The acid phosphatase genes PHO10 and PHO11 in *S. cerevisiae* are located at the telomeres of chromosomes VIII and I

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**ABSTRACT**

Of the three regulated acid phosphatase genes in *S. cerevisiae* (PH05, PHO10 and PHO11) two have previously been cloned (PH05 and PHO11). We have now identified PHO10 and show by restriction mapping that it is highly homologous to PHO11. This homology includes not only the coding sequence but also a stretch of about 2 kb upstream and 2.2 kb downstream of the genes. Analysis of strains in which either gene had been disrupted shows that the two genes are located at the telomeres of two different chromosomes. PHO10 ends about 9 kb from the end of chromosome VIII and PHO11 3.6 kb from the end of chromosome I. This makes PHO11 the gene closest to the end of a chromosome that has been physically mapped so far in *S. cerevisiae*. The organization of the two genes varies strongly from strain to strain consistent with a high incidence of telomere rearrangement. In one of twenty transformants examined a conversion event could be directly demonstrated that resulted in a chromosome VIII which had acquired a copy of the telomere from chromosome I.

**INTRODUCTION**

Starvation of the yeast *S. cerevisiae* for inorganic phosphate leads to the induction of several acid phosphatases. This regulation occurs at the transcriptional level and involves a complex interplay between positive and negative regulatory proteins (for review see 1). Three different inducible acid phosphatases have been identified by in vitro translation of mRNA (2,3), and two of the corresponding genes, PH05 and PH011, have been isolated (2,4-6) and sequenced (7,8). The PH05 gene can be induced to a very high level and accounts for most of the phosphatase activity in phosphate starved cells, while the PH011 gene shows a lower level of inducibility. The third regulated acid phosphatase gene which resembles PH011 in its induction has been referred to as the PHO10 gene (3) but has not yet been identified.
We are studying the role of the chromatin structure in gene regulation in yeast and have shown that the chromatin structure at the PHO5 promoter undergoes a massive change upon induction of the gene (9,10). Interestingly, the promoter of the PHO11 gene also undergoes a transition which is distinctly different, however (W. Hörz, manuscript in preparation). Correlating the similarities and the differences in the chromatin organization with the composition of individual promoters should provide new insights into the role of the chromatin structure in promoter function. It was of obvious interest to us, therefore, to include also the putative PHO10 gene in our investigations. In this report we identify and characterize the PHO10 gene and demonstrate that both, PHO10 and PHO11, are located close to the ends of two different chromosomes.

MATERIALS AND METHODS

Strains. The yeast strain YS18 (α, his3-11, his3-15, leu2-3, leu2-112, ura3-432, ura3-601, canR) (11) was used throughout. The following strains were included when different strains were compared: S288C (12), DBY746 (12), A364A (12), CMY133 (C. Mann), MC45-5A (M. Ciriacy), S173-6B (M. Holland), SC167 (E. Schweizer).

Media. YPD contained 2% Bacto Peptone (Difco), 1% Bacto Yeast Extract (Difco) and 2% glucose. Yeast transformants were selected in YNB (0.67% yeast nitrogen base without amino acids (Difco)), supplemented with the appropriate amino acids.

Yeast DNA. For pulsed field electrophoresis yeast chromosomes were prepared as described by Schwartz et al. (13) with the modifications of Carle and Olson (14). Yeast DNA was prepared according to Struhl et al. (15) and purified by CsCl gradient centrifugation in the presence of ethidium bromide.

Restriction fragments from a plasmid containing a 5 kb EcoRI fragment with the PHO11 gene (8), a gift from A. Hinnen, were subcloned in pBR322 and used as hybridization probes. Subclones containing a KpnI fragment from the CUP1 gene (16) and a XbaI/BglIII fragment from the PYK gene (17) were gifts of K. Breunig and S. Hohmann, respectively. A 3.6 kb KpnI fragment from a Y element next to SUC5 (18) was subcloned in pUC19 by S. Hohmann and kindly donated. It was used to screen for Y elements in the...
vicinity of PH010 and PH011. The Kpnl fragment is identical in its restriction map to the 3.6 kb Kpnl fragment of clone 131B of Chan and Tye (19).

**Gene disruption.** Disruption was accomplished by transformation of yeast cells with a linear DNA fragment according to Rothstein (20). HindIII linkers were attached to the DraI site of the PH011 EcoRI/DraI fragment shown in Fig.2, and also to the HaeIII site of the HaeIII/HpaII fragment from the PH011 coding sequence. BamHI linkers were ligated to the HpaII site of the latter fragment. As shown in Fig.2, a HindIII fragment with the URA3 gene was ligated to the two PH011 fragments via their HindIII sites and the construct cloned into the EcoRI and BamHI sites of pBR322. This subclone was cleaved with EcoRI and BamHI prior to transformation of yeast cells. Competent YS18 cells were obtained by the lithium acetate procedure (21).

**BAL31 digestion.** 33 μg of yeast DNA was digested with 22 U BAL31 (Boehringer, Mannheim) at 30° in 880 μl reaction buffer (20 mM Tris-HCl, 600 mM NaCl, 12.5 mM MgCl2, 12.5 mM CaCl2, 1 mM EDTA, pH 7.2). 80 μl samples were removed at various times and the DNA ethanol precipitated after the addition of 12 μl 100 mM EGTA.

Analyses by restriction nuclease digestion, gel electrophoresis and hybridization were done as previously described (9). Gene Screen Plus membranes were used throughout.

**Pulsed field gel electrophoresis.** Yeast chromosomal DNA was separated by a Biometra Rotaphor apparatus (22). The gel concentration was 1 or 1.3% agarose (Seakem, LE-agarose, FMC, Rockland, ME), and 45 mM Tris-borate, pH 8.3, 1.25 mM EDTA served as electrophoresis buffer. The gel dimensions were 20 x 20 x 0.6 cm, and the gels were run at 12.5° at a voltage of 150 V. The pulse times are specified in the legends to the figures.

**RESULTS**

**Identification of the PH010 gene**

Previous analyses have led to the isolation and characterization of two structural genes encoding inducible acid phosphatases in S. cerevisiae (PH05 and PH011) and of one constitutively expressed gene (PH03) (2, 4-8). PH03 and PH05 are located immediately adjacent to each other on an 8 kb EcoRI fragment (7),
Fig. 1. Identification of the PHO10 and PHO11 genes in genomic Southern blots. 15 μg DNA from strain YS18 was digested with restriction nucleases, DNA was separated in a 1% agarose gel and blotted. Hybridization in panel A was with a probe from the PHO3 coding region (+914 to +1232, ref.7) which crosshybridizes with PHO5, PHO10, and PHO11 and in panel B with probe A from the PHO11 locus (see Fig.2). The latter probe also recognizes the PHO10 gene region. Restriction enzymes used were EcoRI (lanes 1 and 5); BclI (2 and 7); PstI (3 and 8); ScaI (4 and 9); EcoRI/BclI (6). The bands marked with dots in panel A are derived from the PHO10 and PHO11 genes. The molecular weight reference with sizes given in kb is a mixture of fragments consisting of plasmid pJDB207/PHO5,PHO3 (6) linearized with XbaI, and digested with Sali or with EcoRI, and linear pBR322 DNA, and an RsaI digest of pBR322.

and PHO11 was cloned as a 5 kb EcoRI fragment (8). No other EcoRI fragment hybridizes to a probe representing the coding region of PHO5 (2) although there is evidence from protein analyses for a fourth acid phosphatase, the product of a putative PHO10 gene (2-4).

In hybridization experiments with DNA probes from regions flanking the PHO11 gene we occasionally noticed cross hybridization to fragments that did not correspond to the PHO11 gene region. When we pursued this we discovered that there are actually two different 5kb EcoRI fragments with acid phosphatase genes and that the second one contains the PHO10 gene that had been missing so far.

Fig.1 shows a Southern blot analysis of genomic DNA from S.cerevisiae hybridized with either a probe from the PHO3 coding region (lanes 1-4) or from the PHO11 promoter (lanes 5-9). Two 5 kb EcoRI fragments are actually recognized by the PHO11 probe
Fig. 2. Strategy for the disruption of PHO10 and PHO11. Shown at the top are restriction maps of the PHO11 and the PHO10 gene regions with sites that distinguish the two genes in italics. The fragment used for gene disruption is shown at the bottom. It consists of the 1167 bp HindIII fragment of the URA3 gene flanked by an EcoRI-DraI fragment and a HaeIII-HpaII fragment from the cloned PHO11 locus as indicated. Novel restriction sites introduced by the URA3 gene are marked (A). The location of probes A and B used for hybridization is shown.

(lane 5) that differ in the absence or presence of a BclI site (lane 6). The cloned EcoRI fragment with the PHO11 gene (8) does contain a BclI site, and we will therefore refer to the locus lacking the BclI site as the PHO10 locus. That this new locus is different from PHO11 is confirmed by the absence of a Kpnl and a HincI site about 600 bp downstream of PHO10 which are both present in the previously isolated PHO11 gene (5,8) and in the PHO11 copy in our strain. There are only few enzymes, however, by which the two EcoRI fragments can be differentiated: This suggests a high degree of homology extending throughout the entire 5 kb region, and not just confined to the 1.5 kb coding region as in the case of PHO3, PHO5, and PHO11. The homology did stop abruptly, however, when we compared restriction sites at a greater distance of the PHO11 gene region (Fig.1, lanes 7-9, and Fig.2). This strong homology greatly complicates separate ana-
Fig. 3. Identification of the PH010 and PH011 disruptions. DNA was isolated from strain YS18 and from two transformants, digested with Clal, EcoRI or EcoRI/PstI and separated in a 1% gel. The Clal digest was hybridized with probe B and the EcoRI and EcoRI/PstI digests with probe A which gives a weak signal with the disrupted locus (see Fig. 2). A 4.2 kb EcoRI fragment (•) and a 0.4 kb EcoRI/PstI fragment (⁎) can be seen in the transformants. For the molecular weight reference see Fig. 1.

yses of the PH011 or the PH010 gene, especially of their chromatin organization, since all hybridization probes available recognize both genes simultaneously. It was imperative, therefore, to construct strains in which either one of the two genes was disrupted.

Gene disruption of PH010 and PH011

The strategy of the disruption experiment is shown in Fig. 2. Transformation of a ura− strain was carried out with a linear fragment containing the URA3 gene as a selectable marker flanked by a short fragment from the PH011 promoter on one side and a stretch from the PH011 coding region on the other side. Because of the high homology between PH010 and PH011, it was conceivable that both genes would serve as targets for our fragment. URA+ transformants were selected for and analyzed by Southern blotting as shown in Fig. 3. We had established that the two genes differed in the location of an upstream Clal site and could therefore distinguish from the size of the surviving Clal fragment whether the PH010 or the PH011 locus had been disrupted. It turned out that indeed PH010 was missing in some transformants.
and PHO11 in others (Fig.3). That the URA3 gene had actually integrated into the PHO loci was confirmed by including PstI in the Southern analyses since the URA3 gene brings along a PstI site while the two EcoRI fragments both lack sites for PstI (Fig.2). The analyses are fully consistent with a disruption of either the PHO10 or the PHO11 locus by a dual crossover within the two arms of the fragment used for transformation.

Chromosomal location of the PHO10 and the PHO11 gene

We wanted to test if the PHO10 and PHO11 genes are tandemly repeated as previously shown for the other two acid phosphatase genes. In a first effort to do so, we carried out a chromosomal DNA separation by pulsed field gel electrophoresis and used a DNA probe from the PHO11 promoter that recognizes both genes. It turned out that two different chromosomes were lit up in the hybridization (Fig.4A). That this was really due to PHO10 and PHO11 was confirmed by an analysis of the disruptions which lacked the DNA region recognized by the probe. By comparing the ethidium bromide stained DNA pattern to patterns in the literature (e.g. 23) we tentatively assigned the PHO11 gene to chromosome I and the PHO10 gene to either chromosome V or VIII which were not resolved in our strain. In order to confirm the location of PHO11 on chromosome I we rehybridized the blot shown in Fig.4A with a probe from the pyruvate kinase gene which is known to be on chromosome I (24). It can be seen that the same chromosome lights up again as with the PHO11 probe (Fig.4A).

In some of the laboratory strains of S. cerevisiae, chromosome V and VIII can be separated in high resolution conditions (see e.g. 23). When we analyzed such strains it turned out that the PHO10 gene is located on chromosome VIII (Fig.4B) as confirmed independently in a rehybridization experiment using the chromosome VIII specific CUP1 gene (24) as a probe (not shown).

PHO11 is located at the telomere of chromosome I

In order to establish more extensive restriction maps downstream of the PHO10 and PHO11 loci, we subcloned a HindIII/EcoRI fragment on the 3'-side of the PHO11 gene and used it to probe genomic DNA. This probe recognizes both, the PHO10 and the PHO11 gene region, and two additional unidentified loci in the yeast genome.
Fig. 4. Analysis of the chromosomal location of PHO10 and PHO11 by pulsed field gel electrophoresis. Chromosomal DNA from YS18, YS18-1 (phol-) and YS18-2 (pholO-) was separated in a 1% agarose gel at 150 V for 44 h with switching intervals of 130 s, and for an additional 4.5 h with 30 s (A), or in a 1.3% agarose gel at 150 V for 10 days with switching intervals of 70 s (B). Photographs of the ethidium bromide stained gels are shown on the left in panels A and B. The two gels were hybridized with probe B (Fig. 2) to visualize PHO10 and PHO11. In (A) chromosomes I (lower arrow) and V or VIII (upper arrow) hybridize. Shown on the right in panel A is the result of a hybridization experiment of YS18 DNA with a subclone from the pyruvate kinase gene (PYK) (17). In (B), chromosomes V (*) and VIII (**) are marked. DNA from the smaller chromosomes has migrated out of the gel.
Fig. 5. PH011 is located close to the end of chromosome I. DNA from YS18, YS18-2 (pho10⁻), and YS18-3 was digested with BclI, HindIII, BamHI, or SmaI as indicated at the top, separated in a 1% gel, blotted and hybridized to probe C. The diffuse fragments seen with YS18 DNA (arrows) are PH011 specific and derived from the telomere of chromosome I. Fragments marked with dots are derived from PH010. In YS18-3 chromosome I telomere fragments are present in two copies in the restriction nuclease digests (heavy arrows). For the molecular weight reference see Fig. 1.

In certain digests we noticed peculiarly broad bands (see arrows in Fig. 5). Such bands are characteristically obtained from the extreme ends of yeast chromosomes since there is some size heterogeneity at a given telomere due to the presence of a variable number of simple sequence repeats of the type C₁₋₅A (25). In our case the broad bands must be derived from the PH011 gene, since a fragment of the expected size is also generated with BclI, and only the PH011 gene contains a BclI site at this location (see Fig. 2). The sizes of the heterogeneous fragments obtained with BclI, HindIII and BamHI are consistent with the telomere being approximately 3.6 kb away from the end of the PH011 gene (see Fig. 6).

A gene conversion event between chromosome I and VIII

The telomeric location of PH011 raises the question whether PH010 is also located at a telomere. If so, the telomere must be at least 9 kb away from the end of the PH010 gene, since a site for BamHI and also for SmaI both map at that location. For digestion with SmaI we had actually used DNA from YS18-2 (Fig. 5), the strain which contains the URA3 gene at the PH010 locus with its unique SmaI site.
Evidence suggesting that the PHO10 locus is close to the end of a chromosome stems from an analysis of YS18-3, one of the transformants obtained in the disruption experiment. This strain has the URA3 substitution in the PHO10 gene but lacks the restriction fragments typically found downstream of PHO10. Instead it appears to have two copies of the PHO11 specific telomere fragments. Further analyses with restriction nucleases demonstrate that the PHO10 specific genomic situation is clearly present upstream of the URA3 gene (experiments not shown). These results are consistent with a dual event during transformation: integration of the URA3 gene into the PHO10 locus and a gene conversion event resulting in the acquisition of a copy of the chromosome I telomere by chromosome VIII. Restriction nuclease fine mapping of this transformant demonstrates that the transition point where chromosome VIII turns into chromosome I must occur between 0.8 and 1.8 kb downstream of the PHO10 gene region. In fact, the chromosomal analysis in Fig.4, which also
Flg. 7. BAL31 sensitivity of the PH011 (A) and PH010 (B) telomeric fragments. Genomic DNA from strain YS18-2 (pho10-) was incubated with BAL31 for the times indicated (min) and then digested with CiaI (A) or Smal (B). The fragments were separated in a 1% agarose gel, blotted and hybridized to probe C. The largest CiaI fragment in (A) is due to crosshybridization of an unknown region with probe C. For the molecular weight reference see Fig.1.

includes DNA from YS18-3, is also consistent with a small but detectable shortening of chromosome VIII. The resulting slightly higher mobility of this DNA permits its separation from chromosome V and an assignment of PH010 to chromosome VIII also in this strain.

BAL31 digestion proves the telomeric location of PH010 and PH011. It was necessary to show unambiguously that the putative telomeric restriction fragments really represent the ends of the chromosomes. We therefore carried out digestion experiments with BAL31, a double strand specific exonuclease which has been shown to degrade chromosomal DNA from the ends (26).

DNA used for the BAL31 experiments was from strain YS18-2 which has the URA3 integration in the PH010 locus. This provides
a unique Smal restriction site that can be conveniently used in the subsequent mapping (see Fig. 6). After incubation with BAL31, DNA was first digested with Clal and hybridized with probe C (Fig. 7) to visualize a 4.6 kb Clal-Clal fragment from the PHO10 locus and a 4 kb Clal-telomere fragment from the PHO11 locus. During digestion with BAL31 this latter fragment is seen to be continuously decreasing in size at a constant rate of about 150 bp/min. As predicted, the Clal-Clal fragment from PHO10 is not degraded under these conditions and serves as an internal control.

Restriction of the same BAL31 digests with Smal instead of Clal makes it possible to visualize selectively the PHO10 gene while DNA from the PHO11 locus remains of high molecular weight due to the scarcity of Smal sites (see Fig. 5). The DNA fragment is shortened in the course of BAL31 digestion by about 1.5 kb within the first 10 minutes (Fig. 7B). This agrees very well with an initial digestion rate of 150 bp/min calculated for the PHO11 locus. Digestion was allowed to proceed for another 50 min to make the size reduction more pronounced. We conclude from these experiments that PHO10 is indeed at one end of chromosome VIII.

The PHO10/PHO11 loci are highly unstable in yeast

There is a general scarcity of genes in the close vicinity of yeast telomeres and this is undoubtedly related to the high level of recombination events associated with telomeres which makes these regions quite unstable (25). We decided therefore to screen a number of laboratory strains in order to find out how well conserved the chromosomal arrangement of PHO10 and PHO11 were. When we analyzed chromosomal DNA by pulsed field gel electrophoresis (Fig. 8) it turned out that among nine randomly chosen strains only four showed the same chromosomal situation as our YS18 strain. S288C, a commonly used wild type strain, was one of those. Among those that differed, there was one (DBY746) which had lost a copy, apparently the PHO11 gene, and two which have a third copy (S173-6B and MC45-5A), which is most likely on chromosome IX. YAT679 lacks PHO11 on chromosome I but contains sequences complementary to our PHO11 promoter probe on a different chromosome. A364 gives a very strong chromosome I specific hybridization signal and a very weak signal on chromosome VIII.
Fig. 8. Chromosomal DNA analysis of the \textit{PHO10} and the \textit{PHO11} locus in different \textit{S. cerevisiae} strains. DNA from the strains indicated was separated by pulsed field gel electrophoresis as described in Fig. 3A and hybridized to probe A (see Fig. 6). The DNA preparation from S288C was somewhat degraded as judged from ethidium bromide staining which explains the weak signal of chromosome VIII.

We carried out preliminary restriction nuclease analyses of these strains. They showed that in MC45-5A \textit{PHO10} is duplicated and that the homology between the two copies breaks off about 4–5 kb upstream of \textit{PHO10}. In S173-6B it is the \textit{PHO11} copy which is duplicated, again including about 4 kb of upstream sequences. Interestingly, several restriction sites further upstream of the duplicated \textit{PHO10} copy in MC45-5A and upstream of the duplicated \textit{PHO11} copy in S173-6B are identical. This suggests that in these two strains it is the same chromosome (probably chromosome IX) that has acquired a new telomere copied either from chromosome I or from chromosome VIII. The underlying mechanism appears to be analogous to the gene conversion process which we witnessed in transformant YS18-3.

Based on restriction mapping, DBY746 contains a normal copy of the \textit{PHO10} gene. YAT 679 and A364A give restriction patterns that look very different from those of the other strains. It cannot even be decided if they contain a complete copy of either one of the two coding regions.
DISCUSSION

Homology between PHO10 and PHO11

The identification of the PHO10 gene brings the number of genes encoding acid phosphatases that have been characterized in S. cerevisiae to a total of four. As judged from restriction mapping there is a high degree of homology between the PHO10 and the PHO11 locus. This homology is not confined to the coding part of the two genes but extends far upstream and downstream. Over a stretch of 2,000 bp upstream of the initiation codon we have not detected a single restriction site (out of 25 tested) that differed between the two genes. The homology in the restriction maps does end abruptly, however, somewhere between -2000 and -2300. Genomic sequencing within the promoter similarly shows extreme conservation of the DNA sequence between the two genes (not shown).

Downstream of the gene we have detected six differences in the restriction maps of the two genes. The homology continues until shortly after the EcoRI site which is 2.2 kb downstream of the gene and in the case of PHO11 only 1.4 kb away from the end of the chromosome.

Telomeric location of PHO10 and PHO11

To our knowledge no other functional gene has so far been mapped as close to the end of a chromosome as PHO11. Expression of this gene is apparently not adversely affected by its location and reaches similar levels as PHO10 does (2) which is 10.5 kb away from the end. Chromosome I is the smallest yeast chromosome and lacks the repetitive sequences (23) that are characteristic of most other and possibly all large yeast chromosomes (25). We have screened genomic blots with a probe containing a repetitive element of the Y class (19) and could confirm that restriction fragments from the terminal 15 kb of the PHO11 containing telomere of chromosome I do not hybridize to our Y probe.

Our search for Y elements around the PHO10 gene at the telomere of chromosome VIII showed an absence of Y sequences over a stretch of 20 kb from -13,000 up to a XbaI site 6 kb downstream of the gene, which is 2.1 kb away from the end of the chromosome. We have no information as to whether Y sequences are present on the terminal 2.1 kb and can also not exclude the pos-
sible occurrence of highly variant Y sequences that would not crosshybridize under our conditions.

Our probe C (see Fig.6) that is 1.5 kb away from the end of chromosome I does not, however, only recognize the PHO10 and PHO11 loci but two other gene regions in the yeast genome which we have not investigated any further. Together they may constitute a subclass of slightly repetitive telomeric DNA sequences that are specific for certain chromosomes.

Instability of the PHO10 and PHO11 loci

When we separated chromosomal DNA from a number of different S. cerevisiae strains and used a PHO10/PHO11 specific probe for hybridization we found unusually high strain dependent variation of the patterns. One strain (DBY746) lacked the PHO11 gene altogether. This strain was reported to have a duplication of at least 40 kb from chromosome III onto chromosome I (12). This duplication was apparently accompanied by the loss of the telomere of chromosome I. Alternatively, the end of chromosome III with its own telomere might have been duplicated onto chromosome I. This kind of event did actually take place in one of our disruptions. Insertion of the URA3 gene into the PHO10 locus on chromosome VIII was accompanied by a gene conversion event. As a consequence chromosome VIII acquired a copy of the telomere from chromosome I.

Evolution of the PHO10/PHO11 genes

The coding sequence of PHO11 is more than 80% homologous to PHO5, the highly regulated acid phosphatase gene that is located close to the centromere of chromosome II, and also to PHO3 which is located immediately adjacent to PHO5 (7). It seems reasonable that PHO5 and/or PHO3 which themselves presumably arose by a duplication event (6) have served as donors for the generation of additional acid phosphatase genes at telomeric locations. This would not be unique to the PHO gene family. A similar situation has been encountered for the SUC genes in S. cerevisiae (18). SUC2 is apparently universally present in different S.cerevisiae strains. These strains differ, however, in the presence or absence of additional highly homologous SUC loci (SUC1,3,4,5,7) that are located at the telomeres of different chromosomes and are flanked by homologous regions (18). The precise physical
distance of these loci from the ends of the chromosomes has not been determined, however. The MAL gene family constitutes another example of family members located close to telomeres (24) and subject to a high variability as a consequence.

It has been suggested that sequences at the ends of different chromosomes often interact and undergo some sort of gene conversion processes (27). The direct observation of a conversion event in the construction of one of our disruptions underlines the high frequency of processes leading to the dispersal of telomeric DNA to different chromosomes.

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