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review

Regulation of the Yeast *PHO5* and *PHO8* Genes: A Network of Regulatory Proteins, Transcription Factors and Chromatin*

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Summary

The yeast Saccharomyces cerevisiae contains several phosphatases and permeases involved in phosphate uptake and metabolism, the synthesis of which is regulated in response to the phosphate concentration in the growth medium. In phosphate containing media, transcription of these genes is repressed, while phosphate starvation results in strong induction. The most strongly regulated gene of this PHO system is PHO5 which encodes a secreted non-specific acid phosphatase. Repression of PHO5 transcription is achieved through negative regulation of the specific activator, Pho4. Under repressing conditions Pho4 is phosphorylated by the Pho80–Pho85 cyclin-cdk complex, and transcription prevented by its subsequent export out of the nucleus by interaction with the Msn5 receptor. Under phosphate limitation, the Pho80–Pho85 complex is inhibited through the action of the cyclin inhibitor Pho81, which results in the accumulation of non-phosphorylated Pho4 in the nucleus. However, in addition to the regulation of the Pho4 subcellular localization, there is another as yet unclarified mechanism which regulates PHO5 transcription. Activation of PHO5 transcription requires the cooperative interaction of Pho4 with the pleiotropic homeodomain protein Pho2. Pho2 plays a role in increasing both the DNA binding affinity and transactivation potential of Pho4. The PHO5 promoter is also regulated through a repressive chromatin structure. Upon induction, massive, Pho4-dependent remodeling of chromatin occurs, which is a prerequisite for promoter activation. The PHO8 gene, encoding a non-specific alkaline phosphatase, is coordinately regulated with PHO5 through the same set of regulatory proteins and also through chromatin repression. However, in comparison to PHO5, the PHO8 promoter is transcriptionally rather weak. This low level of PHO8 induction can be explained by the inability of Pho4 to accomplish full chromatin remodeling at this promoter. Complexes which influence the ability to remodel chromatin are discussed. These results highlight the importance of chromatin structure in the regulation of promoter activity.

Key words: transcriptional regulation, chromatin, *PHO5*, *PHO8*, *Saccharomyces cerevisiae*

Introduction

When microorganisms grow under conditions of nutrient limitation they respond by transmitting a nutritional signal to the nucleus, which results in the induction of gene transcription and ultimately brings about the increased synthesis of specialised enzyme(s) involved in the metabolism of specific nutrients. The yeast *Saccharomyces cerevisiae* contains several phosphatases and permeases involved in phosphate uptake and meta-

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bolism. The set of genes coding for these enzymes and transporters is coordinately regulated in response to changes in the inorganic phosphate (Pi) concentration of the growth medium. In Pi-containing medium, transcription of the *PHO* genes is efficiently repressed, while phosphate starvation results in induction; the level of induction being different for different genes (*1*). Among them the most strongly regulated is the *PHO5* gene. This gene encodes the major isoenzyme of a repressible, non-specific acid phosphatase (*2*). The enzyme is an oligomeric, heavily glycosylated protein, secreted to the periplasmic space (*3,4*). Under phosphate starvation conditions it plays a role in providing phosphate to the cell.

In addition to acid phosphatase isoenzymes, there are also two species of phosphatases that hydrolyse p-nitrophenylphosphate at alkaline pH. The first, encoded by the *PHO13* gene, is a specific p-nitrophenylphosphatase which is constitutively expressed independent of the Pi concentration in the medium (*5*). The other is a non-specific alkaline phosphatase, encoded by the *PHO8* gene, the expression of which is regulated by the Pi concentration, coordinately with other Pi-regulated genes (*1,6*). The enzyme is located in the vacuole, but its physiological substrate(s) have remained unclear (*7*).

Transcription of both the *PHO5* and the *PHO8* genes is regulated by the same set of regulatory proteins and through chromatin repression of their promoters (see below). However, there is a big difference in the extent of induction between the two genes. Activity of the *PHO5* promoter increases 50–100 fold upon induction (*1*) while activity of the *PHO8* promoter increases only 6–7 fold (M. Münsterkötter *et al.*, manuscript in preparation). Therefore, the two promoters are suitable models for a comparative study of the complex interactions of regulatory proteins, transcription factors and chromatin ultimately determining the strength of the particular promoter.

The Regulators of Pi-repressible Genes

The expression of all Pi-regulated genes is positively regulated by the products of three genes, *PHO2*, *PHO4* and *PHO81*, while the products of *PHO80* and *PHO85* are negative regulators. *PHO2* and *PHO4* encode DNA-binding transcriptional activators, while the *PHO81* gene product acts positively by inhibiting the activity of the negative regulators Pho80 and Pho85, which form a cyclin-cdk complex (*8*).

Pho4 is a specific transcriptional activator of Pi-regulated genes. It is an acidic activator consisting of 312 amino acid residues. Its carboxy-terminal region encodes a basic helix-loop-helix (bHLH) DNA-binding domain (*9*). Pho4 binds to the *PHO5* promoter, as well as to the promoters of other Pi–regulated genes: *PHO8* (*10*), *PHO81* and *PHO84* (*11*). On the other hand, the homeodomain protein Pho2 is a pleiotropic factor which is involved in the regulation of transcription of several genes from diverse regulons besides the *PHO* system, including *HO* (*12*), *HIS4* (*13*) *TRP4* (*14*) and some *ADE* genes (*15*). Although it has been known for a long time that the *PHO2* gene product is strictly required for *PHO5* expression, the mechanism by which Pho2 acts to bring about activation of *PHO5* transcription has only recently been elucidated.

Pho4 and Pho2 act Cooperatively in the Activation of the *PHO5* Promoter

At the *PHO5* promoter two Pho4 binding sites have been mapped by *in vitro* footprinting (*16*), corresponding to the previously identified regulatory elements, UASp1 and UASp2 (*17*). UASp1 and UASp2 contain one copy each of the CACGTT and CACGTG motifs respectively, which are related to the consensus E-box DNA sequence targeted by several bHLH proteins (*18*). Ogawa *et al.* (*11*) compared the sequences of Pho4 binding sites in the promoters of *Pi*-regulated genes and proposed a consensus sequence for two types of Pho4 binding sites. The type 1 sequence, 5'-GCACGTGGG-3', was suggested to be more efficient than the type 2 sequence, 5'-GCACGTTTT-3', with respect to Pho4 binding (*19,20*). Recently, the crystal structure of the Pho4 bHLH domain-DNA complex was reported. It was shown that Pho4 binds to DNA as a homodimer with direct interaction with both the core E-box sequence CACGTG and its 3'-flanking bases, thus providing a mechanistic basis for the differences in binding efficiency for the two types of Pho4 binding sites (*21*).

Pho4 binds to both UASp1 and UASp2 *in vivo* under induced conditions, but no binding under repressive conditions was detected (*22*). Activity of the *PHO5* promoter critically depends on the presence of both sites. Mutation of either one of the sites results in a 10-fold decrease of promoter activity, and a promoter containing mutations in both sites is inactive (*23*). Binding of Pho4 to both UASp1 and UASp2 is also required for the chromatin transition at the *PHO5* promoter to occur (*24*), which appears to be a prerequisite for transcriptional activation (see below). A third, low affinity Pho4 site, located downstream of UASp2, was recently mapped *in vitro* (*25*). Although this site contributes to promoter activity, it is not required for the transition of the chromatin structure, and therefore not critically required for activation of the *PHO5* promoter (23).

Our *in vitro* binding studies (*25*) with purified recombinant Pho2 revealed the presence of multiple Pho2 sites at the *PHO5* promoter of different affinities. Pho2 binding sites at the *PHO5* promoter are located adjacent, or even partially overlapping, to the Pho4 sites (Fig.1.A). Furthermore, it was demonstrated that there is cooperative DNA binding between Pho2 and Pho4 at each Pho4 binding site. *In vivo* experiments show that mutations in the Pho2 sites adjacent to UASp1, as well as those adjacent to UASp2, result in the loss of cooperative binding of the two proteins, and in parallel cause a strong reduction in *PHO5* promoter activity (Fig. 1.B). By the combined mutation of the Pho2 sites adjacent to both UASp1 and UASp2, a dramatic effect on promoter activity was obtained. Activity of this promoter was only slightly higher than that of the promoter variant containing mutated Pho4 sites at both UASp1 and UASp2 (*23*). The functional importance of the mapped Pho2 sites, and the cooperativity between Pho2 and Pho4 *in vivo* was further confirmed by experiments in which the *PHO5* promoter was activated by Pho2 fused to a VP16 activation domain, Pho2-VP16 (*23*), in the presence of a Pho4 derivative lacking an activation domain but capable of DNA binding, Pho4Δ2 (*26*). The efficient activation by Pho2-

VP16 requires not only the presence of Pho4, but also intact Pho2 binding sites (Fig. 1.C). These *in vivo* data strongly support the role of Pho2 in *PHO5* activation as a DNA-binding factor which binds to specific sequences at the *PHO5* promoter cooperatively with Pho4 (23).

Although full activation of the promoter requires intact Pho2 binding sites adjacent to both UASp1 and UASp2, elimination of Pho2 binding around UASp1 has a stronger effect than that around UASp2. This was borne out by a more pronounced decrease in promoter activity and by the extent of the opening of the promoter chromatin structure, which is shown to require efficient binding of Pho4 to both UASp1 and UASp2 (see below). While mutation of Pho2 sites adjacent to UASp1 strongly impaired the transition of the chromatin structure, interference with Pho2 binding around UASp2 was without effect, suggesting that in contrast to UASp1, binding of Pho4 to UASp2 is not absolutely Pho2 dependent, but rather improved by cooperative interactions with Pho2 (23).

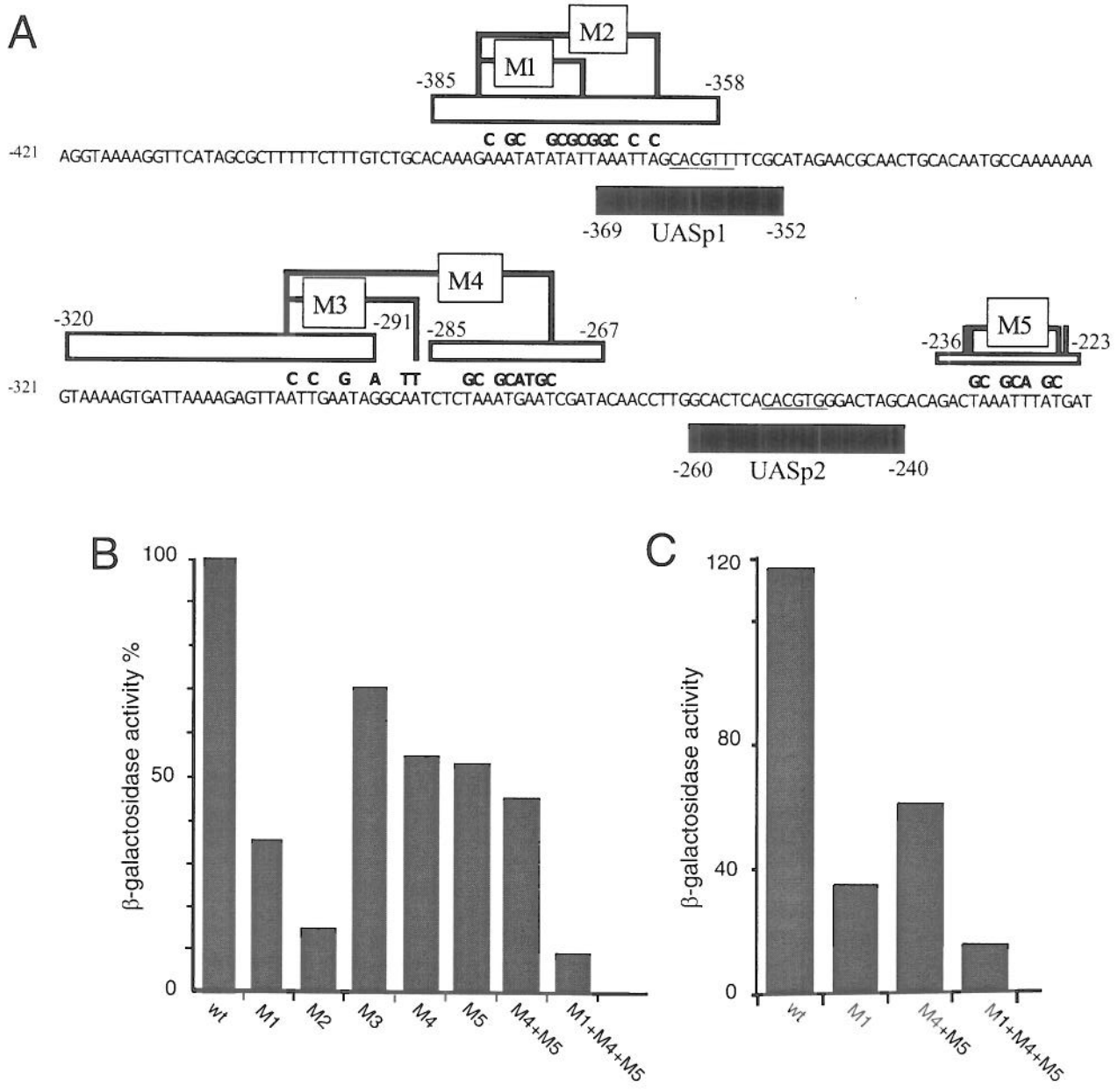

Fig. 1. Mutations in the Pho2 *cis* elements at the *PHO5* promoter differentially affect promoter activity. (A). The locations of the Pho4 sites (UASp1 and UASp2), and the Pho2 sites, as determined by *in vitro* footprinting (25), are indicated by solid and open bars, respectively. The width of the bars corresponds to the relative affinities of the sites for Pho2. Mutated regions are in boxes (M1 to M5) and the changed nucleotides are shown above the wild type sequence. (B). The activity of the *PHO5* promoter variants containing mutations in the Pho2 binding sites were measured by the use of a *lacZ* reporter gene and expressed relative to the activity of the wild type promoter. (C). Activation of *PHO5* promoter variants containing mutated Pho2 binding sites, by a transcriptionally inactive Pho4 derivative, Pho4Δ2, and a hybrid protein containing the VP16 activation domain fused to Pho2, Pho2-VP16, co-expressed in YS27 (*pho4, pho2*).

By using a Pho4 derivative lacking the Pho2 interaction domain, amino acids 200–247 (Pho4Δint), the importance of specific protein-protein interactions for cooperative DNA binding of the two proteins and for promoter activation was clearly shown (*23*). No significant cooperativity between Pho4Δint and Pho2 was observed when binding to a promoter fragment containing either UASp1 or UASp2 was tested, and consequently no appreciable activation of the *PHO5* promoter with this Pho4 derivative was measured. Besides cooperative DNA binding with Pho4, the additional role of Pho2 in transcriptional activation is suggested by the finding that overexpression of Pho4 can almost fully compensate for the absence of Pho2 *cis* elements, but not for the absence of Pho2 *in trans* (*23*). Evidence for a role of Pho2 in transcriptional activation by Pho4 came also from experiments in which the *GAL1* promoter was activated with a hybrid Pho4 protein, containing full length Pho4 fused to the Gal4 DNA binding domain, which was found to be significantly Pho2-dependent. Since a role of Pho2 in increasing the ability of Pho4 to bind DNA was eliminated by using the Gal4 DNA binding domain, this result suggests a role of Pho2 in enhancing the ability of Pho4 to transactivate. It was also shown that a deletion in the Pho4 basic region, which is proposed to mediate functional interactions with Pho2, results in Pho2-independent activation of the *GAL1* promoter by the Gal4-Pho4 protein, a result supported by the discovery of point mutations in the basic domain which produce the same phenotype (*27*). The authors proposed a model in which the Pho4 activation domain interacts with the basic region and is thereby masked. Interactions of Pho4 with Pho2 disrupt these intramolecular interactions and generate a transcriptionally competent molecule with an exposed activation domain. Our activation data obtained with Pho4Δint under more physiological conditions (*23*), are consistent with such a model. It is interesting to note that a dual role of Pho2 in stimulating both DNA binding and activation by another transcriptional activator Bas1 at the *ADE5,7* promoter was recently proposed (*28*).

Pho2, in contrast to Pho4, does not have a known transcriptional activation domain. However, recently described results of protein binding assays revealed that, like Pho4, Pho2 also has affinity to the general transcription factors TBP, TFIIB and TFIIEβ, though somewhat weaker than that of Pho4 (*29*). The authors proposed that the Pho4-Pho2 complex bound to the UAS elements, activates transcription by direct interactions with general transcription factors. It should, however, be pointed out that overexpression of Pho4 in a *pho2* strain results in appreciable activation of *PHO5*, while the opposite is not true (*30*). In addition, no appreciable activation was detected with a Pho4 derivative lacking its activation domain in a *PHO2* strain (*23*), suggesting that Pho4 interactions with general transcription factors, rather than Pho2 interactions, are critical for the transactivation process.

Phosphorylation of Pho4 by the Pho80/85 Complex Regulates its Subcellular Localization

Pho80 and Pho85, negative regulators of *PHO5* expression, form a cyclin-cyclin dependent kinase (cdk) complex, which is shown to phosphorylate Pho4 *in vitro*. Phosphorylation of five serine residues in the Pho4 protein also occurs *in vivo* under repressive conditions, but not when phosphate is limited, indicating that Pho80-Pho85 kinase activity is regulated by the phosphate level (*31*). The phosphate-dependent regulation of Pho80-Pho85 requires the *PHO81* gene product, a positive regulator of *PHO5* expression, which functions *in vitro* as an inhibitor of Pho80-Pho85 activity. A schematic representation of the regulatory network is shown in Fig. 2. Expression of *PHO81* is regulated by the *Pi* concentration in the same way as expression of *PHO5* (*1*). However, it is unlikely that an increase of the Pho81 concentration upon phosphate starvation is required for inhibition of Pho80-Pho85 and consequent *PHO5* transcription, since induction of *PHO5* transcription occurs in the absence of protein synthesis (*2*). The mechanisms by which phosphate starvation signal regulates the Pho81-dependent inhibition of Pho80-Pho85 activity remains unclear.

O'Shea and colleagues have examined the effect of Pho4 phosphorylation on *PHO5* expression and shown that under repressive conditions phosphorylated Pho4 is predominantly localized in the cytoplasm, and therefore unable to activate *PHO5* transcription (*32*). They have recently identified the receptor Msn5, which exports phosphorylated Pho4 out of nucleus (*33*). Under repressive conditions, an unphosphorylatable Pho4 derivative, containing Ser to Ala mutations in the Pho80-Pho85 target sites, is mostly found in the nucleus, showing that phosphorylation of Pho4 by Pho80-Pho85 regulates the subcellular localization of Pho4 and thereby *PHO5* expression. It was also shown that in cells expressing an unphosphorylatable Pho4 derivative, the level of *PHO5* expression under repressing conditions is significantly enhanced, in agreement with the nuclear localization of the Pho4 derivative (*32*). However, under this condition the activity of the Pho4 derivative was only 10 % of that measured with wild type Pho4 at induced conditions. In addition, as the authors mentioned, the activity of *PHO5* in a strain expressing an unphosphorylatable Pho4 mutant still increases 4 times upon phosphate starvation, showing that there is yet a further level to the mechanism regulating *PHO5* expression, which is independent of Pho80-Pho85 phosphorylation of Pho4.

Low level Pho4 – Pho2 Cooperativity is Achieved at the Weakly Regulated *PHO8* Promoter

Promoter deletion analysis indicates the presence of two regulatory regions and an inhibitory region at the *PHO8* promoter (*34*). Our *in vitro* footprinting data showed that the two regulatory regions correspond to Pho4 binding sites (*10*). The upstream Pho4 site, UASp1, is a rather weak site with one mismatch to the consensus sequence of the low affinity type 2 Pho4 binding site, while the second downstream site, UASp2, belongs to the type 1 high affinity class (*11*). However, no significant homology, outside of the consensus hexanucleotide, exists between the *PHO5* and *PHO8* UAS elements (*10*).

It was previously proposed that Pho2 is not involved in the activation of the *PHO8* promoter (1). How-

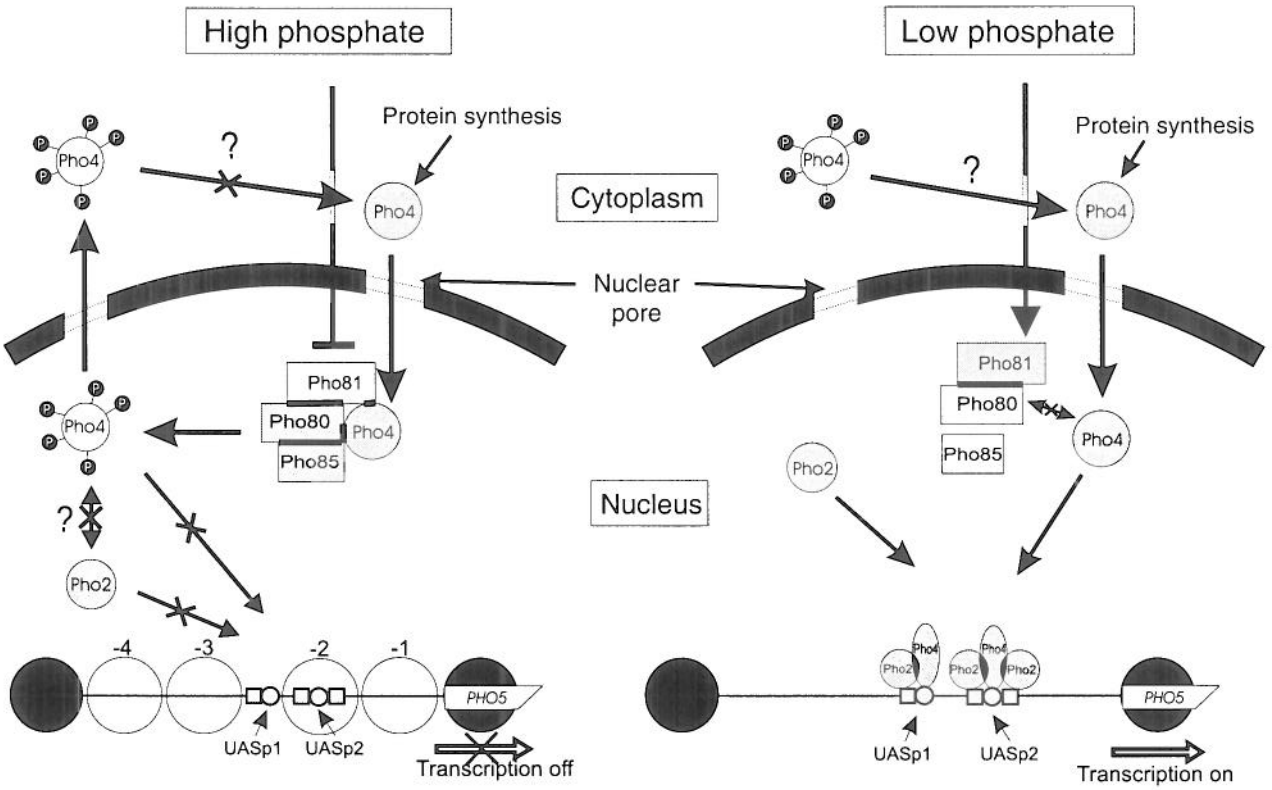

Fig. 2. Molecular mechanism of *PHO5* transcriptional regulation. The white and shaded regulatory proteins represent their inactive and active forms, respectively. The four white circles (marked –1 to –4) present on the promoter at repressive conditions represent nucleosomes which undergo remodeling upon induction. For details see text.

ever, our data show that Pho2, although not absolutely required, contributes to transcriptional activation of the *PHO8* promoter (M. Münsterkötter *et al.*, manuscript in preparation). In the presence of Pho2, activity of the promoter increases 3 fold, showing a much lower degree of cooperativity between Pho4 and Pho2 than observed at the *PHO5* promoter where cooperative interactions of Pho2 with Pho4 increase promoter activity about 50 fold (*23*).

Although both the *PHO5* and *PHO8* promoters are regulated by the same set of regulatory proteins, activity of the *PHO8* promoter increases 6–7 fold upon induction (unpublished data), which is almost 10 times less than measured with *PHO5*. The reason for the lower strength of the *PHO8* promoter can be, at least in part, explained by our recent finding that only the high affinity Pho4 site, UASp2, is involved in the activation of the *PHO8* promoter, while the *in vitro* mapped UASp1 element does not appear to be efficient *in vivo* (M. Münsterkötter *et al.*, manuscript in preparation). In addition, as mentioned above, at the *PHO8* promoter Pho4 and Pho2 are not able to achieve full cooperativity in promoter activation.

Another property of the *PHO8* promoter which distinguishes it from the *PHO5* promoter, is the partial remodeling of the repressive chromatin structure which occurs upon induction (see below). Therefore, the low level induction of the *PHO8* promoter in comparison to *PHO5*, might be explained by the inability of Pho4 to accomplish full relief from chromatin repression, perhaps due to the lack of cooperative interactions between the two Pho4 binding sites, a prerequisite for chromatin remodeling at the *PHO5* promoter.

Repression of Transcription by Chromatin

The eukaryotic genome is packaged into chromatin, a complex of histone and non-histone proteins which serve to compact the DNA of the cell into the nucleus (*35*). This packaging, however, also presents the potential difficulty of access to the underlying sequence as required for the fundamental processes of transcription, recombination and replication. *In vivo* evidence for this repressive function in transcription was provided by experiments in yeast where disruption of nucleosomal structure was shown to result in the activation of a number of promoters (including *HIS3* and *PHO5*) under otherwise non-inducing conditions (*36,37*). Therefore, repressive chromatin structures prevent the transcription machinery from gaining access to the promoter, and this repression must be overcome to allow transcriptional activation.

Role of Chromatin Structure at the Yeast *PHO5* and *PHO8* Promoters

Under repressing conditions the *PHO5* promoter is packaged into a regular array of positioned nucleosomes

interrupted by a short hypersensitive region (*38*). This organization places one of the two UAS elements (UASp1) into the hypersensitive site whereas the other UAS element (UASp2) and the TATA and core promoter are found within positioned nucleosomes (*16,17*). Under repressing conditions then, the chromatin is able to prevent both the transcription factor and the general transcription machinery from accessing the underlying DNA (*22,39*). Upon activation, the two nucleosomes on either side of the hypersensitive site are remodeled and the core promoter made accessible for the general transcription factors (*40*). At the *PHO5* promoter both Pho4 and Pho2 are necessary for this chromatin transition (*24,30*). A schematic model of the *PHO5* promoter chromatin structure under repressing and activating conditions is shown at the bottom of Fig. 2.

The repressed *PHO8* promoter is also organized into an array of nucleosomes. However, the two UAS elements driving transcriptional activation from this promoter are both found within hypersensitive sites. On the other hand, the TATA element is located within a stable positioned nucleosome (*10*). On activation of the promoter under conditions of phosphate starvation, a dramatic Pho4 dependent perturbation of the repressed structure is observed (Fig. 3). This remodeling is quite different to that observed at *PHO5* since the *PHO8* promoter demonstrates only partial accessibility to nucleases and restriction enzymes, consistent with the continued presence of incompletely remodeled or destabilized nucleosomes across the promoter (*10*). Transcription *per se* is not required for this transition, since chromatin remodeling can occur in the absence of transcription while the reciprocal situation has thus far not been documented (*42*). Thus at the phosphate regulated *PHO5* and *PHO8* genes the repressive chromatin serves to silence transcription by limiting both *trans*activator and TBP access to their target sequences on the DNA. This raises the question as to how transcription factors bring about the perturbation of this structure when the time comes to switch on chromatin regulated promoters (*43,44,45*).

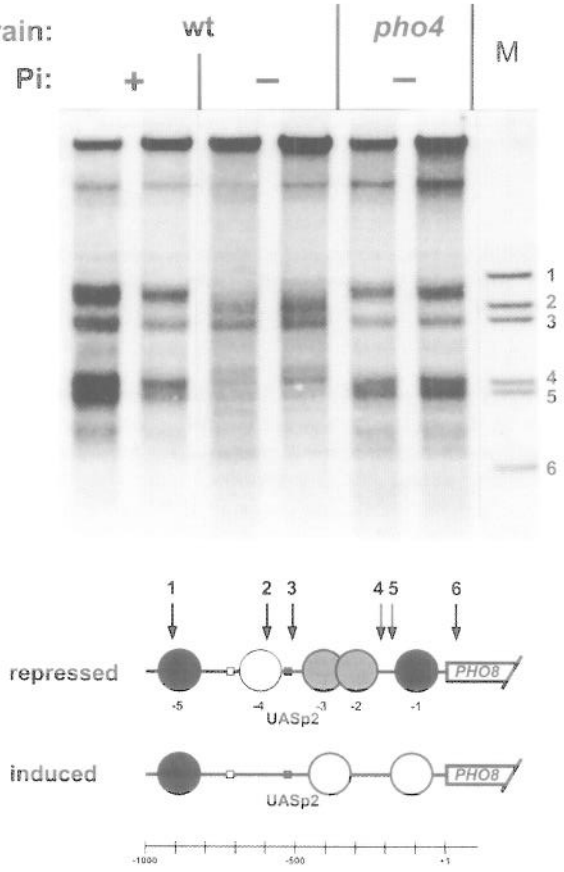

Fig.3. Remodeling of the chromatin structure at the *PHO8* promoter upon induction. DNAse I analysis of chromatin (*41*) at the *PHO8* promoter under repressive (+Pi) and induced (-Pi) conditions in a wild type and a *pho4* strain are shown. The blot was hybridized with a PvuII/XhoI restriction fragment as a *PHO8* specific downstream probe. Restriction nuclease double digests of purified genomic DNA with *Bgl*II and *Eco*RV(1), *Hpa*I(2), *Nhe*I(3), *Rsa*I(4), *Hind*III(5) or *Xho*I(6), serve as marker fragments (M). The nucleosomal structure at the repressed and induced promoter is shown schematically at the bottom. Stable nucleosomes (filled circles), slightly unstable nucleosomes (hatched circles), and highly labilized nucleosomes (open circles) are indicated. Arrows indicate the restriction sites used for markers.

Chromatin Remodeling – the Role of the Activation Domain

Transactivator proteins such as Pho4 play a critical role in the regulation of inducible promoters. Positioned nucleosomes can function to prevent *trans*activator access (*22*), although the ability to bind the DNA may not in itself trigger the remodeling of chromatin. For example, a Pho4 derivative with the acidic activation domain deleted can bind to the accessible UASp1 element at the *PHO5* promoter *in vivo*, but is unable to remodel chromatin (*26*). Importantly, the remodeling process does not require passage through S-phase (*46*), which has been proposed to provide a »window of opportunity« for the transcription factor to access the DNA. Furthermore, as described above, the act of transcription is not in itself required for the chromatin transition to occur.

The activation domain is therefore required to orchestrate chromatin opening, but the mechanism by which this process is achieved is only beginning to be elucidated. Interestingly, for the muscle-specific transcriptional activator MyoD, specific domains separable from the classical activation domain appear to be required for this protein's ability to challenge repressive chromatin (*47*). This, however, has so far not been impossible to achieve at the molecular level with Pho4 (*48*).

Using a Pho4 derivative in which the classical acidic activation domain has been replaced by a domain of the Gal11 protein (a component of the mediator of RNA polymerase II), we have been able to demonstrate that direct recruitment of the basal transcription machinery is sufficient to remodel the chromatin structure of the *PHO5* promoter (*43*). The nature of this remodeling activity remains unknown although once again transcription *per se* is not required for this process.

Acetylation and Active Chromatin

The connection between transcriptionally active chromatin and a higher level of histone acetylation was

first observed some 30 years ago (*49*). However, how acetylation of chromatin exerts its influence on transcription and the molecular activities that are responsible for this post-translational modification were unclear until very recently when the gene for a histone acetylase was cloned from *Tetrahymena thermophila* and shown to be homologous to *GCN5* in yeast, the gene for a transcription factor (*50*). This result formally connected the acetylation of chromatin with the activation of transcription. Importantly, the histone acetyltransferase activity (HAT) of Gcn5 is required for the function of the protein (*51*). Furthermore, by employing antibodies specific for acetylated histones Allis and co-workers have been able to identify a promoter specific increase in the level of histone acetylation on gene activation at the *HIS3* locus (*52*).

When the possibility of a role of Gcn5 at the *PHO5* promoter was examined, we found that the basal level of *PHO5* promoter activity is strongly Gcn5 dependent, while under fully inducing conditions the promoter reaches close to wild type levels of activation (*53*). Induced activity of the promoter is, however, rendered strongly Gcn5 dependent by the deletion of a single UAS element. In addition, the deletion of *GCN5* or mutation of residues critical for HAT function severely impairs the constitutive sub-maximal activation of the promoter in a *Δpho80* strain at high phosphate. DNaseI analysis and restriction enzyme assays of the promoter under these conditions identify a novel chromatin structure consistent with the presence of nucleosomes occupying random positions across the promoter. This structure is indistinguishable in strains deleted for *GCN5* or carrying amino acid substitution mutations in Gcn5 that specifically reduce its histone acetyltransferase activity, demonstrating that the absence of Gcn5 HAT activity is sufficient to generate this unusual structure (*53*). Thus, Gcn5 histone acetylation activity is shown to have direct effects on chromatin remodeling and transcriptional activation at the *PHO5* promoter.

An important message from these results is the redundancy of mechanisms dealing with chromatin repression. Maximal *PHO5* activation can overcome the *gcn5* defect, yet under sub-maximal activation conditions, Gcn5 is critically required. This existence of multiple backup systems has been an initially puzzling outcome of many different studies addressing chromatin repression and chromatin remodeling, but now appears to be a characteristic of this aspect of transcriptional regulation.

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Regulacija kvaščevih gena *PHO5* i *PHO8*: međudjelovanje regulatornih proteina, transkripcijskih faktora i kromatina

Sažetak

Stanice kvasca Saccharomyces cerevisiae sadrže nekoliko fosfataza i permeaza koje su uključene u transport i metabolizam fosfata. Sinteza je tih proteina regulirana ovisno o koncentraciji fosfata u podlozi za uzgoj. U podlozi što sadrži fosfat reprimirana je transkripcija gena koji kodiraju te proteine, dok u podlozi u kojoj nedostaje fosfat dolazi do jake indukcije. Među tim genima, tzv. PHO sustava, gen PHO5, koji kodira nespecifičnu kiselu fosfatazu, pokazuje najvišu razinu regulacije. Represija transkripcije gena PHO5 postiže se negativnom regulacijom specifičnog aktivatora, proteina Pho4. Pod uvjetima represije Pho4 se fosforilira djelovanjem kompleksa ciklin – ciklin ovisna kinaza (Pho80-Pho85), a transkripcija je onemogućena zbog interakcije s Msn5 receptorom, pri čemu fosforilirani Pho4 izlazi iz jezgre. Kada u podlozi nedostaje fosfat, dolazi do inaktivacije Pho80-Pho85 kompleksa djelovanjem ciklin-inhibitora, Pho81, te se nefosforilirani oblik Pho4 proteina akumulira u jezgri. Međutim, osim regulacije lokalizacije Pho4 u stanici, postoji i dodatni, zasad neobjašnjeni mehanizam regulacije transkripcije gena PHO5. Aktivacija transkripcije gena PHO5 zahtijeva kooperativne interakcije Pho4 i pleiotropnog faktora, proteina Pho2. Te interakcije uzrokuju povećani afinitet Pho4 za vezanje na DNA, kao i povećani transaktivacijski potencijal Pho4. Aktivnost promotora gena PHO5 dodatno je regulirana represivnim djelovanjem kromatina. Nakon indukcije gena bitno se mjenja struktura kromatina promotora, koja je ovisna o Pho4 i nužan je preduvjet za aktivaciju promotora. Transkripcija gena PHO8, koji kodira za nespecifičnu alkalnu fosfatazu, regulirana je djelovanjem istih regulatornih proteina uključenih u regulaciju gena PHO5, te također podliježe represiji kromatinom. Međutim, u usporedbi s PHO5, promotor gena PHO8 pokazuje nisku razinu aktivacije transkripcije. Slaba indukcija gena PHO8 mogla bi se objasniti nemogućnošću Pho4 da dovede do nužne promjene strukture kromatina tog promotora. U radu se također raspravlja o ulozi različitih proteinskih kompleksa koji utječu na mogućnost remodeliranja strukture kromatina. Izneseni rezultati pokazuju bitnu ulogu strukture kromatina u regulaciji aktiviranja promotora.