate in the growth medium have been identified (6-8). Recent work by several groups have reported the structure and function of two of the positive elements, PHO4 and PHO2, which are required for derepression of PHO5 transcription (9-12). In this paper, we report the structure of a negative trans-acting factor, PHO80. Previously Toh-e and co-workers have reported similar results (13). Analysis of the expression of PHO80 indicate that the gene is expressed constitutively at a low level, independent

of phosphate concentration. Furthermore, we have cloned and sequenced a temperature-sensitive allele of PHO80 and demonstrated that this is due to a single T to C transition which causes the replacement of a Leu residue with Ser at amino acid 163 of the gene product. Results from gene-disruption and over-expression experiments suggest a mode of action for the PHO80 molecule.

MATERIALS AND METHODS

Strains and Media. Strains of *S. cerevisiae* utilized in this study are listed in Table I. Yeast cells were grown in rich medium consisting of 1% yeast extract, 2% Bacto-peptone (Difco Laboratories) and a 2% carbon source. Minimal medium contained a 2% carbon source, required amino acids and nucleotides at 20 ug/ml. Low Phosphate medium was prepared according to Rubin (14). Yeast transformations were carried out as described by Ito *et al.* (15) using lithium acetate.

Plasmids. A 9.4 Kb Hind III fragment, isolated from strain R95-4A (a S288C derivative) cloned in YRP14/ARSI (p202) containing the centromere to chromosome XV was obtained from Dr. Philip Hieter (16). This fragment, and subsequently a 2.7 Kb BglII-PstI fragment, were found to complement the *pho80* mutation found in strain 29-40. The restriction map of the 2.7 Kb BglII-PstI fragment is shown in Figure 1. The purified 2.7 Kb fragment was subjected to digestion with Bal31 nuclease and a series of DNA deletions isolated. One particular DNA deletion, p801 was found to map from residue -21 (relative to the ATG residue) and lacked the closely linked centromere from chromosome XV. A 1.1 Kb PstI-ClaI fragment (see Figure 1) was used to add the 5'-end of the PHO80 gene to the p801 deletion. This fragment, when subcloned into YEP351 (J. Hill, personal communication), termed p80ΔCEN, reconstructed the wild type PHO80 gene but lacked CENXV and was present in high copy when transformed into an appropriate yeast strain. Plasmid pE602, a PHO5-LacZ fusion plasmid was obtained from Dr. Keith Bostian, and pBM150 was obtained from Dr. Mark Johnston.

DNA Sequence Analysis. The DNA sequence of the wild type PHO80 gene and flanking regions and the temperature-sensitive allele of PHO80 were determined using various purified restriction fragments or DNA deletion mutants cloned into M13 and the dideoxy method of Sanger as illustrated in Figure 1.

Construction of PHO80 Disruption. A 6.9 Kb BamHI-HindIII fragment from p202 was cloned in pBR322 and digested either completely or partially with XbaI. This plasmid was ligated with a 2.2 Kb XbaI fragment containing the LEU2 gene and two plasmids were obtained. p80ΔLEU resulted from a deletion of the internal XbaI fragment in PHO80 (see Figure 1) with insertion of the LEU2 fragment and p80XLEU resulted from an insertion of the LEU2 fragment at the 3'-XbaI site (see Figure 1). The resulting 8.5 Kb (for p80ΔLEU) and 9.0 Kb (for p80XLEU) BamHI-HindIII fragments were purified and used to transform strain YP98/YP102 to leucine

TABLE 1. *S. cerevisiae* strains

Strain	Genotype	Source
YP98	MATa, <i>ura3-52</i> , <i>lys2-801</i> , <i>ade2-101</i> , <i>leu2-Δ1</i> , <i>trp1-Δ1</i>	P. Heiter
YP98/YP102	MATa/MATα, <i>ura3-52/ura3-52</i> , <i>lys2-801/lys2-801</i> , <i>ade2-101/ade2-101</i> , <i>leu2-Δ1/leu2-Δ1</i> , <i>trp1-Δ1/TRP1</i> , <i>HIS3/his3-Δ1</i>	P. Heiter
X3	YP98/YP102, PHO80/PHO80::LEU2 (p80XLEU)	This study
X3.1	MATα, <i>ura3-52</i> , <i>lys2-801</i> , <i>ade2-101</i> , <i>leu2-Δ1</i> , <i>his3-Δ1</i> , PHO80::LEU2 (p80XLEU)	This study
X2.2	MATa, <i>ura3-52</i> , <i>lys2-801</i> , <i>ade2-101</i> , <i>leu2-Δ1</i> , PHO80::LEU2 (p80ΔLEU)	This study
S288C	MATα	R. Kramer
29-40	MATα, <i>pho80-2</i> , <i>trp1</i> , <i>leu2</i> , <i>arg6</i> , <i>ade2</i>	L. Bergman
YAT338	MATa, <i>pho80^{ts}</i>	R. Kramer

auxotrophy. The transformants were screened by Southern analysis and appropriate diploids were sporulated. Haploids were screened for acid phosphatase production on plates containing 50 ug/ml 5-Bromo-4-chloro-3-indolyl phosphate and then subjected to southern analysis.

Cloning of the Temperature-sensitive PHO80 allele. DNA from strain YAT338 was isolated, digested partially with BglII, and subjected to preparative gel electrophoresis using a Hoefer Bull's Eye Unit. Fractions containing the PHO80 gene were localized by Southern analysis and subsequently cloned into the BamHI site of pUC18 (17). From a PHO80 containing clone, the 2.7 Kb BglII-PstI fragment was purified and cloned into YEP351 (termed p351-80TS, as compared to the wild type gene in the same vector, p351-80BP). To localize the temperature-sensitive lesion within the PHO80 gene, various restriction fragments were purified from the wild type or temperature-sensitive allele and used to replace the analogous fragment in the appropriate vector. The plasmids were then used to transform strain X2.2 (see Table I) and the ability to complement the *pho80* allele assayed at both 23° and 37°.

Measurement of β-galactosidase and acid phosphatase. A PHO80-LacZ fusion was constructed using the PHO80 fragment from p80ΔCEN and pMLB1034, essentially as described previously (18). The site of the fusion, as determined by DNA sequence analysis was at nucleotide 367 of the PHO80 gene. The PHO80-LacZ fusion fragment was purified, cloned into the EcoRI site of

Figure 1. Partial restriction map and sequencing strategy of the PHO80 gene. The bold arrow represents the approximate position of the PHO80 transcript. The DNA sequence of the PHO80 gene was determined as described in the Materials and Methods using either purified restriction fragments or several Bal31 nuclease DNA deletion mutants. Key: B = BglII; C = ClaI; L = BclI; E = EcoRV; P = PstI; X = XbaI.

YCP50 and transformed into strain YP98. The measurement of β -galactosidase and acid phosphatase was as described previously (19).

RESULTS

Having obtained a 9.3 Kb HindIII fragment which contained both CENXV and PHO80, subcloning experiments localized both sequences to a 2.7 Kb BglII-PstI fragment, as illustrated in Figure 1. Using both purified restriction fragments and a series of Bal31 nuclease-generated DNA deletion mutants, the DNA sequence of the PHO80 gene product and the 5' and 3' flanking sequences was determined (see Figure 2). The coding sequences encode a protein of 293 amino acids and we find 3 base pair differences from the sequence published by Tohe and Shimanchi (13). These differences are located at the following residues: -94, our sequence lacks an additional G residue; +690 TCT vs TCC, no amino acid change; and +195 TCA vs. ACA, change is a Ser residue vs Thr residue (amino acid 65). There are several interesting features in the nucleotide sequence of the gene and flanking regions. (a) In the 5'-flanking region there are several regions of poly-purine on one strand and poly-pyrimidine on the second strand (-304 to -296, -227 to -209 and -196 to -171 [24 of 26 residues]) (b) there are 3 -TATA- sequences localized at -161, -111 and -76. (c) At the 3'-end of the gene, there is a region of 43 bases of alternating purine/pyrimidine (one exception). (d) Within the coding sequence of the gene, the carboxy-terminal 39 amino acid show an extremely basic nature with 9 residues of either Arg, Lys or His vs 1 Asp residue and 12 of the 39 residues are either Ser or Thr. (e) Finally, there are several smaller regions enriched for basic residues, nucleotides 490-519 and nucleotides 553-579.

We have examined the mRNA and protein levels of PHO80 to determine if there is any transcriptional or translational response to the level of phosphate in the growth medium. Strain S288C was grown in high or low phosphate-containing growth medium, total RNA isolated and analyzed by Northern analysis using the 558 bp XbaI fragment (see Figure 1) as a probe. As

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-360      -350      -340      -330      -320      -310      -300      -290
ATCTCTCTTC ATTGATGAAT AAAGATCAAC TCAGAAAGTT ATTCAGCGTA TATTGGCTTT CGTTTAATCT AATGCCCCCA

-280      -270      -260      -250      -240      -230      -220      -210
AGCCATCATA AATAGCCATA TTACTTTGAA AGTGCTCATC GTATACACTG CAAGTTTCTT TCCTTCTCT TTAGCCGATT

-200      -190      -180      -170      -160      -150      -140      -130
ATAGCTCCGA CGTGTTTTTT TTTCTCTCTT AAGATTATTT ATATTCCAAT TTTATCATCC GTATTGTTTC CAATAGCGTT

-120      -110      -100      -90      -80      -70      -60      -50
ACAGTGATCA GTTATATACT TCGAGAGCAG ATAACCTTTC TTTTATAAG TGTATTACAA ATTAAAGTCT GCAAGCTATC

-40      -30      -20      -10      15      30
ATAAGACGAG GATATCCCTT GGAGACTCAT AGAAATCATC ATG GAA AGC ACA TCA GGA GAA CGT TCC GAA AAT ATA
Met Glu Ser Thr Ser Gly Glu Arg Ser Glu Asn Ile

CAT GAG GAT CAA GGG ATA CCA AAA GTA ATT CTG CCC GCT GAT TTT AAT AAA TGC TCT AGA ACT GAC CTA GTG
His Glu Asp Gln Gly Ile Pro Lys Val Ile Leu Pro Ala Asp Phe Asn Lys Cys Ser Arg Thr Asp Leu Val
120      135      150      165      180
GTG CTC ATA TCA CGA ATG TTA GTA TCG CTG ATA GCA ATC AAT GAA AAT TCA GCA ACA AAG AAA TCT GAT GAC
Val Leu Ile Ser Arg Met Leu Val Ser Leu Ile Ala Ile Asn Glu Asn Ser Ala Thr Lys Lys Ser Asp Asp
195      210      225      240      255
CAA ATT ACT TTA TCA CGA TAC CAT TCT AAG ATT CCT CCA AAC ATA TCA ATC TTC AAC TAT TTC ATA CGA CTG
Gln Ile Thr Leu Ser Arg Tyr His Ser Lys Ile Pro Pro Asn Ile Ser Ile Phe Asn Tyr Phe Ile Arg Leu
270      285      300      315      330
ACA AAG TTT TCC TCT TTA GAA CAT TGT GTG CTT ATG ACA TCA CTC TAT TAT ATC GAT TTA TTG CAA ACT GTG
Thr Lys Phe Ser Ser Leu Glu His Cys Val Leu Met Thr Ser Leu Tyr Tyr Ile Asp Leu Leu Gln Thr Val
345      360      375      390      405
TAT CCT GAT TTT ACG CTT AAT TCG TTG ACT GCC CAT AGG TTT TTA TTA ACA GCC ACC ACA GTC GCA ACA AAA
Tyr Pro Asp Phe Thr Leu Asn Ser Leu Thr Ala His Arg Phe Leu Leu Thr Ala Thr Thr Val Ala Thr Lys
420      435      450      465      480
GGC TTA TGT GAT TCG TTC TCA ACA AAC GCC CAT TAT GCA AAA GTT GGA GGA GTA CGA TGT CAC GAA TTG AAT
Gly Leu Cys Asp Ser Phe Ser Thr Asn Ala His Tyr Ala Lys Val Gly Gly Val Arg Cys His Glu Leu Asn
495      510      525      540      555
ATA CTG GAG AAC GAT TTT TTA AAG AGA GTA AAC TAC AGA ATC ATT CCG CGG GAT CAT AAC ATT ACG TTA TGT
Ile Leu Glu Asn Asp Phe Leu Lys Arg Val Asn Tyr Arg Ile Ile Pro Arg Asp His Asn Ile Thr Leu Cys
570      585      600      615      630
AGT ATA GAG CAA AAA CAG AAA AAG TTT GTC ATA GAT AAA AAC GCA TTA GGG TCT CTC GAT TTG GAT TCT TAT
Ser Ile Glu Gln Lys Lys Lys Phe Val Ile Asp Lys Asn Ala Leu Gly Ser Leu Asp Leu Asp Ser Tyr
645      660      675      690      705
TCT TAC GTT AAT CGT CCA AAA AGT GGA TAT AAT GTT CTA GAT AAA TAC TAT CGA AGA ATA GTT CAG CTG GTG
Ser Tyr Val Asn Arg Pro Lys Ser Gly Tyr Asn Val Leu Asp Lys Tyr Tyr Arg Arg Ile Val Gln Leu Val
720      735      750      765      780
GGT TCT TTT AAC GCT TCA CCT GAT AAG AGT AGA AAG GTT GAT TAT GTT CTC CCG CCA AAT ATT GAT ATA GTG
Gly Ser Phe Asn Ala Ser Pro Asp Lys Ser Arg Lys Val Asp Tyr Val Leu Pro Pro Asn Ile Asp Ile Val
795      810      825      840      855
AGT GAA AGT GGC TCC CAA ACT ACT CAA CTA AAG GGG TCG TCA TCA CCC AAT TCT CAC TCT TCA CAA AAG CGA
Ser Glu Ser Gly Ser Gln Thr Thr Gln Leu Lys Gly Ser Ser Ser Pro Asn Ser His Ser Ser Gln Lys Arg
870      885      900      910      920
TAT TCT GAG GCA AAG GAC GCA CAT ATC TAT AAC AAG CGA TCA AAG GCA GAT TAA TGA TTTTTCGTGG
Tyr Ser Glu Ala Lys Asp Ala His Ile Tyr Asn Lys Arg Ser Lys Ala Asp *

900      910      920      930      940      950      960      970
GGTATTATGA AAGCAATCAT GATTGAGCAA AACTTTCCAT TTGTACATAT ATATATATAT ATATATATAT ATCTGCTGTG

980      990
GTATAATAAG CAATTGGG

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Figure 2. DNA sequence of the PHO80 gene and the 5' and 3' flanking sequences. The A residue of the ATG initiator methionine is designated +1. The poly-purine/ poly-pyrimidine tracts and the site of the temperature sensitive mutation are underlined.

illustrated in Figure 3, the level of PHO80 mRNA is essentially the same in either growth condition suggesting that the gene is expressed constitutively. The size of the transcript is approximately 1.2 Kb. Furthermore, we have examined the level of expression of a PHO80-LacZ fusion protein, cloned on a CEN vector and transformed into strain YP98. Table II demonstrates that the fusion protein is expressed at approximately the same low basal level under either growth

Figure 3. PHO80 mRNA levels isolated from cells grown in either high- (H) or low- (L) phosphate-containing growth medium. Total RNA was isolated from strain S288C, fractionated on a 1.5% agarose gel containing 2.2M formaldehyde, transferred to nitrocellulose and hybridized with a [32 P]-labeled 558 bp XbaI-XbaI fragment (see Figure 1).

condition when compared to a PHO5-LacZ fusion protein in the same strain, which demonstrates a phosphate-mediated response.

Two plasmids were constructed in an attempt to disrupt the PHO80 gene on chromosome XV. The first, p80XLEU, has the 2.2 Kb LEU2 fragment inserted at the 3'-XbaI site (see Figure 1) which corresponds to amino acid 215 (of 293 total) but deletes no PHO80 sequences. The second, p80ΔLEU, results from the replacement of the 588 bp XbaI fragment with the LEU2 fragment thus the PHO80 molecule retains only the N-terminal 30 amino acids. (There is a frame-shift at the 3'-XbaI site thus eliminating the C-terminal 77 amino acids.) Fragments which span CENXV, from both plasmids were purified and used to transform strain YP98/YP102. Leu⁺ transformants were screened by Southern analysis and diploid strains showing an integration at PHO80 were sporulated. Leu⁺ haploid strains which exhibit increased production of acid phosphatase in high phosphate growth conditions were selected for further analysis. As seen in Figure 4, Southern analysis confirms the replacement of the genomic PHO80 sequence with the disrupted copy.

The presence of a wild type PHO80 gene results in low amounts of acid phosphatase production when the strain is grown in medium containing high levels of inorganic phosphate and derepression of enzyme production in low phosphate growth medium. As demonstrated in Figure 5, haploid strains which contain either disruption, both show high levels of acid phosphatase production in high phosphate growth medium. Interestingly, the haploid strains containing the PHO80 disruption exhibited approximately 2.5-3.0 higher levels of acid phosphatase as compared to either wild type haploid or diploid (homozygous or heterozygous for PHO80) strains when grown in low phosphate growth medium.

To further investigate the role of the PHO80 gene product in the repression/derepression of PHO5 transcription, we have examined the effect of over-expression of PHO80 on the level of acid phosphatase. This has been approached in 2 ways. The first utilizes a DNA deletion which

Figure 4. Southern analysis of DNA from yeast strains containing the PHO80 disruption. DNA was isolated from the various strains, digested with BglII, subjected to Southern analysis using a 4.5 kb BglII-BglII fragment containing the PHO80 gene as a probe. Lane A, strain YP98; lane B, strain YP98/YP102; lane C, strain X3; lane D, strain X3.1 (see Table 1).

Figure 5. Acid phosphatase levels from cells grown in high phosphate (top panel) or low phosphate (bottom panel) growth medium. (●) strain YP98/YP102; (○) strain X3; (△) strain X3.1; (□) strain X2.2. The dashed line represents the composite data for strains YP98/YP102 and X3.

Figure 6. Acid phosphatase levels from cells grown in high phosphate medium (top panel) or low phosphate medium (bottom panel). (●) strain 29-40; (■) strain 29-40 transformed with plasmid 351-80BP; (▲) strain 29-40 transformed with plasmid 351-80ΔCEN.

removes the closely-linked CENXV. The resulting PHO80 fragment was cloned in YEP351 and transformed into strain 29-40. As seen in Figure 6A, the presence of the multi-copy plasmid (copy number is approximately 40 as determined by DNA dot blot analysis, data not shown) completely represses the synthesis of acid phosphatase in high phosphate growth medium whereas the presence of the single copy vector, 351-80BP, shows slightly increased levels of enzyme activity (i.e. the single copy vector does not completely block expression in a *pho80* strain). Interestingly, under low phosphate growth conditions (Figure 6B), the presence of the multi-copy plasmid causes an approximate 3-4 fold reduction in the level of acid phosphatase produced as compared to the single copy vector. Similar results for 351-80ΔCEN are observed in transformation of a wild type cell grown under derepressing growth conditions (data not shown). Our second approach has been to utilize the PHO80 deletion (p801) which starts 21 base pairs

Figure 7. Acid phosphatase levels from cells grown in either high phosphate (top panel) or low phosphate (bottom panel) growth medium. Circles represent strain X2.2 transformed with pBM150 and grown with either glucose (○) or galactose (●) as sole carbon source. Triangles represent strain X2.2 transformed with p150-801 and grown with either glucose (△) or galactose (▲) as sole carbon source.

upstream of the ATG residue and link this deletion to the highly inducible GAL10 promoter in the vector pBM150 (20). When this vector, p150-801 is transformed into strain X2.2, as seen in Figure 7A, the synthesis of acid phosphatase is repressed under high phosphate growth conditions only when galactose is utilized as the sole carbon source. Furthermore, the level of acid phosphatase produced is approximately 4-fold lower than the level seen in strain X2.2 transformed with pBM150 alone. These results suggest that even under derepressing growth conditions, increased levels of PHO80 may cause a reduction in the level of acid phosphatase produced.

To begin to study those regions of the PHO80 molecule which are required for the repression of acid phosphatase expression, we have cloned a temperature-sensitive allele of PHO80 present in strain YAT338. This allele represses synthesis of acid phosphatase in high

PLASMIDS	Enzyme Activity (Units x 10 ⁻² /OD ₆₀₀ x l)		APase 37°
	23°	37°	APase 23°
2.14	1.67	0.78
	4.15	17.11	4.12
	1.32	1.74	1.32
	2.95	10.19	3.45
	3.16	14.55	4.63
	3.42	3.11	0.91
X2.2	25.00	26.51	1.06
YAT 338	1.53	19.28	12.60

Figure 8. Schematic representation of the various plasmid constructions used to localize the temperature sensitive mutation. In the plasmids, the solid line represents sequences from the wild type allele and the dashed line represents sequences from the temperature-sensitive allele. In each case, YEP351-URA3 containing the PHO80 derivative was used to transform strain X2.2. The acid phosphatase activities are shown and the ratio of the activity at 37° C/activity at 23° C at an OD(600) = 1 was determined.

phosphate conditions at 23°C but fails to repress if the cells are grown at 37°C (see Figure 8). DNA from strain YAT338 was isolated, digested partially with BglII and fractionated by preparative gel electrophoresis. Appropriate fractions were identified by Southern analysis, ligated to pUC18 (cleaved with BamHI) and the PHO80^{ts} clones identified by colony hybridization. From a positively hybridizing clone, a 2.7 Kb BglII-Pst fragment was purified and cloned into YEP351 (p351-80TS). This plasmid was then transformed into strain X2.2 and, as seen in Figure 8, complements the *pho80* mutation when assayed at 23°C but fails to repress acid phosphatase synthesis at 37°C (as compared to the PHO80 vector, p351-80BP). Whereas the strain containing the temperature-sensitive allele (YAT338) shows approximately 12-fold higher levels of acid phosphatase activity at 37°C vs. 23°C, when the cloned temperature sensitive gene is placed on a replicating vector the level of enzyme activity is only approximately 3-6 fold higher at 37°C vs. 23°C. This appears to reflect higher levels of acid phosphatase at 23°C and may be due to instability of the plasmid. (The strain into which the plasmid is transformed, X2.2, contains a disruption of the PHO80 gene conferring high levels of enzyme activity at either temperature.) To localize the temperature-sensitive mutation, various restriction fragments were purified from either the wild type or temperature-sensitive allele and used to replace the analogous fragment in the opposite plasmid. As depicted in Figure 8, the temperature sensitive mutation maps to the 344 bp ClaI-XbaI fragment (see Figure 1, also). This fragment (and the corresponding wild type fragment) were then subjected to DNA sequence analysis using M13 vectors and the dideoxy sequencing method. As illustrated in Figure 9, the temperature-sensitive mutation result from a

Figure 9. DNA sequence of a region of the wild type (B) and the temperature-sensitive (A) PHO80 alleles. The arrow indicates the location of the single base change. The DNA sequence of the coding strand is being read on the gel in the 3' to 5' direction from the XbaI site and proceeding towards the ClaI site (see Figure 1). The deduced amino acid sequences of the two gene products from amino acid 161-166 are shown.

single base pair change of nucleotide 488 from T to C. This causes a change from a Leu residue to a Ser residue to the protein product.

DISCUSSION

The synthesis of phosphate-repressible acid phosphatase involves the complex interaction of a number of both positive and negative trans-acting factors. To begin to elucidate the mechanism of transcriptional repression/derepression, our laboratory has analyzed the structure and expression of PHO80, a factor required to repress the synthesis of acid phosphatase under high phosphate growth conditions. Taking advantage of the close linkage of PHO80 and CENXV, we obtained a DNA clone which contained both CENXV and complemented a *pho80* mutation. Subsequent restriction analysis, subcloning experiments and finally DNA sequence analysis localized the PHO80 gene. As revealed in Figure 2, we have determined the DNA

TABLE 2. β -galactosidase Activities of PHO80- and PHO5-Fusion Proteins

Plasmid		Enzyme Activity (Units per mg protein)	
		High P_i	Low P_i
YCP-80-26Z	(PHO80)	55 Units	49 Units
E602-CEN	(PHO5)	8 Units	1762 Units

sequence of the PHO80 gene product and 360 bp at the 5'-end of the gene and 127 bp at the 3'-end of the gene. The PHO80 gene encodes a protein of 293 amino acids. Where a direct comparison is possible, we find 3 base pair changes from the sequence published by Toh-e and Shimauchi (13), one in the 5'-flanking sequences, one "silent" third base change in the coding region, and finally a "conservative" change of a Thr residue to a Ser residue at amino acid 65 in the protein. These changes may only represent differences in that the genes were isolated from 2 different laboratory yeast strains. At the 5'-end of the gene, there are several regions of poly-pyrimidine tracts. These sequences are similar to the constitutive promoter elements described by Struhl (21). Analysis of PHO80 mRNA levels (see Figure 3) has indicated that the gene is expressed at similar levels under both high and low phosphate conditions. Furthermore, expression of a PHO80-LacZ fusion protein appears to be independent of the phosphate concentration (see Table II). This rules out the possibility that the derepression of PHO5 transcription is associated with a repression of PHO80 expression. Similar constitutive expression, independent of phosphate concentration, is observed for two positive factors, PHO4 (9) and PHO2 (11). Furthermore, consistent with other yeast transcription factors, the coding region exhibits an unbiased codon usage (22), most likely also contributing to the low level of expression of the PHO80 gene product.

At the present time, it is not known which regions of the PHO80 gene product are required for the functioning of the molecule. Of particular interest is the observation that the carboxy-terminal 39 amino acids are extremely rich in basic residues and additionally, approximately 30% of the residues in that region are either Ser and Thr. Interestingly, insertion of the LEU2 at amino acid 216, which removes the carboxy-terminal 77 amino acids, causes a disruption of PHO80 function when integrated into the chromosome (see Figures 4 and 5). Finally, molecular analysis of a temperature-sensitive allele of PHO80 has revealed that the phenotype of this strain is due to a change of amino acid 163 from Leu to Ser in the PHO80 gene product. This suggest that residues present in this region are required for the normal function of PHO80. Utilization of this mutant gene product may prove instrumental in elucidating the function of PHO80.

Analysis of the effect of over-expression of PHO80 gene product has indicated that under derepressing growth conditions, the increased level of the PHO80, either by increased gene copy

number (Figure 6) or synthesis under control of the highly inducible GAL10 promoter (Figure 7) leads to a significant reduction in the level of acid phosphatase produced. Consistent with this result is the observation that decreasing the gene copy number by gene disruption causes an significant increase in the level of acid phosphatase produced (Figure 5). Interestingly, recent work by Bostian and co-workers (23) suggested that excess PHO4 gene product is sufficient for positive control of acid phosphatase production. These results suggest that even under derepressed growth conditions the PHO80 molecule may still interact with the necessary components to cause repression of PHO5 transcription. This potentially rules out the possibility that either PHO80 or one of the positive factors is irreversibly modified to allow derepression to occur but rather suggests that PHO80 and presumably PHO4 compete for utilization of some common site. Whether that site is on the PHO5 upstream activator sequence (6-8) or on one of the molecules itself may only be determined by further experimentation.

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