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Transcriptional Control of Phosphate-regulated Genes in Yeast: the Role of Specific Transcription Factors and Chromatin Remodeling Complexes *in vivo*

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Summary

Gene specific regulation of transcription is of fundamental importance to cell survival. When the yeast, *Saccharomyces cerevisiae* is challenged by growth under conditions of nutrient limitation the cell must respond rapidly to stimulate expression of the necessary gene products and thus efficiently counter this environmental stress. The *PHO* system of yeast is an example of such a regulatory pathway. It contains several phosphatases and permeases the expression of which being determined by the phosphate concentration of the growth medium. In phosphate containing medium the transcription of these genes is prohibited by the negative regulation of the *PHO* specific *trans*activator Pho4. These repressing conditions witness the phosphorylation of Pho4 by the Pho80-Pho85 cyclin-CDK complex and its subsequent Msn5 dependent export from the nucleus, thus spatially precluding transcription. Under conditions of phosphate limitation the activity of the Pho80-Pho85 complex is blocked through the action of the cyclin-CDK inhibitor, Pho81, leading to the accumulation of unphosphorylated Pho4 in the nucleus and hence transcriptional activation of *PHO* specific genes such as *PHO5* and *PHO8*. Pho4 brings about gene activation in a co-operative manner with the pleiotropic factor Pho2. Phosphorylation of Pho4 also serves to prevent this protein-protein interaction, and thus regulate the activation potential of Pho4 at a second level. Finally, to bring about the activation of transcription Pho4 must effectively challenge the repressive chromatin structures found in the promoter of its target genes. To alleviate this repression the cell has evolved dedicated complexes which locally alter the structure of chromatin, thus facilitating gene specific release from nucleosomal repression. Thus the *PHO* system provides an ideal model for the study of the interplay between gene specific transcription factors and chromatin modifying complexes in the regulation of transcription.

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

Introduction

When the yeast *Saccharomyces cerevisiae* grows under conditions of phosphate starvation it responds by specifically inducing the expression of a set of specialized genes encoding the proteins involved in phosphate uptake and metabolism. In phosphate-containing medium, however, these genes are strongly and efficiently re-

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pressed (*1*). To bring about the expression of the P_i-regulated genes the cell employs the co-ordinate action of two DNA-binding proteins, the phosphatase system specific activator Pho4 and a pleiotropic factor Pho2 (*2*). Of these proteins Pho4 is itself negatively regulated through phosphorylation by the cyclin/cyclin-dependent kinase (CDK) complex, Pho80/Pho85 (*3*). In turn the kinase activity of Pho80/Pho85 complex is regulated in response to the phosphate concentration through the CDK inhibitor Pho81 (*4*) which is activated in repsonse to phosphate starvation signal. This leads to the expression of all Pi regulated genes including *PHO81* itself. The phosphatase system is therefore unique in its use of a dedicated cyclin/CDK kinase for a regulatory process other than cell-cycle control.

Our current understanding of the mechanisms involved in the transcriptional control of the phosphate-regulated genes comes primarily from studies of *PHO5* gene regulation (*5*,*6*). *PHO5* encodes the major isoenzyme of acid phosphatase (*7*), an oligomeric, heavily glycosylated extracellular enzyme, which provides cells with phosphate by hydrolyzing phosphomonoesters scavenged from the environment (*8*). The *PHO5* promoter is strongly regulated with the level of transcription increasing some 100-fold upon phosphate starvation (*1*). There are two regulatory elements at the *PHO5* promoter, UASp1 and UASp2, corresponding to the two major binding sites for Pho4, and adjacent Pho2 binding sites (*9*,*10*). Pho4 binds to these sites in a cooperative manner with Pho2 (*10*,*11*).

Binding of Pho4 to the promoter is triggered by phosphate starvation (*12*), and causes an extensive perturbation of the promoter chromatin structure (*13*). This remodeling of promoter chromatin is a prerequisite for promoter activation (*6*), requiring the transcriptional activation domain of Pho4 (*14*), but not transcription itself (*15*). Interestingly, attempts to separate the activation and chromatin remodeling functions of Pho4 have not been successful (*16*), suggesting that these two processes are intimately linked with one another.

The vacuolar alkaline phosphatase encoded by the *PHO8* gene is coregulated with *PHO5* in response to the phosphate signal (*17*). In accord with the *PHO5* results (see above) induction of this promoter is similarly accompanied by the alteration of its chromatin structure (*18*). Interestingly the chromatin remodeling process at these two promoters exhibits different extents of promoter opening and differential requirements for *trans*factors and chromatin remodeling complexes (*19*,*20*). This system of two coordinately regulated genes employing subsets of the same transcription factors to achieve expression has provided a powerful tool for uncovering the interplay between the transcription factors and the chromatin remodeling machinery necessary for control of eukaryotic gene transcription.

Multiple Levels of Regulation of Pho4 through Phosphorylation

The helix-loop-helix protein, Pho4, is the specific transcriptional activator of phosphate-responsive genes (*1*). The ability of this protein to bring about gene activation is regulated by its phosphorylation through the Pho80/Pho85 cyclin/CDK complex (see Introduction). Under conditions of high phosphate the Pho80/Pho85 complex phosphorylates Pho4 on five specific serines residues (see Fig. 1A). Phosphorylated Pho4 is negatively regulated by this covalent modification. Moreover, the specific phosphorylation of particular serine residues affects the different potential fates of the activator. Phosphorylation of Ser_{223}, which is located in the Pho2 interaction domain of Pho4 (*11*), prevents interaction of Pho4 with Pho2 (*21*). Thus, Pho4 cannot bind to the Pho2 dependent *PHO* system target promoters and is therefore transcriptionally inactive. On the other hand, phosphorylation of Serines 114 and 128 facilitates Pho4 recognition by the nuclear export protein Msn5 and brings about the export of the activator to the cytoplasm, thus physically and spatially inhibiting its activation function. Furthermore, phosphorylation of a fourth serine residue located at position 152 within the nuclear localization signal of Pho4, prevents its interaction with the import receptor Pse1, inhibiting its re-import into the nucleus (*22*). Thus, specific phosphorylation of particular serine residues independently affects Pho2 interaction, export and import of Pho4.

Phosphate starvation results in the inhibition of the Pho80/Pho85 complex by the CDK inhibitor Pho81 (*4*). This leads to the accummulation of Pho4 in the nucleus, since non-phosphorylated Pho4 is inhibited in its interaction with the exportin Msn5. Furthermore, in its non-phosphorylated state Pho4 may bind to its target promoters in a cooperative manner with the pleiotropic factor Pho2 to activate transcription (see Fig. 1B). The rapidity of the response to low phosphate medium is inconsistent with induction through newly synthesized Pho4 alone. Rather phosphorylated Pho4, which is localized to the cytoplasm under repressing conditions, could be actively dephosphorylated in response to the phosphate starvation signal. However, the phosphatase presumed to be necessary for this dephosphorylation remains unknown. Therefore, the transcriptional activity of Pho4 is negatively regulated by two distinct mechanisms: its subcellular localization and its ability to bind DNA *via* cooperative interactions with Pho2.

Why would the cell wish to regulate Pho4 at multiple levels? Layers of Pho4 regulation could be important for selective repression of a subset of Pho4-activated genes. While the export of Pho4 to the cytoplasm would negatively affect all Pho4-regulated promoters, the inability of phosphorylated Pho4 to interact with Pho2 would only be of critical importance for the class of Pho2-dependent genes. For example, partial phosphorylation of Pho4 would result in a strong repression of *PHO5*, but at the same time, could still allow an albeit reduced level of transcription from the *PHO8* promoter to which Pho4 binds in a Pho2-independent manner. Additionally, the challenge of limiting inorganic phosphate in the environment must be rapidly met to achieve optimal cell growth and survival. Therefore, a mechanism that allows a swift alteration in gene expression would be of considerable value. In this regard the regulatory pathway described requires no additional and potentially rate limiting expression of the *trans*activator,

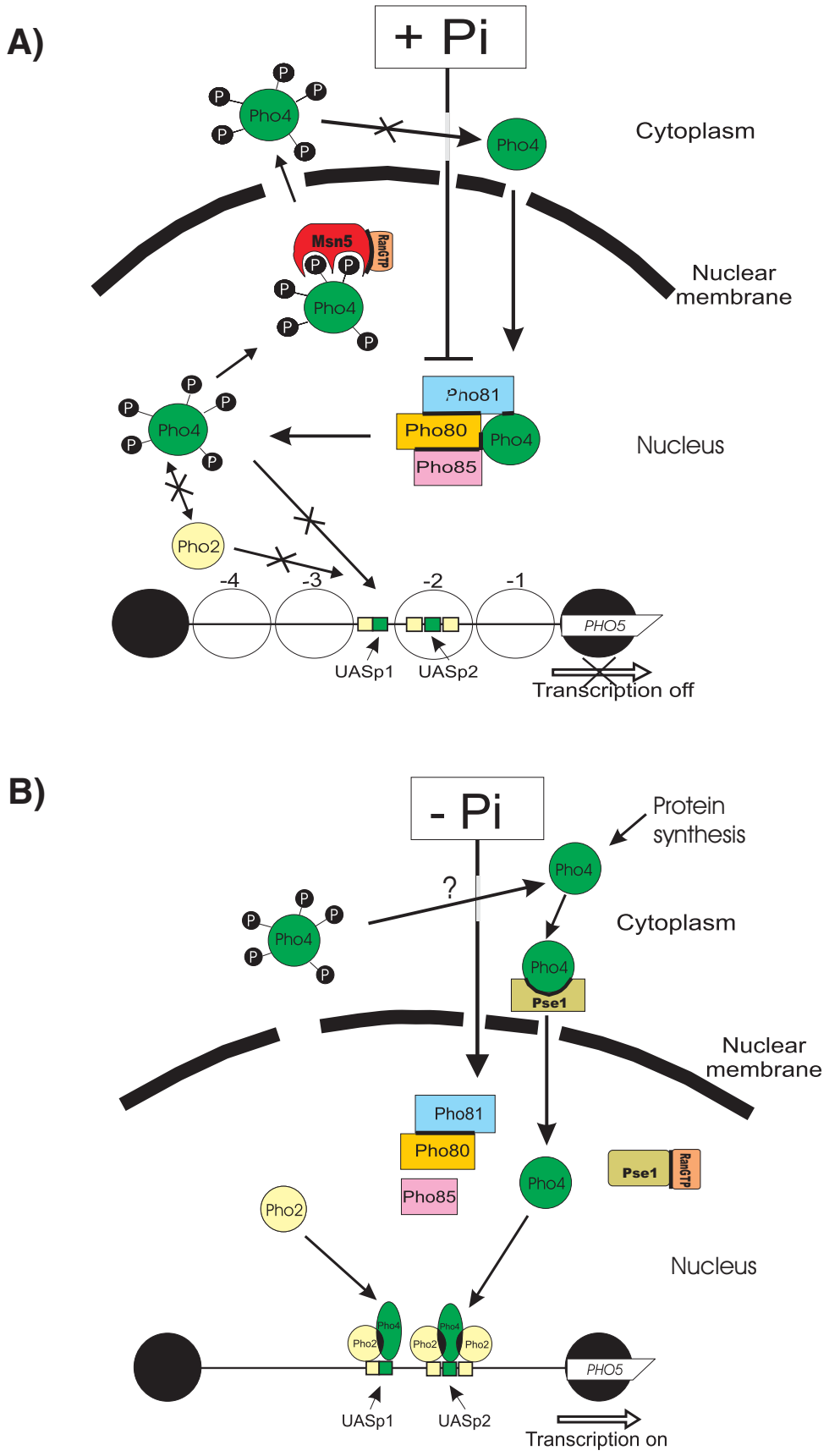

Fig. 1. Multiple levels of *PHO5* transcription regulation. A) repressive conditions, **+Pi**; **B**) inducing conditions, **-Pi**. The four white circles (marked –1 to –4) present on the *PHO5* promoter under repressive conditions represent nucleosomes which undergo remodeling upon induction, while black circles represent stable nucleosomes. For the role of transcriptional regulators and other details see text

thus ensuring a timely response to the new environmental signal.

Differential Roles for Pho2 at the *PHO5* and *PHO8* Promoters

The homeodomain protein, Pho2 (*23*), is a pleiotropic factor which is involved in the transcriptional regulation of a number of different genes from divergent regulatory pathways such as *PHO5* (*24*), *HIS*4 (*25*), *TRP*4 (*26*), *HO* (*27*) and *ADE*5, 7 (*28*). This broad role for Pho2 places the protein in an ideal situation for coordinating the regulation of different sets of genes in different regulons. There is, however, no evidence to date that the activity of Pho2 is itself regulated in response to the low phosphate signal.

What role does Pho2 play in *PHO* regulation? At the *PHO5* promoter the primary role of Pho2 is to facilitate the binding of Pho4 to UASp1, which is achieved through cooperative interaction of these two proteins on the DNA (*10*). Indeed, in the absence of Pho2 the level of Pho4 binding is insufficient to bring about chromatin opening and activation at the *PHO5* promoter. Importantly, however, this requirement can be compensated by overexpression of Pho4 (*29*), demonstrating that Pho4, and not Pho2, is the chromatin modulator and supporting a role for Pho2 in increasing the binding affinity of Pho4. Additionally Pho2 plays a more direct role in the transactivation process (*11*,*30*).

In contrast to the situation described above for *PHO5*, DMS footprinting (*31*) reveals binding of Pho4 to the *PHO8* promoter in the absence of Pho2 and Pho4 derivative lacking the Pho2-interaction domain, Pho4Δint, is also able to bind to this promoter (Fig. 2). Furthermore, Pho2 is not required for the Pho4-dependent chromatin remodeling at the *PHO8* promoter (*18*). However, the absence of Pho2 significantly reduces promoter activity (*32*). Therefore, at the *PHO8* promoter Pho2 plays a role in the activation potential of Pho4 but is not necessary for Pho4 binding.

As mentioned before, transcriptional repression of the *PHO* genes is controlled in part through the negative regulation of Pho4-Pho2 interactions *via* the phosphorylation of Pho4. However, not all *PHO* system promoters exclusively require the presence of Pho2 for appreciable activation (*32*). This differential Pho2 requirement of specific promoters could provide the cell with the ability to »fine tune« the regulation of their basal transcription.

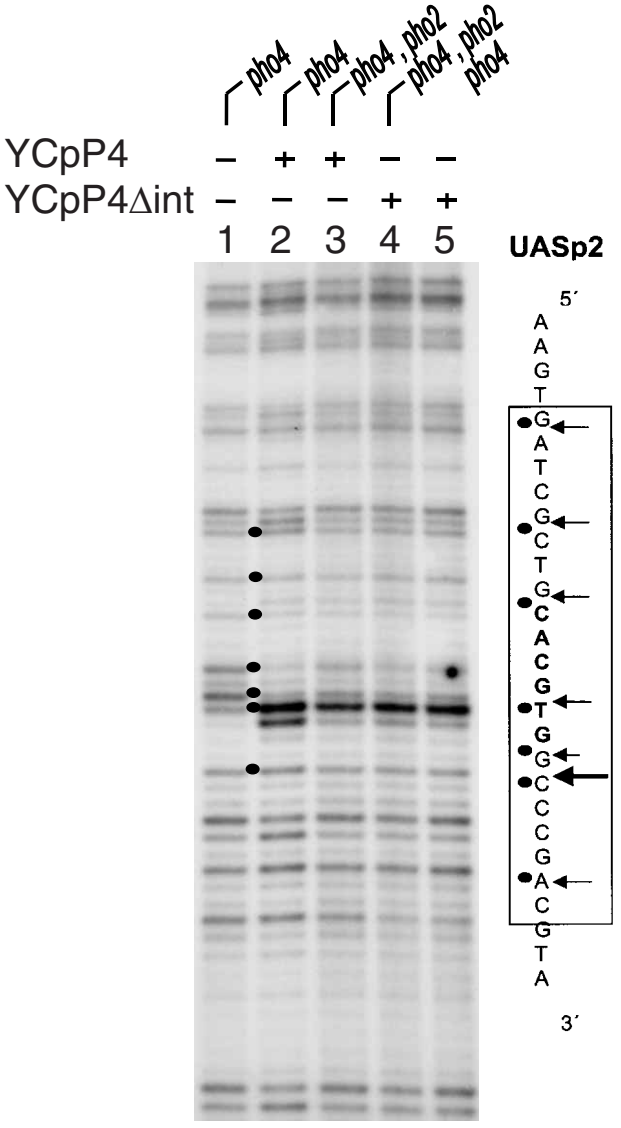

Fig. 2. Binding of Pho4 to the *PHO8* UASp2 element does not require interaction with Pho2. Binding of Pho4, or a Pho4 derivative lacking the Pho2-interaction domain, Pho4Δint, to the *PHO8* UASp2 element under inducing conditions, in a wt or a *pho2* strain, was analyzed by the DMS footprint technique (*31*). All strains carry a *PHO4* deletion and wild type Pho4 or Pho4Δint were expressed from centromeric expression plasmids. The sequence of the Pho4 binding site determined by DNaseI footprinting (*18*) is shown on the side. Guanines are marked by dots and arrows: small arrow indicates a guanine that is protected by Pho4, the big arrow a guanine that becomes hypersensitive to DMS, and medium arrows denote guanines whose reactivity with DMS is not changed

The Extent of Chromatin Remodeling Determines the Level of Gene Expression at *PHO8*

Despite being under the control of the same set of transcriptional regulators the *PHO5* and *PHO8* promoters are remarkably different in their relative strength, with *PHO5* being some 10-fold more active than *PHO8*. Mutational analysis of the two *in vitro* defined Pho4 binding sites at the *PHO8* promoter (*18*) showed that in contrast to the *PHO5* promoter, where two UAS elements act in a cooperative manner in promoter activation (*6*,*11*), the *PHO8* promoter is activated through only one UAS element, UASp2 (*32*). This finding suggested that the difference in strength between the two promoters could be a consequence of the number and/or quality of their UAS elements. However, the introduction of the *PHO5* UASp1 into the *PHO8* promoter as an additional UAS element increased the activity of the hybrid promoter, but only 2-fold (Fig. 3). More surprisingly, replacement of the native *PHO8* UASp2 with the corresponding element from *PHO5* practically eliminated promoter activity, even though Pho4 binds to this

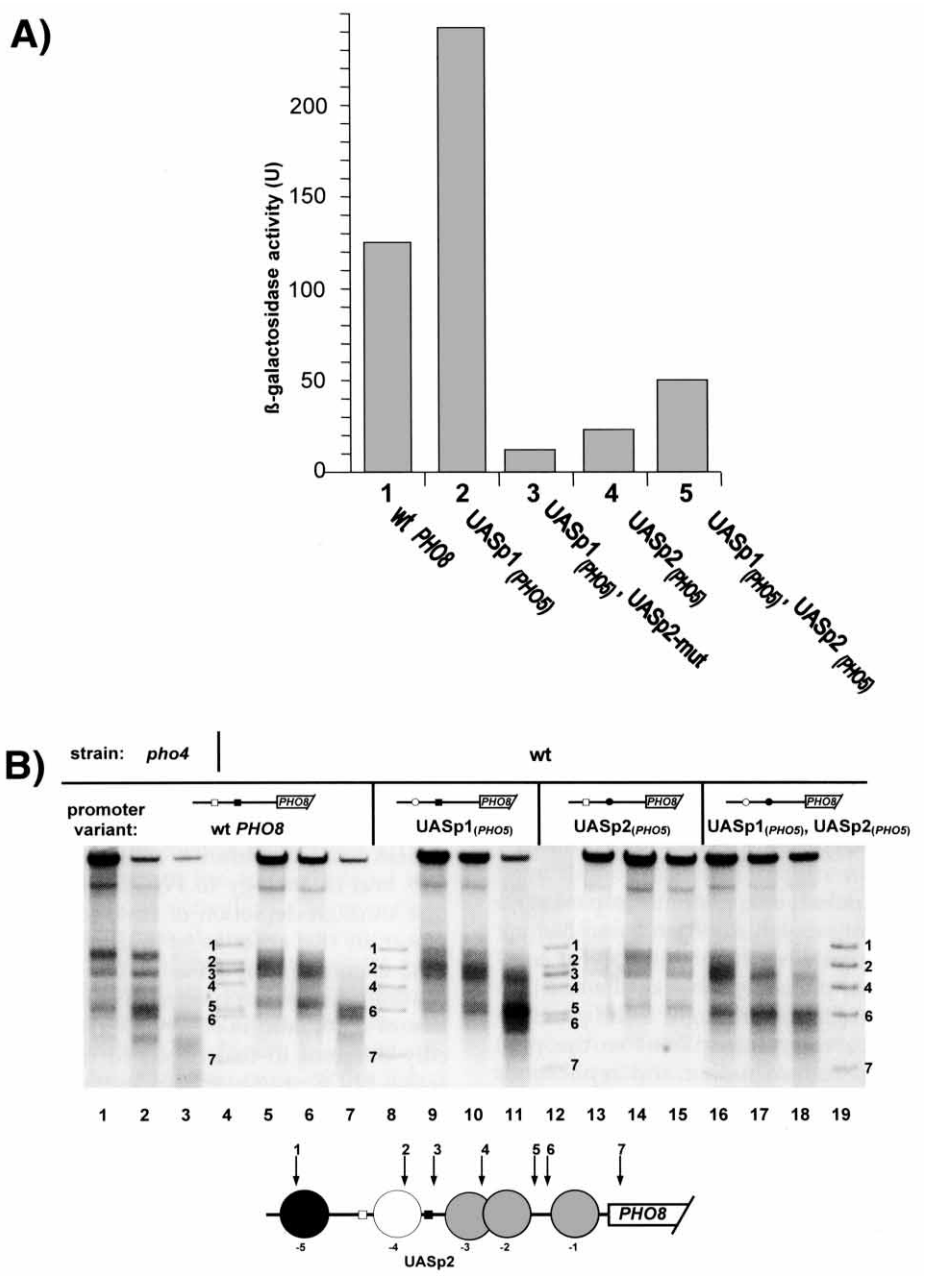

Fig. 3. The Activity of *PHO8* promoter variants containing *PHO5* UAS elements correlates with the extent of chromatin remodeling at the promoter. A) Activities of *PHO8* promoter variants containing *PHO5* UAS elements as indicated in the figure; **B)** The chromatin structure of the *PHO8* promoter variants indicated at the top was probed by DNaseI digestion. Nuclei isolated from the strains containing various *PHO8* promoter variants were treated with increasing DNase I concentration, DNA was isolated and analyzed as described (*56*). The marker lanes contain restriction nuclease double digests of purified genomic DNA. The nucleosomal structure of the promoter under repressive conditions is shown at the bottom with the positions of the restriction sites used to generate marker fragments indicated. Nucleosome –5 (black circle) does not undergo remodeling upon induction, nucleosomes –1, –2, and –3 (gray circles) undergo partial, and nucleosome –4 (white circle) complete remodeling (*18*)

newly added UAS element equally well as to the identical element in the native *PHO5* promoter (*32*). Thus neither the number of the binding sites nor their quality can adequately explain the apparent weakness of the *PHO8* promoter.

Chromatin structure analysis of hybrid promoter constructs demonstrated a striking correlation between the level of promoter activity and the extent of chromatin remodeling (Fig. 3). The most active promoter variant, *i.e.* that containing the UASp1 from *PHO5*, showed a more extensive alteration of the chromatin structure, particularly within the region which in the native *PHO8* promoter remains partially resistant to remodeling (nucleosomes –3 and –2). Deletion of this region in an otherwise native promoter resulted in a 2-fold increase in activity, consistent with a repressive role of underlying nucleosomes (*32*). Interestingly, the chromatin structure

of the inactive hybride promoter containing the *PHO5* UASp2 element was indistinguishable from that of the repressed promoter, despite the fact that Pho4 binds strongly to this element. This remarkable finding demonstrates that the mere binding of the *trans*activator needs not lead to transcription, and suggests that the neighboring nucleosomes and/or overall promoter structure could modulate the ability of Pho4 to trigger chromatin perturbation and consequent promoter activation. In this respect it is worthy of note that nucleosome perturbation at the native *PHO8* promoter is abolished in the absence of certain chromatin remodeling complexes (see below), although Pho4 is similarly bound to the promoter. This might suggest that the manner in which Pho4 interacts with the defective UASp2 of *PHO5*, when placed in the *PHO8* context, prevents its association with chromatin remodeling machinery.

Taken together these data suggest that the relatively low level of activity of the *PHO8* promoter is determined by a balance between chromatin repression and the activation potential of Pho4. The presence of a single UAS element and the necessity to overcome a region of repressive chromatin structure combine to render *PHO8* significantly weaker than the co-regulated *PHO5* promoter.

Regulation of Transcription through Chromatin

The DNA of the eukaryotic genome is packaged into chromatin, a complex of histone and non-histone proteins, which serve to achieve the high degree of compaction necessary to compress the DNA of the cell into the nucleus (*33*). Although once thought to be purely a structural matrix, apparently transparent to the processes of transcription, recombination and replication, this highly condensed structure presents the cell with the problem of access to the underlying DNA sequence and its genetic information.

In transcription, this repressive function for chromatin was elegantly demonstrated by experiments in yeast where disruption of nucleosomal structure by histone depletion was shown to result in the activation of a number of promoters, including *HIS3* and *PHO5*. This activation did not require the UAS elements normally responsible for gene activation and occurred under otherwise non-inducing conditions (*34*,*35*). Thus a repressive chromatin structure was shown to prevent the basal transcription machinery from gaining access to the proximal promoter.

To activate transcription within this repressive environment two classes of chromatin modifying enzymes are currently known to be employed. The first are the ATP-dependent chromatin remodeling machines exemplified by the SWI/SNF complex and the second the histone acetyltransferases exemplified by the SAGA complex. Although the biochemical action of these two classes of chromatin modifiers are quite different, both have been shown to play a role in the regulation of a subset of yeast promoters including those of *PHO5* and *PHO8* (see below).

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

The nucleosomal barrier to transcription has been the focus of much work over the last decade, and the basic principle of repression outlined above holds true for the phosphate regulated *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 (*13*). This organization places one of the two UAS elements (UASp1) into the hypersensitive site whereas the other UAS element (UASp2) and, importantly, the core promoter is found within a positioned nucleosome (*9*,*36*). Thus, under repressing conditions, the nucleosomal structure of the promoter prevents both the specific transcription factor Pho4 and the general transcription machinery from accessing the underlying DNA (*12*,*37*). Upon activation, a dramatic remodeling of the promoter chromatin ensues resulting in the apparent loss of two nucleosomes on either side of UASp1, and consequently the core promoter is made accessible for the general transcription factors (*38*). At the *PHO5* promoter both Pho4 and Pho2 are necessary for this chromatin transition (*6*,*29*).

The repressed *PHO8* promoter is also organized into an array of nucleosomes. In contrast to *PHO5*, however, the two UAS elements at this promoter are both found within hypersensitive sites. The TATA element is nevertheless located within a stable positioned nucleosome (*18*), and in analogy to *PHO5* loss of nucleosome structure through depletion of histone H4 activates this promoter in the absence of inducing conditions and/or UAS elements (*20*). On activation of the promoter under conditions of phosphate starvation, a striking perturbation of chromatin is observed. This remodeling is markedly different to that observed at *PHO5* since the fully active *PHO8* promoter demonstrates only partial accessibility to nucleases and restriction enzymes, consistent with the continued presence of incompletely remodeled or partially destabilized nucleosomes across the promoter (*18*). 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 (*39*). Thus, at both *PHO5* and *PHO8*, the repressive chromatin serves to silence transcription by limiting transcription factor 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 chromatin regulated promoters are switched on.

*Trans*activators and Chromatin Perturbation

*Trans*activator proteins such as Pho4 play a critical role in the regulation of inducible promoters. Positioned nucleosomes can function to prevent *trans*activator access (*12*), 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 (*14*). Importantly, the remodeling process does not

require passage through S-phase (*40*), which has been proposed to provide a »window of opportunity« for a transcription factor to access the DNA. It should be noted, however, that forcing cells to express genes in defined stages of the cell cycle is able to alter the precise requirements a particular gene has for chromatin remodeling factors. Indeed, the number of genes which require the SWI/SNF remodeling machine for activity is considerably higher for those genes which must be expressed during mitosis (*41*).

The activation domain is required to orchestrate chromatin opening (*14*), 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 (*42*). This, however, does not seen to be true for Pho4 (*16*).

More recent data have implicated acid activation domains directly in the recruitment of large multi-subunit machines necessary for the remodeling of chromatin (*43*,*44*). For example, the acidic activation domain of Gcn4 has been shown to interact independently with the Mediator, SWI/SNF and SAGA complex (*45*). Thus one critical role of the activation domain is to bring such entities to a specific promoter where they may then facilitate the activation of transcription.

One interesting question then is if artificial activators designed to directly recruit the transcriptional machinery can also bring about activation. 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 indeed sufficient to remodel the chromatin structure of the *PHO5* promoter (*46*). The nature of this remodeling activity remains unknown, although once again transcription *per se* is not required for this process. Interestingly, Morse and co-workers have found that the artificial recruitment of TBP to specific promoters through targeted Gal4-TBP fusions was unable to bring about chromatin remodeling if the TATA element of the promoter was located within a nucleosome (*47*). Thus in these experiments the direct recruitment of the transcription machinery is clearly not sufficient to bring about chromatin perturbation and activation, further supporting the general importance of dedicated chromatin remodeling activities.

ATP-dependent Chromatin Remodeling Complexes

Chromatin remodeling complexes typified by the SWI/SNF complex, are able to use the energy of ATP hydrolysis to alter the structure of the nucleosome and therefore bring about gene activation (for recent reviews see *48*,*49*). Although the precise nature of this modification is still undefined, it is likely that these complexes in some way alter the path of the DNA as it wraps around the histone octamer, thus releasing the DNA from nucleosomal repression and assisting factor binding to sites within chromatin. Furthermore, *in vivo* experiments have demonstrated a requirement for the SWI/SNF complex dependent upon the strength and position of the UAS element with respect to its nucleosomal location, suggesting that a primary function of SWI/SNF can be to assist factors which must bind to weak nucleosomal sites (*50*). Interestingly, at the *PHO8* promoter (although not at *PHO5*) the SWI/SNF complex has been shown to play a critical role in the opening of chromatin *in vivo* (Fig. 4).

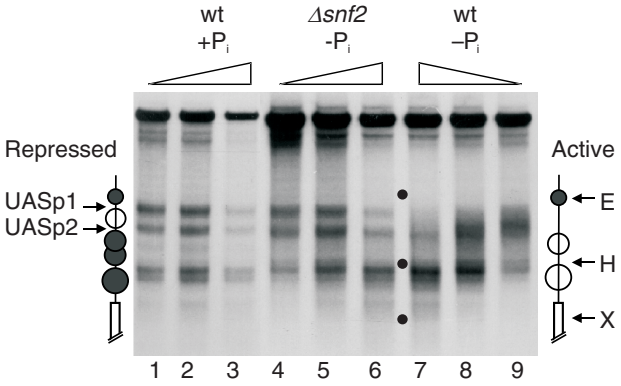

Fig. 4. The SWI/SNF complex is required for chromatin opening of the *PHO8* promoter *in vivo*. The chromatin structure of the *PHO8* promoter from wt (CY337) or Δ*snf*2 (CY407) strains grown in the presence or absence of phosphate was probed by DNase I digestion (see the legend of Fig. 3). The approximate positions of size marker fragments obtained by restriction nuclease double digests of purified genomic DNA are indicated by solid circles in between lanes 6 and 7. Schematic representations of the repressed and active promoters with respect to the position of the UAS elements and marker fragments are shown to the right and left of the figure, respectively. For nucleosome numbering and shading see Fig. 3

This occurs despite the fact that the UAS element responsible for activation of the *PHO8* promoter is non-nucleosomal (*18*), and we have been able to demonstrate that Pho4 is indeed bound to this site *in vivo* independently of both the presence or absence of the SWI/SNF complex (*20*). Thus, at the *PHO8* promoter SWI/SNF is clearly shown to have an effect on promoter remodeling at a stage subsequent to activator binding.

Histone Acetyltransferases

The association of transcriptionally active chromatin with a higher level of histone acetylation was first observed over three decades ago (*51*). However, the molecular activities that are responsible for this post-translational modification were unknown until recently when the gene for a histone acetylase from *Tetrahymena thermophila* was identified and shown to be homologous to the transcription cofactor *GCN5* in yeast (*52*). This result formally connected the acetylation of chromatin with the activation of transcription. Importantly, the histone acetyltransferase HAT activity of Gcn5 is required for the function of the protein (*19*,*53*). Furthermore, by em-

ploying antibodies specific for acetylated histones Allis and coworkers have been able to identify a promoter specific increase in the level of histone acetylation on gene activation at the *HIS3* locus (*54*,*55*).

A role for Gcn5 was examined initially at the *PHO5* promoter, where we found the basal level of *PHO5* promoter activity to be strongly Gcn5 dependent, while fully inducing conditions allowed the promoter to reach effectively wild type levels of activation (*19*). The deletion of a single UAS element, however, rendered the promoter strongly Gcn5 dependent also at the inducing conditions. In addition, the deletion of *GCN5* or mutation of residues critical for HAT function severely impairs the constitutive sub-maximal activation of the promoter at repressing conditions in a strain lacking the negative regulator Pho80. DNaseI analysis and restriction enzyme assays of the promoter under these conditions identified for the first time an effect of Gcn5 on chromatin structure since a novel chromatin organization was generated at the *PHO5* promoter, consistent with the presence of nucleosomes occupying random positions across the promoter. This structure is indistinguishable in strains carrying amino acid substitutions in Gcn5 that specifically reduce its HAT activity, demonstrating that specifically the absence of Gcn5 HAT activity is sufficient to generate this unusual structure (*19*). Thus, Gcn5 histone acetylation activity is shown to have direct effects on chromatin remodeling and transcriptional activation at the *PHO5* promoter. Interestingly, the Gcn5 dependence of the *PHO8* promoter was found to be much stronger since even under fully inducing conditions the *PHO8* promoter remains strongly Gcn5 dependent (*20*). At this promoter the absence of Gcn5 almost completely abolished chromatin remodeling, allowing only a minor widening of the hypersensitive site adjacent to the UASp2 binding site for Pho4. This result confirmed a direct effect of histone acetyltransferases upon the ability to remodel chromatin *in vivo*. Importantly, however, Pho4 was still able to bind to its UAS element in the promoter. Thus as for the SWI/SNF complex, at the *PHO8* promoter SAGA is required for chromatin modification at a stage after activator binding.

Conluding Remarks

The precise regulation of transcription is of fundamental importance to all eukaryotic cells. The *PHO* system of yeast provides an outstanding opportunity to study the regulation of transcription within a highly defined regulatory cascade. Furthermore, the ability to simultaneously study the co-regulated *PHO5* and *PHO8* promoter has already provided valuable insights into the function of the various *PHO* specific DNA-binding *trans*factors and regulatory proteins, general co-factors and remodelling machines necessary to effectively and efficiently challenge repression through chromatin and achieve controled gene activation.

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Transkripcijska kontrola gena reguliranih fosfatom u kvascu Uloga specifičnih faktora transkripcije i kompleksa koji pregrađuju kromatin *in vivo*

Sažetak

Regulacija transkripcije specifičnih gena osobito je važna za preživljavanje stanice. Kada je kvasac *Saccharomyces cerevisiae* prisiljen rasti u uvjetima nedovoljne ishrane, stanica mora brzo reagirati stimulirajući ekspresiju specifičnih gena i time se djelotvorno suprotstaviti stresu u okolišu. Sustav PHO gena u kvascu primjer je takva regulacijskog sustava. Taj sustav sadržava nekoliko fosfataza i permeaza, čija ekspresija ovisi o koncentraciji fosfata u podlozi. U podlozi koja sadržava fosfat spriječena je transkripcija tih gena negativnom regulacijom PHO-specifičnog transaktivatora Pho4. Pod uvjetima represije Pho4 je fosforiliran ciklin-CDK kompleksom Pho80-Pho85, a zatim transportiran iz jezgre s pomoću Msn5, čime je transkripcija prostorno spriječena. U uvjetima kada nedostaje fosfata, blokirana je aktivnost Pho80/Pho85 kompleksa djelovanjem ciklin-CDK inhibitora Pho81, što dovodi do nakupljanja nefosforiliranog Pho4 u jezgri, te aktivacije *PHO* gena kao što su *PHO5* i *PHO8*. Pho4 aktivira transkripciju kooperativnim međudjelovanjem s pleiotropnim faktorom Pho2. Fosforilacija Pho4 ujedno sprječava interakciju ovih dvaju proteina i time dodatno regulira aktivacijski potencijal Pho4. Konačno, da bi se provela aktivacija transkripcije Pho4, mora se uspješno suprostaviti reprimirajućem djelovanju kromatina u promotorima koje aktivira. U tu svrhu stanica se služi proteinskim kompleksima koji dovode do lokalne, specifične promjene strukture kromatina u promotorskim regijama odgovarajućih gena, čime se uklanja reprimirajuće djelovanje nukleosoma. Tako PHO sustav predstavlja idealni model za proučavanje međudjelovanja specifičnih faktora transkripcije i kompleksa koji dovode do promjene u strukturi kromatina u procesu regulacije transkripcije.