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Interpathway regulation of the *TRP4* gene of yeast

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Two regulatory proteins, PHO2 and the general control regulator GCN4, bind *in vitro* to the promoter of the tryptophan biosynthetic *TRP4* gene; the *TRP4* gene product catalyses the phosphoribosylation of anthranilate. PHO2 binds specifically to the *TRP4* promoter, but does not bind to any other *TRP* promoter. PHO2 and GCN4 proteins bind in a mutually exclusive manner to the same sequence, UAS_1, one of two GCN4 binding sites in the *TRP4* promoter. UAS_1 is the major site for GCN4-dependent *TRP4* activation. The second GCN4 binding site, UAS_2, interacts with GCN4 alone. PHO2 binding interferes with the general control response of *TRP4* under low phosphate conditions and simultaneous amino acid starvation and thus the PHO2 regulatory protein connects phosphate metabolism and amino acid biosynthesis in yeast. The GCN4 protein mediates the response of the transcriptional apparatus to the environmental signal 'amino acid limitation', while PHO2 seems to be the phosphate sensor that adjusts the response to the availability of phosphate precursors.

Key words: amino acid biosynthesis/DNA binding proteins/phosphate metabolism/*Saccharomyces cerevisiae*/ transcriptional regulation

Introduction

The living cell adjusts its pattern of transcription in response to environmental signals. Transcriptional regulation is mediated by site-specific DNA binding proteins. Specific regulatory sites in the promoters of the target genes allow binding of the cognate regulatory proteins. The presence of these regulators bound to promoters enables or inhibits initiation of transcription. In complex cases, activators and repressors can bind to the same DNA sequence as described for the phage lambda repressor and cro protein (Ptashne *et al.*, 1980) and for the yeast activator HAP1 and a factor of unknown function, RC2 (Pfeifer *et al.*, 1987).

In *Saccharomyces cerevisiae* the regulator protein GCN4 is necessary for the response to amino acid starvation and turns on transcription of numerous amino acid biosynthetic genes (Hinnebusch and Fink, 1983). The optimal promoter binding site for the GCN4 protein is the well characterized palindrome 5'ATGA(C/G)TCAT3' (Hope and Struhl, 1985; Hill *et al.*, 1986; Arndt and Fink, 1986; Hope and Struhl, 1987). The GCN4 protein shares homology to the jun oncoprotein and the human *trans*-activator protein AP-1 (Struhl, 1987; Bohmann *et al.*, 1987). All three proteins contain the hypothetical 'leucine zipper' structure that may represent a characteristic property of a new category of DNA binding proteins (Landschulz *et al.*, 1988).

Two other regulator proteins PHO2 and PHO4 induce the acid phosphatase genes *PHO5* and *PHO11* of yeast under conditions of phosphate starvation (Oshima, 1982; Yoshida *et al.*, 1987). The *PHO2* gene has been cloned and sequenced (Sengstag and Hinnen, 1987) and encodes a homeobox protein (Bürglin, 1988) as has been found for genes in developmental regulation in many different species (Gehring, 1987). Recently it has been shown that PHO2 protein (which is identical to BAS2) also binds the *HIS4* promoter and is necessary for basal expression of this amino acid biosynthetic gene (Arndt *et al.*, 1987).

In this paper we investigate the influence of GCN4 and PHO2 proteins on the general control regulated *TRP4* gene (Furter *et al.*, 1986, 1988). The *TRP4* gene encodes the enzyme anthranilate phosphoribosyl transferase (PRtransferase EC 2.4.2.18) that catalyses the second step in tryptophan biosynthesis and uses phosphoribosyl pyrophosphate (PRPP) and anthranilate as substrates.

Our results indicate that GCN4 and PHO2 proteins bind *in vitro* to the *TRP4* promoter. The GCN4 protein binds to two upstream activation sites, UAS_1 and UAS_2. The PHO2 protein binds specifically to the UAS_1 site of the *TRP4* promoter but does not bind to any other *TRP* promoter. The PHO2 protein/*TRP4* promoter binding site overlaps completely with the GCN4/UAS_1 binding site and both factors interact competitively in binding to this sequence. UAS_1 is the major regulatory site for GCN4-dependent *TRP4* activation. Studies on *TRP4* expression reveal that PHO2 binding modulates the general control response in low phosphate medium. The implications of these findings for regulation of gene expression in a cellular environment, where the end product and a substrate of a biosynthetic pathway are limited, are discussed.

Results

GCN4 protein binds to two sites of the TRP4 promoter

Four of the five tryptophan biosynthetic genes, including *TRP4*, are regulated by the general control regulatory system under amino acid starvation (Miozzari *et al.*, 1978); *TRP1* is the only unregulated *TRP* gene (Braus *et al.*, 1988). The *TRP4* promoter contains two putative general control UAS sequences: UAS_1 comprises a single GCN4 binding sequence (−246)ATGACTAAT(−238) and UAS_2 comprises two adjacent repeats (−166)TTGACTCTC (−158) and (−151)ATGATTCAT(−143) relative to the translational start site (Furter *et al.*, 1986).

In order to show binding of the general control regulator GCN4 to the *TRP4* promoter *in vitro*, we employed DNase I protection experiments (Galas and Schmitz, 1978). GCN4 protein was produced in *Escherichia coli* as described by

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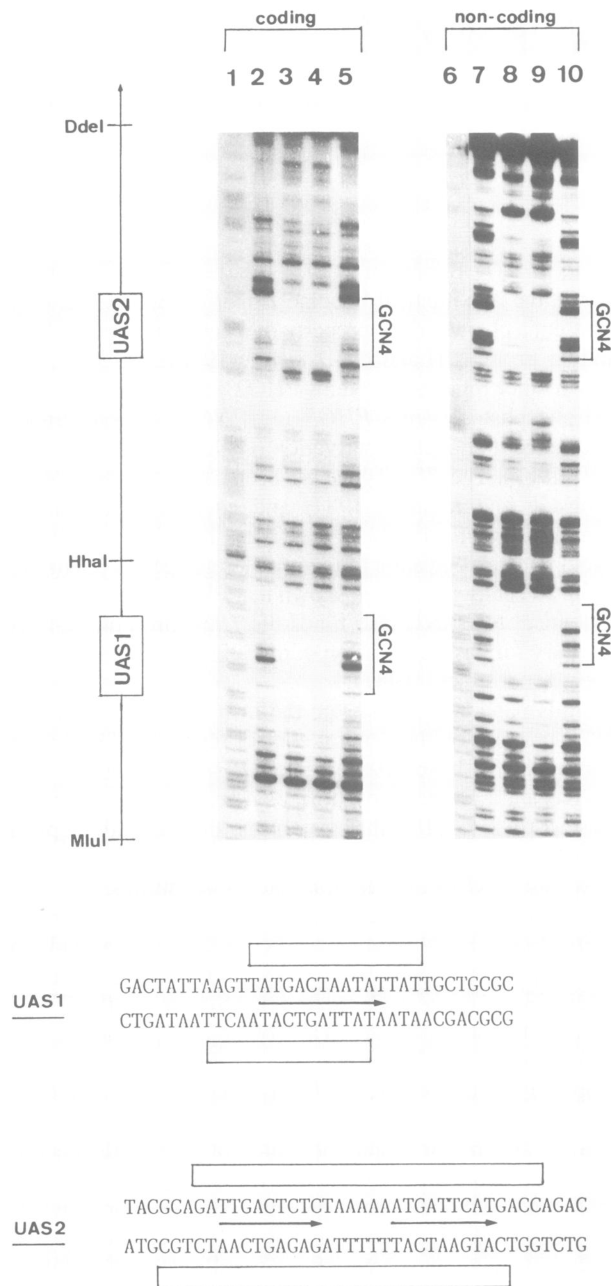

Fig. 1. DNase I footprint analysis of the *TRP4*–GCN4 complex. DNase I protection of the coding and the non-coding strands of the *TRP4* promoter was performed using GCN4 protein produced in *E.coli*. The coding and the non-coding strands were radiolabeled at the *Acc*I site (−448 relative to translation start) and restricted at the *Dde*I site (−52). The DNA probes were incubated with *E.coli* extracts containing GCN4 protein (5 μg in **lanes 3** and **8**, 10 μg in **lanes 4** and **9**) or without GCN4 protein (**lanes 2, 5, 7** and **10**). After treatment with DNase I the samples were separated on a standard sequencing gel. An A/G sequence ladder (**lanes 1** and **6**) was used as size marker. Protected sequences are bracketed. The corresponding DNA sequences are indicated below: UAS_1 containing region, sequence −258 to −225; UAS_2 containing region, sequence −174 to −135.

Arndt and Fink (1986). The data are summarized in Figure 1: UAS_1 is protected from DNase I cleavage in the region from position −251 to −237 on the coding and −247 to −233 on the non-coding strand. The two repeats of UAS_2 bind GCN4 protein from position −171 to −142 on the coding and −168 to −139 on the non-coding strand. From DNase I footprint titrations we estimate that ~60% of the GCN4 protein binds to the single repeat of UAS_1 and ~40% to the double repeat of UAS_2 (our unpublished data).

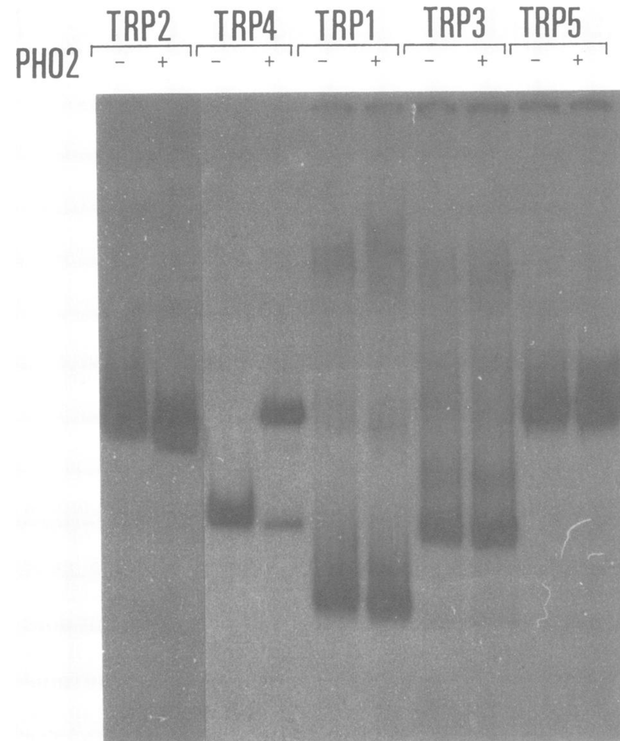

Fig. 2. Promoter-specific DNA binding activity of PHO2 protein. Radiolabelled promoter fragments of all *TRP* promoters were analysed for complex formation wth *E.coli* produced PHO2 protein. Lanes marked by a (−) are *E.coli* extracts containing no PHO2 protein as controls. *TRP2*, *Xba*I–*Cla*I fragment (−777 to −43 relative to the start of translation); *TRP4*, *Mlu*I–*Bcl*I fragment (−279 to +195); *TRP1*, *Hinc*II–*Xba*I fragment (−278 to +85); *TRP3*, *Stu*I–*Nar*I fragment (−375 to +103); *TRP5*, *Bam*HI–*Eco*RV fragment (−490 to +149). The same results were found using various other *TRP* promoter fragments (data not shown).

The regulatory protein PHO2 binds to the TRP4 promoter, but does not bind to any other TRP promoter

The PHO2 protein is involved in phosphate metabolism and in maintaining a basal level of expression of the *HIS4* gene. *HIS4* encodes a trifunctional histidine biosynthetic enzyme that needs phosphoribosyl pyrophosphate (PRPP) as a precursor. PHO2 (also called BAS2) was proposed to be a multiple global regulator and binds directly to the *PHO5* and *HIS4* promoters (Arndt *et al.*, 1987).

Since PRPP is also necessary for the biosynthesis of the aromatic amino acid tryptophan, we asked whether PHO2 protein was able to bind any *TRP* promoter. Different sets of overlapping DNA fragments of the promoters of all five *TRP* genes were tested for their ability to bind PHO2 protein *in vitro* and assayed for DNA–protein complex formation by gel retardation (Fried and Crothers, 1981; Garner and Revzin, 1981). PHO2 protein was produced using *Escherichia coli* as an expression system (Rosenberg *et al.*, 1983) as described in Materials and methods. Figure 2 demonstrates that PHO2 protein binds only to the *TRP4* promoter and does not bind to any other *TRP* promoter in gel retar-

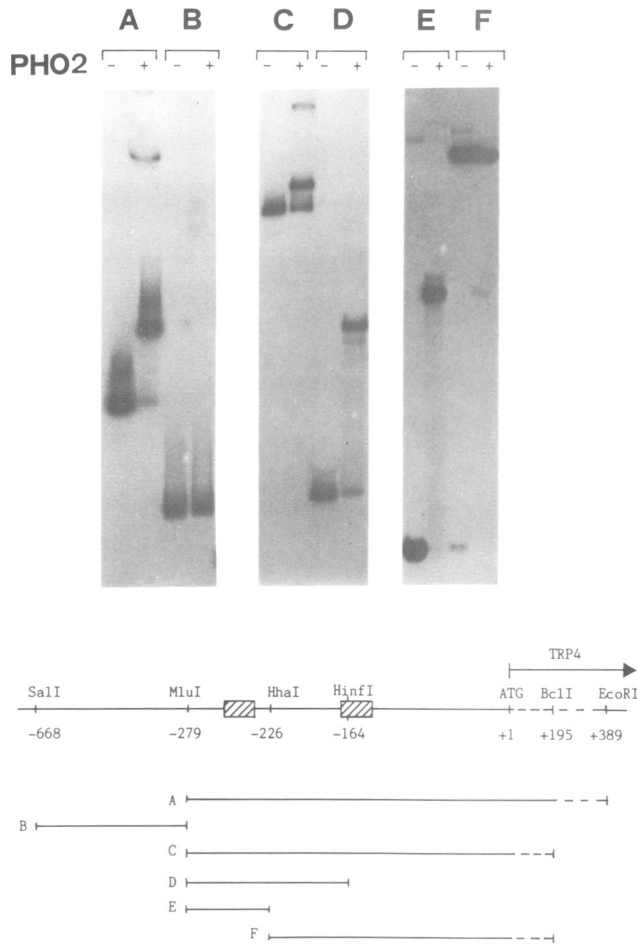

Fig. 3. Localization of the PHO2 binding site at the *TRP4* promoter. A set of radiolabelled *TRP4* promoter fragments was analysed for complex formation with *E.coli* produced PHO2 protein (+). Lanes marked by a (−) are *E.coli* extracts containing no PHO2 protein as controls. The *TRP4* promoter fragments are indicated below. The boxes represent the GCN4 binding sites, UAS_1 and UAS_2.

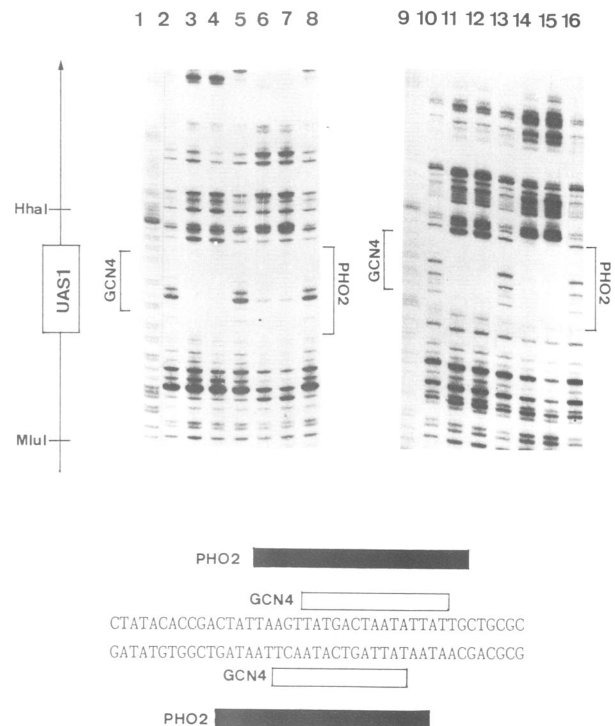

Fig. 4. DNase I footprint analysis of the *TRP4*−UAS_1 site and the PHO2 protein. DNase I protection of the coding and the non-coding strands of the *TRP4* promoter was performed using either PHO2 or GCN4 protein produced in *E.coli*. The coding and the non-coding strands were radiolabelled at the *Acc*I site (−448 relative to translation start) and restricted at the *Hin*fI site (−164). The DNA probes were incubated with *E.coli* extracts containing PHO2 protein (5 μg in **lanes 6** and **14**, 10 μg in **lanes 7** and **15**). In **lanes 3, 4, 11** and **12** GCN4−UAS_1 footprints were performed as described in Figure 1 as controls. In **lanes 2, 5, 8, 10, 13** and **16** no *E.coli* extracts were added. After treatment with DNase I the samples were separated on a standard sequencing gel. An A/G sequence ladder was used as a size marker (**lanes 1** and **9**). Protected sequences are bracketed. The corresponding DNA sequence from position −267 to −225 including the protected regions of PHO2 and GCN4, respectively, is indicated below.

dation experiments. The *TRP4* gene product anthranilate phosphoribosyl (PR) transferase (EC 2.4.2.18) is the *TRP* enzyme that needs PRPP as one of its substrates.

To localize the PHO2 binding site on the *TRP4* promoter a refined set of different radiolabelled *TRP4* promoter fragments was incubated with PHO2 protein. Figure 3 demonstrates that the PHO2 binding site is located in the region between −279 and −226 of the *TRP4* promoter, a region which contains UAS_1, one of the two GCN4 binding sites.

These results suggest that PHO2 protein effects gene expression as a specific and not as a general regulator by binding to the promoters of selective genes.

PHO2 and GCN4 compete in binding to the UAS_1 site of the TRP4 promoter

The PHO2 binding site was probed in more detail by DNase I protection analysis (Galas and Schmitz, 1978). Figure 4 indicates the nucleotides in the region between −256 and −236 on the coding and −251 and −233 on the non-coding strand relative to the translational start site that are inaccessible to DNase I cleavage because of the binding of PHO2. These data correlate with the results obtained by gel retardation experiments (Figure 3). The PHO2 protected region of ~20 nt is completely overlapping with the GCN4 protected UAS_1 region that is shown as a reference (Figure 4).

To test if the protein GCN4 and PHO2 interfere in binding UAS_1, competition band shifts (Figure 5) and a competition DNase I footprint (Figure 6) were carried out. Both proteins, GCN4 and PHO2, were produced in *E.coli*.

In Figure 5A increasing amounts of GCN4 and a constant amount of PHO2 were added to radiolabelled UAS_1-DNA (53-bp *Mlu*I−*Hha*I fragment from position −279 and −226); in Figure 5B the amount of GCN4 was kept constant and the amount of PHO2 was increased. In the band shift experiments no additional DNA−protein complex that would indicate simultaneous binding of both factors could be detected. Both factors can be titrated out by each other.

In the DNase I competition footprint in Figure 6 UAS_1-DNA was first incubated with PHO2 protein and then increasing amounts of GCN4 protein were added prior to DNase I cleavage. The nucleotide at position −231 that is exclusively protected from cleavage by the PHO2 protein can be used as an indicator for competition between the two protein factors: it disappears when PHO2 only is present

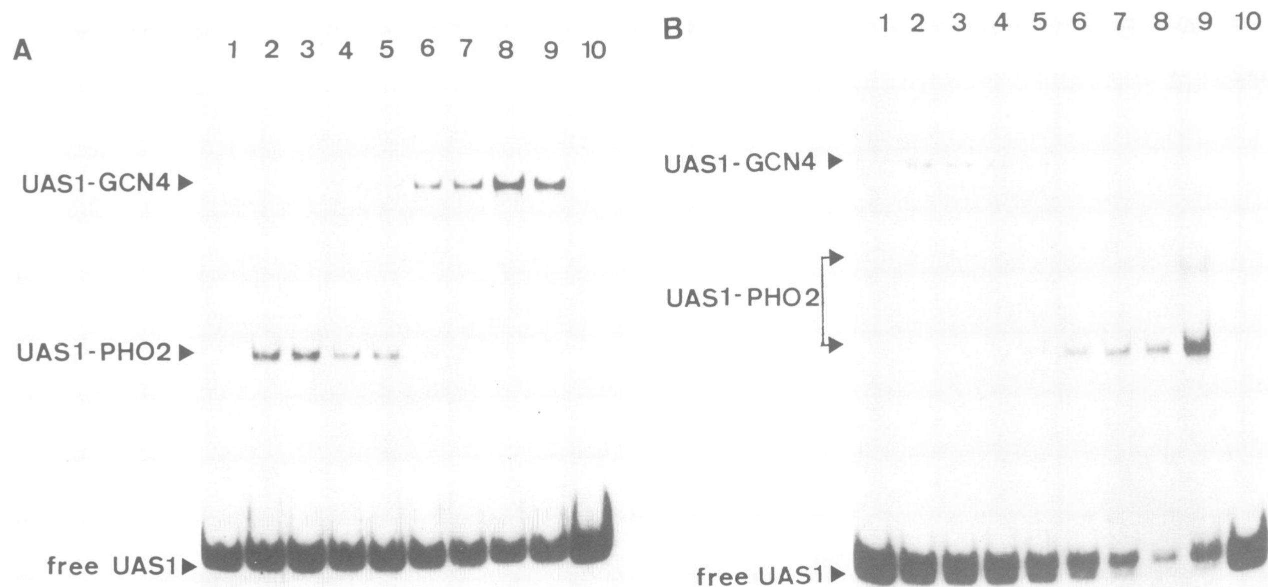

Fig. 5. Competition bandshift analysis of the *TRP4*–UAS_1 promoter site. The radiolabelled 53-bp *TRP4*–UAS_1 fragment (from the *Mlu*I restriction site at position −279 to the *Hha*I site at position −226) was pre-bound with 2 μg partially purified PHO2 protein (**A**) or 0.2 μg partially purified GCN4 protein (**B**). Afterwards *E.coli* extracts containing GCN4 protein (**A**, from 0.05 μg in **lane 3** to 1.6 μg in **lane 8**, the concentration was doubled) or PHO2 protein (**B**, from 0.16 μg in **lane 3** to 5 μg in **lane 8**) were added. Different amounts of BSA were added to keep the total amount of protein in all lanes equal. **Lanes 1** and **10** show free *TRP4*–DNA. In **lanes 2** (**A**) and 9 (**B**) the *TRP4*–DNA was only incubated with PHO2 protein, in **lanes 2** (**B**) and **9** (**A**) only with GCN4 protein.

and appears again by adding increasing amounts of GCN4. High amounts of GCN4 protein titrate out the pre-bound UAS_1–PHO2 complex and the DNase I footprint becomes indistinguishable from a DNase I footprint using only GCN4 protein (Figure 6, lanes 11 and 12). These data show that GCN4 and PHO2 bind to the UAS_1–*TRP4* promoter site in a mutually exclusive manner.

PHO2 interferes with the general control response of TRP4 gene expression

Binding of the PHO2 protein is required for both derepression of the *PHO5* gene under conditions of phosphate starvation (Oshima, 1982) and a basal level of transcription of the *HIS4* gene (Arndt *et al.*, 1987). These functions indicate that PHO2 binding to the *TRP4* promoter might also interact with *TRP4* transcription. To determine the physiological role of the specific PHO2–*TRP4* binding, the influence of phosphate and amino acid starvation on *TRP4* gene expression was studied. *TRP4* gene expression was determined by assaying the specific enzyme activiy of the *TRP4* gene product PRtransferase. PRtransferase enzyme activity correlates well with the change in transcription rate induced by amino acid starvation and the assay is more sensitive than quantitative RNA measurements (Furter *et al.*, 1988). No tryptophan requirement and no significant drop in *TRP4* gene expression could be observed in the *PHO2 GCN4* wild-type (wt), the *pho2* deficient or in the *pho2 gcn4* double mutant strain, when grown on high or low phosphate medium (Figure 7). These results imply that besides PHO2 additional *cis*- or *trans*-acting factors are involved in regulating the basal level of *TRP4* transcription and that these factors might be able to replace PHO2.

In order to detect whether PHO2 protein can influence the general control response of *TRP4*, we determined the specific enzyme activity of the *TRP4* gene product PRtransferase in cells grown under amino acid starvation

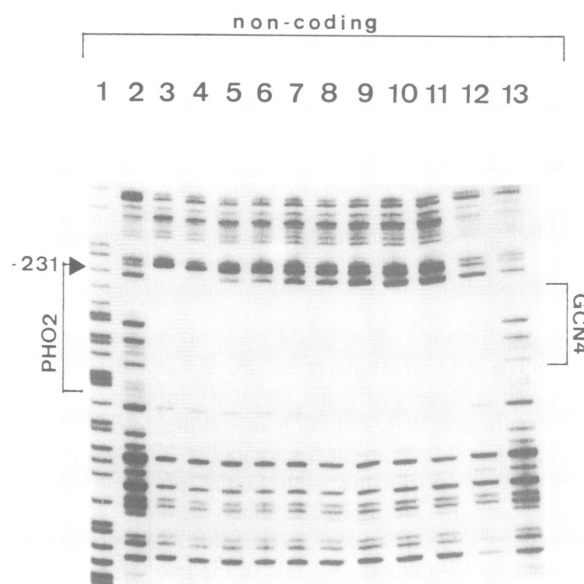

Fig. 6. Competition DNase I footprint analysis of the *TRP4*–UAS_1 promoter site. The radiolabelled non-coding strand of *TRP4* was pre-bound with 5 μg of *E.coli* extract containing partially purified PHO2 protein. Before DNase I treatment *E.coli* extracts containing partially purified GCN4 protein were added (from 0.13 μg in **lane 4** to 16 μg in **lane 11**, the concentration was doubled). Different amounts of BSA were added to keep the total amount of protein in all lanes equal. In **lane 3** the *TRP4*–DNA was incubated with 5 μg of PHO2 protein only. In **lane 12** with 16 μg of GCN4 protein only. In **lane 2** and **13** no protein was added. The nucleotide at position −231 that is only protected by the PHO2 protein is indicated. An A/G sequence ladder (**lane 1**) was used as a size marker.

induced by the presence of an amino acid analogue. Whereas in high phosphate medium in the *PHO2 GCN4* wt strain the *TRP4* could be derepressed by a factor of 2.5, only poor derepression was found when the same strain was starved

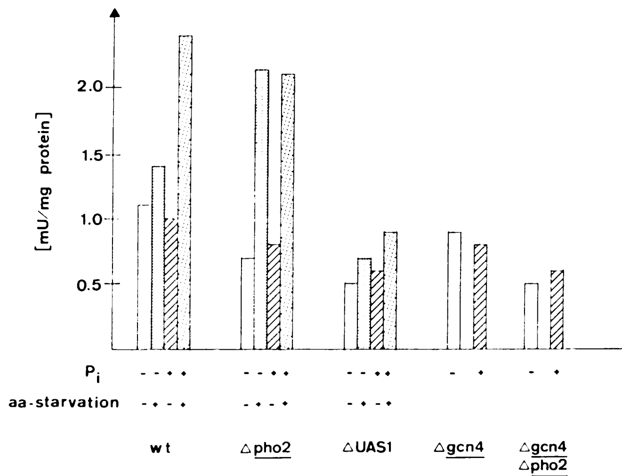

Fig. 7. Effect of phosphate and amino acid starvation on *TRP4* gene expression. High (+) and low (−) phosphate minimal media were supplemented with arginine, adenine and uracil; for the *gcn4 pho2* double mutation histidine as additional supplement was necessary. Amino acid starvation (+) was achieved in media containing the histidine analogue 3-aminotriazole. *TRP4* gene expression was determined by assaying the specific enzyme activity of the gene product PRtransferase. The given values are means of four independent cultivations, each one measured twice (the standard deviation did not exceed 20%).

for amino acids on low phosphate medium. In none of the other *TRP* enzymes tested as control was the general control response influenced by the phosphate content of the growth medium (data not shown). In the absence of PHO2 protein, in the *pho2* deficient strain, *TRP4* expression can be derepressed to a similar extent on low and high phosphate medium. Therefore a full general control response of *TRP4* under phosphate starvation is feasible only in the absence of the PHO2 regulator protein.

To characterize the *TRP4*−UAS_1 site further, a 38-bp deletion of the UAS_1-site of the *TRP4* promoter (from position −275 to −238) was introduced into yeast. There is no tryptophan requirement for the yeast strain carrying the UAS_1 deletion and there is only poor derepression of the *TRP4* gene under amino acid starvation. These data show that UAS_1 is the major regulatory site of the GCN4-dependent *TRP4* activation. In accordance with these results there is no more effect on *TRP4* gene expression by the phosphate concentration of the cultivation medium. Since the basal level of *TRP4* expression is reduced in the UAS_1 deleted *TRP4* promoter but not in the *gcn4 pho2* mutant strain, we assume that other factors are able to bind in this region and may contribute to the basal level of *TRP4* expression.

Discussion

Gene expression can be regulated by increasing or decreasing the rate of transcription initiation. Several protein factors in yeast, like GAL4 or GCN4, have been identified that can discriminate between different promoters and bind selectively to specific DNA sequences (Giniger *et al.*, 1985; Hope and Struhl, 1985). GAL4 and GCN4 activate transcription of sets of genes involved in galactose metabolism and amino acid biosynthesis, respectively (Klar and Halvorson, 1974; Hinnebusch and Fink, 1983). Other transcription factors, like RAP1, may play a role in either repression or activation of transcription, depending on the context of their binding site (Shore and Nasmyth, 1987. In more complex yeast systems multiple loci act in *trans* to control a regulatory response at a single site, e.g. the combined action of MATα2 and MATα1 turn off haplo-specific genes (Strathern *et al.*, 1981; Hall and Johnson, 1987). The physiological function of most transcription factors described so far remains to be elucidated.

The GCN4 regulator protein is required for activation of the tryptophan biosynthetic gene *TRP4* under conditions of amino acid limitation. *In vitro* GCN4 binds both putative upstream activation sites of the *TRP4* promotor, UAS_1 and UAS_2. UAS_1 (starting at position −246) contains a single repeat with eight of nine nucleotides matching with the well-characterized GCN4 consensus sequence (Hope and Struhl, 1987). UAS_2 is located eight helix turns more downstream (starting at position −166) and contains two adjacent repeats of the GCN4 recognition element with six nucleotides in between; six nucleotides of the first and eight nucleotides of the second repeat match with the GCN4 consensus sequence. From our *in vitro* data we estimate that the UAS_1 element has a higher affinity to GCN4 than the complete UAS_2 element. A *TRP4* promoter with a deletion of UAS_1 can hardly be activated by the general control system and shows *in vivo* that UAS_1 is necessary for the GCN4-dependent activation of transcription of *TRP4*.

In this report, we have shown that a second protein PHO2, originally identified as a phosphate metabolism regulator (Sengstag and Hinnen, 1987), is also able to bind the *TRP4* promoter. PHO2 protein binds specifically to the *TRP4* promoter and does not bind to any other *TRP* promoter pointing to a specific link between tryptophan biosynthetic pathway and phosphate metabolism. An important point in this context seems to be that the *TRP4* gene product PRtransferase is the only tryptophan biosynthetic enzyme that needs the phosphate derivative PRPP as one of the substrates.

PHO2 protein binds to the GCN4 binding site UAS_1 and the DNase I footprint experiments show a complete overlap of both binding sites. No binding of the PHO2 protein could be observed at the other GCN4 binding site UAS_2. Our competition experiments demonstrate that the PHO2 and the GCN4 protein bind UAS_1 in a mutually exclusive manner. Since the precise concentrations of active DNA binding proteins present in both GCN4 and PHO2 preparations are not known, we can estimate the relative affinity of both binding factors only roughly: according to Figures 5 and 6 and our other unpublished titration bandshifts we estimate that GCN4 in comparison with PHO2 has a 2- to 4-fold higher affinity to UAS_1. Addition of PRPP or of inorganic phosphate does not improve the affinity of PHO2 protein to its DNA binding site (our unpublished results). Another competitive interaction of two DNA binding factors for one promoter site has been reported in yeast for the *CYC1* promoter. The activator protein HAP1 requires haem for optimal binding to the *CYC1*−UAS_1 site and competes *in vitro* with RC2, a factor of unknown function, for this binding sequence (Pfeifer *et al.*, 1987). It was speculated that RC2 might be either a repressor of the *CYC1*−UAS_1 site or might be necessary for activation of the more downstream *CYC1*−UAS_2 sequence that is activated by the heteromeric complex HAP2−HAP3 (Hahn and Guarente, 1988).

Neither yeast strains carrying a *pho2* mutation nor a *pho2*

gcn4 double mutation nor a deletion in the *TRP4*–UAS_1 sequence are auxotrophic for tryptophan. Only in the yeast strains carrying the UAS_1 deletion the basal level of *TRP4* expression is affected significantly. Therefore we conclude that, if there is any contribution of the PHO2–UAS_1 interaction to the basal level of *TRP4* transcription, it can be substituted by the interaction of UAS_1 with another transcription factor. Since there is considerable homology to the *TRP4*–UAS_1 sequence e.g. with the binding sequence of the yAP-1 protein (C/GTGACTC/AA; Harshman *et al.*, 1988), this protein could be a possible candidate to bind UAS_1. The yAP-1 protein was identified and purified by its ability to bind the AP-1 recognition element of the SV40 enhancer and its function in yeast is not yet known.

Our results show that the PHO2 protein interferes with the general control activation system. The data presented suggest that the interplay of both DNA binding proteins with the UAS_1 promoter site of the yeast PHO2 gene takes place under simultaneous phosphate and amino acid starvation. The reduced degree of derepression of the *TRP4* gene product anthranilate phosphoribosyl transferase under conditions of amino acid and phosphate limitation can be explained by the competitive binding of GCN4 and PHO2 protein at UAS_1, the major site of GCN4-dependent activation of *TRP4*. The second GCN4 binding site UAS_2 seems to be only a minor upstream activation site *in vivo*. The data indicate that the PHO2 protein is involved in a modulation of the general control response of *TRP4* expression under phosphate limitation but does not directly activate or repress *TRP4* transcription. Whereas GCN4 mediates the response of the transcriptional apparatus to the environmental signal 'amino acid limitation', PHO2 could be the phosphate sensor that adjusts the response to the availability of phosphate precursors for tryptophan biosynthesis.

The mode of action of PHO2 protein seems to differ depending on the context of the binding sites of the corresponding target genes: PHO2 and PHO4 are both necessary for the activation of PHO5 under phosphate limitation (Oshima, 1982; Yoshida *et al.*, 1987; Sengstag and Hinnen, 1987). Furthermore PHO2 participates in maintaining a basal level of *HIS4* expression (Arndt *et al.*, 1987). In case of *TRP4* it modulates the general control response under simultaneous amino acid and phosphate limitation. The partial adenine requirement of *pho2 gcn4* mutant strains reported by Arndt *et al.* (1987) suggests an additional role of the PHO2 protein in adenine biosynthesis. The involvement of PHO2 protein in phosphate metabolism and in the biosynthesis of tryptophan, histidine and adenine, that use PRPP as a common precursor, suggests that PHO2 is one of the key DNA binding proteins that coordinate the phosphate regulation of diverse metabolic pathways. The connection of phosphate, tryptophan, histidine and adenine metabolism on the level of a *trans*-acting factor demonstrates an example of the complex interpathway control of gene regulation in the physiological network of the living cell.

Materials and methods

Yeast strains

All yeast strains are derivatives of the *Saccharomyces cerevisiae* laboratory strain S288C. The *gcn4-103* deletion was as described earlier (Hinnebusch, 1985). Deletions in the *PHO2* gene were obtained by evicting the *PHO2* wt gene by gene disruption. To delete the *TRP4* UAS_1 element in a first step the complete *TRP4* promoter was evicted and substituted by the *URA3* gene. In a second step a *TRP4* promoter with a 38-bp UAS_1 deletion from position −275 (*Mlu*I site) to position −237 (*Ssp*I) was re-introduced instead of the *URA3* gene. Yeast transformation was performed using the Li-acetate treatment (Ito *et al.*, 1983).

Preparation of GCN4 and PHO2 protein

GCN4 protein was produced using *E.coli* as expression system as described earlier (Arndt and Fink, 1986). The plasmid pAB100 that contains the *GCN4* gene under the control of the P_L promoter and the strain AR68 were a gift from K.Arndt and G.R.Fink.

PHO2 protein was produced in a similar way to GCN4 from an *E.coli* expression system. Therefore the *PHO2* gene (Sengstag and Hinnen, 1987) was cloned behind the P_L promoter of the pLC24 derivative pPLmu (Remaut *et al.*, 1981) and a different ribosome binding site was introduced (K.Vogel, W.Hörz and A.Hinnen, in preparation). As described for the GCN4 protein PHO2 protein was partially purified after heat induction from a soluble cell lysate (Arndt and Fink, 1986) using either a phosphocellulose or a heparin column. In our hands the yield of *E.coli* produced GCN4 protein in comparison to PHO2 protein was ~5-fold higher.

Gel retardation assay

The gel retardation method was described earlier (Fried and Crothers, 1981; Garner and Revzin, 1981). The promoter fragments of the *TRP* genes derived from the following plasmids: pMA33 (*TRP1*; Dobson *et al.*, 1983), pME514 (*TRP2*; Braus *et al.*, 1985), pME503 (*TRP3*; Aebi *et al.*, 1984), pME511 (*TRP4*; Furter *et al.*, 1986), pYAS-1 (*TRP5*; Niederberger *et al.*, 1984).

For the DNA binding assay with *E.coli* produced GCN4 or PHO2 protein, radiolabelled DNA fragments (5000 c.p.m.) were incubated with 1−4 μg of enriched GCN4 protein or 3−10 μg of enriched PHO2 protein for 25 min at 25°C in 10 mM Tris (pH 7.5), 0.1 mM EDTA, 100 mM NaCl, 0.8 mM DTT and 150 μg/ml poly[dIdC] in a 25 μl assay. The samples were separated on a native 6% PAGE gel. Afterwards the gel was fixed, dried and autoradiographed. As a control for unspecific binding extracts of *E.coli* cells carrying the original vector without the corresponding gene were prepared and applied. For the competition experiments the total protein concentration was kept constant by adding various amounts of bovine serum albumin (BSA).

DNase I footprint analysis

DNase I protection analysis was performed with modifications as described (Galas and Schmitz, 1978). 10 000−20 000 c.p.m. of either 5′ or 3′ end-labelled *TRP4* promoter fragments and 1−5 μg of partially purified GCN4 or 5−10 μg partially purified PHO2 protein were incubated in 20 mM Hepes (pH 7.0), 8% glycerol, 40 mM KCl, 0.1 mM EDTA, 5 mM $MgCl_2$ and 2 mM β-mercaptoethanol in a 50 μl assay. After 20 min incubation on ice, DNase I was added to a final concentration of 10 ng/μl and the reaction was terminated after 90 s by adding 125 μl of 0.12% SDS, 12 mM EDTA, 0.36 M NaAc containing 5 μg of yeast tRNA. Samples were separated on a standard sequencing gel and autoradiographed. A G/A sequencing ladder was used as size marker (Maxam and Gilbert, 1980).

Media and assays

Yeast strains were cultivated in low and high phosphate medium as described by Meyhack *et al.* (1982). Adenine, uracil, arginine and histidine were added to a final concentration of 40 μg/ml, 3-aminotriazol to 10 mM. PRtransferase activity (Furter *et al.*, 1986) and other *TRP* enzyme activities (Miozzari *et al.*, 1978) were determined as described. Acid phosphatase activity was determined by a staining assay (Meyhack *et al.*, 1982).

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