Possible Cross-Regulation of Phosphate and Sulfate Metabolism in *Saccharomyces cerevisiae*

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ABSTRACT

CP1 (encoded by the gene *CEP1*) is a sequence-specific DNA binding protein of *Saccharomyces cerevisiae* that recognizes a sequence element (CDEI) found in both yeast centromeres and gene promoters. Strains lacking CP1 exhibit defects in growth, chromosome segregation and methionine biosynthesis. A YEp24-based yeast genomic library was screened for plasmids which suppressed the methionine auxotrophy of a *cep1* null mutant. The suppressing plasmids contained either *CEP1* or DNA derived from the *PHO4* locus. Subcloning experiments confirmed that suppression correlated with increased dosage of *PHO4*. *PHO4*^{c}, *pho80* and *pho84* mutations, all of which lead to constitutive activation of the PHO4 transcription factor, also suppressed *cep1* methionine auxotrophy. The suppression appeared to be a direct effect of PHO4, not a secondary effect of *PHO* regulon derepression, and was *PHO2*-dependent. Spontaneously arising extragenic suppressors of *cep1* methionine auxotrophy were also isolated; approximately one-third of them were alleles of *pho80*. While *PHO4* overexpression suppressed the methionine auxotrophy of a *cep1* mutant, *CEP1* overexpression failed to suppress the phenotype of a *pho4* mutant; however, a *cep1* null mutation suppressed the low inorganic phosphate growth deficiency of a *pho84* mutant. The results may suggest that phosphate and sulfate metabolism are cross-regulated.

CP1 is a sequence-specific DNA binding protein of *Saccharomyces cerevisiae* that recognizes the degenerate octanucleotide sequence RTCACRTG (R = purine). While CP1 binding sites are scattered throughout the yeast genome, their occurrence in two locations appears to have functional relevance. The site is present in all *S. cerevisiae* centromeres, where it comprises the 100% conserved *c*entromere *D*NA *e*lement I (CDEI) (FITZGERALD-HAYES, CLARKE and CARBON 1982; HIETER *et al.* 1985). CDEI motifs are also found in the promoter regions of many yeast genes, including *GAL2*, *TRP1*, nuclear genes encoding imported mitochondrial proteins and methionine biosynthetic genes (BAKER, FITZGERALD-HAYES and O'BRIEN 1989; BRAM and KORNBERG 1987; DORSMAN, VAN HEESWIJK and GRIVELL 1988; THOMAS, CHEREST and SURDIN-KERJAN 1989). The wide distribution of CDEI sites and the abundance of CP1 (greater than 500 molecules per cell) led BRAM and KORNBERG (1987) to speculate that CP1 might act as a general facilitator of protein-DNA interactions and be involved in a variety of chromatin-related processes. This idea was reinforced by the finding that strains carrying disruptions of the gene encoding CP1–designated *CEP1* (also *CBF1* and *CPF1*)–exhibit multiple defects. The *cep1* mutant phenotype includes increased rates of mitotic chromosome loss, decreased growth rate, and methionine auxotrophy (BAKER and MASISON 1990; CAI and DAVIS 1990; MELLOR *et al.* 1990).

Several studies have shown that optimal centromere function requires an intact CDEI. Mutation or deletion of CDEI from the centromere results in 3–70-fold increases in mitotic chromosome loss rates (CUMBERLEDGE and CARBON 1987; GAUDET and FITZGERALD-HAYES 1989; HEGEMANN *et al.* 1988), and the magnitude of the effect is correlated with decreased CP1 binding affinity (BAKER, FITZGERALD-HAYES and O'BRIEN 1989; CAI and DAVIS 1989). The *trans* mutation, *i.e.*, disrupting *CEP1*, has a quantitatively similar consequence; the mitotic chromosome loss rate is increased 9–25-fold (BAKER and MASISON 1990; CAI and DAVIS 1990). When the effects of *cis* and *trans* mutation are compared directly, they are found to be equivalent and nonadditive (BAKER and MASISON 1990). Biochemical experiments have shown that CDEI sites are protein-bound *in vivo* and that CP1 is required to maintain normal chromatin structure in the CDEI region (DENSMORE, PAYNE and FITZGERALD-HAYES 1991; MELLOR *et al.* 1990). Taken together, these results indicate that the role of CDEI in the assembly and/or function of the *S. cerevisiae* centromere (kinetochore) is mediated through CP1 and that lack of CP1 interaction at centromeric CDEI sites accounts for the chromosome loss phenotype of *cep1* null mutants.

A second role for CP1 may be inferred from its amino acid sequence. CP1 belongs to the helix-loop-helix (HLH) family of DNA-binding proteins (CAI and DAVIS 1990), a family predominated by known or suspected transcriptional regulators (*e.g.*, MyoD, *myc*,

daughterless, E12/E47, AP-4, USF) (GREGOR, SAWADOGO and ROEDER 1990; HU *et al.* 1990; SUN and BALTIMORE 1991). Members of this class of proteins all share a region of homology spanning 60 amino acid residues, predicted to form two amphipathic helices separated by a loop (MURRE, MCCAW and BALTIMORE 1989). Most also contain a region rich in basic amino acid residues immediately preceding the HLH domain (DAVIS *et al.* 1990). All HLH family members which bind DNA recognize the core consensus sequence CANNTG (CAI and DAVIS 1990; LASSAR *et al.* 1989); this element is contained within CDEI. *S. cerevisiae* appears to contain other HLH factors. One of them, the product of the gene *PHO4*, contains an HLH-adjacent basic region highly similar to CP1 (BERBEN *et al.* 1990; DANG *et al.* 1992; FISHER, JAYARAMAN and GODING 1991; MELLOR *et al.* 1990). PHO4 protein is a positive activator of genes involved in phosphate metabolism and appears to be a transcription factor in the conventional sense, *i.e.*, it binds to its cognate site in DNA and, interacting directly or indirectly with RNA polymerase II, stimulates transcription initiation (HAYASHI and OSHIMA 1991; OGAWA and OSHIMA 1990; VOGEL, HÖRZ and HINNEN 1989).

CDEI sites are found in the promoter regions of a number of genes; however, the occurrence of CDEI sites near genes involved in methionine biosynthesis is particularly striking. Almost every *MET* gene sequenced to date contains at least one CDEI site in its 5′-flanking DNA. These genes include *MET2*, *MET3*, *MET25*, *MET8*, *MET4*, *MET14* and *MET16*, as well as *SAM2* which encodes S-adenosylmethionine synthetase (CHEREST, THOMAS and SURDIN-KERJAN 1990; KORCH, MOUNTAIN and BYSTRÖM 1991; THOMAS, BARBEY and SURDIN-KERJAN 1990; THOMAS, CHEREST and SURDIN-KERJAN 1989; THOMAS, JACQUEMIN and SURDIN-KERJAN 1992). Given that strains lacking CP1 are methionine auxotrophs and that CP1 appears to bind the *MET25* CDEI sites *in vivo* (MELLOR *et al.* 1990), a circumstantial case exists for CP1 acting as a transcriptional regulator of *MET25* and the other coordinately controlled *MET* genes. Recently, direct evidence to confirm this hypothesis has been obtained. THOMAS, JACQUEMIN and SURDIN-KERJAN (1992) have discovered that the *MET16* gene, which encodes phosphoadenylylsulfate (PAPS) reductase, requires CP1 for its expression. Neither MET16 enzyme activity nor *MET16* mRNA are detectable in *cp1* mutants, suggesting that CP1 regulation is exerted at the level of transcription. These authors also report a threefold decrease in MET25 (homocysteine synthase) activity and a corresponding decrease in *MET25* mRNA levels. MELLOR *et al.* (1991) also observed decreased *MET25* mRNA levels in *cp1* mutants. The *cp1* strains additionally lack sulfate permease activity, but this deficiency may be secondary to the lesion blocking PAPS reductase expression since *met16* mutants themselves lack sulfate permease activity (BRETON and SURDIN-KERJAN 1977; THOMAS, JACQUEMIN and SURDIN-KERJAN 1992).

Here we describe two genetic approaches we have taken to characterize the methionine auxotrophy of *cp1* null mutants. In one case, we screened a yeast multicopy plasmid gene bank for plasmids which rescued methionine prototrophy, in the other, we isolated spontaneously arising Met^+ pseudorevertants. Rather than leading to one or more *MET* genes, both lines of investigation led to genes regulating phosphate metabolism and in particular the key regulator *PHO4*. Our results indicated that the transcription factor encoded by *PHO4* can functionally substitute for CP1 in regulating methionine biosynthesis and that CP1 levels effect expression of the *PHO* regulon. We discuss the possibility that phosphate and sulfate metabolism may be cross-regulated.

MATERIALS AND METHODS

Strains, media and general methods: Yeast strains used in this study are listed in Table 1. Strain YPH98 (SPENCER *et al.* 1990) was obtained from P. HIETER, strains NBW7, NBD4-1 and NBD82-1 (OGAWA and OSHIMA 1990) from Y. OSHIMA, and strains h-A and 5-43 from L. BERGMAN. The *pho3* and *pho5* mutations present in diploid K52 originated from strain GG100-14D (BERGMAN 1986) obtained from D. TIPPER. All other strains were constructed in our laboratory using standard genetic methods. Yeast transformations were performed by the lithium acetate procedure (ITO *et al.* 1983) as modified by SCHIESTL and GIETZ (1989). *Escherichia coli* strain RR1 was the host for isolating and maintaining all plasmids. Plasmids were rescued from yeast transformants using a modification of the procedure of BIRNBOIM and DOLY (1979) as follows. Cells from 1.5 ml of a selectively grown culture were pelleted and resuspended in 100 μl of 1.2 M sorbitol-0.12 M K_2HPO_4-0.033 M citric acid (pH 5.9) containing 2.5 mg/ml Zymolyase-100T. After incubating 10–30 min to obtain spheroplasts, the BIRNBOIM and DOLY procedure was followed from the alkaline lysis step.

Media were as described (BAKER and MASISON 1990) except for inorganic phosphate (P_i)-depleted YEPD which was prepared as described by RUBIN (1974) and adjusted to pH 4.7. Synthetic media used for growing cells for acid phosphatase assays contained 0.17% P_i-depleted yeast nitrogen base (lacking amino acids and ammonium sulfate), 25 mM sodium citrate (pH 4.7), and 2% glucose. Amino acids (40 μg/ml), adenine (20 μg/ml) and uracil (20 μg/ml) were added as needed. For the experiments reported in Tables 2 and 3, the same medium, supplemented with methionine and differing only in phosphate content, was used for all strains. High phosphate, 3/5 phosphate, and low phosphate media contained 1500 mg, 900 mg and 20 mg KH_2PO_4 per liter, respectively. In addition, KCl was added to 3/5 phosphate, and low phosphate media at 600 mg and 1,500 mg per liter, respectively. Inorganic phosphate was depleted from yeast nitrogen base by precipitation as $MgNH_4PO_4$ as follows. For a 10 × stock solution, 8.5 g of yeast nitrogen base was dissolved in 400 ml water. Fifty milliliters each of 1 M $MgSO_4$ and concentrated NH_4OH were added and the solution stirred at room temperature for 30 min. The precipitate was removed by filtering the solution two successive

TABLE 1

Strains

Strain	Genotype[a]
D1-1C	*MATα cry1 his4-580 lys2 trp1 SUP4-3 ade2-1 leu2 ura3-52 ade3 cep1::URA3-11*
D1-6C	*MATα cry1 his4-580 lys2 trp1 SUP4-3 ade2-1 leu2 ura3-52 ade3*
SMAF13α	*MATα cry1 his4-580 lys2 trp1 SUP4-3 ade2-1 leu2 ura3-52 ade3 cep1::URA3-11 sma1-F13*
R31-3BR	*MATα leu2Δ1 lys2-801 trp1Δ1 ura3-52 ade2-101 his3Δ200 cep1::TRP1*
R31-5C	*MATa leu2Δ1 lys2-801 trp1Δ1 ura3-52 ade2-101 his3Δ200 cep1::TRP1 CFVII (RAD2.d.YPH277)*
R31-1A	*MATα leu2Δ1 lys2-801 trp1Δ1 ura3-52 ade2-101 his3Δ200 CFVII (RAD2.d.YPH277)*
h-A	*MATα leu2Δ1 lys2-801 trp1Δ1 ura3-52 ade2-101 his3Δ200 pho80::LEU2*
5-43	*MATα leu2Δ1 lys2-801 trp1Δ1 ura3-52 ade2-101 his3Δ200 pho2::LEU2*
NBD82-1	*MATa leu2-3,112 pho3-1 trp1-289 ura3-1,2 can1 PHO4^c-1 ade2 his3-532*
K22-T8	*MATa leu2-3,112 pho3-1 trp1-289 ura3-1,2 can1 PHO4^c-1 ade2 his3-532 cep1::TRP1*
NBD4-1	*MATa leu2-3,112 pho3-1 trp1-289 ura3-1,2 can1 ade2 his3-532 pho4::HIS3*
NBW7	*MATa leu2-3,112 pho3-1 trp1-289 ura3-1,2 can1 ade2 his3-532*
K43-T1	*MATa leu2-3,112 pho3-1 trp1-289 ura3-1,2 can1 ade2 his3-532 cep1::TRP1*
K6	*MATa/MATα cry1/cry1 HIS4/his4-580 lys2/lys2 SUP4-3/SUP4-3 ade2-1/ade2-1 leu2/leu2 ura3-52/ura3-52 ade3/ade3 trp1::LEU2/TRP1 can1/CAN1 TYR1/tyr1 cep1::ura3/cep1::URA3-11 cyh2/CYH2 SMA1/sma1-F13*
K23	*MATa/MATα leu2Δ1/leu2Δ1 lys2-801/lys2-801 trp1Δ1/trp1Δ1 ura3-52/ura3-52 ade2-101/ade2-101 his3Δ200/ his3Δ200 cep1::TRP1/CEP1 pho80::LEU2/PHO80 CFVII (RAD2.d.YPH277)*
R33R63	*MATa/MATα leu2Δ1/leu2Δ1 lys2-801/lys2-801 trp1Δ1/trp1Δ1 ura3-52/ura3-52 ade2-101/ade2-101 his3Δ200/HIS3 cep1::TRP1/cep1::TRP1*
K37	*MATa/MATα leu2Δ1/leu2Δ1 lys2-801/lys2-801 trp1Δ1/trp1Δ1 ura3-52/ura3-52 ade2-101/ade2-101 his3Δ200/ his3Δ200 cep1::TRP1/cep1::TRP1 sma1-1c/SMA1 CYH2/cyh2 PHO81/pho81::HIS3 CFVII (RAD2.d.YPH277)*
K39	*MATa/MATα leu2Δ1/leu2Δ1 lys2-801/lys2-801 trp1Δ1/trp1Δ1 ura3-52/ura3-52 ade2-101/ade2-101 his3Δ200/ his3Δ200 cep1::TRP1/cep1::TRP1 PHO2/pho2::LEU2 PHO80/pho80::LEU2 CFVII (RAD2.d.YPH277)*
K45	*MATa/MATα leu2Δ1/leu2Δ1 lys2-801/lys2-801 trp1Δ1/trp1Δ1 ura3-52/ura3-52 ade2-101/ade2-101 his3Δ200/ his3Δ200 cep1::TRP1/CEP1 pho80::LEU2/PHO80 PHO84/pho84::URA3*
K47	*MATa/MATα cry1/CRY1 HIS3/his3Δ200 his4-580(?)/HIS4 lys2/lys2-801 TRP1/trp1Δ1 SUP4-3/sup4$^+$ ade2-1/ade2-101 leu2/leu2Δ1 ura3-52/ura3-52 can1/CAN1 ade3/ADE3 cep1::URA3-11/cep1::TRP1 PHO80/pho80::LEU2 sma1-F13/SMA1*
K52	*MATa/MATα leu2Δ1/leu2Δ1 lys2-801/lys2-801 trp1Δ1/trp1 ura3-52/ura3-52 ade2-101/ADE2 his3Δ200/his3 cep1::TRP1/CEP1 pho80::LEU2/PHO80 pho3pho5/PHO3PHO5 CFVII (RAD2.d.YPH277)*

[a] *CFVII (RAD2.d.YPH277)* is a supernumerary chromosome fragment derived from chromosome *VII* and carrying *URA3* and *SUP11* (SPENCER *et al.* 1990).

times through Whatman No. 1 filter paper, and the filtrate was adjusted to pH 4.7 with concentrated HCl.

Gene disruptions: The *cep1::URA3* allele was described previously (BAKER and MASISON 1990). A *cep1::TRP1* disruption allele was constructed by replacing 620-bp of *CEP1* coding region [between SspI sites at nucleotides 409 and 1029 (BAKER and MASISON 1990)] with the *Eco*RI/*Bgl*II restriction fragment of the yeast *TRP1* gene using *Xho*I linkers. This construct (contained in plasmid pRB101) was used to replace the endogenous *CEP1* gene in strains YPH98, NBW7 and NBD82-1 by homologous recombination (ROTHSTEIN 1983) to create strains R31-3B, K43-T1 and K22-T8, respectively.

To construct the *pho84* disruption allele, a segment of the *PHO84* gene extending from the *Hin*dIII site at position 644 to the *Xba*I site at position 1674 (BUN-YA *et al.* 1991) was obtained by amplification of yeast genomic DNA using the polymerase chain reaction and inserted into the polylinker of pUC18. The segment extending from the *Hpa*I site (position 867) to the *Bgl*II site (position 1465) was then removed and replaced with the yeast *URA3* gene. The resulting *pho84::URA3* disruption allele was excised from the polylinker and used to transform diploid yeast strain K23, selecting for uracil prototrophy. Tetrad analysis of one of the transformants (strain K45-T1) indicated that one of the two *PHO84* loci had been replaced by the disrupted version, and Ura$^+$ segregants were Pho$^-$, *i.e.*, they fail to grow on P_i-depleted medium.

Isolation of plasmid suppressors: Plasmid suppressors were isolated in two separate screens of the YEp24-based (*URA3*) yeast genomic library of CARLSON and BOTSTEIN (1982). In the first screen, diploid R33R63 was transformed to uracil prototrophy and Met$^+$ colonies were identified by replica plating onto media lacking both uracil and methionine. Plasmid dependence of the Met$^+$ phenotype was tested by plating the transformants on medium containing 5-fluoro-orotic acid to select for cells having lost the plasmid (BOEKE, LACROUTE and FINK 1984) and then redetermining the Met phenotype. Three of 8200 transformants had acquired a Met$^+$ phenotype that was plasmid-dependent. The three plasmids were rescued into *E. coli*. Restriction enzyme analysis revealed that two of the three plasmids were identical and all contained *CEP1*.

In the second screen, haploid strain R31-3BR was transformed and transformants were selected directly on medium lacking both uracil and methionine. An aliquot of the transformation mix was plated on uracil single drop-out medium to estimate transformation frequency. Of a predicted 11,300 transformants, four grew on double selection medium. When tested for plasmid-dependence, the Met$^+$ and Ura$^+$ phenotypes cosegregated in all four cases. Restriction analysis of the rescued plasmids revealed that two of the plasmids (pMAC2-1, pMAC2-2) were identical and contained an insert which overlapped that of the third plasmid (pMAC3-2). The fourth plasmid (pMAC1-3) was unique. None of the plasmids contained *CEP1*. Upon retransformation, pMAC1-3 was not able to reproducibly confer the Met$^+$ phenotype, and it was not analyzed further.

Isolation of Met$^+$ pseudorevertants: Pseudorevertants were isolated in two different strain backgrounds, D1-1C (*cep1::URA3*) and R31-5C (*cep1::TRP1*). In the first screen, cells from 12 independent stocks of D1-1C were spread onto methionine dropout plates. Met$^+$ colonies arose after 3–4 days at a frequency of $1–4 \times 10^{-5}$. Eighteen isolates (12 independent) were chosen for analysis. Each pseudorevertant was backcrossed to test for dominance, and the resulting diploids were sporulated to recover all 18 suppressors in a background of the opposite mating type. A complete complementation matrix was obtained. All 18 suppressors were recessive, and 6 of them defined a single complementation group that we designated *sma1* (*s*uppressor of *cep1* *m*ethionine *a*uxotrophy). The remaining suppressors were weak and appeared to be under mating type control, because suppression was only observed in a haploid genetic background. That is, *MAT***a** *smaX* and *MATα smaX* strains were Met$^+$, but *MAT***a**/*MATα smaX*/*smaX* diploids were Met$^-$.

In a second screen, cells from a single culture of R31-5C were plated on methionine dropout plates, and Met$^+$ colonies arose at a frequency of 4×10^{-5}. Twenty-three isolates (not necessarily independent) were analyzed as before. Thirteen of the 23 suppressors were were recessive, and 8 of them failed to complement *sma1*. The non-*sma1* suppressors were weak and they were not characterized further. Pseudorevertants were customarily maintained as patches on methionine dropout plates; when spread at low cell density (*e.g.*, streaking for single colonies) plating efficiency was poor.

Acid phosphatase assays: Acid phosphatase activity was quantitated using whole cells as the enzyme source. Strains were pregrown to near saturation in media containing 3/5 the normal amount of phosphate. Cells were then diluted at least 100-fold into either high or low phosphate media and grown to an OD_{650} of between 0.5 and 4.5. Phosphatase activity was assayed using *p*-nitrophenylphosphate as substrate. The reaction mixture contained 0.1 M sodium acetate (pH 4.2), 4.5 mg/ml *p*-nitrophenylphosphate (Sigma), and up to 0.1 ml culture in a total reaction volume of 0.50 ml. After incubation for 10 min at 37°, reactions were terminated by the addition of 0.72 ml saturated sodium carbonate and the cells removed by centrifugation. The amount of *p*-nitrophenol produced was determined by measuring absorbance at 420 nm. One unit of activity is defined as 1 μmol of *p*-nitrophenol liberated per min.

RESULTS

A multicopy suppressor of *cep1* methionine auxotrophy: In an attempt to identify the gene or genes limiting the growth of *cep1* disruption strains on medium lacking methionine, we screened for yeast plasmids which would suppress *cep1* methionine auxotrophy when present at high copy. In two screens of a YEp24-based gene bank (Carlson and Botstein 1982), four different plasmids were obtained which conferred methionine prototrophy (see Materials and Methods). Restriction analysis revealed that two of the suppressing plasmids contained *CEP1*. The other two plasmids (pMAC2-1 and pMAC3-2) contained overlapping inserts apparently unrelated to *CEP1*. A hybridization probe prepared from the insert of pMAC2-1 was used to probe a blot of electrophoretically separated yeast chromosomes. The probe hybridized to chromosome *VI* (not shown). A subsequent survey of cloned chromosome *VI* genes revealed a striking similarity between the restriction map of the 8.6-kbp pMAC2-1 insert and that of the *CDC26-PHO4-UCR6* locus (Figure 1A). Several pMAC2-1 subclones were tested, and the Met$^+$ phenotype was found to correlate with the presence of *PHO4* (Figure 1A). Suppression does not require many additional copies of *PHO4*, because a subclone of *PHO4* carried on a low copy centromere-containing vector (pKO27) was sufficient to rescue methionine prototrophy (Figure 1C). To verify that pKO27 carried a functional*PHO4*, allele, we tested its ability to complement a *pho4* mutation. Strain NBD4-1 (*pho4::HIS3*) was transformed with plasmid pKO27, a multicopy *CEP1* plasmid (pMAC3-1), and a vector control (pRIP1). Only pKO27 rescued the ability to grow on medium depleted of inorganic phosphate (Figure 1B). Thus, 1–2 extrachromosomal copies of *PHO4* are sufficient to suppress the methionine auxotrophy of a *cep1* mutant, but multiple copies of *CEP1* are unable to suppress the Pho$^-$ phenotype of a *pho4* mutant.

The Met$^+$ phenotype correlates with derepression of the *PHO* regulon: *PHO4* is part of a regulatory network consisting of several genes (Toh-e 1989; Vogel and Hinnen 1990). *PHO80* is a negative regulatory element whose gene product is thought to function by sequestering PHO4 protein in an inactive form when P_i is not limiting. When P_i becomes limiting, the repressive effect of PHO80 is relieved and PHO4 becomes free to activate transcription of several target genes. The response to P_i limitation requires the product of *PHO81*, which appears to be a sensor of intracellular P_i levels. Among the genes activated by PHO4 are *PHO5* and *PHO84*. *PHO5* encodes a repressible acid phosphatase (rAPase) and *PHO84* a low K_m phosphate permease (Bun-Ya *et al.* 1991). The expression of both *PHO5* and *PHO84* requires a second factor encoded by *PHO2* (also known as *BAS2* or *GRF10*) (Arndt, Styles and Fink 1987; Tamai, Toh-e and Oshima 1985; Yoshida, Ogawa and Oshima 1989).

To determine if *PHO5* was derepressed in *cep1* strains carrying extrachromosomal copies of *PHO4*, rAPase levels were measured in transformants of a *cep1* strain carrying single or multicopy *PHO4* plasmids and grown in high (repressing) P_i media (Table 2). The host strain used for these experiments also carried a *pho3* mutation, eliminating interference from the constitutive acid phosphatase encoded by this gene. Transformants carrying the CEN plasmid pKO27 expressed normal repressed levels of rAPase, but cells carrying the multicopy *PHO4* plasmid pKO17 expressed significantly elevated levels of rAPase. Therefore, overexpression of *PHO4* in these strains leads to derepression of *PHO5* and presumably other genes activated by PHO4. Also, while overexpression of *PHO4* suppresses *cep1* methionine auxotrophy, the elevated level of PHO4 apparently is insufficient to

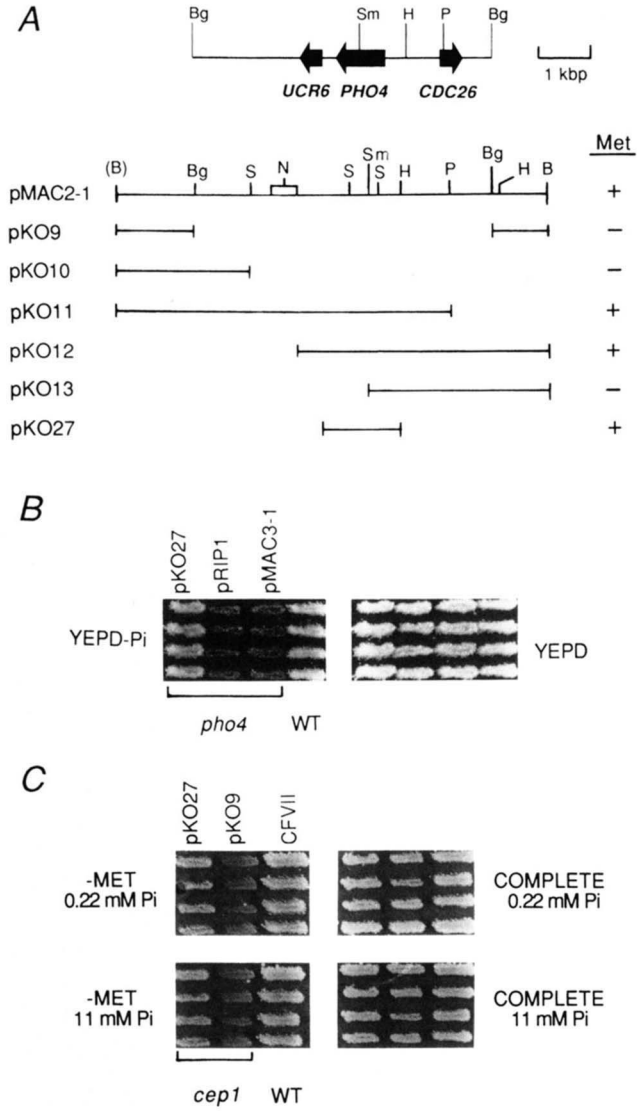

FIGURE 1.—Analysis of plasmid suppressor pMAC2-1. (A) Restriction maps of the pMAC2-1 insert and the *PHO4* locus (OGAWA and OSHIMA 1990). Subclones tested for suppressor function are diagrammed below the pMAC2-1 map. The lines represent the DNA present in each construct. Plasmids pKO9, pKO10, pKO11, pKO12, and pKO13 were obtained by digesting pMAC2-1 with *Bgl*II, *Sph*I, *Pvu*II, *Nhe*I, and *Sma*I respectively, and religating. Plasmid pKO17 was obtained by inserting the 3.0 kbp *Nhe*I-*Pvu*II fragment of pKO11 between the *Xba*I and *Sma*I sites of YEp352 (HILL *et al.* 1986). Plasmid pKO27 contains the 1.5-kbp *Acc*I-*Hin*dIII fragment (*Acc*I end filled in by Klenow polymerase) inserted between the *Hin*dIII and *Sma*I sites of pRIP1 (PARKER and JACOBSON 1990). Restriction sites: B, *Bam*HI; Bg, *Bgl*II; H, *Hin*dIII; N, *Nhe*I; P, *Pvu*II; S, *Sph*I; and Sm, *Sma*I. (B) Complementation of a *pho4* mutation. The host strain NBD4-1 (*pho4::HIS3*) was transformed with the CEN plasmids pKO27 and pRIP1 (vector control), and the episomal plasmid pMAC3-1 which carries *CEP1*. Transformants were tested for growth on YEPD medium depleted of inorganic phosphate (YEPD-P_i). (C) Growth of *cep1* strains in media lacking methionine at high and low P_i concentrations. Host strain R31-3BR (*cep1*) was transformed with either pKO27 or pKO9 (control), and transformants tested on methionine dropout plates containing the indicated amounts of P_i. The isogenic wild-type strain (WT) is R31-1A which carries a chromosome fragment (CFVII) marked with *URA3* (Table 1). The plates were photographed after 3 days of growth at 30°.

TABLE 2

rAPase activity of *PHO4* plasmid-bearing strains

Strain	Genotype [plasmid]	Growth conditions	APase activity[a]
NBW7	*pho3*	Hi P_i	0.92 ± 0.09 (3)
NBW7	*pho3*	Lo P_i	84.1 ± 2.8 (3)
K43-T1	*pho3 cep1*	Hi P_i	0.60 ± 0.05 (4)
K43-T1	*pho3 cep1*	Lo P_i	39.7 ± 4.2 (4)
K43-T1	*pho3 cep1* [pRIP1]	Hi P_i	1.13 ± 0.89 (4)
K43-T1	*pho3 cep1* [pKO27]	Hi P_i	0.60 ± 0.15 (4)
K43-T1	*pho3 cep1* [YEp352]	Hi P_i	0.34 ± 0.08 (4)
K43-T1	*pho3 cep1* [pKO17]	Hi P_i	5.48 ± 1.2 (4)

[a] mU/OD_{660} cells; mean ± SD (No. determinations).

TABLE 3

rAPase activities

Strain	Genotype[a]	APase activity[b]
NBW7	*pho3*	0.92 ± 0.09 (3)
NBD82-1	*pho3 PHO4^c*	10.7 ± 1.2 (3)
K22-T8	*pho3 PHO4^c cep1*	5.85 ± 1.3 (5)
K23-4A	Wild-type	4.84 ± 0.54 (4)
K23-4B	*pho80*	171 ± 16 (6)
K23-4C	*cep1*	6.47 ± 0.37 (4)
K23-4D	*cep1 pho80*	88.6 ± 11 (6)
D1-6C	Wild-type	4.82 ± 2.0 (3)
K6-25B	*cep1*	5.38 ± 1.1 (3)
K6-25A	*cep1 sma1*	53.1 ± 5.7 (3)
K45T1-4B	*pho84*	73.9 ± 8.1 (4)
K45T2-1D	*cep1 pho84*	39.6 ± 4.5 (4)

Growth conditions: high P_i.
[a] Complete genotypes given in Table 1.
[b] mU/OD_{660} cells; mean ± SD (No. determinations).

derepress *PHO5* to the level achievable through P_i limitation.

The rAPase derepression in cells overexpressing *PHO4* probably occurs due to an imbalance between the level of PHO4 and that of its negative regulator PHO80 (YOSHIDA, OGAWA and OSHIMA 1989). Since mutations in either *PHO80* or *PHO4* can also cause inappropriate *PHO* gene derepression (OGAWA and OSHIMA 1990), we tested two such mutations to determine if they would suppress *cep1* methionine auxotrophy. Strain NBD82-1 carries the *PHO4^c-1* allele. Repressible APase activity in this strain is derepressed in high phosphate medium by approximately 10-fold compared to the wild-type strain NBW7 (Table 3). A *PHO4^c-1 cep1::TRP1* double mutant was obtained by disrupting *CEP1* in NBD82-1. The disruptant (K22-T8) was phenotypically Met$^+$, although not to the full extent of a wild-type strain (Figure 2B), and it expressed rAPase constitutively (Table 3). The level of rAPase expression in the *cep1 PHO4^c* double mutant was only about half that of the *CEP1 PHO4^c* strain NBD82-1. When K22-T8 was backcrossed to a *cep1::URA3* strain, Met$^+$ and Pho^c phenotypes cosegregated (not shown). To prove that suppression was genetically linked to *PHO4^c-1*, K22-T8 (*PHO4^c-1 cep1::TRP1*) was mated with a *pho4::HIS3 cep1::URA3*

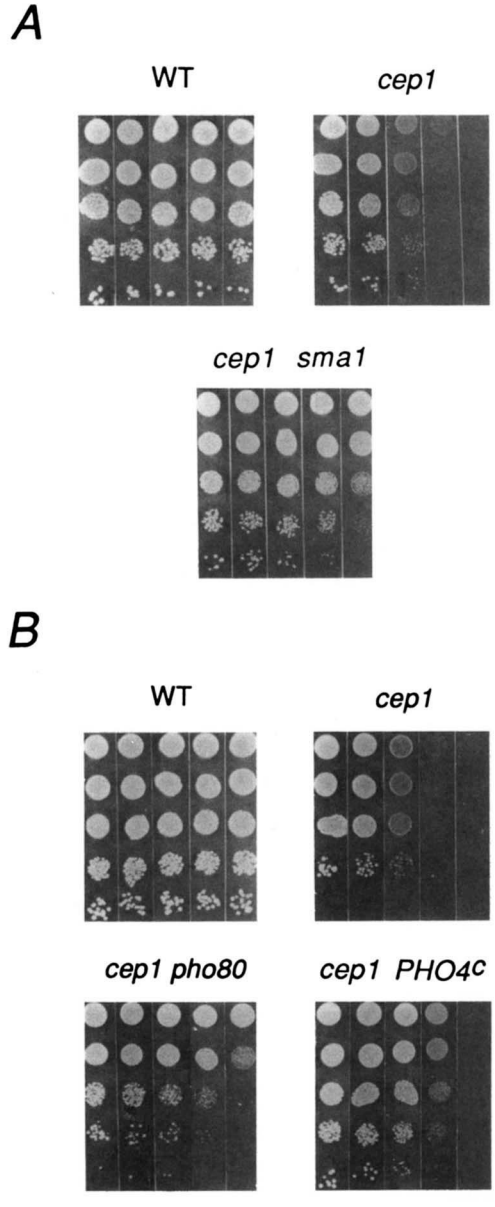

FIGURE 2.—Met phenotypes of suppressor strains. After growth in complete synthetic media to near saturation, cells were pelleted and resuspended in water to a density of 2×10^7 cells/ml. Tenfold serial dilutions of each were prepared and 10-μl aliquots of the undiluted and diluted samples were spotted on plates containing various concentrations of methionine. Plates were photographed after 3 days of growth at 30°. Each panel is a composite photograph for each strain. From left to right in each panel, medium containing 300, 30, 3, 0.3 and 0 μM methionine. Normal synthetic medium contains 270 μM methionine. (A) D1-6C (wild-type), D1-1C (*cep1::URA3*), F13α (*cep1::URA3 sma1*). (B). R31-1A (wild-type), R31-3BR (*cep1::TRP1*), K23-4D (*cep1::TRP1 pho80::LEU2*), K22-T8 (*cep1::TRP1 PHO4c-1*).

strain and segregation analysis performed. Fifty of 51 His^- segregants were Met^+, while 50 of 50 His^+ segregants were Met^-, demonstrating tight linkage between suppression and *PHO4c*.

Next we tested whether loss of the negative regulator PHO80 would result in suppression. Diploid K23 is heterozygous for null alleles of both *cep1* and *pho80*. Tetrad analysis of K23 revealed that the Met^+ phenotype segregated predominantly $3^+:1^-$ (Table 4), as would be expected if the *pho80::LEU2* allele suppressed the methionine auxotrophy of the *cep1::TRP1* segregants, *i.e.*, all of the *CEP1* and half of the *cep1::TRP1* segregants are Met^+. All spores cosegregating *cep1::TRP1* and *pho80::LEU2* (*i.e.*, Trp^+ Leu^+) were Met^+ (30/30). The suppressed phenotype of a typical *pho80::LEU2 cep1::TRP1* segregant is shown in Figure 2B. The phosphatase activities of spores obtained from a tetratype K23 tetrad are given in Table 3. Again, derepression of rAPase correlates with suppression of *cep1* methionine auxotrophy (K23-4D), and the level of rAPase in the *cep1* background is approximately half that of the wild-type segregant (compare strains K23-4D and K23-4B). The higher background activity of acid phosphatase in these strains (about 5 mU/OD_{660} cells) was due to the presence of an active *PHO3* allele.

The prototrophy of *cep1 PHO4c* and *cep1 pho80* double mutants indicated that a single chromosomal copy of *PHO4* was sufficient to suppress *cep1* methionine auxotrophy when negative regulation by *pho80* was abrogated. Next we asked if the auxotrophy could be suppressed under normal physiological conditions, *i.e.*, by limiting phosphate. A master plate containing wild-type and *cep1* strains was replicated onto a series of plates containing various concentrations of methionine and P_i. As shown in Figure 1C, the wild-type strain and a *cep1* strain carrying *PHO4* on a CEN plasmid (pKO27) were able to grow in the absence of added methionine at both high and low P_i concentrations, but neither of the conditions tested allowed growth of a *cep1* strain carrying only the control plasmid (pKO9). Colony staining (TOH-E and OSHIMA 1974) demonstrated that rAPase was indeed derepressed in all strains on the low P_i plate, so while sufficient to derepress *PHO5*, these conditions were insufficient to achieve suppression of *cep1* methionine auxotrophy by PHO4.

Spontaneous suppressors of *cep1* methionine auxotrophy: In working with *cep1* deletion mutants, we had observed that patches of cells replicated to methionine dropout plates frequently gave rise to Met^+ papillae. Since true reversion would be impossible, these pseudorevertants were presumed to contain unlinked suppressor mutations. To analyze the phenomenon in more detail, a number of independent pseudorevertants, isolated in two different *cep1* genetic backgrounds, were characterized (see MATERIALS AND METHODS). The pseudorevertants arose at a frequency of $1\text{–}4 \times 10^{-5}$, and approximately one-third of them defined a single recessive complementation group we named *sma1* (*s*uppressor of *cep1* *m*ethionine *a*uxotrophy). Meiotic mapping revealed that *sma1* was tightly centromere-linked, displaying a second division segregation frequency of only 0.7% (ditype:tetratype, 157:2) when scored against *trp1*. [The second division

TABLE 4

Segregation analysis

Strain	Genotype[a]	Tetrad class (Met^+:Met^-)				
		4:0	3:1	2:2	1:3	0:4
K23	*cep1/CEP1 pho80/PHO80*	5	20	3	0	0
K47	*cep1/cep1 sma1/SMA1 pho80/PHO80*	18[b]	1	0	0	0
K37	*cep1/cep1 sma1/SMA1 pho81/PHO81*	0	0	15	0	0
K39	*cep1/cep1 pho80/PHO80 pho2/PHO2*	0	0	2	16	1
K52	*cep1/cep1 pho80/PHO80 pho3pho5/PHO3PHO5*	0	0	15	0	0
K45	*cep1/CEP1 pho80/PHO80 pho84/PHO84*	23	28	1	0	0

[a] Complete genotypes given in Table 1.
[b] Pho^c segregated 4:0 in 19/19 tetrads.

segregation of *trp1* is itself 0.9% (MORTIMER and HAWTHORNE 1969).] Suppression by *sma1* was quantitatively similar to that observed with *PHO4*^{c} and *pho80::LEU2* (Figure 2). The non-*sma1* suppressor mutations conferred a very weak Met^+ phenotype which made further analysis difficult, and they were not studied further.

Acid phosphatase assays revealed that *sma1* strains had a *Pho*^{c} phenotype; rAPase levels were derepressed about 10-fold under high P_i growth conditions (Table 3, K6–25B *vs.* K6–25A). This suggested that *sma1* might act through PHO4. Genetic tests confirmed that suppression by *sma1* was *PHO4*-dependent; *cep1 sma1 pho4* triple mutants were Met^- (not shown). The recessive Pho^c phenotype, *PHO4*-dependence, and tight centromere linkage led us to suspect that *sma1* mutations might be alleles of *pho80*. To test this, a *cep1 sma1* strain was crossed to a *cep1 pho80::LEU2* strain (diploid K47). The Pho^c phenotype segregated 4^+:0^- in 19 of 19 tetrads and 75 of 76 segregants were Met^+ (Table 4). Since *sma1* and *pho80* did not cosegregate, we concluded that they are allelic.

Epistasis studies: The preceding experiments demonstrated a clear correlation between derepression of the *PHO* regulon (assessed by rAPase levels) and the suppression of *cep1* methionine auxotrophy. But are these two phenotypes independent, or are they causally linked? To address this question, we investigated whether or not *PHO* genes other than *PHO4* were required for the observed suppression. First we tested the regulatory genes *PHO81* and *PHO2*. Diploid K37 is homozygous for *cep1* and heterozygous for both *sma1* and *pho81::HIS3*. K37 tetrads segregated methionine prototrophy 2:2 (Table 4), and about half (16/30) of the His^+ spores were also Met^+. This result indicated that suppression by *sma1* (*pho80*) was independent of *PHO81*. *PHO2* dependence was tested in the same manner. Diploid K39 is homozygous for *cep1* and heterozygous for *pho80::LEU2* and *pho2::LEU2*. In contrast to the *pho81* heterozygote, K39 segregated methionine prototrophy mostly 1^+:3^-, and all (38/38) *pho2* spores were Met^- (Table 4). [*PHO2* was scored by assaying growth on P_i-depleted medium.] This segregation pattern indicated that suppression by *pho80* required cosegregation of the wild-type *PHO2* allele. In the single tetrad where Leu^+ segregated 2:2 and the two Leu^+ spores were necessarily *pho80::LEU2 pho2::LEU2* double mutants, both were Met^-. These results demonstrate that the gene product of *PHO2* but not *PHO81* is required for the *PHO4*-dependent suppression of *cep1* methionine auxotrophy.

Next we tested *PHO5* and *PHO84*, two downstream targets of *PHO4*. While it was not obvious how *PHO5* (rAPase) derepression could affect methionine biosynthesis, prototrophy correlated perfectly with high rAPase levels and *PHO5* transcription was known to be *PHO2*-dependent. Tetrad analysis of diploid K52 (*cep1/cep1 pho80::LEU2/PHO80 pho5/PHO5*) ruled out the formal possibility that suppression required *PHO5*. Methionine prototrophy segregated 2:2, and all (30/30) *pho80* (Leu^+) spores were Met^+ regardless of their allele at *pho5* (Table 4). The rationale for testing *PHO84* stemmed from the finding of THOMAS, JACQUEMIN and SURDIN-KERJAN (1992) that *cep1* mutants lacked sulfate permease activity. Since *PHO84* encodes a phosphate permease, one possible suppression mechanism would be gratuitous sulfate transport via the *PHO84* permease. Also, *PHO84* expression is *PHO2*-dependent. To test for *PHO84*-dependence of suppression, *PHO84* was disrupted in strain K23. K23 is heterozygous for both *cep1::TRP1* and *pho80::LEU2* and segregates Met^+ mostly 3^+:1^- (above). The resulting strain (K45) was thus triply heterozygous-*pho84::URA3*/+, *cep1::TRP1*/+, and *pho80::LEU2*/+. If *PHO84* were not required for suppression (by *pho80*), K45 should yield tetrads in which methionine prototrophy segregates 3^+:1^- (as for K23), and all Leu^+ spores would be Met^+. If, on the other hand, *PHO84* were required for suppression, the frequency of Met^+ spores would be reduced, and the proportion of tetrads segregating Met 3^+:1^- would be significantly decreased. Unexpectedly, the segregation pattern observed corresponded to neither of these predictions. Methionine prototrophy segregated mostly 4^+:0^- and 3^+:1^- (Table 4), suggesting that *pho84* was itself a suppressor of *cep1* methionine auxotrophy. Indeed,

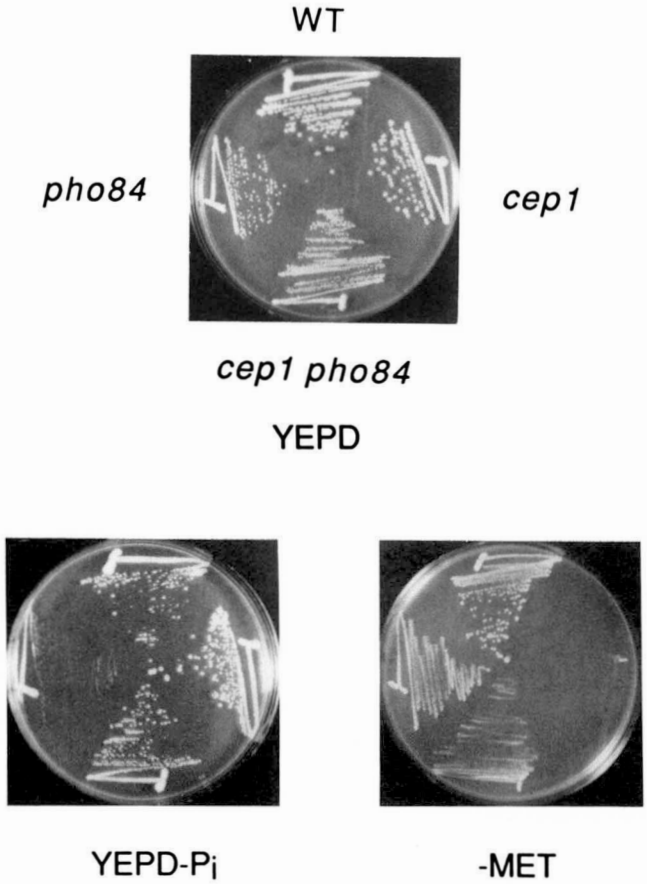

FIGURE 3.—Mutual suppression by *cep1* and *pho84*. Selected segregants of K45 were streaked on synthetic media lacking methionine and YEPD medium depleted of P_i. Plates were incubated at 30° for 3 days (YEPD and YEPD-P_i) or 5 days (-Met).

all (104/104) Leu$^+$ (*pho80*) spores were Met$^+$ regardless of their *PHO84* allele, and virtually all (103/104) Ura$^+$ (*pho84*) spores were Met$^+$ regardless of their *CEP1* allele. Again suppression correlated with derepression of the *PHO* regulon, as all Ura$^+$ segregants had elevated rAPase activities. Acid phosphatase activities of two *pho84::URA3* segregants are reported in Table 3. As before, the presence of the *cep1* allele reduced rAPase activity by about fifty percent. A second surprise was that *cep1 pho84* double mutants were Pho$^+$, indicating that *cep1* suppressed *pho84* for the ability to grow on P_i-depleted medium. The mutual suppression of *cep1* and *pho84* is shown in Figure 3.

DISCUSSION

Three separate lines of evidence lead to the conclusion that activation of the transcription factor PHO4 in *cep1* null mutants results in suppression of *cep1* methionine auxotrophy. First, the presence of one or more extrachromosomal copies of *PHO4* in the *cep1* background confers the Met$^+$ phenotype; the resulting overexpression of *PHO4* presumably upsets the balance between PHO4 and its negative regulator PHO80, creating a pool of active transcription factor. Second, mutations which disrupt or eliminate *PHO80* regulation (*e.g.*, *PHO4^c*, *pho80*) also suppress *cep1* methionine auxotrophy, and a significant portion (about 30%) of independent spontaneously arising suppressors are alleles of *pho80*. Third, disruption of *PHO84*, which results in lowered intracellular P_i levels and constitutive activation of PHO4, suppresses *cep1* auxotrophy. In all cases (except for the weak suppression observed when *PHO4* is carried on a CEN plasmid), suppression correlates with increased rAPase expression indicative of *PHO* regulon derepression. However, suppression appears to be a direct effect of PHO4. Neither *PHO5* (rAPase) nor *PHO84* (phosphate permease), two downstream targets of PHO4, are required for the *PHO4*-dependent suppression. The most straightforward interpretation of these findings is that an active PHO4 transcription factor can functionally substitute for CP1 in regulating methionine biosynthesis.

One condition where PHO4 activation does not result in suppression is when *cep1* mutants are grown in medium containing derepressing concentrations of P_i. Although rAPase is derepressed, no growth occurs in the absence of methionine. Quantitation of acid phosphatase levels reveals that *cep1* strains grown in complete synthetic media at low P_i concentrations contain rAPase activities higher than isogenic strains carrying a *PHO4^c* mutation or extra plasmid copies of *PHO4*. Thus, if rAPase levels are a reliable measure of PHO4 activity, cells starved for P_i appear to possess the requisite PHO4 activity. It is possible that under certain circumstances rAPase activity fails to accurately reflect PHO4 activity. Cultures of plasmid-bearing cells are heterogeneous with respect to plasmid copy number; therefore, the rAPase activity determined for a strain grown under selection for the plasmid may underestimate the true activity of the methionine prototrophs. In the case of the *PHO4^c* strain (K22-T8), it is possible that an unlinked mutation affects *PHO5* expression without affecting suppression. Consistent with this idea, when K22-T8 was backcrossed to a *cep1 PHO4$^+$* strain, the diploid gave rise to some Met$^+$ segregants with very high phosphatase activities (data not shown). Alternatively, it is possible that the rAPase activity determined for *cep1* strains grown under derepressing conditions overestimates the actual activity which would be present in cells grown in the absence of methionine. Since *cep1* strains do not grow in the absence of methionine, rAPase activity had to be measured for cells grown in complete medium.

The biochemical basis of *cep1* methionine auxotrophy is not completely understood, but *cep1* null mutants lack two activities which are essential for methionine biosynthesis, sulfate permease and PAPS reductase. Both deficiencies are explained by the inability of *cep1* cells to transcribe *MET16*, the gene encoding

PAPS reductase (THOMAS, JACQUEMIN and SURDIN-KERJAN 1992). While the cis regulatory sequences of *MET16* have not been identified, the gene 5′-flanking DNA contains a CP1 binding site, and one obvious model for CP1 action would be that CP1 binds to the *MET16* promoter and directly stimulates transcription by RNA polymerase II. The finding that PHO4 can functionally substitute for CP1 in activating methionine biosynthesis is consistent with this notion. Both proteins are members of the HLH DNA binding protein family and recognize similar sites in DNA. Recently, domain-swap experiments have demonstrated that the basic region of the c-*Myc* protein can functionally substitute for the corresponding domains of both CP1 and PHO4 (DANG *et al.* 1992; FISHER, JAYARAMAN and GODING 1991). All three proteins bind the sequence CACGTG which is found in the *MET16* promoter. The basic region of AP-4, which recognizes the sequence CAGCTG, could not substitute for the basic region of CP1. These results imply that specific recognition of CACGTG by CP1 (or PHO4) is required for methionine prototrophy.

Other results argue against CP1's playing a direct role in transcription activation. CP1, unlike PHO4, appears to lack a transcription activation domain. Specifically, lexA-CP1 fusion proteins fail to activate transcription of a lexA binding site-driven reporter gene (THOMAS, JACQUEMIN and SURDIN-KERJAN 1992). Also, PHO4 and CP1 are not interchangeable, otherwise *CEP1* overexpression should suppress the Pho^- phenotype of a *pho4* mutant. While lack of mutual suppression could be explained by differences in binding specificity, it could also indicate a fundamental difference in the mechanism by which CP1 and PHO4 activate transcription.

CP1 may not be a transcription factor in the traditional sense. CP1 is a member of a growing class of yeast DNA-binding proteins known as general regulatory factors. Other examples include the gene products of *RAP1*, *REB1* and *ABF1*. All of these factors are moderately abundant (100–1000 copies per cell), bind at diverse genomic locations, and in many cases activate or repress transcription (BUCHMAN *et al.* 1988; JU, MORROW and WARNER 1990). RAP1 protein is required for *GCN4*- or *BAS1/BAS2*-driven transcription of *HIS4*, and *HIS4* activation by either mechanism appears to require a chromatin structure ordered by RAP1 (DEVLIN *et al.* 1991). [RAP1 is not required when *GCN4* and *BAS1/BAS2* pathways are both intact.] REB1 and ABF1 proteins appear to stimulate transcription synergistically in concert with other activators (BUCHMAN and KORNBERG 1990; CHASMAN *et al.* 1990). As with RAP1, the mechanism is probably tied to chromatin configuration, since REB1 (also known as GRF2) is a strong positioner of nucleosomes (FEDOR, LUE and KORNBERG 1988). CP1 may act similarly to stimulate transcription at *MET* gene promoters. The yeast *MET4* gene encodes a leucine zipper transcription factor which interacts with a UAS element in the *MET25* promoter (THOMAS, JACQUEMIN and SURDIN-KERJAN 1992). THOMAS *et al.* find that this UAS contains a CP1 binding site and that *MET4* activation is strongly facilitated by CP1. It is not yet known whether the MET4 factor binds directly to the UAS or whether additional factors are required, but additional study of this promoter and the strongly CP1-dependent *MET16* promoter should help us to understand the role of CP1 in transcription activation and elucidate the mechanism of suppression by PHO4.

The *PHO2*-dependence of *cep1* suppression is interesting. While the *PHO2* requirement may be *PHO4*-specific (*e.g.*, stabilization of PHO4 binding), it may also reflect the general regulatory role of PHO2 itself. PHO2 is the same as BAS2, which was originally identified as a factor required for basal level transcription of *HIS4* and probably one or more genes involved in adenine biosynthesis (ARNDT, STYLES and FINK 1987). BRAUS *et al.* (1989) have shown that *PHO2* modulates transcription of *TRP4*, and they suggest that PHO2 is a general regulator of cellular metabolism in response to phosphate availability. Perhaps it is not coincidental then that HIS4 and TRP4 enzymes catalyze reactions involving phosphorylated substrates. Methionine biosynthesis requires two phosphorylated intermediates, adenylylsulfate (APS) and phosphoadenylylsulfate (PAPS). PAPS is a general sulfate donor and is the direct substrate of PAPS reductase (*MET16*), the enzyme absent in *cep1* mutants. The finding that suppression of *cep1* methionine auxotrophy requires *PHO2* may be a consequence of normal *MET16* regulation by *PHO2*.

These results may suggest the existence of regulatory cross-talk between the biochemical pathways utilizing sulfate and phosphate. Under every condition of *PHO* regulon derepression examined–*PHO4*^{c}, *pho80*, *pho84* mutations and low P_i growth conditions–rAPase activity was reduced approximately twofold in the *cep1* genetic background; therefore, *cep1* gene disruption leads not only to a block in sulfate assimilation but also a perturbation in *PHO* gene expression. The *cep1* null mutation also suppresses the Pho^- phenotype of a *pho84* (phosphate permease) mutant. Since transport is the first step in metabolite utilization, it is well suited as a regulatory point. The mutual suppression of *cep1* and *pho84* may reflect coordination of the two pathways at this step. *PHO84* expression is *PHO2*-dependent, and if *PHO2* also regulates sulfate permease, this could explain the *PHO2* dependence of *cep1* suppression. None of our results rule out the possibility that *PHO4* itself coregulates *MET* genes. HLH factors are known to form heteromers with other HLH family members (BRAUN *et al.* 1990; DAVIS *et al.* 1990; MURRE *et al.* 1989), and heteromerization

can alter DNA binding specificity and/or transcriptional activation potential (BENEZRA *et al.* 1990; BLACKWELL and WEINTRAUB 1990; SUN and BALTIMORE 1991). PHO4 might regulate *MET* gene transcription as a heterodimer with CP1. The PHO4 component would provide transcription activation function and the CP1 component would block PHO80 interaction and direct binding to *MET* gene promoters. In *pho4* mutants (which are not methionine auxotrophs), *MET* gene activation could still be effected by the CP1 homodimer, while in *cep1* mutants, PHO4 homodimers would substitute when negative regulation by PHO80 is relieved. We have noticed that the *MET25* UAS identified by THOMAS, CHEREST and SURDIN-KERJAN (1989) contains the sequence AAATGGCACGT which, allowing a one nucleotide insertion, matches the PHO4 binding site UAS_P1 in the *PHO5* promoter at 10 of 11 positions (VOGEL, HÖRZ and HINNEN 1989). We are currently interested in testing if this homology has any functional significance, and whether *MET* and *PHO* genes utilize common transcription factors.

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