Genes Genet. Syst. (1997) **72**, p. 323–334

REVIEW

The phosphatase system in *Saccharomyces cerevisiae*

Yasuji Oshima

Department of Biotechnology, Faculty of Engineering, Kansai University, 3-3-35 Yamatecho, Suita-shi, Osaka 564, Japan

The yeast *Saccharomyces cerevisiae* has at least six species of acid and alkaline phosphatases with different cellular localizations, as well as inorganic phosphate (P_i) transporters. Most of the genes encoding these enzymes are coordinately repressed and derepressed depending on the P_i concentration in the growth medium. The P_i signals are conveyed to these genes through a regulatory circuit consisting of a set of positive and negative regulatory proteins. This phosphatase system is interested as one of the best systems for studying gene regulation in *S. cerevisiae* due to the simplicity of phenotype determination in genetic analysis. With this methodological advantage, considerable amounts of genetic and molecular evidence in phosphatase regulation have been accumulated in the past twenty-five years. This article summarizes the current progress of research into this subject.

INTRODUCTION

Saccharomyces cerevisiae has several phosphatases, with different specificities and cellular locations, and permeases, used in inorganic phosphate (P_i) uptake. The set of genes responsible for these activities is coordinately regulated repressively by P_i concentration in the growth medium. This phosphatase (*PHO*) system, consisting of several genes and regulators (Table 1), has been studied extensively due to numerous technical advantages (Oshima, 1991). Acid phosphatase activity is easily detected for each colony by specific staining, because the enzyme is located on the cell surface. Enzyme synthesis is regulated in response to a simple effector, P_i, in the medium. The same regulatory system (with minor differences) also controls the synthesis of alkaline phosphatase, located in the cell envelope, whose activity is also detectable in colonies by staining after permeabilizing the cells to the substrate. There is a 500-fold difference between the repressed and derepressed levels of acid phosphatase activity. With the above technical easiness in the mutant selection, we were able to isolate various mutants that were deficient in or constitutive for synthesis of repressible acid and alkaline phosphatases. Genetic and the subsequent molecular analysis of the *PHO* system with these mutants over the twenty-five years has provided insight into the mechanism of signal transduction for gene regulation. In this article, I attempt to summarize the current understanding of the molecular mechanism in phosphatase regulation, especially in signal transduction between negative and positive regulators and *cis*-acting regulatory sites in the *PHO* promoters. For more details on the initial genetic study of the *PHO* system, see review by Oshima (1982), and that by Johnston and Carlson (1992) on the subsequent molecular analysis.

THE GENES AND PROTEINS CONCERNING PHOSPHOROUS METABOLISM

The genes that are regulated by P_i are *PHO5*, encoding the major fraction of repressible acid phosphatase, designated p60 (rAPase; EC 3.1.3.2; Arima et al., 1983; Bajwa et al., 1984; Bostian et al., 1980; Lemire et al., 1985; Toh-e et al., 1975b), *PHO10* (or *PHO12*; de Steensma et al., 1989) and *PHO11* encoding p58 and p56 isozymes of the p60 enzyme respectively (Bostian et al., 1980; Lemire et al., 1985), *PHO8* encoding a non-specific repressible alkaline phosphatase (rALPase; EC 3.1.3.1; Kaneko et al., 1982, 1987; Toh-e et al., 1976), *PHO84* encoding a P_i-transporter (Bunya et al., 1991), and *PHO81* encoding one of the regulatory proteins in the *PHO* system (Ogawa et al., 1993; Yoshida et al., 1989b). These three rAPase isozymes, p60, p58, and p56, (optimum pH 3–4) are glycoproteins (Boer and Steyn-Parve, 1966; Onishi et al., 1979) and located in the cell wall or the periplasmic space (Arnold, 1972; Linnemans et al., 1977; Lopandic et al., 1987) and are probably responsible for acquiring phosphate from diverse extracellular sources. rALPase (optimum pH 8) encoded by *PHO8*, which is also a glycoprotein, is located in the vacuole (Clark et al., 1982) and cleaves diverse substrates to retrieve phosphate from intracellular products. It was suggested that the most important substrate of Pho8p is fructose-2, 6-bisphosphate (Plankert et al., 1991).

S. cerevisiae also has other phosphatases; one is an acid phosphatase (T-rAPase), encoded by *PHO3*, which may func-

Table 1. Genes and their encoding proteins or functions in the *PHO* system

Gene	Chromosome location	Function of gene-product	References
Structural genes			
PHO3	2R	Thiamine phosphatase (T-rAPase)	Nosaka (1990)
PHO5	2R	rAPase, p60	Toh-e et al. (1975b) Arima et al. (1983)
PHO10	8R	rAPase, p58	Bostian et al. (1980) Lemire et al. (1985) Venter and Hörz (1989)
or *PHO12*	8R	rAPase	de Steensma et al. (1989)
PHO11	1R	rAPase, p56	Bostian et al. (1980) Lemire et al. (1985) Venter and Hörz (1989) de Steensma et al. (1989)
PHO8	4R	rALPase Fructose-2, 6-bisphosphate 6-phosphatase	Kaneko et al. (1987) Plankert et al. (1991)
PHO9 (*PEP4*)	16L	Protease for Pho8p maturation	Kaneko et al. (1982)
PHO13	4L	Specific *p*-nitrophosphatase	Kaneko et al. (1989)
PHO84	13L	P_i-transporter	Bun-ya et al. (1991)
PHO86	4R	P_i-transporter associate	Yompakdee et al. (1996a)
PHO87	3R	P_i-transporter associate	Bun-ya et al. (1996)
PHO88	2R	P_i-transporter associate	Yompakdee et al. (1996b)
GTR1	13L	P_i-transporter associate	Bun-ya et al. (1992)
Regulatory genes			
PHO2 (*BAS2*)	4L	DNA-binding transcriptional activator	Sengstag and Hinnen (1987) Yoshida et al. (1989a)
PHO4	6R	DNA-binding transcriptional activator	Yoshida et al. (1989a) Ogawa and Oshima (1990)
PHO6 (*THI2*)	2R	Required for *PHO3* expression	Nishimura et al. (1992a,b)
PHO7		Required for *PHO3* expression	Toh-e et al. (1975a)
THI3	4	Required for *PHO3* expression	Nishimura et al. (1992b)
PHO80 (*TUP7*)	15L	Cyclin; inhibitor of Pho4p	Toh-e and Shimauchi (1986) Kawasaki et al. (1991) Uesono et al. (1992) Kaffman et al. (1994)
PHO81	7R	Inhibitor of Pho80p/Pho85p	Schneider et al. (1994) Ogawa et al. (1993, 1995a)
PHO85	16L	Cyclin-dependent kinase; inhibitor of Pho4p	Uesono et al. (1987) Toh-e et al. (1988) Kaffman et al. (1994)

tion to acquire thiamine (Nosaka, 1990; Schweingruber et al., 1986), and another alkaline phosphatase encoded by *PHO13* exhibiting specificity only for *p*-nitrophenylphosphate (Kaneko et al., 1989). The *PHO3* gene, which is closely linked with *PHO5* on the right arm of chromosome II (Toh-e and Oshima, 1975), is only expressed when *PHO5* is not expressed (Tait-Kamradt et al., 1986) and modestly repressed by thiamine (Nosaka, 1990; Schweingruber et al., 1986) under the positive regulation of the proteins encoded by *PHO6* (= *THI2*) (Nishimura et al., 1992a; Toh-e et al., 1975a) and *PHO7* (Toh-e et al., 1975a) or *THI3* (Nishimura et al., 1992b). The *PHO13* gene is expressed constitutively (Toh-e et al., 1976).

Beside phosphatases, *S. cerevisiae* has at least two systems for P_i uptake; one with a low K_m value (8.2 μM) and the other with a high K_m value (770 μM) for external P_i (Tamai et al., 1985). The low K_m system is repressed by P_i via the same system as that for rAPase regulation and requires the *PHO84* gene product for its function. From the nucleotide sequence of cloned *PHO84*, it was predicted that Pho84p is a 65-kDa 596-amino acid protein homologous to that of several sugar transporters of mammalian, yeast, and bacterial origin (Bun-ya et al., 1991). Pho84p has 12 putative transmembrane segments, each consisting of 21 amino acid residues and the majority of which are hydrophobic. These putative transmembrane segments are separated into two groups by a 74-amino acid segment, which is rich in charged amino acid residues and is located between the sixth and seventh segments. These architectural characteristics and the amino acid sequences of sev-

eral segments of Pho84p are highly similar to those of proteins in the human glucose transporter superfamily (Mueckler et al., 1985).

During the sequence determination of *PHO84*, another open reading frame (ORF) was detected in a cloned *PHO84* fragment at a head-to-head position of the *PHO84* ORF (Bun-ya et al., 1992). This ORF, designated *GTR1*, encodes a 310-amino acid protein containing the characteristic tripartite consensus elements for binding GTP, conserved in GTP-binding proteins of the *ras* family, in its N-terminal region. Disruption of *GTR1* resulted in a slow-growth phenotype at 30°C and no growth at 15°C, and conferred the phenotypes of constitutive synthesis of rAPase, reduced P_i uptake activity, and resistance to arsenate (a toxic P_i analog) on the cells, similar to the *pho84* mutant. Therefore, Gtr1p encoded by *GTR1* might be associated with the P_i transport mechanism.

Two other genes that may be involved in the P_i transport mechanism were detected in a mutant exhibiting arsenate resistance and constitutive synthesis of rAPase (Bun-ya et al., 1996). The mutant was shown to have double mutations designated *pho86* and *pho87*. This double mutant transcribes *PHO84* even in a high-P_i medium but has a severe defect in P_i uptake. The *pho86* single mutation conferred a substantial reduction of P_i uptake on the cells, while the *pho87* single mutant or a disruptant of it showed no apparent difference in its phenotypes with those of the wild-type cells. Sequence determination of a partial *PHO87* coding region revealed that it is identical to *YCR524* on the right arm of chromosome III (Thierry et al., 1990). The published nucleotide sequence of *YCR524* predicts that *PHO87* encodes a 923-amino acid protein with a highly charged N-terminal half and a C-terminal half consisting of twelve transmembrane segments (Bun-ya et al., 1996). The Pho86p protein, deduced from the nucleotide sequence of *PHO86*, is a 34-kDa membrane protein consisting of 311 amino acid residues with two strongly hydrophobic segments in its N-terminal half (Yompakdee et al., 1996a). Thus, Pho86p and Pho87p are likely to be involved in the P_i transport mechanism along with Pho84p and Gtr1p. Both disruption (Yompakdee et al., 1996a) and increased dosage (Yompakdee et al., 1996b) of *PHO86* activates P_i-inhibited Pho81p produced under the control of the *GAL1* promoter (described below).

Another putative membrane protein, Pho88p, was identified by screening for a multicopy suppressor of the rAPase$^-$ phenotype caused by the P_i inhibition of Pho81p (Yompakdee et al., 1996b). The nucleotide sequence of the cloned fragment revealed that the gene, encoding Pho88p, is identical to *YBR106w* (Feldmann et al., 1994). The hydrophobicity profile of the amino acid sequence predicted from the published nucleotide sequence of *YBR106w* and the cellular location suggested that Pho88p is a membrane protein. Both disruption and high dosage of *PHO88*, similarly to *PHO86*, resulted in the activation of P_i-inhibited Pho81p and reduced P_i uptake. The double disruption of *pho86* and *pho88* resulted in a somewhat higher level of rAPase activity in a high-P_i medium and conferred a higher arsenate resistance on the cells than on cells with single disruptants of these genes. These characteristics of Pho88p strongly suggest that it is also involved in P_i uptake and, together with Pho86p, modulates Pho81p activity as a primary receptor for P_i signals from the environment.

REGULATORY CIRCUITS

For the regulation of the activities of the above enzymes and the P_i uptake system, a complex interplay between numerous genes and proteins is required. In a current model for phosphatase regulation (Fig. 1), a positive regulator (or positive factor) Pho4p, coded for by *PHO4*, is indispensable for the transcriptional activation of the above *PHO* structural genes by binding at a specific cis-acting regulatory site(s), termed upstream activation site (UAS),

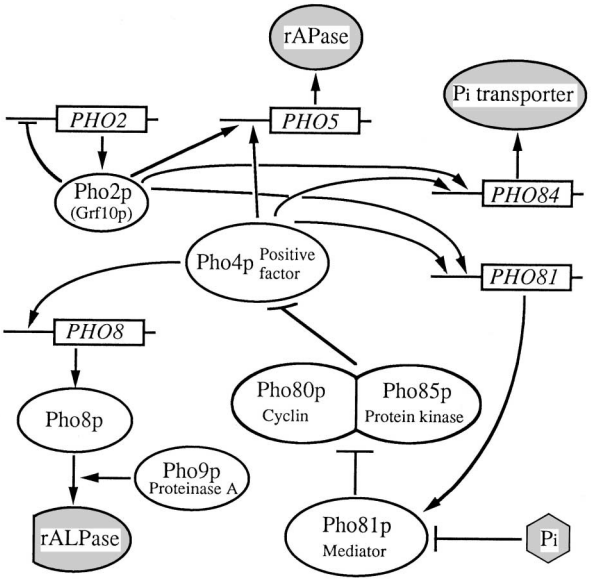

Fig. 1. The genetic regulatory circuit for phosphatase genes. The protein factors, enzymes, and enzyme precursors in the regulatory circuit are shown in open ovals, and the genes regulated by these proteins are shown in boxes. The factors in the shaded hexagon and ovals, P_i, rAPase (repressible acid phosphatase), rALPase (repressible alkaline phosphatase), and P_i-transporter are the input signal to and outputs of the regulatory circuit, respectively. The regulatory factor, for which the corresponding gene is not shown, is known to be produced constitutively in all cases, except *PHO9* (= *PEP4*), the regulation mechanism of which is unknown. The arrowheads in the regulatory circuit indicate a positive or stimulating function of the factors, and when replaced by the bars, they indicate repressive or inhibitory functions. Pho9p (= Pep4p, proteinase A) is required for processing of the Pho8p polypeptide. The *PHO3, PHO6* (= *THI2*), *PHO7, THI3, PHO10, PHO11, PHO86, PHO87, PHO88*, and *GTR1* genes and relevant proteins were not shown for simplicity.

in the promoter. An additional DNA binding protein, Pho2p (also known as Bas2p or Grf10p; Yoshida et al., 1989b), is involved in the transcription of all the *PHO* structural genes except *PHO8*, but it does not have a direct function in the transduction of P_i signals. Pho2p activates the transcription of numerous other genes, including *HIS4* (Arndt et al., 1987; Tice-Baldwin et al., 1989), *TRP4* (Braus et al., 1989), *CYC1* (Sengstag and Hinnen, 1988), genes for adenine biosynthesis (Arndt et al., 1987; Daignan-Fornier and Fink, 1992), and *HO* involved in the mating-type switching mechanism (Brazas and Stillman, 1993a,b) in combination with a cognate positive regulator.

In a high-P_i medium, a complex of negative regulators, Pho80p (cyclin) and Pho85p (cyclin-dependent protein kinase), phosphorylates five serine residues of Pho4p (Kaffman et al., 1994). The complex then turns off the transcription of *PHO* genes by reducing the nuclear location of Pho4p (O'Neill et al., 1996). When the P_i concentration in the medium is sufficiently low, Pho86p and Pho88p may stimulate Pho81p activity (Yompakdee et al., 1996b). Then the activated Pho81p inhibits the function of the Pho80p/Pho85p complex (Ogawa et al., 1995a; Schneider et al., 1994), thus allowing Pho4p, in collaboration with Pho2p, to activate the transcription of the *PHO* genes (Fig. 2; see review, Lenburg and O'Shea, 1996).

The *PHO4* (Yoshida et al., 1989a), *PHO80* (Madden et al., 1990), and *PHO85* (Madden et al., 1990; Uesono et al., 1992) genes are transcribed constitutively at low levels. *PHO2* transcription is also at a low level but is self-regulated (Yoshida et al., 1989a). The transcription of *PHO86* is under relaxed regulation of P_i, while that of *PHO87* is constitutive (Bun-ya et al., 1996), and that of *PHO88* is not affected by the P_i concentration in the medium (Yompakdee et al., 1996b). *GTR1* is transcribed at an extremely low level independent of the P_i concentration in the medium, while it shares the promoter region with *PHO84* but in the reverse direction (Bun-ya et al., 1992). In contrast, the transcription of *PHO81*, encoding Pho81p, is repressed by P_i via the same *PHO* regulatory system, indicating that the *PHO* regulatory circuit forms a positive feedback loop (Ogawa et al., 1993; Yoshida et al., 1989b).

Positive regulators Pho2p and Pho4p are DNA binding proteins. The nucleotide sequence of *PHO4* suggests that Pho4p consists of 312 amino acid residues (Yoshida et al., 1989a). It has the characteristics of a basic region beside an amphipathic helix-loop-helix (bHLH) structure known as a DNA binding domain and homodimer formation (Murre and Baltimore, 1992) in the 85-amino acid C-terminal region (Ogawa and Oshima, 1990), and binds at a specific UAS sequences in *PHO* promoters (Ogawa et al., 1994). The N-terminal region (amino acids 1 to 109), which is rich in acidic amino acids, was suggested to act as the activation domain for gene transcription and a region between amino acids 203 to 227 might be involved in oligomer formation.

The nucleotide sequence of *PHO2* predicts that Pho2p is composed of 519 (Yoshida et al., 1989a) or 559 (Sengstag and Hinnen, 1987) amino acid residues, and contains a homeodomain sequence between amino acids 76 to 135 or between amino acids 77 to 136 (Bürglin, 1988). A portion of the homeodomain sequence of Pho2p has a similar amino acid sequence to that of a short stretch found in Pho4p. Pho2p also has a stretch of glutamine residues (14 of 18 amino acids) on the N-terminal side of the homeodomain sequence. It was shown that Pho2p forms a ternary complex with Pho4p on a *PHO* promoter (Barbarić et al., 1996) by binding at A/T-rich segment(s) beside the UAS site (Magbanua et al., 1997a). It is thought that the Pho2p/Pho4p/UAS ternary complex may interact with the TBP, TFIIB, and TFIIEβ, and initiate transcription of the *PHO* genes (Magbanua et al., 1997b).

Proteins involved in the transduction of P_i signals to Pho4p. Genetic evidence indicated that the negative regulators, Pho80p and Pho85p, inhibit Pho4p function but not the expression of *PHO4* or *PHO2* (Oshima, 1982; Yoshida et al., 1989a). Pho80p, deduced from the nucleotide sequence of *PHO80*, is a 34-kDa protein (Uesono et al., 1992) composed of 293 amino acid residues (Toh-e and Shimauchi, 1986). A coordinated increase of *PHO80* dosage was found to cancel the dosage effect of *PHO4* on the expression of *PHO5* (Yoshida et al., 1989b) and a mutation occurring in *PHO80* suppressed a specific *PHO4*^{c} mutation (Okada and Toh-e, 1992). Based on these findings, it was thought that Pho80p directly interacts with Pho4p for the transduction of P_i signals. On the other hand, Pho85p, encoded by *PHO85* which contains an intron at the 6th codon, is a 36-kDa 305-amino acid protein known as a homologue of Cdc28p, a cyclin-dependent protein kinase of *S. cerevisiae* (Toh-e et al., 1988; Uesono et al., 1987, 1992). It was thought that Pho85p kinase phosphorylates Pho80p, resulting in Pho80p activation (Uesono et al., 1992). Recent reexamination of the amino acid sequence of Pho80p, however, has revealed that it has significant similarities to those of two yeast cyclins, Hcs26p (Ogas et al., 1991) and OrfDp (Fröhlich et al., 1991) in a conserved region of the cyclins (Kaffman et al., 1994). Pho80p and Pho85p form a complex and this complex, but neither protein alone, efficiently phosphorylates Pho4p in vitro and in vivo (Kaffman et al., 1994).

It was believed that the role of the cyclins and cyclin-dependent kinases is in cell cycle control. Pho80p and Pho85p, however, are thought to be unrelated to the cell cycle. This suggests a broader biochemical repertoire for cyclins and cyclin-dependent kinases in yeast (O'Neill and O'Shea, 1995). Pho85p kinase activity is apparently not restricted to the *PHO* system. A cyclin determines the specificity of the partner cyclin-dependent kinase, and Pho85p seems to associate with two other cyclins, Hsc26p and

OrfDp, besides Pho80p (Lenburg and O'Shea, 1996). Since both of these cyclins have been implicated in cell cycle regulation, Pho85p might therefore link cell-cycle events to the cell's nutritional need for phosphate.

According to the genetic analysis, the *pho81* mutation is hypostatic under the *pho80* and/or *pho85* mutation (Oshima, 1982). This fact indicates that Pho81p functions in the upstream of the Pho80p/Pho85p complex (Fig. 1). The nucleotide sequence of *PHO81* suggests that Pho81p is a 134-kDa 1179-amino acid protein which has six repeats of a 33-amino acid sequence homologous to the ankyrin repeat (Lux et al., 1990) between amino acids 424 to 656 and an asparagine-rich region (26 of 33 amino acid residues) between amino acids 222 to 254 (Ogawa et al., 1993). The transcription of *PHO81* is regulated by P_i via the *PHO* regulatory system including both Pho4p and Pho2p (Fig. 1). Experiments with a chimeric gene consisting of the *PHO81* ORF connected with the *GAL1* promoter revealed that stimulation of the *PHO* regulatory system requires both increased transcription of *PHO81* and P_i-starvation conditions (Ogawa et al., 1995a). These findings suggest that Pho81p is inactive under high-P_i conditions.

In a low-P_i medium, Pho81p binds with and inhibits the kinase activity of the Pho80p/Pho85p complex (Ogawa et al., 1995a; Schneider et al., 1994), and allows underphosphorylated Pho4p to be imported into the nucleus and to turn on the transcription of *PHO* genes (Fig. 2). In the high-P_i condition, Pho81p is, however, inactive as an inhibitor. Thus, the Pho80p/Pho85p complex is active for hyperphosphorylation of Pho4p and turns off the transcription of *PHO* genes, as described above. Hirst et al. (1994), however, claimed that Pho81p interacts directly with Pho4p, along with Pho80p, under conditions of *PHO* repression and that Pho80p and Pho81p dissociate from Pho4p under derepressive conditions.

The minimum amino acid sequence required for the above Pho81p function in vivo was narrowed down to a 141-amino acid segment (amino acids 584 to 724), which contains the fifth and sixth repeats of the ankyrin-like motif (Ogawa et al., 1995a). Schneider et al. (1994) also demonstrated that a similar short fragment of Pho81p (amino acids 400 to 720) inhibits the kinase activity of the Pho80p/Pho85p complex in vivo. The third to sixth repeats of the ankyrin-like motif of Pho81p have significant similarities to that of $p16^{INK4}$ which inhibits the activity of the human cyclin D/CDK4 kinase complex (Serrano et al., 1993). Deletion analyses revealed that the N- and C-terminal regions of Pho81p function as negative and positive regulatory domains, respectively, for the minimal 141-amino acid region (Ogawa et al., 1995a). The negative regulatory activity of the N-terminal domain was antagonized by a C-terminal segment of Pho81p supplied in trans. All four known classes of *PHO81*^{c} mutations that conferred significant rAPase activity on cells grown in a high-P_i medium occur in the N-terminal half of Pho81p.

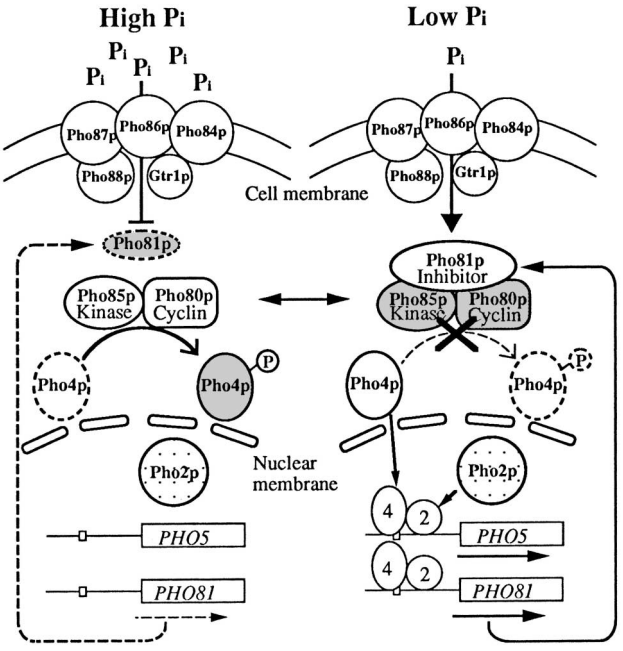

Fig. 2. The molecular mechanism for the transduction of extracellular P_i signals to the *PHO* structural genes in the nucleus. The shaded regulatory proteins are the inactivated forms of the relevant one. The arrows shown underneath the *PHO5* and *PHO81* genes indicate transcription of these genes. The broken arrow from the *PHO81* to Pho81p indicates basal expression of *PHO81*. For the detailed explanation, see text. The other structural genes in the *PHO* system were eliminated from the figure for simplicity.

Specific UAS sequence for Pho4p binding. Genetic evidence suggests that Pho2p and Pho4p bind to or interact with a specific UAS in the promoter region of the *PHO* structural genes (Oshima, 1982). Such sites of *PHO5* have been studied in several laboratories (Bergman et al., 1986; Nakao et al., 1986; Rudolph and Hinnen, 1987). The proposed nucleotide sequences for the UAS function by these authors were, however, in conflict with each other. To clarify the Pho4p-binding site, we investigated the *PHO8* promoter, because only Pho4p, and not Pho2p, is required for *PHO8* transcription. We found that a 6-bp sequence, CACGTG, at nucleotide position –535 relative to the ATG start codon (Fig. 3) is the Pho4p binding site in the *PHO8* promoter, as determined by deletion, modification, and gel retardation analyses (Hayashi and Oshima, 1991). The protection of this CACGTG sequence by purified Pho4p from DNase I digestion in vitro was also shown (Barbarić et al., 1992; Ogawa et al., 1994).

Two putative UASs in the *PHO5* promoter, designated UAS_p1 in the region from nucleotide positions –373 to –347 and UAS_p2 located from nucleotide positions –262 to –239 (Fig. 3), have been detected by in vitro binding of Pho4p (Vogel et al., 1989). These regions contain one copy each of, respectively, the CACGTT and CACGTG motifs (Fig. 4). We confirmed that purified Pho4p protects these sites

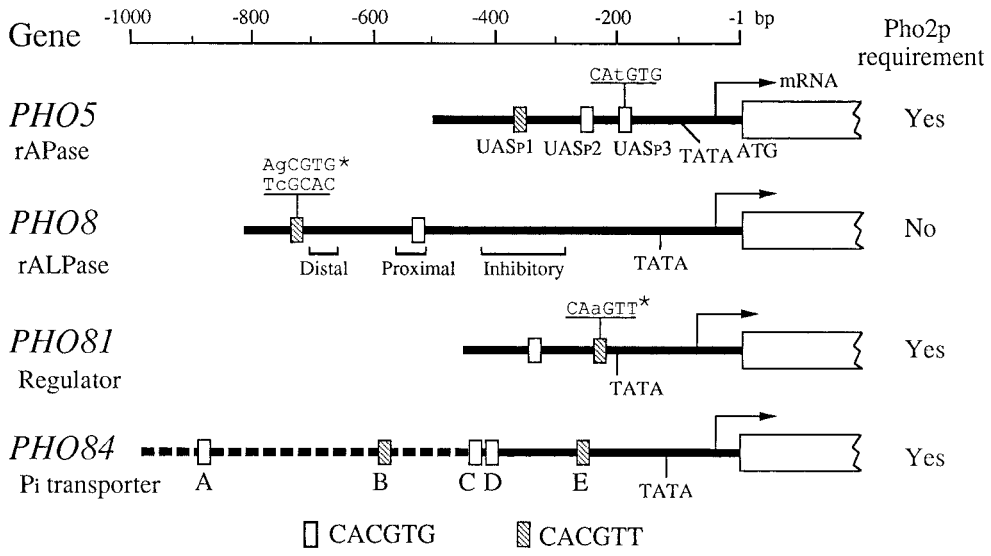

Fig. 3. Distribution of the 6-bp motif, CACGTK (K = G or T), in the promoter regions of the *PHO* genes. The open box at the right end of individual genes represents an ORF. Short open and hatched boxes indicate CACGTG and CACGTT motifs, respectively. Three 6-bp sequences, analogous to the 6-bp motif, shown in the *PHO5, PHO8*, and *PHO81* promoters are indicated with their sequences (lowercase letters indicate deviation from the 6-bp motifs). The region in the *PHO84* promoter shown with a broken line bearing sites A and B is dispensable for regulatory function. The function and Pho4p binding ability of the sequence analogous to the 6-bp motif in the *PHO8* promoter (marked with an asterisk) were described by Barbarić et al. (1992), but that of the *PHO81* promoter has not yet been examined.

from DNase I digestion in vitro (Ogawa et al., 1994). In the same experiment, we found that another 21-bp region, bearing a CATGTG sequence, in the *PHO5* promoter from nucleotide positions –202 to –182 (Figs. 3 and 4) was also protected weakly by Pho4p. Deletion of the above 21-bp region resulted in lower activity of *PHO5* transcription (Rudolph and Hinnen, 1987). Thus, this 6-bp sequence, designated as UAS_p3, might also be involved in the *PHO5* regulation.

We also examined UASs in the *PHO81* promoter (Ogawa et al., 1993). Results of a deletion analysis showed that a 95-bp region, extending from nucleotide positions –385 to –291, could sense the P_i signals. A 19-bp region in the 95-bp fragment was protected by Pho4p from DNase I digestion in vitro (Fig. 4). We found one copy of the CACGTG motif in the 19-bp region and no other CACGTG or CACGTT motifs in the 95-bp region. It is known that *PHO81* transcription is severely reduced in a *pho2* mutant (Yoshida et al., 1989b). The above 95-bp fragment, however, could not respond to a *pho2* disruption allele, but a 488-bp fragment, bearing a region of *PHO81* DNA from nucleotide positions –385 to +103, could respond to the *pho2* disruption on a YCp-type plasmid (Ogawa et al., 1993). This suggests that a specific site for interaction with Pho2p might be located in a region downstream of nucleotide position –290. A CAAGTT sequence was found at nucleotide position –233 in the *PHO81* promoter (Fig. 3), but the function of this sequence remains to be examined.

The promoter region of *PHO84* bears five copies of the 6-bp motifs at nucleotide positions –880 (site A), –587 (site B), –436 (site C), –414 (site D), and –262 (site E) relative to the ATG start codon (Fig. 3). Binding of Pho4p to these five copies of the 6-bp motif was confirmed by DNase I footprinting (Ogawa et al., 1994). We found the highest affinity for Pho4p at site D, whereas sites C and E had somewhat weaker affinities than site D. The A and B sites also had some affinity but much weaker than site E. Analyses of the promoter with deletion and base substitutions in the above 6-bp motifs revealed that the 6-bp motifs at site C or D and E are essential and sufficient for the full regulation of *PHO84* transcription, whereas sites A and B are dispensable (Ogawa et al., 1995b).

Eight effective UAS sites, each one located in the middle of the 19 to 22 bp footprinting regions, protected by Pho4p from DNase I digestion in vitro, in the promoters of *PHO5*, *PHO8*, *PHO81*, and *PHO84* (Ogawa et al., 1994), were compared along with a sequence found at the distal site in the *PHO8* promoter (Barbarić et al., 1992). We found that six of the UASs have the CACGTG sequence (type 1; Fig. 4) and three have the CACGTT sequence (type 2).

The 6-bp CACGTG core sequence has been found to have similarity to a highly conserved octanucleotide, RTCACRTG (where R is a purine residue) called CDEI, located in centromeres and in the promoters of various genes for methionine synthesis in *S. cerevisiae* (Baker and Masison, 1990; Cai and Davis, 1990; Mellor et al., 1990). The CDEI-binding protein (i.e., Cpf1p, CP1 or Cbf1p) has a bHLH structure in its DNA-binding domain. Although crosstalk was not detected between the phosphatase and methionine genes in wild-type cells, overexpression of *PHO4* was found

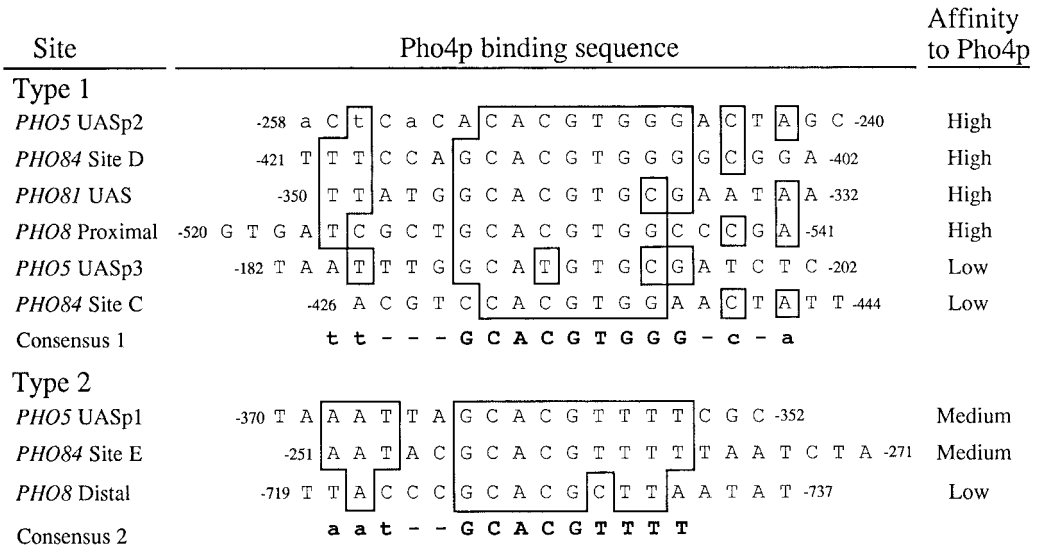

Fig. 4. Regions in the *PHO* promoters protected from DNase I digestion in vitro by Pho4p. The protected sequences observed in previous studies (Ogawa et al., 1993, 1994) are listed for strands having sequences conforming most closely to GCACGTGGG or GCACGTTTT along with the data of Barbarić et al. (1992) for the similar sequences at the distal site in the *PHO8* promoter. These sequences were classified into two groups depending on whether they contained the CACGTG (type 1) or CACGTT (type 2) sequence in the core region. The relative affinity of each sequence to Pho4p was estimated from the amount of Pho4p protein required for protection from DNase I digestion (Ogawa et al., 1994). The numerals at both ends of the sequences indicate nucleotide position of the 5'- and 3'-ends of the sequences in the promoter relative to the translation initiation codon of the respective genes.

to suppress the methionine auxotrophy of a *cep1* (= *cpf1*) mutant (O'Connell and Baker, 1992), under conditions of methionine starvation (O'Connell et al., 1995). In contrast, *CEP1* overexpression failed to suppress the rAPase$^-$ phenotype of a *pho4* mutant (O'Connell and Baker, 1992). In this context, we speculated that the 6-bp sequence, CACGTG, is not sufficient for the specific recognition of a cognate positive regulator, and that the nucleotide sequences flanking the 6-bp sequence might have important functions in the discrimination of Pho4p and Cpflp. This view was supported by the findings that flanking by a T residue at the 5'-end of the CACGTG motif is preferable for Cpf1p interaction but not for Pho4p interaction (Fisher and Goding, 1992). We found that the 6-bp motif regulated by Pho4p was flanked by a G residue at the 5' end and GG (for type 1 core sequence) or TT (for type 2 core sequence) residues at the 3' end (Fig. 4). Therefore, we proposed that the GCACGTGGG and GCACGTTTT motifs are consensus sequences in the *PHO* promoter (Ogawa et al., 1995b). Recent analysis of a complex of the bHLH domain of Pho4p with UAS DNA by X-ray crystallography supported the significance of the G and GG residues flanking, respectively, at 5'- and 3'-ends of the CACGTG motif (Shimizu et al., 1997). The observation that *CEP1* overexpression could not suppress a *pho4* mutation (O'Connell and Baker, 1992) suggests that Cpf1p has no affinity to the GCACGTTTT sequence of UAS_p1 in the nucleosome-free region (Almer and Hörz, 1986) of the *PHO5* promoter.

Pho2p/Pho4p binding is aided by A/T-rich segment(s). The transcription of all these *PHO* structural genes, except *PHO8*, is dependent on the other positive regulator, Pho2p. Thus, Pho2p might also be involved in the *PHO* regulation. It was demonstrated by use of the two-hybrid system that Pho2p interacts with Pho4p depending on the P_i concentration in the cultivation medium (Hirst et al., 1994) and the accessibility of the activation domain of Pho4p to the transcriptional machinery is enhanced by the interaction with Pho2p (Shao et al., 1996). Barbarić et al. (1996) reported that Pho4p and Pho2p form a ternary complex with UAS DNA and that a possible Pho2p binding site partially overlaps the UAS_p1 site with a GCACGTTTT motif. We could also detect direct interaction between Pho4p and Pho2p using immunoprecipitation and a protein binding assay (Magbanua et al., 1997b). This interaction results in an increased binding affinity of Pho4p to the UAS. Both the Pho4p and Pho2p proteins bind or interact with the basal transcription factors TBP, TFIIB, and TFIIEβ. These observations suggest that the *PHO* gene transcription is initiated by direct contact of the Pho4p/Pho2p complex with the transcription machinery.

In the evaluation of the above 9-bp motifs on their transcriptional activity, we observed that a short A/T-rich segment(s) flanking the 9-bp motif has a role in the binding of Pho2p (Magbanua et al., 1997a), as suggested by Parent et al. (1994). Various 30-bp fragments of different *PHO* promoters, each bearing a 9-bp motif in the middle, and connected with 6-bp *Xho*I cohesive ends, were inserted

into a *CYC1-lacZ* fusion gene on a YCp- or YEp-type plasmid. Then the β-galactosidase activities of the yeast cells with the wild-type or *pho2* disruptant allele and harboring one of the above plasmids were examined. Although the *CYC1* promoter was reported to respond to a *pho2* mutation (Sengstag and Hinnen, 1988), we confirmed that the *CYC1* promoter fragment used was unresponsive to Pho2p and P_i signals. No expression of the *lacZ* gene was detected with the above 36-bp fragment bearing UAS_p2 of *PHO5* which possessed the ACACGTGGG sequence (Magbanua et al., 1997a). Similar 36-bp fragments each bearing UAS_p1 (GCACGTTTT) of *PHO5*, site D (GCACGTGGG) or E (GCACGTTTT) of *PHO84* showed UAS activity in response to P_i concentration in the medium in the wild-type cells, but not in the *pho2* disruptant cells. We found that the Pho2p-responsive UASs are flanked by one or two copies of an A/T-rich segment, whereas UAS_p2 is not (Fig. 5). Gel retardation and competition experiments with Pho2p, tagged with T7 epitope on N-terminal and six histidine residues at C-terminal, showed that the chimeric protein binds to the 36-bp fragments bearing A/T-rich segment(s) but not appreciably to that without A/T-rich segment(s). These facts strongly suggest that the A/T-rich segments flanking the *PHO* UASs are Pho2p binding sites and play an important role in *PHO* regulation.

Pho2p also binds to the *HIS4, ADE2, ADE5,7* and *TRP4* promoters. The Bas1p and Bas2p (= Pho2p) proteins are both required for activation of basal transcription of the *HIS4* gene. The results of DNase I footprinting experiments (Tice-Baldwin et al., 1989) demonstrated that Pho2p binding site, which is rich in A/T pairs, partially overlaps over the Bas1p binding site (Fig. 5). It was known that the core TGACTC sequence alone, to which Bas1p binds, is not sufficient for the proper regulation of the *ADE2* and *ADE5,7* promoters unless a Pho2p binding site is also present (Daignan-Fornier and Fink, 1992). The Bas1p binding sites, shown by the above authors in the *ADE2* and *ADE5,7* promoters, are flanked by one or two copies of A/T-rich segment(s) (Fig. 5). Although the Pho2p binding of the *ADE1* and *ADE8* promoters has not been demonstrated

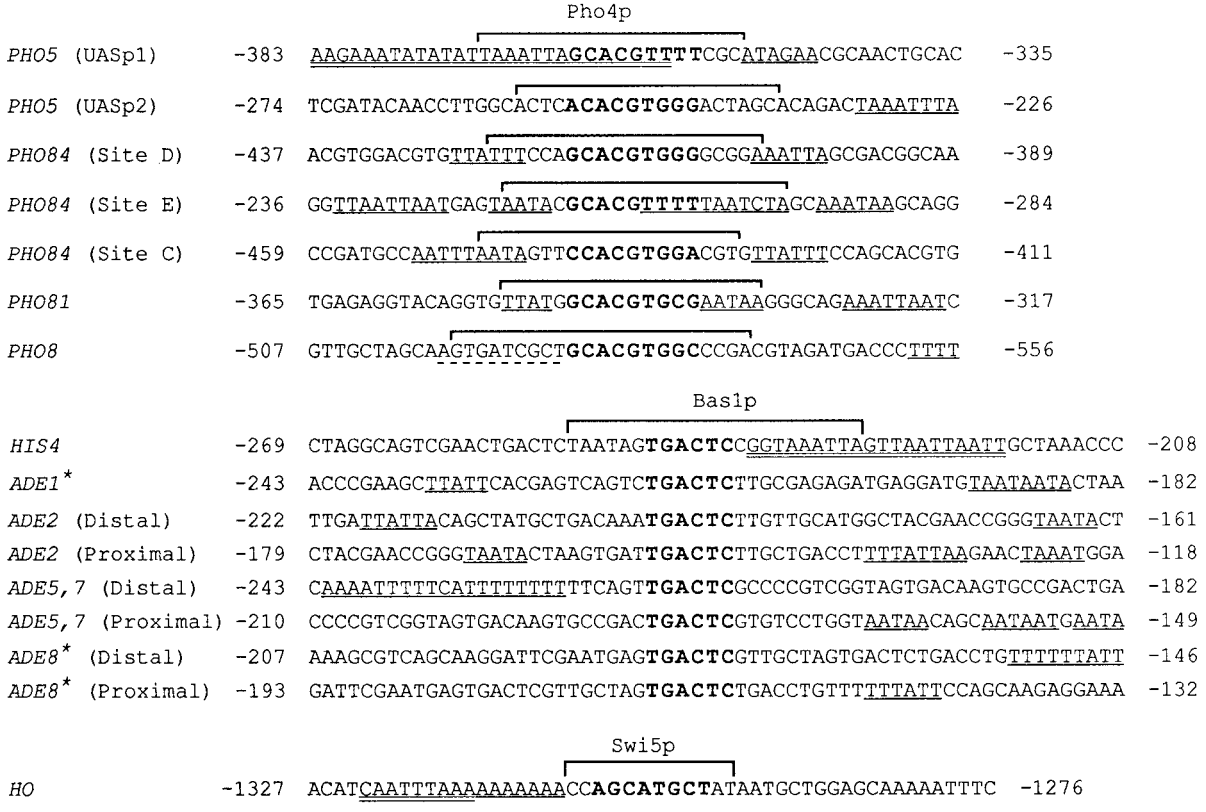

Fig. 5. Alignment of UAS sequences to which relevant regulatory proteins bind together with Pho2p. The consensus sequences in the promoter for Bas1p (Daignan-Fornier and Fink, 1992), Pho4p (Barbarić et al., 1996; Ogawa et al., 1994, 1995b), and Swi5p (Brazas and Stillman, 1993b) were as described. The core UAS sequences for binding of the indicated proteins are written in boldface letters and the regions protected in the footprinting experiments are indicated by brackets. The asterisks indicate that the Bas1p binding sites in the *ADE1* and *ADE8* promoters were identified by sequence comparison (Daignan-Fornier and Fink, 1992), Pho2p binding sites identified in footprinting experiments are indicated by double underlines, while A/T-rich segments are singly underlined. The broken underline indicates the palindromic sequence flanking the 9-bp UAS motif of *PHO8*. The numerals at both ends of the sequences indicate nucleotide position of the 5'- and 3'-ends of the sequences in the promoter of the respective genes.

in vitro, the alignment of their promoter sequences indicates the presence of A/T-rich segment(s) flanking the TGACTC motif (Fig. 5). Pho2p also binds to the promoter of the tryptophan biosynthetic *TRP4* gene at the A/T rich UAS_1 site, to which the general control regulator, Gcn4p, binds (Braus et al., 1989). In this case, Pho2p and Gcn4p, however, bind to the UAS_1 site in a mutually exclusive manner. Pho2p recognizes similar A/T-rich segments at a region adjacent to the Swi5p binding site in the *HO* promoter (Brazas and Stillman, 1993a,b). This was also supported by the experiments with increased spacing between putative Pho2p and Swi5p binding sites with A/T pair of nucleotides in the *HO* promoter (Brazas et al., 1995).

The 36-bp *PHO81* UAS fragment which was found to have 4- or 5-bp A/T segments flanking the 9-bp UAS motif had weak affinity to Pho2p and could not exhibit appreciable UAS activity under the normal dosage of Pho4p (Magbanua et al., 1997a). We speculated that the 4- and 5-bp A/T segments at the *PHO81* UAS are covered completely by Pho4p as it protects a 19-bp region, bearing these A/T segments, from the DNase I digestion (see Fig. 5). Thus, it may result in a reduced level of UAS activity due to the ineffective binding of Pho4p and/or Pho2p to the *PHO81* UAS. The GCAAGTTTT sequence at nucleotide position –234 in the *PHO81* promoter (Ogawa et al., 1993) is flanked with a long A/T-rich segment at the 5' side, but not on the 3' side. In contrast, the similar 36-bp *PHO8* UAS fragment has no apparent A/T-rich segment and no appreciable binding affinity to Pho2p was observed. However, the level of expression of the *CYC1-lacZ* fusion gene inserted with the 36-bp *PHO8* UAS fragment under high-P_i conditions was relatively high and the differential ratio of the *lacZ* expression in low- and high-P_i media was about 2.5 (Magbanua et al., 1997a) as in the case of the genomic *PHO8* expression (Kaneko et al., 1987; Toh-e et al., 1976). This may be due to the fact that the 9-bp UAS of *PHO8* has a 10-bp palindromic sequence, AGTGATCGCT, adjacent to it (Fig. 5). This sequence may respond to another unknown regulatory protein.

CONCLUDING REMARKS

We can now attain substantial understanding into the broad outline of function of the regulatory genes and proteins in the *PHO* system. However, many questions remain unanswered. One of the major unsolved mechanisms is of how extracellular P_i signals are detected. In the recent genetic study of mutants exhibiting a defect in P_i uptake, we could identify three putative membrane proteins, Pho86p, Pho87p, and Pho88p, which might be involved in the P_i uptake mechanism in association with Pho84p and Gtr1p (Bun-ya et al., 1996; Yompakdee et al., 1996a,b). Among them, Pho86p and Pho88p are particularly interesting as they were suggested to modulate Pho81p function in accordance with extracellular P_i concentration.

Another question remaining to be addressed is "what is the consequence of the Pho4p/Pho2p/UAS ternary complex formation in recruiting the transcriptional machinery to the *PHO* genes?". It was argued that nucleosome remodeling is evidently a direct consequence of the recruitment of the transcriptional machinery (Ptashne and Gann, 1997). Hörz and his colleagues have examined this idea using the *PHO5* gene (see review, Svaren and Hörz, 1997). These authors proposed that the *PHO5* promoter is protected by positioned nucleosomes in repressed conditions, except for the UAS_p1 site which is positioned at a nucleosome-free site. Upon derepression of *PHO5*, disruption of nucleosomes occurs due to the binding of Pho4p at the UAS_p1 site, possibly in association with Pho2p. Then the binding of an additional Pho4p molecule at another site, UAS_p2, induces removal of four nucleosomes from the promoter, thereby facilitating the binding of the transcriptional machinery to the promoter. Thus, these authors claimed that the chromatin disruption at the promoter is the prerequisite for transcription of the *PHO5* gene. The powerful combination of genetic and biochemical studies available in *S. cerevisiae* will lead to a more detailed and comprehensive understanding of the above questions in the near future.

Our studies cited in this article were carried out in the Department of Biotechnology (formerly Department of Fermentation Technology), Osaka University in collaboration with A. Toh-e, S. Harashima, Y. Kaneko, K. Yoshida, N. Ogawa, N. Hayashi, M. Bunya, and many students, and were supported by grants for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

REFERENCES

Almer, A., and Hörz, W. (1986) Nuclease hypersensitive regions with adjacent positioned nucleosomes mark the gene boundaries of the PHO5/PHO3 locus in yeast. EMBO J. **5**, 2681–2687.

Arima, K., Oshima, T., Kubota, I., Nakamura, N., Mizunaga, T., and Toh-e, A. (1983) The nucleotide sequence of the yeast *PHO5* gene: a putative precursor of repressible acid phosphatase contains a signal peptide. Nucleic Acids Res. **11**, 1657–1672.

Arndt, K., Styles, C., and Fink, G. R. (1987) Multiple global regulators control *HIS4* transcription in yeast. Science **237**, 874–880.

Arnold, W. N. (1972) Location of acid phosphatase and β-fructofuranosidase within yeast cell envelopes. J. Bacteriol. **112**, 1346–1352.

Bajwa, W., Meyhack, B., Rudolph, H., Schweingruber, A. -M., and Hinnen, A. (1984) Structural analysis of the two tandemly repeated acid phosphatase genes in yeast. Nucleic acids Res. **12**, 7721–7739.

Baker, R. E., and Masison, D. C. (1990) Isolation of the gene encoding the *Saccharomyces cerevisiae* centromere-binding protein CP1. Mol. Cell. Biol. **10**, 2458–2467.

Barbarić, S., Fascher, K. -D., and Hörz, W. (1992) Activation of the weakly regulated *PHO8* promoter in *S. cerevisiae*: chromatin transition and binding sites for the positive regulatory protein PHO4. Nucleic Acids Res. **20**, 1031–1038.

Barbarić S., Münsterkötter, M., Svaren, J., and Hörz, W. (1996) The

homeodomain protein Pho2 and the basic-helix-loop-helix protein Pho4 bind DNA cooperatively at the yeast *PHO5* promoter. Nucleic Acids Res. **24**, 4479–4486.

Bergman, L. W., McClinton, D. C., Madden, S. L., and Preis, L. H. (1986) Molecular analysis of the DNA sequences involved in the transcriptional regulation of the phosphate-repressible acid phosphatase gene (*PHO5*) of *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **83**, 6070–6074.

Boer, P., and Steyn-Parve, E. P. (1966) Isolation and purification of an acid phosphatase from baker's yeast (*Saccharomyces cerevisiae*). Biochim. Biophys. Acta **128**, 400–402.

Bostian, K. A., Lemire, J. M., Cannon, L. E., and Halvorson, H. O. (1980) *In vitro* synthesis of repressible yeast acid phosphatase: identification of multiple mRNAs and products. Proc. Natl. Acad. Sci. USA **77**, 4504–4508.

Braus, G., Mösch, H. -U., Vogel, K., Hinnen, A., and Hütter, R. (1989) Interpathway regulation of the *TRP4* gene of yeast. EMBO J. **8**, 939–945.

Brazas, R. M., Bhoite, L. T., Murphy, M. D., Yu, Y., Chen, Y., Neklason, D. W., and Stillman, D. J. (1995) Determining the requirements for cooperative DNA binding by Swi5p and Pho2p (Grf10p/Bas2p) at the *HO* promoter. J. Biol. Chem. **270**, 29151–29161.

Brazas, R. M., and Stillman, D. J. (1993a) Identification and purification of a protein that binds DNA cooperatively with the yeast SWI5 protein. Mol. Cell. Biol. **13**, 5524–5537.

Brazas, R. M., and Stillman, D. J. (1993b) The Swi5 zinc-finger protein and Grf10 homeodomain proteins bind DNA cooperatively at the yeast *HO* promoter. Proc. Natl. Acad. Sci. USA **90**, 11237–11241.

Bun-ya, M., Harashima, S., and Oshima, Y. (1992) Putative GTP-binding protein, Gtr1, associated with the function of the Pho84 inorganic phosphate transporter in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **12**, 2958–2966.

Bun-ya, M., Nishimura, M., Harashima, S., and Oshima, Y. (1991) The *PHO84* gene of *Saccharomyces cerevisiae* encodes an inorganic phosphate transporter. Mol. Cell. Biol. **11**, 3229–3238.

Bun-ya, M., Shikata, K., Nakade, S., Yompakdee, C., Harashima, S., and Oshima, Y. (1996) Two new genes, *PHO86* and *PHO87*, involved in inorganic phosphate uptake in *Saccharomyces cerevisiae*. Curr. Genet. **29**, 344–351.

Bürglin, T. R. (1988) The yeast regulatory gene *PHO2* encodes a homeo box. Cell **53**, 339–340.

Cai, M., and Davis, R. W. (1990) Yeast centromere binding protein CBF1, of the helix-loop-helix protein family, is required for chromosome stability and methionine prototrophy. Cell **61**, 437–446.

Clark, D. W., Tkacz, J. S., and Lampen, J. O. (1982) Asparagine-linked carbohydrate does not determine the cellular location of yeast vacuolar nonspecific alkaline phosphatase. J. Bacteriol. **152**, 865–873.

Daignan-Fornier, B., and Fink, G. R. (1992) Coregulation of purine and histidine biosynthesis by the transcriptional activators BAS1 and BAS2. Proc. Natl. Acad. Sci. USA **89**, 6746–6750.

de Steensma, H. Y., de Jonge, P., Kaptein, A., and Kaback, D. B. (1989) Molecular cloning of chromosome I DNA from *Saccharomyces cerevisiae*: localization of a repeated sequence containing an acid phosphatase gene near a telomere of chromosome I and chromosome VIII. Curr. Genet. **16**, 131–137.

Feldmann, H., Aigle, M., Aljinovic, G., André, B., Baclet, M.C., Barthe, C., Baur, A., Bécam, A. -M., Biteau, N., Boles, E., Brandt, T., Brendel, M., Brückner, M., Bussereau, F., Christiansen, C., Contreras, R., Crouzet, M., Czieplucha, C., Démolis, N., Delaveau, Th., Doignon, F., Domdey, H., Düsterhus, S., Dubois, E., Dujon, B., El Bakkoury, M., Entian, K. -D., Feuermann, M., Fiers, W., Fobo, G. M., Fritz, C., Gassenhuber, H., Glansdorff, N., Goffeau, A., Grivell, L. A., de Haan, M., Hein, C., Herbert, C. J., Hollenberg, C. P., Holmstrøm, K., Jacq, C., Jacquet, M., Jauniaux, J. C., Jonniaux, J. -L., Kallesøe, T., Kiesau, P., Kirchrath, L., Kötter, P., Korol, S., Liebl, S., Logghe, M., Lohan, A. J. E., Louis, E. J., Li, Z. Y., Maat, M. J., Mallet, L., Mannhaupt, G., Messenguy, F., Miosga, T., Molemans, F., Müller, S., Nasr, F., Obermaier, B., Perea, J., Piérard, A., Piravandi, E., Pohl, F. M., Pohl, T. M., Potier, S., Proft, M., Purnelle, B., Ramezani Rad, M., Rieger, M., Rose, M., Schaaff-Gerstenschläger, I., Scherens, B., Schwarzlose, C., Skala, J., Slonimski, P. P., Smits, P. H. M., Souciet, J. L., Steensma, H. Y., Stucka, R., Urrestarazu, A., van der Aart, Q. J. M., van Dyck, L., Vassarotti, A., Vetter, I., Vierendeels, F., Vissers, S., Wagner, G., de Wergifosse, P., Wolfe, K. H., Zagulski, M., Zimmermann, F. K., Mewes, H. W., and Kleine, K. (1994) Complete DNA sequence of yeast chromosome II. EMBO J. **13**, 5795–5809.

Fisher, F., and Goding, C. R. (1992) Single amino acid substitutions alter helix-loop-helix protein specificity for bases flanking the core CANNTG motif. EMBO J. **11**, 4103–4109.

Fröhlich, K.-U., Fries, H.-W., Rüdiger, M., Erdmann, R., Botstein, D., and Mecke, D. (1991) Yeast cell cycle protein CDC48p shows full-length homology to the mammalian protein VCP and is a member of a protein family involved in secretion, peroxisome formation, and gene expression. J. Cell Biol. **114**, 443–453.

Hayashi, N., and Oshima, Y. (1991) Specific *cis*-acting sequence for *PHO8* expression interacts with PHO4 protein, a positive regulatory factor, in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11**, 785–794.

Hirst, K., Fisher, F., McAndrew, P. C., and Goding, C. R. (1994) The transcription factor, the Cdk, its cyclin and their regulator: directing the transcriptional response to a nutritional signal. EMBO J. **13**, 5410–5420.

Johnston, M., and Carlson, M. (1992) Regulation of carbon and phosphate utilization: In: The Molecular and Cellular Biology of the Yeast *Saccharomyces*: Gene Expression (eds.: E. W. Jones, J. R. Pringle, and J. R. Broach), pp. 193–281. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Kaffman, A., Herskowitz, I., Tjian, R., and O'Shea, E. K. (1994) Phosphorylation of the transcription factor PHO4 by a cyclin-CDK complex, PHO80-PHO85. Science **263**, 1153–1156.

Kaneko, Y., Hayashi, N., Toh-e, A., Banno, I., and Oshima, Y. (1987) Structural characteristics of the *PHO8* gene encoding repressible alkaline phosphatase in *Saccharomyces cerevisiae*. Gene **58**, 137–148.

Kaneko, Y., Toh-e, A., Banno, I., and Oshima, Y. (1989) Molecular characterization of a specific *p*-nitrophenylphosphatase gene, *PHO13*, and its mapping by chromosome fragmentation in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **220**, 133–139.

Kaneko, Y., Toh-e, A., and Oshima, Y. (1982) Identification of the genetic locus for the structural gene and a new regulatory gene for the synthesis of repressible alkaline phosphatase in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **2**, 127–137.

Kawasaki, H., Matsuzaki, H., Nakajima, R., and Oshima, Y. (1991) The *PHO80/TUP7* locus in *Saccharomyces cerevisiae* is on the left arm of chromosome XV: mapping by chromosome engineering. Yeast **7**, 859–865.

Lemire, J. M., Willcocks, T., Halvorson, H. O., and Bostian, K. A. (1985) Regulation of repressible acid phosphatase gene transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **5**, 2131–2141.

Lenburg, M. E., and O'Shea, E. K. (1996) Signaling phosphate starvation. Trends Biochem. Sci. **21**, 383–387.

Linnemans, W. A. M., Boer, P., and Elbers, P. F. (1977) Localization of acid phosphatase in *Saccharomyces cerevisiae*: a clue to cell wall formation. J. Bacteriol. **131**, 638–644.

Lopandic, K., Deana, A. D., Barbarić, S., and Pinna, L. A. (1987)

Biochemical and genetic evidence that yeast extracellular protein phosphatase activity is due to acid phosphatase. Biochem. Int. **14**, 627–633.

Lux, S. E., John, K. M., and Bennett V. (1990) Analysis of cDNA for human erythrocyte ankyrin indicates a repeated structure with homology to tissue-differentiation and cell-cycle control proteins. Nature **344**, 36–42.

Madden, S. L., Johnson, D. L., and Bergman, L. W. (1990) Molecular and expression analysis of the negative regulators involved in the transcriptional regulation of acid phosphatase production in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**, 5950–5957.

Magbanua, J. P. V., Fujisawa, K., Ogawa, N., and Oshima, Y. (1997a) The homeodomain protein Pho2p binds at an A/T-rich segment flanking the binding site of the basic-helix-loop-helix protein Pho4p in the yeast *PHO* promoters. Yeast **13**, 1299–1308.

Magbanua, J. P. V., Ogawa, N., Harashima, S., and Oshima, Y. (1997b) The transcriptional activators of the *PHO* regulon, Pho4p and Pho2p, interact directly with each other and with components of the basal transcription machinery in *Saccharomyces cerevisiae*. J. Biochem. **121**, 1182–1189.

Mellor, J., Jiang, W., Funk, M., Rathjen, J., Barnes, C. A., Hinz, T., Hegemann, J. H., and Philippsen, P. (1990) CPF1, a yeast protein which functions in centromeres and promoters. EMBO J. **9**, 4017–4026.

Mueckler, M., Caruso, C., Baldwin, S. A., Panico, M., Blench, I., Morris, H. R., Allard, W. J., Lienhard, G. E., and Lodish, H. F. (1985) Sequence and structure of a human glucose transporter. Science **229**, 941–945.

Murre, C., and Baltimore, D. (1992) The helix-loop-helix motif: structure and function: In: Transcriptional Regulation (eds.: S. L. McKnight and Y. R. Yamamoto), pp. 861–879. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Nakao, J., Miyanohara, A., Toh-e, A., and Matsubara, K. (1986) *Saccharomyces cerevisiae PHO5* promoter region: location and function of the upstream activation site. Mol. Cell. Biol. **6**, 2613–2623.

Nishimura, H., Kawasaki, Y., Kaneko, Y., Nosaka, K., and Iwashima, A. (1992a) Cloning and characteristics of a positive regulatory gene, *THI2* (*PHO6*), of thiamine biosynthesis in *Saccharomyces cerevisiae*. FEBS Lett. **297**, 155–158.

Nishimura, H., Kawasaki, Y., Kaneko, Y., Nosaka, K., and Iwashima, A. (1992b) A positive regulatory gene, *THI3*, is required for thiamine metabolism in *Saccharomyces cerevisiae*. J. Bacteriol. **174**, 4701–4706.

Nosaka, K. (1990) High affinity of acid phosphatase encoded by *PHO3* gene in *Saccharomyces cerevisiae* for thiamin phosphates. Biochim. Biophys. Acta **1037**, 147–154.

O'Connell, K. F., and Baker, R. E. (1992) Possible cross-regulation of phosphate and sulfate metabolism in *Saccharomyces cerevisiae*. Genetics **132**, 63–75.

O'Connell, K. F., Surdin-Kerjan, Y., and Baker, R. E. (1995) Role of the *Saccharomyces cerevisiae* general regulatory factor CP1 in methionine biosynthetic gene transcription. Mol. Cell. Biol. **15**, 1879–1888.

Ogas, J., Andrews, B. J., and Herskowitz, I. (1991) Transcriptional activation of *CLN1, CLN2*, and a putative new G1 cyclin (*HCS26*) by SWI4, a positive regulator of G1-specific transcription. Cell **66**, 1015–1026.

Ogawa, N., Hayashi, N., Saito, H., Noguchi, K., Yamashita, Y., and Oshima, Y. (1994) Regulatory circuit for phosphatase genes in *Saccharomyces cerevisiae*: specific *cis*-acting sites in *PHO* promoters for binding the positive regulator Pho4p: In: Phosphate in Microorganisms: Cellular and Molecular Biology (eds.: A. Torriani-Gorini, E. Yagil, and S. Silver), pp. 56–62. American Society for Microbiology, Washington, D. C.

Ogawa, N., Noguchi, K., Sawai, H., Yamashita, Y., Yompakdee, C., and Oshima, Y. (1995a) Functional domains of Pho81p, an inhibitor of Pho85p protein kinase, in the transduction pathway of P_i signals in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **15**, 997–1004.

Ogawa, N., Noguchi, K., Yamashita, Y., Yasuhara, T., Hayashi, N., Yoshida, K., and Oshima, Y. (1993) Promoter analysis of the *PHO81* gene encoding a 134 kDa protein bearing ankyrin repeats in the phosphatase regulon of *Saccharomyces cerevisiae*. Mol. Gen. Genet. **238**, 444–454.

Ogawa, N., and Oshima, Y. (1990) Functional domains of a positive regulatory protein, PHO4, for transcriptional control of the phosphatase regulon in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10**, 2224–2236.

Ogawa, N., Saitoh, H., Miura, K., Magbanua, J.P.V., Bun-ya, M., Harashima, S., and Oshima, Y. (1995b) Structure and distribution of specific *cis*-elements for transcriptional regulation of *PHO84* in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **249**, 406–416.

Okada, H., and Toh-e, A. (1992) A novel mutation occurring in the *PHO80* gene suppresses the *PHO4*^{c} mutations of *Saccharomyces cerevisiae.* Curr. Genet. **21**, 95–99.

O'Neill, E. M., Kaffman, A., Jolly, E.R., and O'Shea, E. K. (1996) Regulation of PHO4 nuclear localization by the PHO80-PHO85 cyclin-CDK complex. Science **271**, 209–212.

O'Neill, E. M., and O'Shea, E. K. (1995) Cyclins in initiation. Nature **374**, 121–122.

Onishi, H. R., Tkacz, J. S., and Lampen, J. O. (1979) Glycoprotein nature of yeast alkaline phosphatase: formation of active enzyme in the presence of tunicamycin. J. Biol. Chem. **254**, 11943–11952.

Oshima, Y. (1982) Regulatory circuits for gene expression: the metabolism of galactose and of phosphate: In: The Molecular Biology of the Yeast Saccharomyces: Metabolism and Gene Expression (eds.: J. N. Strathern, E. W. Jones, and J. R. Broach), pp. 159–180. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Oshima, Y. (1991) Impact of the Douglas-Hawthorne model as a paradigm for elucidating cellular regulatory mechanisms in fungi. Genetics **128**, 195–201.

Parent, S. A., Justice, M. C., Yuan, L. -W., Hopper, J. E., and Bostian, K. A. (1994) Protein-DNA and protein-protein interactions regulating the phosphatase multigene family of *Saccharomyces cerevisiae*: In: Phosphate in Microorganisms: Cellular and Molecular Biology (eds.: A. Torriani-Gorini, E. Yagil, and S. Silver), pp. 63–69. American Society for Microbiology, Washington, D. C.

Plankert, U., Purwin, C., and Holzer, H. (1991) Yeast fructose-2,6-bisphosphate 6-phosphatase is encoded by *PHO8*, the gene for nonspecific repressible alkaline phosphatase. Eur. J. Biochem. **196**, 191–196.

Ptashne, M., and Gann, A. (1997) Transcriptional activation by recruitment. Nature **386**, 569–577.

Rudolph, H., and Hinnen, A. (1987) The yeast *PHO5* promoter: phosphate-control elements and sequences mediating mRNA start-site selection. Proc. Natl. Acad. Sci. USA **84**, 1340–1344.

Schneider, K. R., Smith, R. L., and O'Shea, E. K. (1994) Phosphate-regulated inactivation of the kinase PHO80-PHO85 by the CDK inhibitor PHO81. Science **266**, 122–126.

Schweingruber, M. E., Fluri, R., Maudrell, K., Schweingruber, A. -M., and Dumermuth, E. (1986) Identification and characterization of thiamin repressible acid phosphatase in yeast. J. Biol. Chem. **261**, 15877–15882.

Sengstag, C., and Hinnen, A. (1987) The sequence of the *Saccharomyces cerevisiae* gene *PHO2* codes for a regulatory protein with unusual aminoacid composition. Nucleic Acids Res. **15**, 233–

246.

Sengstag, C., and Hinnen, A. (1988) A 28-bp segment of the *Saccharomyces cerevisiae PHO5* upstream activator sequence confers phosphate control to the *CYC1-lacZ* gene fusion. Gene **67**, 223–228.

Serrano, M., Hannon, G. J., and Beach, D. (1993) A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature **366**, 704–707.

Shao, D., Creasy, C. L., and Bergman, L. W. (1996) Interaction of *Saccharomyces cerevisiae* Pho2 with Pho4 increases the accessibility of the activation domain of Pho4. Mol. Gen. Genet. **251**, 358–364.

Shimizu, T., Toumoto, A., Ihara, K., Shimizu, M., Kyogoku, Y., Ogawa, N., Oshima, Y., and Hakoshima, T. (1997) Crystal structure of PHO4 bHLH domain-DNA complex: flanking base recognition. EMBO J. **16**, 4689–4697.

Svaren, J., and Hörz, W. (1997) Transcription factors vs nucleosomes: regulation of the *PHO5* promoter in yeast. Trends Biochem. Sci. **22**, 93–97.

Tait-Kamradt, A. G., Turner, K. J., Kramer, R. A., Elliott, Q. D., Bostian, S. J., Thill, G. P., Rogers, D. T., and Bostian, K. A. (1986) Reciprocal regulation of the tandemly duplicated *PHO5*/*PHO3* gene cluster within the acid phosphatase multigene family of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**, 1855–1865.

Tamai, Y., Toh-e, A., and Oshima, Y. (1985) Regulation of inorganic phosphate transport systems in *Saccharomyces cerevisiae*. J. Bacteriol. **164**, 964–968.

Thierry, A., Fairhead, C., and Dujon, B. (1990) The complete sequence of the 8.2 kb segment left of *MAT* on chromosome III reveals five ORFs, including a gene for a yeast ribokinase. Yeast **6**, 521–534.

Tice-Baldwin, K., Fink, G. R., and Arndt, K. T. (1989) BAS1 has a Myb motif and activates *HIS4* transcription only in combination with BAS2. Science **246**, 931–935.

Toh-e, A., Kakimoto, S., and Oshima, Y. (1975a) Two new genes controlling the constitutive acid phosphatase synthesis in Saccharomyces cerevisiae. Mol. Gen. Genet. **141**, 81–83.

Toh-e, A., Kakimoto, S., and Oshima, Y. (1975b) Genes coding for the structure of the acid phosphatase in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **143**, 65–70.

Toh-e, A., Nakamura, H., and Oshima, Y. (1976) A gene controlling the synthesis of non specific alkaline phosphatase in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **428**, 182–192.

Toh-e, A., and Oshima, Y. (1975) Regulation of acid phosphatase synthesis in *Saccharomyces cerevisiae*: In: Proc. 1st Intersectional Congress of IAMS, Vol. 1 (ed.: T. Hasegawa), pp. 396–399. Science Council of Japan, Tokyo.

Toh-e, A., and Shimauchi, T. (1986) Cloning and sequencing of the *PHO80* gene and *CEN15* of *Saccharomyces cerevisiae*. Yeast **2**, 129–139.

Toh-e, A., Tanaka, K., Uesono, Y., and Wickner, R. B. (1988) *PHO85*, a negative regulator of the PHO system, is a homolog of the protein kinase gene, *CDC28*, of *Saccharomyces cerevisiae.* Mol. Gen. Genet. **214**, 162–164.

Uesono, Y., Tanaka, K., and Toh-e, A. (1987) Negative regulators of the PHO system in *Saccharomyces cerevisiae*: isolation and structural characterization of *PHO85*. Nucleic Acids Res. **15**, 10299–10309.

Uesono, Y., Tokai, M., Tanaka, K., and Toh-e, A. (1992) Negative regulators of the PHO system of *Saccharomyces cerevisiae*: characterization of *PHO80* and *PHO85*. Mol. Gen. Genet. **231**, 426–432.

Venter, U., and Hörz, W. (1989) The acid phosphatase genes PHO10 and PHO11 in *S. cerevisiae* are located at the telomeres of chromosomes VIII and I. Nucleic Acids Res. **17**, 1353–1369.

Vogel, K., Hörz, W., and Hinnen, A. (1989) The two positively acting regulatory proteins PHO2 and PHO4 physically interact with *PHO5* upstream activation regions. Mol. Cell. Biol. **9**, 2050–2057.

Yompakdee, C., Bun-ya, M., Shikata, K., Ogawa, N., Harashima, S., and Oshima, Y. (1996a) A putative new membrane protein, Pho86p, in the inorganic phosphate uptake system of *Saccharomyces cerevisiae*. Gene **171**, 41–47.

Yompakdee, C., Ogawa, N., Harashima, S., and Oshima, Y. (1996b) A putative membrane protein, Pho88p, involved in inorganic phosphate transport in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **251**, 580–590.

Yoshida, K., Kuromitsu, Z., Ogawa, N., and Oshima, Y. (1989a) Mode of expression of the positive regulatory genes *PHO2* and *PHO4* of the phosphatase regulon in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **217**, 31–39.

Yoshida, K., Ogawa, N., and Oshima, Y. (1989b) Function of the *PHO* regulatory genes for repressible acid phosphatase synthesis in *Saccharomyces cerevisiae*. Mol. Gen. Genet. **217**, 40–46.