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# Structure and Function of the *PHO82-pho4* Locus Controlling the Synthesis of Repressible Acid Phosphatase of *Saccharomyces cerevisiae*

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*pho4* mutants of *Saccharomyces cerevisiae*, although rare among phosphatase-negative mutants isolated from wild-type strains, were isolated efficiently from *pho80*, *pho85*, or *pho80 pho85* strains. The distribution of these *pho4* mutants over the *pho4* locus was determined by analyzing random spores of two- and three-factor crosses. The *pho4-4* mutation confers temperature-sensitive synthesis of repressible acid phosphatase. An intragenic suppressor for the *pho4-12* allele results in the temperature-sensitive synthesis of repressible acid phosphatase. Recombination between these sites occurs at 1.0 to 3.0%, the highest for any pair of sites within the *pho4* locus. All these results strongly indicate that the information of the *pho4* locus is translated into a protein. The *PHO82* site was mapped inside the *pho4* locus by random spore analysis. The order *met10-pho4-1PHO82-1-pho4-9* on the right arm of chromosome VI was confirmed by tetrad analysis. Doubly heterozygous diploids, *pho3 PHO82$^c$ PHO4$^+$/pho3 pho82$^+$ pho4*, produce variable amounts of repressible acid phosphatase under repressive conditions depending on the combination of *PHO82$^c$* and *pho4* alleles. This phenomenon may reflect the constitutive production of the *pho82$^+$-pho4* product in the repressed condition, which interferes with the function of the *PHO82$^c$-PHO4$^+$* product. The earlier model for the function of the *PHO82-pho4* cluster, in which the *PHO82* site acts as an operator of the *pho4* gene, has been revised to a model in which the *PHO82* site codes for the part of the *pho4* protein that has affinity for the regulatory protein encoded by the *pho80* and *pho85* genes.

Many strains of *Saccharomyces cerevisiae* have two species of acid phosphatase (E.C. 3.1.3.2): one is a constitutive enzyme coded for by the *pho3* (previous designation was *phoC*) gene, and the other is a repressible enzyme encoded by the *pho5* (*phoE* or *acp1*) gene (4, 14, 17, 20). The *pho3* and *pho5* genes form a cluster on chromosome II. The *pho5* or *acp1* locus has been mapped between *tsm134* and *lys2* by Hansche et al. (4). Besides the structural gene, at least five genes, *pho2* (*phoB*), *pho4* (*phoD*), *pho81* (*phoS*), *pho80* (*phoR*), and *pho85* (*phoU*), have been found to be involved in the synthesis of repressible acid phosphatase (20, 23). Another gene, *pho84* (*phoT*), originally identified as a mutant showing constitutive synthesis of acid phosphatase, seems to be defective in taking up inorganic phosphate (22). Recently, *pho80* was mapped near the centromere of chromosome XV (V. Beres, personal communication), *pho2* was mapped on the left arm of chromosome IV, *pho4* was mapped on the right arm of chromosome VI, and *pho85* was located on the left arm of chromosome XVI (16). Strains with recessive mutations in *pho2*, *pho4*, or *pho81* do not produce the repressible acid phosphatase, even under low-phosphate conditions where repression is normally released. The *pho4* and *pho81* mutations also result in a defect in the derepression of both nonspecific alkaline phosphatase (18) and inorganic phosphate uptake (Y. Tamai et al., unpublished data). The *pho2* mutations also result in the inability to take up inorganic phosphate (Y. Tamai, unpublished data). Recessive mutations occurring in the *pho80* or *pho85* loci result in the constitutive synthesis of repressible acid and alkaline phosphatases. From the analysis of various double mutants containing one constitutive mutation and one phosphatase-negative mutation, we suggested a possible function for each gene: (i) *pho80* and *pho85* code for polypeptides which together form a repressor protein exerting negative control, (ii) *pho2* and *pho4* genes code for certain proteins working as positive effectors for the expression of the structural gene, and (iii) the *pho81* gene product controls the activity of

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the repressor encoded by the *pho80* and *pho85* genes. A *PHO82* (*PHOO*) mutation conferring a dominant constitutive phenotype was found very near to the *pho4* locus (19). Since the expression of the *PHO82* phenotype needs an active *pho4* allele in *cis* position, the *PHO82* site was believed to be an operator of the *pho4* gene (19). This circumstance resembles that of the *GAL81-gal4* locus in the *gal* system of yeast (3).

In this communication, the *PHO82-pho4* locus is studied further by making a fine-structure map of this locus and by analyzing the synthesis of acid phosphatase by *PHO82$^c$ PHO4$^+$/pho82$^+$ pho4* diploids. Mapping of the *PHO82* mutation sites in relation to the *pho4* mutation sites was carried out by random spore analysis as well as tetrad analysis, and the *PHO82* sites were found in a narrow region located inside the *pho4* locus. Acid phosphatase activity shown by *PHO82$^c$ PHO4$^+$/pho82$^+$ pho4* diploids grown under the repressing conditions varied depending on the combination of the *PHO82* and *pho4* alleles. These results strongly suggest that the *PHO82* site does not represent an operator locus of the *pho4* gene, but that it encodes a special region of *pho4* product which may interact with the regulatory factor produced by *pho80* and *pho85* genes.

## MATERIALS AND METHODS

**Strains.** The principal strains used in this study are listed in Table 1. The nomenclature system of yeast genetic markers recommended by Plischke et al. (9) is used except for mating types (designated a and $\alpha$). The conventional naming system is shown in parentheses as appropriate. Since all the strains were marked with the *pho3-1* mutant allele to eliminate the activity of the constitutive acid phosphatase, *pho3-1* is omitted from the description of the genotype of the strains used here. Mutants were isolated from auxotrophic strains to facilitate complementation tests. After these mutants were assigned to loci, the auxotrophic markers were crossed out by backcrossing each mutant with the wild-type strain, since the presence of an auxotrophic marker sometimes reduces acid phosphatase activity (unpublished data).

TABLE 1. *Strains of S. cerevisiae*

| Strain | Genotype | Source or reference |
|---|---|---|
| YAT61 | a *pho3-1 his7* | This study |
| YAT62 | $\alpha$ *pho3-1 his7* | This study |
| YAT129 | a *pho3-1 met10* | This study |
| AX-26-4A | a *pho3-1 leu*[a] his** | This study |
| P-137-1A | $\alpha$ *pho3-1 cyh* | Cycloheximide-resistant mutant derived from K-4-13D (17) |
| P-135-8B | a *pho3-1 pho4-4* | This study |
| R6-3C | a *pho3-1 PHO4-12R6* | This study |
| P-144-2D | $\alpha$ *pho3-1 pho4-1 arg6* | This study |
| AL-21-2A | $\alpha$ *pho3-1 pho4-1* | Our stock culture (18) |
| Q-101-3B | a *pho3-1 pho80 pho85* | Our stock culture (23) |
| Q-101-3C | a *pho3-1 pho80 pho85* | Our stock culture (23) |
| P-159-1B | a *pho3-1 PHO82-1* | Our stock culture (19) |
| P-122-2A | a *pho3-1 pho4-1 PHO82-1* | This study |
| P-227-2A | a *pho3-1 pho4-9 PHO82-1* | This study |
| P-240-1A | a *pho3-1 pho4-5 PHO82-1* | This study |

[a] The locus is not known.

Additional mutations at the *PHO82-pho4* locus were isolated spontaneously or after mutagenesis with ethyl methane sulfonate according to Lindegren et al. (5). Mutant or recombinant colonies were detected by the staining method described previously (2, 19).

*PHO82$^c$ pho4* double mutants were prepared by dissecting diploids *PHO82-1 PHO4$^+$/pho82$^+$ pho4*. Tetratype asci showing 2+:2− segregation on low-$P_i$ medium and 1+:3− on high-$P_i$ medium with respect to the acid phosphatase trait were analyzed further: one of the two acid phosphatase nonproducers on low-$P_i$ medium may be *PHO82$^c$ pho4*, and the other may be *pho82$^+$ pho4*. These two spore clones can be distinguished by crossing each with a wild-type strain and testing the segregation of clones having the *PHO82* phenotype. Diploids *PHO82-1 pho4/pho82$^+$ PHO4$^+$* will segregate rare constitutive clones, but diploids *pho82$^+$ pho4/pho82$^+$ PHO4$^+$* will not. The *cyh* mutant allele, which confers resistance to cycloheximide in P-137-1A, was introduced into one of the parents to counterselect unsporulated diploid cells when random spore analysis was carried out.

**Media.** Nutrient medium contained 10 g of polypeptone (Daigo Eiyo Chemicals, Japan), 5 g of yeast extract (Daigo Eiyo Chemicals), 40 g of glucose, 5 g of $KH_2PO_4$, and 2 g of $MgSO_4 \cdot 7H_2O$ per liter. The high-$P_i$ medium was the same as Burkholder's synthetic medium in which L-asparagine alone is used as nitrogen source. The low-$P_i$ medium (1.5 g of $KH_2PO_4$ in the high-$P_i$ medium was replaced by 30 mg of $KH_2PO_4$ and 1.5 g of KCl) was described previously (20). Another form of the low-$P_i$ medium was also prepared by precipitating inorganic phosphate from YPAD (20 g of peptone [Difco], 10 g of yeast extract [Difco], 0.4 g of adenine, and 20 g of glucose per liter) according to Rubin (11), and this low-$P_i$ medium was used for scoring the Pho phenotypes under derepressing conditions. Sporulation medium and omission medium were prepared according to the formula described in *Methods in Yeast Genetics* (F. Sherman, G. R. Fink, and C. W. Lawrence, 1974, Cold Spring Harbor Laboratory). Solid media were prepared by adding 2% agar. Culture conditions were described previously (20). Cells were grown at 30°C unless otherwise stated. Cell growth in liquid medium was monitored by reading optical density of the culture at 660 nm.

**Random spore analysis.** Diploids to be tested by random spore analysis are heterozygous for *cyh*. Although the *cyh* locus was not determined, the absence of linkage between *cyh* and *pho4* was confirmed by tetrad analysis (52:46:165 ratio of parental ditype to nonparental ditype to tetratype tetrads). The methods for sporulation and collection of spores were described previously (17). Appropriate dilutions were spread on high-$P_i$ or low-$P_i$ medium containing 1 $\mu$g of cycloheximide per ml.

**Determination of acid phosphatase activity.**

Since acid phosphatase is located outside the cell membrane (13, 15), activity of this enzyme can be assayed by using cell suspensions. Enzyme activity was assayed according to the method described by Torriani (21) with some modifications. The reaction mixture (1 ml), containing 0.128 mg of *p*-nitrophenyl-phosphate, 0.05 M acetate buffer (pH 4.0), and 0.05 ml of cell suspension, was incubated at 35°C for 10 min. The reaction was stopped by the addition of 1 ml of 10% trichloroacetic acid. Then 2 ml of a saturated solution of $Na_2CO_3$ was added to the mixture. Liberated *p*-nitrophenol was determined by reading optical density at 420 nm after centrifugation of the mixture at 3,000 rpm for 10 min. One unit of enzyme activity was defined as the amount of enzyme that liberated 1 μmol of *p*-nitrophenol per min under the conditions described above.

## RESULTS

**Isolation of *pho4* mutants.** To construct a fine-structure map of the *PHO82-pho4* locus, we isolated additional mutations at this locus. Although *pho4* mutants arise only infrequently, we tried to isolate such mutants from the wild-type strain, AX-26-4A (a *his leu*), after ethyl methane sulfonate mutagenesis. Thirty-seven phosphatase-negative mutants were crossed with each of the authentic *pho2*, *pho4*, *pho5*, and *pho81* strains to determine the mutant locus. Only one was a *pho4* mutant (*pho4-7*). The *pho85* strains segregated acid phosphatase-negative papillae spontaneously on nutrient medium, and most of them were found to be *pho85 pho4* double mutants (unpublished data). Preferential segregation of *pho4* papillae was also found in the *pho80* and *pho80 pho85* mutants. Although the reason why the *pho85*, *pho80*, and *pho80 pho85* strains segregate *pho4* mutants is not known, this fact enabled us to isolate *pho4* mutants efficiently. Four independent spontaneous *pho4* mutants, *pho4-2* and *pho4-3* from Q-101-3B (a *pho80 pho85*) and *pho4-4* and *pho4-5* from Q-101-3C (a *pho80 pho85*), were isolated, and each of these *pho4* mutations was separated from the *pho80* and *pho85* mutant genes by backcrosses with the wild-type strain. The *pho85* mutant (O106-M43) isolated from AX-26-4A was treated with ethyl methane sulfonate, and three *pho4* mutants were obtained: *pho4-9*, *pho4-10*, and *pho4-11*. The *pho4-12* mutation was isolated from O105-M23, a *pho80* mutant derived from AX-26-4A, by ethyl methane sulfonate treatment.

The *pho4-4* strain (P-135-8B) was found to be temperature sensitive with respect to repressible acid phosphatase synthesis. Inability to produce acid phosphatase at 35°C was not due to the inactivation of the enzyme at this temperature, since enzyme formed at the permissive temperature (25°C) was stable at 35°C (data not shown). One of the revertants from a *pho4-12*/*pho4-12* diploid also showed temperature sensitivity with respect to the synthesis of acid phosphatase. The reversion site, designated *PHO4-12R6*, was found to occur in the vicinity of the *pho4-12* mutation site as shown later. The revertant diploid was sporulated and dissected to obtain a haploid *PHO4-12R6* strain, R6-3C. The time course of the appearance of acid phosphatase in the culture of the temperature-sensitive (ts) *pho4* mutants in low-$P_i$ medium is shown in Fig. 1. These two *pho4*(Ts) mutants grew well at 35°C. The *pho4-12* allele was sensitive to extragenic suppressors which suppress an ochre non-

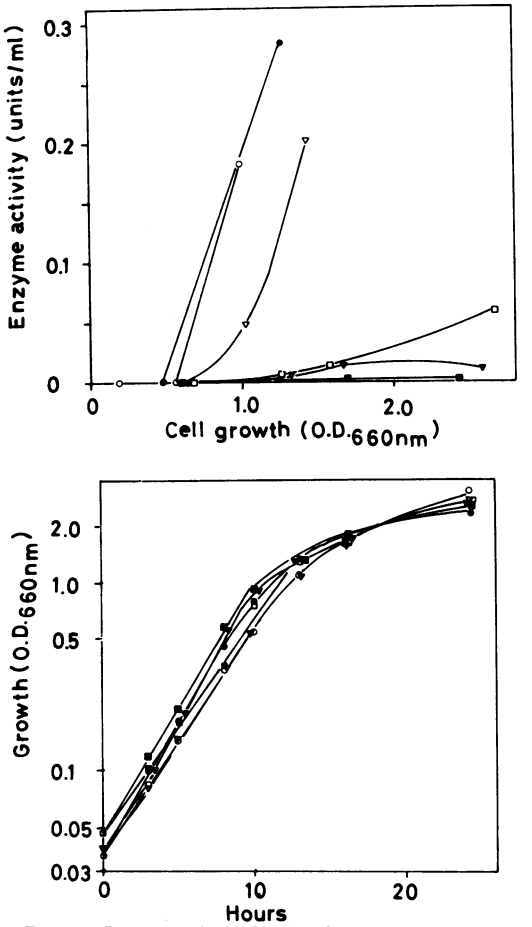



FIG. 1. *Derepression of acid phosphatase activity in temperature-sensitive pho4 mutants. Cells from each strain to be tested were grown in nutrient medium overnight at 30°C. Cells were harvested, washed, and suspended in the original volume of sterile water. One milliliter of this suspension was inoculated into 100 ml of low-$P_i$ medium, and the cultures were shaken at the indicated temperature. P-28-24C (wild type) at 25°C (○) and at 35°C (●); R6-3C (PHO4-12R6) at 25°C (▽) and at 35°C (▼); P-135-8B (pho4-4) at 25°C (□) and at 35°C (■).*

sense allele such as *gal4-2*. Further characterization of these suppressors is under way.

**Isolation of *PHO82* mutants.** Mutants producing repressible acid phosphatase constitutively were isolated from AX-26-4A after ethyl methane sulfonate mutagenesis. Of 376 constitutive mutants, 67 isolates were dominant. Mutations at at least three loci are known to confer the dominant constitutive phenotype in a *pho3* strain: they are *PHO82*$^c$, *PHO81*$^c$, and *PHO3*$^+$ back-mutations (16, 17). The remarkable difference between the *PHO82*$^c$ mutation and the other two mutations is that the former occurs at a site very close to the *pho4* locus. If a mutation conferring dominant constitutivity is closely linked to the *pho4* locus, segregation of the Pho trait from diploids constructed by crossing the mutant with a *pho4* strain should be 2+:2− on low-$P_i$ medium, where both acid phosphatase producers are constitutive. Each mutant showing the dominant constitutivity was crossed with strain P-144-2D (*α pho4-1 arg6*), and the resulting diploids were dissected. Approximately 10 asci from each cross were tested for their ability to produce acid phosphatase on both high-$P_i$ and low-$P_i$ medium. Seven *PHO82*$^c$ mutant alleles, designated *PHO82-3* through *PHO82-9*, were found. A segregant carrying appropriate genetic markers was selected from each cross described above for further study.

The time course of the synthesis of acid phosphatase by some of the *PHO82*$^c$ mutants was followed during growth in high-$P_i$ and low-$P_i$ media (Fig. 2). All *PHO82*$^c$ strains tested showed acid phosphatase activity in high-$P_i$ medium, whereas the wild-type strain did not. The synthesis of acid phosphatase by the wild-type strain grown in low-$P_i$ medium was derepressed when the cell density at 660 nm exceeded 0.5. In contrast, the onset of derepression in *PHO82*$^c$ strains occurred much earlier than in the wild-type strain, i.e., at ⅛ of the cell density at which the wild-type strain started producing the enzyme.

**Two-factor crosses between *pho4* mutants.** Diploids *pho4-x/pho4-y* were constructed and sporulated. Diploids homozygous for the *pho4* allele were also constructed as controls. Spores were released by sonication after treatment of asci with Zymolyase (Zymolyase 5,000, Kirin Brewry Co.), and an appropriate dilution was spread on low-$P_i$ medium containing 1 μg of cycloheximide per ml. After 3 to 5 days of incubation at 30°C, each of 8,174 to 23,000 colonies was scored for its acid phosphatase phenotype by staining in each cross (Table 2). Figure 3 shows a map drawn using the data of Table 2. Ten *pho4* mutant alleles were localized to four separate subregions of this locus: *pho4-1*, *pho4-5*, *pho4-7*, *pho4-10*, and *pho4-11* comprised one subregion; *pho4-3*, *pho4-4*, and *pho4-9* were another; and *pho4-2* and *pho4-12* were each one other site. Crosses containing the *pho4-1* allele tended to give a higher frequency of recombination. This may be the reason why the map distance between the *pho4-1* site and the *pho4-3* site is larger than that between the *pho4-3* site and the *pho4-12* site. The revertant from the *pho4-12* strain, the *PHO4-12R6* strain, was crossed with the wild-type strain, and among 21,000 colonies tested only 1 colony showed the *pho4* phenotype. This result indicates that the reversion site in the *PHO4-12R6* strain is close to the *pho4-12* site.

**Two-factor crosses between *PHO82* mutants.** Diploids *PHO82-x/PHO82-y* were constructed and analyzed by random spore analysis. Appropriate dilutions of spore suspensions were spread on high-$P_i$ medium containing 1 μg of cycloheximide per ml. After 3 to 5 days of incubation at 30°C, colonies that appeared on the plates were stained for acid phosphatase activity. Colonies which could not be stained were scored as recombinants. From 5,171 to 25,000 colonies were tested, and the recombination frequency in each cross is summarized in Table 2. Since the recombination value was less than 0.1%, the *PHO82* sites seem to occupy a single subregion.

**Two-factor crosses between *PHO82* and *pho4* mutants.** Diploids constructed from the cross *PHO82*$^c$ *PHO4*$^+$ × *pho82*$^+$ *pho4* were sporulated, and an appropriate dilution of the spore suspension was plated out on high-$P_i$ medium containing 1 μg of cycloheximide per ml. After 3 to 5 days at 30°C, colonies on the plates were replica-plated onto low-$P_i$ medium, and both plates were incubated at 30°C for another day. The colonies were then stained to test for acid phosphatase activity, and the number of wild-type recombinant colonies showing acid phosphatase activity on low-$P_i$ medium but not on high-$P_i$ medium was determined. In each cross, 9,892 to 12,922 colonies were tested, and the recombination frequency is shown in Table 2. The data can be most easily explained by placing the *PHO82* site inside the *pho4* locus as shown in Fig. 3.

**Three-factor cross: *PHO82-1 pho4-x/ pho82*$^+$ *pho4-y*.** To examine the location of the *PHO82* region in relation to the *pho4* mutation sites, *PHO82-1 pho4-x/pho82*$^+$ *pho4-y* diploids were analyzed by random spore analysis. Diploids were constructed by crossing the *PHO82-1 pho4-1 cyh* strain with the *pho4-2*, *pho4-9*, or *pho4-12* strain, and spore suspensions were prepared as described above. Spore clones appearing on low-$P_i$ medium containing 1 μg of cyclo-

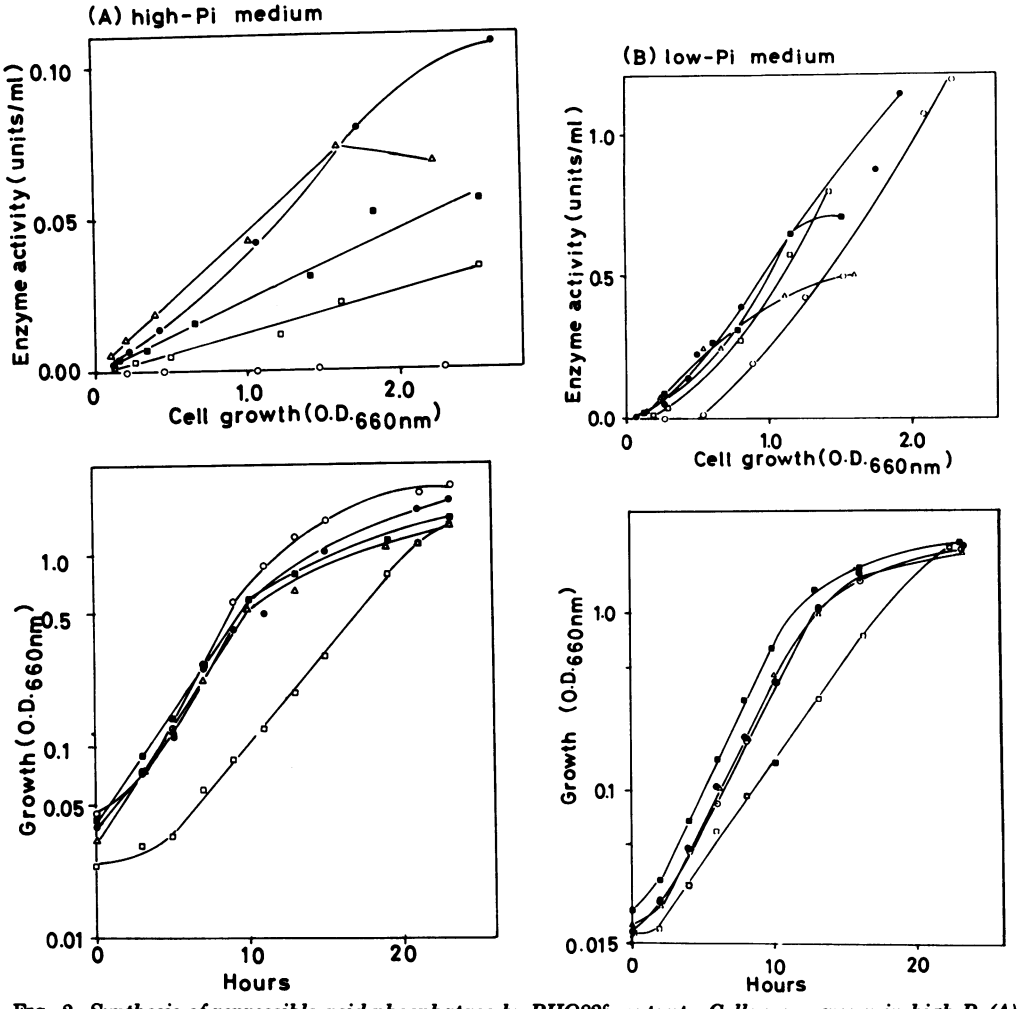



FIG. 2. *Synthesis of repressible acid phosphatase by PHO82$^c$ mutants. Cells were grown in high-$P_i$ (A) or low-$P_i$ medium (B).* (○) *P-28-24C (wild type);* (□) *P-159-1B (PHO82-1);* (■) *P-157-1B (PHO82-4);* (△) *P-178-6C (PHO82-7);* (●) *P-172-1A (PHO82-9).*

heximide per ml were stained for acid phosphatase activity. Colonies producing acid phosphatase were replica-plated onto high-$P_i$ medium to test for their constitutivity. Crosses *PHO82-1 pho4-1/pho82$^+$ pho4-2* and *PHO82-1 pho4-1/ pho82$^+$ pho4-9* gave rise to a comparable number of wild-type and constitutive recombinants (Fig. 4); 86 wild-type and 44 constitutive recombinants were found among 15,000 colonies from the former cross, and 121 wild-type and 38 constitutive recombinants were found among 7,300 colonies from the latter cross. In contrast, the majority of recombinants from cross *PHO82-1 pho4-1/pho82$^+$ pho4-12* were wild type; among 20,000 colonies tested, 16 were wild type and 1 was constitutive. A control cross, *PHO82-1 pho4-1/pho82$^+$ pho4-1*, gave no recombinants among 10,000 colonies tested. The *PHO82-1 pho4-5* double mutant was made and crossed with *pho4-2*, *pho4-12*, or *pho4-5*. A random spore analysis was then carried out to test for both wild-type and constitutive recombinants. Diploid *PHO82-1 pho4-5/pho82$^+$ pho4-2* gave 38 wild-type and 17 constitutive recombinants. On the other hand, diploid *PHO82-1 pho4-5/ pho82$^+$ pho4-12* gave only wild-type recombinants: 28 wild-type recombinants out of 39,900 colonies tested. Diploid *PHO82-1 pho4-5/ pho82$^+$ pho4-5* gave no recombinants among 11,100 colonies tested. The *PHO82-1 pho4-9* double mutant was also constructed and crossed with the *pho4-2* strain. When the diploid was tested for segregation of recombinants, only constitutive recombinants (five recombinants) were

TABLE 2. *Data of two-factor crosses*

| Cross | | No. of colonies tested | No. of recombinants[a] | No. of recombinants per $10^4$ colonies tested |
|---|---|---|---|---|
| α | a | | | |
| *pho4-1* | *pho4-1* | 17,000 | 0 | 0 |
| *pho4-1* | *pho4-2* | 20,584 | 297 | 144 |
| *pho4-1* | *pho4-3* | 22,461 | 511 | 228 |
| *pho4-1* | *pho4-4* | 11,312 | 311 | 275 |
| *pho4-1* | *pho4-5* | 19,410 | 0 | 0 |
| *pho4-1* | *pho4-7* | 26,517 | 0 | 0 |
| *pho4-1* | *pho4-9* | 9,881 | 306 | 310 |
| *pho4-1* | *pho4-10* | 18,384 | 2 | 1 |
| *pho4-1* | *pho4-11* | 12,812 | 1 | 1 |
| *pho4-1* | *pho4-12* | 10,026 | 9 | 9 |
| *pho4-2* | *pho4-2* | 23,805 | 0 | 0 |
| *pho4-2* | *pho4-3* | 16,370 | 21 | 13 |
| *pho4-2* | *pho4-4* | 17,338 | 23 | 13 |
| *pho4-2* | *pho4-5* | 11,408 | 82 | 72 |
| *pho4-2* | *pho4-7* | 18,027 | 93 | 52 |
| *pho4-2* | *pho4-9* | 16,939 | 13 | 8 |
| *pho4-3* | *pho4-3* | 22,446 | 0 | 0 |
| *pho4-3* | *pho4-4* | 13,844 | 1 | 1 |
| *pho4-3* | *pho4-5* | 17,234 | 323 | 187 |
| *pho4-3* | *pho4-9* | 12,384 | 0 | 0 |
| *pho4-4* | *pho4-4* | 18,982 | 0 | 0 |
| *pho4-4* | *pho4-5* | 14,878 | 165 | 111 |
| *pho4-4* | *pho4-7* | 14,091 | 174 | 123 |
| *pho4-4* | *pho4-9* | 12,294 | 0 | 0 |
| *pho4-4* | *pho4-10* | 8,174 | 206 | 252 |
| *pho4-4* | *pho4-11* | 9,375 | 172 | 183 |
| *pho4-4* | *pho4-12* | 10,115 | 207 | 205 |
| *pho4-4* | *pho4-12R6* | 19,415 | 228 | 117 |
| *pho4-5* | *pho4-4* | 13,506 | 137 | 101 |
| *PHO82-1* | *PHO82-1* | 14,035 | 0 | 0 |
| *PHO82-1* | *PHO82-3* | 16,526 | 2 | 1 |
| *PHO82-1* | *PHO82-4* | 12,195 | 9 | 7 |
| *PHO82-1* | *PHO82-5* | 9,574 | 2 | 2 |
| *PHO82-1* | *PHO82-6* | 13,659 | 3 | 2 |
| *PHO82-1* | *PHO82-7* | 11,420 | 3 | 3 |
| *PHO82-1* | *PHO82-8* | 9,227 | 1 | 1 |
| *PHO82-3* | *PHO82-3* | 18,000 | 1 | 1 |
| *PHO82-3* | *PHO82-4* | 14,419 | 0 | 0 |
| *PHO82-3* | *PHO82-5* | 11,721 | 0 | 0 |
| *PHO82-3* | *PHO82-6* | 8,392 | 6 | 7 |
| *PHO82-3* | *PHO82-7* | 5,171 | 0 | 0 |
| *PHO82-3* | *PHO82-8* | 7,574 | 1 | 1 |
| *PHO82-3* | *PHO82-9* | 6,928 | 2 | 3 |
| *PHO82-4* | *PHO82-4* | 21,778 | 1 | 1 |
| *PHO82-4* | *PHO82-5* | 8,904 | 0 | 0 |
| *PHO82-4* | *PHO82-6* | 10,675 | 10 | 10 |
| *PHO82-4* | *PHO82-7* | 10,417 | 0 | 0 |
| *PHO82-4* | *PHO82-8* | 20,260 | 7 | 4 |
| *PHO82-4* | *PHO82-9* | 11,686 | 2 | 2 |
| *PHO82-5* | *PHO82-5* | 25,000 | 0 | 0 |
| *PHO82-5* | *PHO82-6* | 15,209 | 1 | 1 |
| *PHO82-5* | *PHO82-7* | 11,595 | 0 | 0 |
| *PHO82-5* | *PHO82-8* | 5,930 | 0 | 0 |
| *PHO82-5* | *PHO82-9* | 10,040 | 0 | 0 |
| *pho4-1* | *PHO82-4* | 11,029 | 33 | 29 |
| *pho4-2* | *PHO82-4* | 12,922 | 86 | 67 |
| *pho4-2* | *PHO82-5* | 11,501 | 85 | 74 |
| *pho4-4* | *PHO82-4* | 9,892 | 97 | 98 |
| *pho4-4* | *PHO82-5* | 11,634 | 113 | 97 |

[a] $Pho^+$ colonies were scored as recombinants from two-factor crosses between *pho4* mutants. $Pho^-$ colonies were scored as recombinants from two-factor crosses between *PHO82*[c] mutants. Colonies showing the wild-type phenotype ($Pho^+$ on low-$P_i$ medium and $Pho^-$ on high-$P_i$ medium) were scored as recombinants from two-factor crosses between *pho4* and *PHO82*[c] mutants.

found among 12,900 colonies tested. All these data are consistent with the order shown in Fig. 3 and 4.

**Ordering the *PHO82* and *pho4* mutation sites by tetrad analysis.** To substantiate the above conclusion, the order of the *PHO82-1*, *pho4-1*, and *pho4-9* sites was further examined by tetrad analysis. Since the *PHO82-pho4* locus is located 6.5 centimorgans centromere-distal to *met10* on chromosome VI (16), the order of the *PHO82-1*, *pho4-1*, and *pho4-9* sites can be determined in relation to the *met10* locus. Three diploids, W619 (*pho82*$^+$ *PHO4*$^+$ *met10/PHO82-1 pho4-9 MET10*$^+$) and W639 and W640 (*pho82*$^+$ *PHO4*$^+$ *met10/PHO82-1 pho4-1 MET10*$^+$), were analyzed. Of 146 tetrads from W619, 6 contained a recombinant or a convertant. Three of these tetrads contained a constitutive clone, which can be explained by a reciprocal recombination between the *PHO82-1* and *pho4-9* sites or a gene conversion of *pho82*$^+$ to *PHO82-1* and a crossover between *PHO82-1* and *met10* if the order shown in Fig. 5 is correct. These two possibilities can be distinguished by testing whether the $Pho^-$ segregants in these tetrads contain the *PHO82-1* allele. If a gene conversion of *pho82*$^+$ to *PHO82-1* occurred in the ascus, both $Pho^-$ clones would carry the *PHO82-1* allele. However, if the constitutive ascus resulted, instead, from a reciprocal recombination, then only one of the $Pho^-$ clones in this ascus must have the *PHO82-1* allele. The pair of $Pho^-$ clones from each ascus were analyzed. Diploids were constructed by crossing each of these $Pho^-$ strains with a wild-type strain, and acid phosphatase-constitutive progeny were detected by analyzing random spores. As shown in Table 3, one $Pho^-$ clone in each pair can give rise to constitutive segregants in the above cross. This result indicates that all three tetrads analyzed above resulted from reciprocal recombinations between the *PHO82-1* and *pho4-9* sites.

By analyzing the segregation of *met10* in these tetrads, the order *pho4-9–PHO82-1–met10* was deduced as shown in Fig. 5. Two convertants can be explained by coconversion of *PHO82-1 pho4-9* to *pho82*$^+$ *PHO4*$^+$, and the rest are ex-

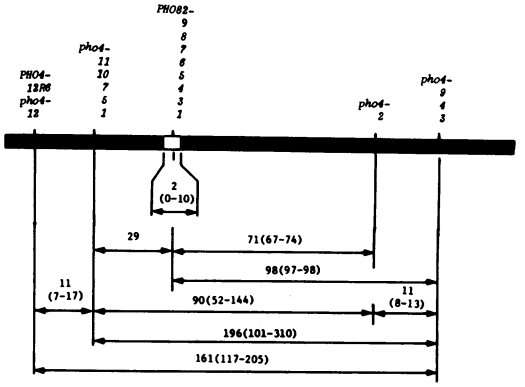
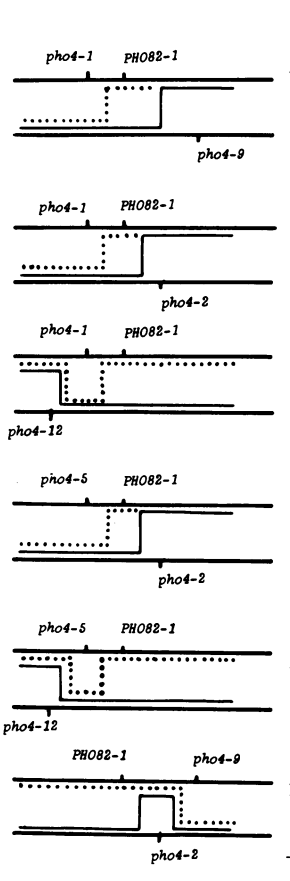



FIG. 3. *Mapping of the PHO82-pho4 cluster. The map was constructed from the data listed in Table 2. Averaged distance between each site is expressed as the number of recombinants per $10^4$ colonies tested. The minimum and maximum distances are shown in parentheses.*

plained by a conversion of *pho82*$^+$ *PHO4*$^+$ to *PHO82-1 pho4-9* or *pho82*$^+$ *pho4-9*. The genotype shown in the bottom of Fig. 5 is consistent with the crossover model shown at the top of Fig. 5.

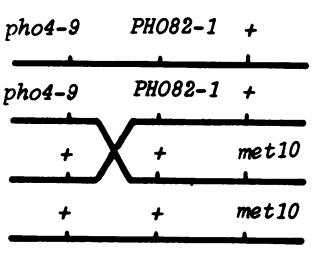
A total of 148 asci were dissected from cross W639, and 79 asci were obtained from W640. The irregular asci can be classified into three types which can be explained in the following way: in nine asci, a coconversion of *PHO82-1 pho4-1* to *pho82*$^+$ *PHO4*$^+$; in eight asci, a coconversion of *pho82*$^+$ *PHO4*$^+$ to *PHO82-1 pho4-1* or *pho82*$^+$ *pho4-1*; and in four of them, a reciprocal recombination producing a constitutive clone had occurred. Three tetrads from W639 containing a constitutive clone were analyzed in the same way as W619 above (Table 3). The result indicates that these tetrads resulted from a reciprocal recombination between the *PHO82-1* site and the *pho4-1* site. The segregation of *met10* confirmed the order of *PHO82-1–pho4-1–met10* (Fig. 6). Conversion of *PHO82-1 pho4-1* to *pho82*$^+$ *PHO4*$^+$ occurred more frequently than *PHO82-1 pho4-9* to *pho82*$^+$ *PHO4*$^+$. This result suggests closer linkage between the *PHO82-1* site and the *pho4-1* site than between the *PHO82-1* site and the *pho4-9* site. Combined tetrad data of W619 and W639 indicate the order of *met10–pho4-1–PHO82-1–pho4-9*. This result again places the *PHO82-1* site inside the *pho4* gene.

**Synthesis of acid phosphatase by diploids *PHO82*$^c$ *PHO4*$^+$/*pho82*$^+$ *pho4*.** Various diploids doubly heterozygous with respect to the *PHO82*$^c$ *and pho4* mutant alleles were constructed, and acid phosphatase activity was followed during their growth in high-$P_i$ and low-$P_i$ media. Representative data are shown in Fig. 7 and 8. The wild-type diploid (*pho82*$^+$ *PHO4*$^+$/*pho82*$^+$ *PHO4*$^+$) behaved like its haploid parent in both high-$P_i$ and low-$P_i$ media. All the diploids containing the *PHO82* mutant allele began to produce acid phosphatase in low-$P_i$ medium earlier than the wild-type strain did (Fig. 2). The synthesis of acid phosphatase by these diploids was tested in high-$P_i$ medium. Homozygous diploids (*PHO82-4 PHO4*$^+$/*PHO82-4 PHO4*$^+$, *PHO82-7 PHO4*$^+$/*PHO82-7 PHO4*$^+$, and *PHO82-9 PHO4*$^+$/*PHO82-9 PHO4*$^+$) showed the same pattern of enzyme synthesis as shown by the respective haploid strains. Doubly heterozygous diploids, *PHO82*$^c$ *PHO4*$^+$/*pho82*$^+$ *pho4*, showed variable amounts of acid phosphatase activity depending on the combination of *PHO82*$^c$ and *pho4* mutant alleles. Table 4 summarizes three sets of such experiments. The *PHO82*$^c$/*pho82*$^+$ diploid showed 6 to 12% of the




| No. of spores tested | No. of recombinants: Wild type | No. of recombinants: Constitutive |
|---|---|---|
| 7,300 | 120 | 38 |
| 15,100 | 86 | 44 |
| 19,900 | 16 | 1 |
| 15,900 | 38 | 17 |
| 39,900 | 28 | 0 |
| 12,900 | 0 | 5 |

FIG. 4. *Three-factor crosses between the PHO82-1 site and pho4 mutation sites. Solid lines show a recombinational event which results in a wild-type recombinant. The broken line shows a recombination which results in a constitutive recombinant.*




| Segregation: Acid phosphatase in medium, High $P_i$ | Segregation: Acid phosphatase in medium, Low $P_i$ | Segregation: Met | Genotype |
|---|---|---|---|
| - | - | + | *PHO82-1 pho4-9 MET10* |
| + | + | + | *PHO82-1 PHO4 MET10* |
| - | - | - | *pho82 pho4-9 met10* |
| - | + | - | *pho82 PHO4 met10* |

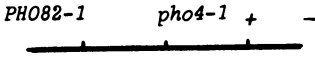
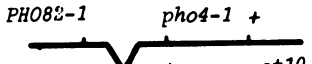
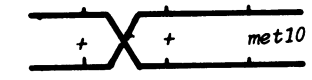
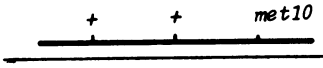
| Segregant | Segregation: Acid phosphatase in medium, High $P_i$ | Segregation: Acid phosphatase in medium, Low $P_i$ | Segregation: Met | Genotype |
|---|---|---|---|---|
| W619-30A | - | - | - | *pho82 pho4-9 met10* |
| 30B | - | - | + | *PHO82-1 pho4-9 MET10* |
| 30C | + | + | + | *PHO82-1 PHO4 MET10* |
| 30D | - | + | - | *pho82 PHO4 met10* |
| W619-90A | + | + | + | *PHO82-1 PHO4 MET10* |
| 90B | - | - | + | *PHO82-1 pho4-9 MET10* |
| 90C | - | - | - | *pho82 pho4-9 met10* |
| 90D | - | + | - | *pho82 PHO4 met10* |
| W619-142A | - | - | - | *pho82 pho4-9 met10* |
| 142B | + | + | + | *PHO82-1 PHO4 MET10* |
| 142C | - | + | - | *pho82 PHO4 met10* |
| 142D | - | - | + | *PHO82-1 pho4-9 MET10* |

FIG. 5. *Tetrads from cross W619 that contain a PHO82-1 recombinant. The genotypes of Pho⁻ clones in these tetrads were determined by the experiments shown in Table 3.*

acid phosphatase activity of the homozygous *PHO82*$^c$/*PHO82*$^c$ diploid. This result suggests that the presence of the wild-type allele in the diploid suppresses the synthesis of acid phosphatase. The *pho4-9* allele was least effective in suppressing enzyme synthesis in these heterozygous diploids.

## DISCUSSION

The *pho4-4* and *PHO4-12R6* alleles confer a temperature-sensitive phenotype with respect to the synthesis of repressible acid phosphatase. Although the presence of a temperature-sensitive mutant alone does not prove that a locus

| | Segregation | | | |
|---|---|---|---|---|
| | Acid phosphatase in medium | | Met | Genotype |
| | High $P_i$ | Low $P_i$ | | |
|  *PHO82-1* *pho4-1* + | - | - | + | *PHO82-1 pho4-1 MET10* |
|  *PHO82-1* *pho4-1* + | - | - | + | *pho82 pho4-1 MET10* |
|  + + *met10* | + | + | - | *PHO82-1 PHO4 met10* |
|  + + *met10* | - | + | - | *pho82 PHO4 met10* |

| Segregant | Segregation | | | Genotype |
|---|---|---|---|---|
| | Acid phosphatase in medium | | Met | |
| | High $P_i$ | Low $P_i$ | | |
| W639-9A | - | + | - | *pho82 PHO4 met10* |
| 9B | + | + | - | *PHO82-1 PHO4 met10* |
| 9C | - | - | + | *PHO82-1 pho4-1 MET10* |
| 9D | - | - | + | *pho82 pho4-1 MET10* |
| W639-54A | - | + | - | *pho82 PHO4 met10* |
| 54B | + | + | - | *PHO82-1 PHO4 met10* |
| 54C | - | - | + | *PHO82-1 pho4-1 MET10* |
| 54D | - | - | + | *pho82 pho4-1 MET10* |
| W639-71A | - | + | - | *pho82 PHO4 met10* |
| 71B | + | + | - | *PHO82-1 PHO4 met10* |
| 71C | - | - | + | *pho82 pho4-1 MET10* |
| 71D | - | - | + | *PHO82-1 pho4-1 MET10* |

FIG. 6. *Tetrads from cross W639 that contain a PHO82-1 recombinant. The genotypes of Pho⁻ clones in these tetrads were determined by the experiments shown in Table 3.*

codes for a protein (10), the presence of two temperature-sensitive mutants, coupled with the fact that the *pho4-12* allele is suppressible by a nonsense suppressor, strongly suggests that the *pho4* locus codes for some protein.

By using *pho80*, *pho85*, or *pho80 pho85* mutants as parental strains, we have devised an efficient method for isolating *pho4* mutants. However, the mechanism underlying this phenomenon remains unknown. Mapping of these *pho4* mutants by the two-factor crosses revealed that the *pho4* mutants isolated by this method are distributed over the *pho4* locus.

The *PHO82-4* and *PHO82-5* mutations are located between *pho4-1* and *pho4-4* as judged by *PHO82-4* or *PHO82-5* × *pho4* two-factor crosses (Table 2 and Fig. 3). The same conclusion was reached from the analysis of *PHO82-1 pho4-x/*

TABLE 3. *Crosses between Pho⁻ segregants from asci containing a constitutive clone and wild-type strain*[a]

| Cross | Segregation of constitutive clones[b] |
|---|---|
| W619-30A × YAT61 | − |
| W619-30B × YAT62 | + |
| W619-90B × YAT62 | + |
| W619-90C × YAT61 | − |
| W619-142A × YAT62 | − |
| W619-142D × YAT62 | + |
| W639-9C × YAT62 | + |
| W639-9D × YAT61 | − |
| W639-54C × YAT62 | + |
| W639-54D × YAT61 | − |
| W639-71C × YAT61 | − |
| W639-71D × YAT62 | + |

[a] A dense spore suspension from each cross was plated on nutrient medium after treatment of the sporulated culture with snail gut juice (suc d'Helix pomatia stabilisé, Industrie Biologique Française). After 2 days at 30°C, staining solution was overlaid to see whether there were clones with acid phosphatase activity.

[b] Acid phosphatase producers were scored on nutrient medium. +, Acid phosphatase producers were found among the spore clones. −, No acid phosphatase producer was found.

*pho82⁺ pho4-y* three-factor crosses (Fig. 4). Since the two-factor crosses of the *PHO82ᶜ* mutants revealed that all *PHO82ᶜ* mutation sites are clustered in a narrow region showing recombination frequency of at most 0.1% (Table 2), each *PHO82ᶜ* site must be located inside the *pho4* locus. The order of *met10-pho4-1-PHO82-1-pho4-9* was also confirmed by tetrad analysis (Fig. 5 and 6).

The fact that the *PHO82* site is flanked by two *pho4* sites which apparently encode a protein argues against the model that the *PHO82* site defines the operator of the *pho4* gene. If the *PHO82* site is not the operator but codes for a part of the *pho4* protein, then the expression of the *pho4* gene is probably not regulated by phosphate at the transcriptional level. The fact that the degree of repression in *PHO82ᶜ PHO4⁺/pho82⁺ pho4* diploids varies depending on the combination of the *PHO82* and *pho4* alleles (Table 4) supports the idea that the *pho4* gene is expressed even in high-$P_i$ medium. Although the *pho4-12* allele is suppressible by a nonsense suppressor, the *pho4-12* allele still affected the synthesis of acid phosphatase in the diploid *PHO82ᶜ PHO4⁺/pho82⁺ pho4-12*. Since it was anticipated that the position of the *pho4-12* al-

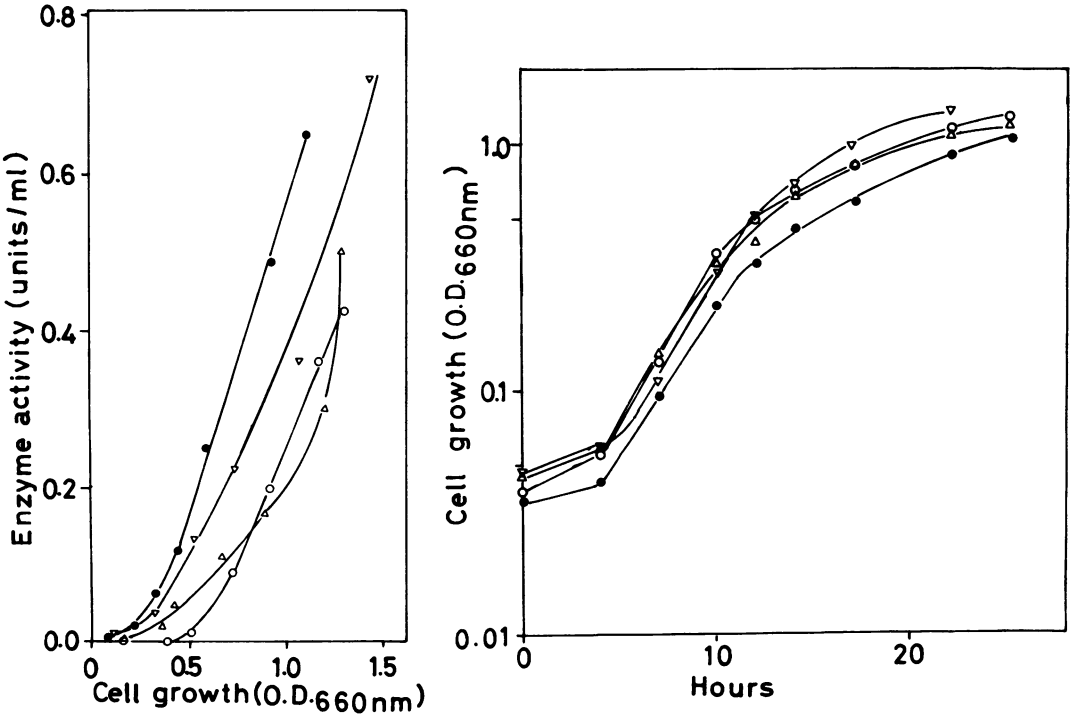



FIG. 7. *Derepression of acid phosphatase activity in heterozygous diploids PHO82ᶜ PHO4⁺/pho82⁺ pho4. Each strain was grown in low-$P_i$ medium at 30°C with shaking.* (○) *+/+ (wild type);* (△) *PHO82-9/pho4-1;* (●) *PHO82-9/+;* (▽) *PHO82-9/pho4-9.*

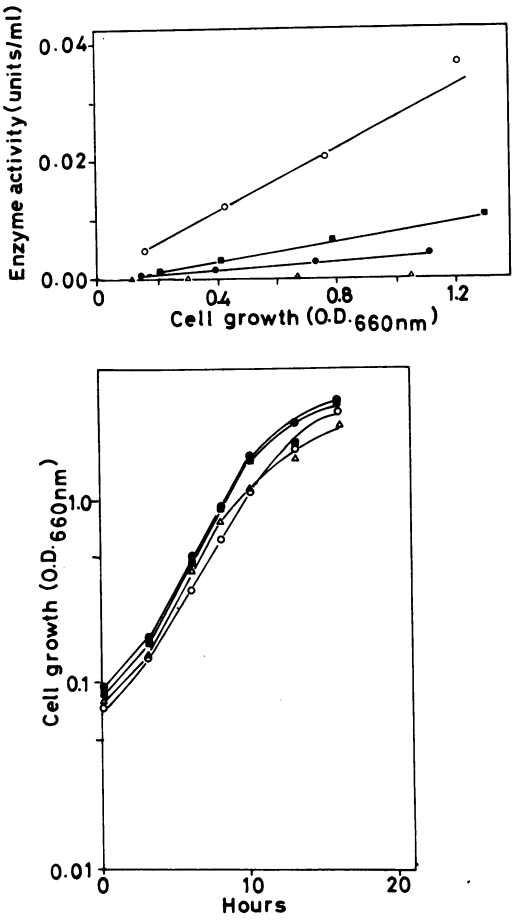



FIG. 8. *Constitutive synthesis of acid phosphatase by PHO82$^c$ PHO4$^+$/pho82$^+$ pho4 heterozygous diploids. Each strain was grown in high-P$_i$ medium at 30°C with shaking.* (○) *PHO82-9/PHO82-9;* (■) *PHO82-9/pho4-9;* (●) *PHO82-9/+;* (△) *+/+.*

lele in the *pho4* locus affects this kind of analysis, the explanation of this result will require further characterization of the *pho4-12* mutation.

In view of these new results, the protein encoded by *pho80* and *pho85* does not function by recognizing a special sequence of DNA. Instead, its control over the *pho4* product is probably exerted via protein-protein interaction: interaction between the *pho80-pho85* product and the part of the *pho4* protein encoded by the *PHO82* site.

In another well-characterized system in *S. cerevisiae*, the *gal* system, the *GAL81-gal4* complex had been believed to be an operon in which *GAL81* was the operator and *gal4* was the structural gene. However, Matsumoto et al. (7) characterized a temperature-sensitive *gal4* mutant and found that the expression of the *gal4* gene is not regulated by the inducer galactose. Perlman and Hopper (8) reached the same conclusion after showing that the levels of galactokinase and transferase mRNA increased after addition of galactose in the presence of cycloheximide. Matsumoto et al. (6) substantiated this further by genetic methods showing that (i) constitutive *GAL81* type mutants could be isolated directly from *gal4* mutants, (ii) the *GAL81* site maps inside the *gal4* gene, and (iii) two suppressor-sensitive alleles of the *gal4* mutant were found in the *GAL81* site.

Although the analyses of the *GAL81-gal4* locus and the *PHO82-pho4* locus revealed that neither of them comprises the classical operon, there are some possibilities other than those described above to be considered to reach the final conclusion. Mapping the regulatory site inside the structural gene alone does not prove that the regulatory site does not function at the transcriptional levels. Sakonju et al. (12) and Bogenhagen et al. (1) found that the start signal for the transcription of 5S RNA gene of *Xenopus borealis* is located in the middle of the gene. Even if a regulatory site is proved to be translated into protein, as in the case of *GAL81-gal4*, there still remain the possibilities that the regulatory site may function either as an antenna for RNA polymerase entry or as an attenuator. If it is not known whether the regulatory site is translated or not, the possibility that an intervening sequence may cover the regulatory site

TABLE 4. *Variable constitutivity of PHO82$^c$ mutation in the diploids PHO82-x PHO4$^+$/pho82$^+$ pho4-y or PHO82-x PHO4$^+$/pho82$^+$ PHO4$^+$* [a]

| Genotype | Sp act (mU/OD$_{660}$ per ml) | Relative activity (%) |
|---|---|---|
| *PHO82-9 × PHO82-9* | 29.0 | 100 |
| *PHO82-9 × pho82$^+$* | 3.5 | 12.1 |
| *PHO82-9 × pho4-1* | 2.0 | 7.0 |
| *PHO82-9 × pho4-9* | 8.0 | 28.0 |
| *PHO82-9 × pho4-12* | 4.8 | 16.6 |
| *pho82$^+$ × pho82$^+$* | 0.0 | 0.0 |
| *PHO82-7 × PHO82-7* | 96.0 | 100 |
| *PHO82-7 × pho82$^+$* | 5.8 | 6.0 |
| *PHO82-7 × pho4-9* | 16.0 | 16.7 |
| *PHO82-7 × pho4-12* | 11.0 | 11.5 |
| *PHO82-4 × PHO82-4* | 31.0 | 100 |
| *PHO82-4 × pho82$^+$* | 2.2 | 7.1 |
| *PHO82-4 × pho4-9* | 8.0 | 25.8 |
| *PHO82-4 × pho4-12* | 4.1 | 13.2 |

[a] Diploid cells were grown in high-P$_i$ medium, and the time course of acid phosphatase formation was followed as seen in Fig. 8. Measurements of specific activity were made on culture with an optical density at 660 nm (OD$_{660}$) of around 1.0.

must be considered. To distinguish these possibilities, more precise work on the structure of the regulatory gene and on its expression is required.

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## LITERATURE CITED

1. **Bogenhagen, D. F., S. Sakonju, and D. D. Brown.** 1980. A control region in the center of the 5S RNA gene directs specific initiation of transcription. II. The 3' border of the region. Cell **19**:27-35.
2. **Dorn, G.** 1965. Genetic anaysis of the phosphatases in *Aspergillus nidulans*. Genet. Res. **6**:13-26.
3. **Douglas, H. C., and D. C. Hawthorne.** 1966. Regulation of genes controlling synthesis of the galactose pathway enzymes in yeast. Genetics **54**:911-916.
4. **Hansche, P. E., V. Beres, and P. Lange.** 1978. Gene duplication in *Saccharomyces cerevisiae*. Genetics **88**: 673-687.
5. **Lindegren, G., Y. L. Hwang, Y. Oshima, and C. C. Lindegren.** 1965. Genetical mutants induced by ethyl methanesulfonate in *Saccharomyces*. Can. J. Genet. Cytol. **8**: 491-499.
6. **Matsumoto, K., Y. Adachi, A. Toh-e, and Y. Oshima.** 1980. Function of positive regulatory gene *gal4* in the synthesis of galactose pathway enzymes in *Saccharomyces cerevisiae*: evidence that the *GAL81* region codes for part of the *gal4* protein. J. Bacteriol. **141**:508-527.
7. **Matsumoto, K., A. Toh-e, and Y. Oshima.** 1978. Genetic control of galactokinase synthesis in *Saccharomyces cerevisiae*: evidence for constitutive expression of the positive regulatory gene *gal4*. J. Bacteriol. **134**: 446-457.
8. **Perlman, D., and J. E. Hopper.** 1979. Constitutive synthesis of the *gal4* protein, a galactose pathway regulator in *Saccharomyces cerevisiae*. Cell **16**:89-95.
9. **Plischke, M. E., R. C. von Borstel, R. K. Mortimer, and W. E. Cohn.** 1975. Genetic markers and associated gene products in *Saccharomyces cerevisiae*, p. 765-832. *In* G. D. Fasman (ed.), Handbook of biochemistry and molecular biology, 3rd ed., vol. II. Chemical Rubber Co. Press, Cleveland, Ohio.
10. **Rasse-Messenguy, F., and G. R. Fink.** 1973. Temperature sensitive nonsense suppressors in yeast. Genetics **75**:459-464.
11. **Rubin, G. M.** 1974. Three forms of the 5.8S ribosomal RNA species in *Saccharomyces cerevisiae*. Eur. J. Biochem. **41**:197-202.
12. **Sakonju, S., D. F. Bogenhagen, and D. D. Brown.** 1980. A control region in the center of the 5S RNA gene directs specific initiation of transcription. I. The 5' border of the region. Cell **19**:13-25.
13. **Schmidt, G. M., G. Bartsch, M. C. Lamont, T. Herman, and M. Liss.** 1963. Acid phosphatase of baker's yeast: an enzyme of the external cell surface. Biochemistry **2**:126-131.
14. **Schurr, A., and E. Yagil.** 1971. Regulation and characterization of acid and alkaline phosphatase in yeast. J. Gen. Microbiol. **65**:291-303.
15. **Suomalainen, H., M. Linko, and E. Oura.** 1960. Changes in the phosphatase activity of baker's yeast during the growth phase and location of the phosphatases in the yeast cell. Biochim. Biophys. Acta **37**:482-490.
16. **Toh-e, A.** 1980. Genetic mapping of the *pho2*, *PHO82-pho4*, and *pho85* loci of yeast. Genetics **94**:923-932.
17. **Toh-e, A., S. Kakimoto, and Y. Oshima.** 1975. Genes coding for the structure of the acid phosphatases in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **143**:65-70.
18. **Toh-e, A., H. Nakamura, and Y. Oshima.** 1976. A gene controlling the synthesis of nonspecific alkaline phosphatase in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **428**:182-192.
19. **Toh-e, A., and Y. Oshima.** 1974. Characterization of a dominant, constitutive mutation, *PHO0*, for the repressible acid phosphatase synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **120**:608-617.
20. **Toh-e, A., Y. Ueda, S. Kakimoto, and Y. Oshima.** 1973. Isolation and characterization of acid phosphatase mutants in *Saccharomyces cerevisiae*. J. Bacteriol. **113**: 727-738.
21. **Torriani, A.** 1960. Influence of inorganic phosphate on the formation of phosphatases by *Escherichia coli*. Biochim. Biophys. Acta **38**:460-479.
22. **Ueda, Y., and Y. Oshima.** 1975. A constitutive mutation, *phoT*, of repressible acid phosphatase synthesis with inability to transport inorganic phosphate in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **136**:255-259.
23. **Ueda, Y., A. Toh-e, and Y. Oshima.** 1975. Isolation and characterization of recessive mutations for repressible acid phosphatase synthesis in *Saccharomyces cerevisiae*. J. Bacteriol. **122**:911-922.