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ISSN 1330-9862
(FTB-2488)

review

Yeast *PHO* Genes: An Excellent Model for Elucidation of Chromatin-Remodelling Mechanisms

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Received: March 29, 2010
Accepted: July 5, 2010

Summary

Nucleosomes, the basic units of chromatin structure, repress transcription by restricting access of transcription factors to promoter *cis*-regulatory elements. It has recently become clearly evident that nucleosomes are highly dynamic and that there is, especially in yeast, a constant histone turnover mediated by a variety of chromatin-modifying and -remodelling multiprotein complexes. The yeast *PHO5* promoter has been a very useful model in elucidating the relationship between chromatin structure remodelling and gene regulation, showing that chromatin remodelling is replication-independent and is not a consequence of, but a prerequisite for the gene transcription. Also, chromatin remodelling at the *PHO5* promoter was the first *in vivo* demonstrated example of histone eviction in *trans*, a mechanism that operates also at the two other coregulated *PHO* promoters, *PHO8* and *PHO84*, and has recently been revealed to occur genome-wide. Despite the fact that chromatin remodelling at all three promoters eventually leads to nucleosome disassembly, they show differential cofactor requirements. At the *PHO5* promoter, an essential chromatin factor has not been identified yet and there is a redundancy of remodelling pathways involved. On the contrary, remodelling of the *PHO8* nucleosomes is critically dependent on Snf2, but still another remodeller is involved as well. Interestingly, the two neighbouring nucleosomes at the *PHO84* promoter demonstrate different stringency of remodeller dependency. Parallel *in vitro* studies of nucleosome stability and *in vivo* studies of cofactor requirements for their remodelling have shown that differential stringency of chromatin cofactor requirements is, at least to a large degree, determined by different intrinsic stabilities of individual promoter nucleosomes. As an already well characterized and established model system, the *PHO* promoters are a favourable system for parallel studies of remodelling events *in vivo* and mechanism of chromatin remodelling *in vitro*, which are of essential importance for our further understanding of the mechanisms of chromatin remodelling.

Key words: transcriptional regulation, chromatin remodelling, yeast *PHO* genes

Introduction

On the first level of compaction, nuclear eukaryotic DNA is assembled with histone proteins to form the nucleosome, the basic unit of chromatin (*1*). It has long been acknowledged that the assembly of eukaryotic genes into chromatin generally represses transcription by inhibiting the binding and therefore the function of transcription factors and components of the general transcriptional apparatus. Extensive explorations in the last decade have resulted in the discovery and characterization of a large number of different chromatin-related complexes as pro-

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[#]S.B. would like to dedicate this paper to the late Prof Marić, a respected colleague and dear friend

moter-associated transcriptional coactivators or corepressors. It has also become evident that nucleosomes are highly dynamic and that there is a constant replication--independent turnover of histones, especially in yeast promoters (*2–4*), mediated by chromatin-modifying and -remodelling complexes, histone chaperones, and histone variants (*5,6*). Modulation of nucleosome occupancy in the promoter region influences the usage of factor binding sites and thus provides an important level of transcriptional regulation.

Protein complexes that modify chromatin can be classified into two groups based on their modes of action. The first class, so-called chromatin-remodelling complexes, represents complexes that possess ATPase activity and use the energy of ATP hydrolysis either to slide nucleosomes along the DNA, to alter the nucleosome structure providing more accessible DNA, to exchange canonical histones for variant histones, or to disassemble nucleosomes and evict the histones from the promoter DNA (*7–9*). Several families of these remodelling complexes can be distinguished on the basis of the sequence homology of their ATPase subunit and different families are specialized for certain basic functions in chromatin-remodelling (for extensive review see references *7–11*). Remodellers in the SWI/SNF family, best exemplified by the yeast SWI/SNF complex and its homologues from *Drosophila* and humans, provide access to nucleosomal factor binding sites by nucleosomal movement (*12*) or ejection (*13*). Their functions are mostly correlated with promoter activation (*5,14*), but the roles of these complexes in transcriptional repression have also been reported (*5,7,14*). On the other hand, complexes of the ISWI family function in chromatin organization and nucleosome positioning (*15,16*), while those of the SWR1 family exchange canonical histones for histone variants (*17*).

The second class of chromatin-related factors, termed chromatin modifiers, involves proteins with different enzymatic activities that bring about covalent modifications of histones and thereby alter the chromatin structure. In particular, acetylation of the histone N-terminal tails has been known to be associated in a correlative way with transcriptional activation for more than 4 decades and histone acetyltransferase (HAT) activity of Gcn5, a subunit of the yeast SAGA complex, is the most extensively studied one *in vitro* and *in vivo* (for review see references *18–20*). Besides acetylation and deacetylation, histone tails undergo other covalent modifications which have come into focus during the last years and include phosphorylation, methylation and ubiquitination. The interplay between these histone modifications, the so called 'histone code', has been suggested to codetermine the transcriptional state of a gene (for review see *21* and references therein). It is not fully clear if covalent histone modifications are always involved in chromatin structure transitions, but several cases were reported where chromatin modifiers and remodellers collaborate to alter promoter chromatin structure, resulting in promoter activation (*6,22–25*).

The inducible yeast *PHO5* promoter was the first and still is one of the classic, best characterized examples of a massive chromatin transition concomitant with promoter activation. Transcription of the *PHO5* gene, which encodes an extracellular nonspecific acid phosphatase (*26*), is activated in response to a phosphate-starvation signal *via* the specific transcriptional activator Pho4 (*27*). Under repressive conditions, *i.e.* in phosphate-containing media, Pho4 is inactivated by multiple phosphorylations through the action of Pho80/Pho85, a cyclin/cyclin-dependent kinase complex, resulting in the export of phosphorylated Pho4 from the nucleus. The phosphate starvation signal brings about activation of Pho81, which inhibits kinase activity of Pho80/Pho85, and unphosphorylated Pho4 accumulates in the nucleus (*28*). Pho4-Pho2 interactions then result in the cooperative binding of the two proteins to the promoter and consequent activation of the *PHO5* gene transcription (*29*).

Under repressive conditions, the *PHO5* promoter region is covered by four positioned nucleosomes, interrupted only by a 70-bp hypersensitive region containing one of the two Pho4 binding sites (*30*). Upon promoter induction, Pho4 triggers a process of massive remodelling of the promoter chromatin structure (*31*), resulting in a 600-bp long nucleosome-free region (NFR). This allows binding of Pho4 to the second high affinity site, as well as binding of other required protein factors to the promoter and eventually the transcriptional activation.

The yeast *PHO8* promoter is coregulated by the same transactivator as *PHO5*, Pho4, and also shows a pronounced but distinct modulation of chromatin structure upon induction by phosphate depletion (*32*). Very recently our group and others have demonstrated that the strongest promoter of the *PHO* regulon, *PHO84*, is also regulated on the level of its chromatin structure modulation (*33,34*), but again distinct chromatin architecture and chromatin transition pattern were observed.

The fact that three *PHO* promoters are regulated by the same transactivation mechanism, *i.e. via* common specific activator Pho4, but use apparently distinct remodelling pathways for chromatin structure transition from a repressed to an active state, makes them an excellent model for elucidation of logic and basic mechanism involved in the modulation of promoter chromatin structure. In this review, current knowledge about their distinct chromatin architectures and differential chromatin-remodelling and -modifying cofactor requirements are summarized and discussed in terms of causal relationship between inherent promoter chromatin structure and remodelling pathways involved at particular promoter.

Transcriptional Regulation by Promoter Chromatin Structure Remodelling: Pioneer Studies with the *PHO5* Promoter

The transition of chromatin structure at the *PHO5* promoter from a repressed to an active state was clearly demonstrated to be accompanied with transcriptional activation by pioneer work of Wolfram Hoerz almost 25 years ago. Under repressive conditions, *i.e.* in a phosphate-containing media, the *PHO5* promoter region is covered by positioned nucleosomes, but there is a short hypersensitive stretch of about 70 bp between nucleosomes –2 and –3. There are two regulatory elements in the *PHO5* promoter corresponding to the specific activator Pho4 binding sites. Importantly, one of the two

Pho4 binding sites, UASp1, lies in this hypersensitive region, while the second Pho4 binding site, UASp2, is covered by nucleosome –2. Upon induction, a 600-bp region, covered by nucleosomes –1 to –4 in the repressed promoter, becomes extremely sensitive to DNase I (*30*). By a more quantitative analysis, using restriction nuclease digestion (*35*), it was found that the central part of this long hypersensitive region was almost fully accessible, while in the inactive promoter the accessibility of all restriction sites contained within positioned nucleosomes was not more than 10 %. This chromatin transition uncovers TATA box as well as UASp2 element, allowing Pho4 binding to this site (see below). In clear contrast to nucleosomes –1 to –4, restriction enzyme analysis showed that the accessibility of DNA covered by nucleosomes –5 and +1 did not significantly change under induction conditions, indicating that the structure of these nucleosomes is not altered upon promoter activation (*36*).

A rather important result revealed by studies with *PHO5* promoter in Hoerz's laboratory was the finding that upon induction, disruption of the four nucleosomes at the promoter occurs even if transcription is prevented by a deletion of the TATA box, clearly demonstrating that the chromatin transition is a prerequisite for the subsequent promoter activation rather than its consequence (*31*), and that chromatin opening mechanism is independent of interactions with the components of transcriptional machinery. Another finding of general importance in the field was that nucleosome disruption upon induction of the *PHO5* promoter also occurred in the absence of DNA replication (*37*). The regulatory role of the chromatin structure in the *PHO5* transcriptional activation was also demonstrated by a rather different approach. Elegant studies from the Grunstein's laboratory showed that depletion of H4 histone levels, which prevents the formation of intact nucleosomes, results in the partial activation of the *PHO5* promoter, under otherwise repressive conditions (*38*).

Early studies in Hoerz's laboratory, concentrating on the factors involved in remodelling of chromatin structure at the *PHO5* promoter, showed that this process critically required the transcriptional activator Pho4 (*39*,*40*). The requirement for coactivator Pho2 is probably an indirect effect *via* Pho4, since the binding of Pho4 to the promoter requires cooperative interactions with Pho2 (*29*,*41*). It was also shown that the absence of Pho2 can be compensated for by the overexpression of Pho4, but not *vice versa* (*39*). More detailed studies show that Pho4 triggers a process of chromatin remodelling through its activation domain and an attempt to separate a possible 'chromatin remodelling domain' from the transactivation domain has failed (*42*,*43*), suggesting that at least one of the roles of the activation domain was to recruit chromatin-modulating coactivator complexes to the promoter. This was confirmed by the later *in vitro* studies in Workman's laboratory with several acidic activators, including Pho4, showing the direct interactions of these activators with multiple SWI/SNF subunits (*44*). They also showed that yeast HAT complexes interact with similar sets of acidic activators like SWI/SNF (*45*,*46*). However, recruitment of chromatin-modulating complexes *via* the mediator complex and/or the holoenzyme could still be an alternative, redundant pathway (*47*).

As already mentioned, the *PHO5* promoter contains two binding sites for specific activator Pho4: one accessible low-affinity site, UASp1, localized in the extended linker region between nucleosomes –2 and –3 and another, high-affinity site UASp2, covered by nucleosome –2 under repressive conditions. Binding of Pho4 to UASp2 is of critical importance for *PHO5* activation (*40*). Pho4 binding to UASp2 under conditions of full induction in wild-type (wt) strain has only been detected at the open *PHO5* promoter, after the Pho4 bound to UASp1 induced remodelling that uncovered the UASp2 element (*48*). Moreover, the activation domain of Pho4 was critically important for Pho4 to access UASp2 (*42*), suggesting a pivotal role for chromatin remodelling by recruited factors. Activation of the *PHO5* promoter was therefore, expected to be critically dependent on chromatin-modulating activities.

A Network of Redundant Mutually Independent Remodelling Pathways Leads to Chromatin Structure Transition at the *PHO5* Promoter

As mentioned before, the similarity between interactions of Pho4 and other activation domains with SWI/SNF and SAGA complexes observed *in vitro* suggested overlapping functions of these two complexes *in vivo* (*49*). However, early findings showed that full induction of *PHO5* upon phosphate depletion was largely independent of both Gcn5 and Snf2 activities (*47*,*50*). On the other hand, the absence of Gcn5 or the inactivation of its HAT activity has been found to strongly reduce the promoter activity under repressive conditions, or under artificial conditions of submaximal promoter activation. Under such conditions and in the absence of Gcn5, nucleosome positions were randomized, suggesting that histone acetylation by Gcn5 must play a certain role in chromatin remodelling at the *PHO5* promoter (*50*). When we reexamined the effect of Gcn5, a strong decrease in the rate of chromatin remodelling in the absence of its HAT activity was observed, demonstrating an important novel contribution of Gcn5 in increasing the rate of gene induction, rather than affecting the final steady-state expression levels (*51*). Using chromatin immunoprecipitation, we found that SAGA is recruited to the *PHO5* promoter under induction conditions and only in the presence of Pho4 (*52*). Furthermore, it was shown that induction of *PHO5*, when remodelling was delayed in the absence of Snf2 (see below), resulted in localized increases in histone acetylation (*23*). This demonstrates that histones at the *PHO5* promoter are indeed modified by the HAT activity of SAGA in a targeted fashion and that the observed delay in chromatin remodelling in a *gcn5* strain might be due to the lack of hyperacetylation.

The finding that the absence of Gcn5 affects the rate rather than the final level of chromatin remodelling was a clear suggestion to examine a possible effect of Snf2 on the kinetics of *PHO5* induction. Indeed, the absence of Snf2, or the inactivation of the Snf2 ATPase activity, strongly delayed remodelling at the *PHO5* promoter, even more pronounced than the absence of Gcn5 (*53*).

As already mentioned, chromatin remodelling at the *PHO5* promoter occurs in the absence of transcription (*31*) and moreover, we have also demonstrated the complete independence of chromatin opening kinetics from *PHO5* transcription, not only under wt conditions, but even in the absence of Snf2 or Gcn5 (*53*). The rate of *PHO5* induction in the cells deleted for both *GCN5* and *SNF2* showed a synthetic phenotype, indicating a functional interplay of the two activities in modulation of the promoter chromatin structure (*53*). Elegant *in vitro* studies demonstrated that following recruitment through transcription factors, SWI/SNF is stably anchored to hyperacetylated nucleosomes *via* its bromodomain (*54*), suggesting that at least one of the roles of a prior histone hyperacetylation at the *PHO5* promoter could be the stabilization of SWI/SNF on the promoter chromatin. However, much stronger delay observed in the *gcn5 snf2* double mutant than in the *gcn5* strain indicated that Snf2 was involved in chromatin remodelling also in the absence of Gcn5.

The fact that the *PHO5* promoter could eventually be remodelled even in a strain lacking a functional SWI/SNF complex and Gcn5 suggested that an alternative remodelling pathway had to be involved. This was rather intriguing, especially since it had previously been shown that the *PHO8* promoter, which is activated with the same specific activator, was strictly dependent on remodelling activity of SWI/SNF complex and on HAT activity of Gcn5 (*55*; see below). We therefore performed a comprehensive search for additional remodelling machines involved in chromatin structure transition at the *PHO5* promoter. Practically all viable chromatin-remodeller mutants were examined and the main outcome of this *in vivo* study was that none of the tested chromatin cofactor mutations prevented *PHO5* promoter remodelling and the same was true even for several double mutants (*53*). Besides *snf2* deletion mutant, only *ino80* cells showed a strong delay in chromatin remodelling kinetics. The *snf2 ino80* double mutation had a synthetic kinetic effect, but eventually a high level of the *PHO5* induction was achieved. We could, therefore, conclude that Snf2 and Ino80 both participated independently of each other in remodelling process at the *PHO5* promoter. Moreover, high level of remodelling eventually achieved in the absence of both Snf2 and Ino80 suggested that even additional remodelling activities could be involved. Apparently, a complex network of redundant, mutually independent parallel remodelling pathways is involved in chromatin transition at the *PHO5* promoter (see Table 1; *23,33,53,55–57*).

Table 1. Summary of the effects on chromatin remodelling at *PHO5*, *PHO8* and *PHO84* promoters in chromatin cofactor mutants under different induction conditions

Mutation	Induction conditions	*PHO* promoters				Reference
		PHO5	*PHO8*	*PHO84*		
				upstream nucleosome	downstream nucleosome	
snf2	full induction	kinetic delay	no remodelling	no remodelling	kinetic delay	*23,33,53,55*
	submaximal induction	no remodelling	n.d.	no remodelling	fully remodelled	*33,53*
gcn5	full induction	kinetic delay	local remodelling	kinetic delay	kinetic delay	*33,51,53,55*
	submaximal induction	no remodelling	n.d.	n.d.	n.d.	*50*
snf2gcn5	full induction	synthetic kinetic delay	n.d.	no remodelling	final remodelling not affected	*33,53*
ino80	full induction	kinetic delay	kinetic delay	kinetic delay	kinetic delay	*33,53*
	submaximal induction	no remodelling	n.d.	no remodelling	fully remodelled	*33,53*
snf2ino80	full induction	synthetic kinetic delay	n.d.	no remodelling	final remodelling not affected	*33,53*
ino80gcn5	full induction	synthetic kinetic delay	n.d.	n.d.	n.d.	*53*
asf1	full induction	kinetic delay	kinetic delay	subtle effect (strain dependent)	subtle effect (strain dependent)	*33,56*
	submaximal induction	no remodelling	no remodelling	n.d.	n.d.	*57*
snf2asf1	full induction	synthetic kinetic delay	n.d.	n.d.	n.d.	*56*

n.d. – not determined

The RSC complex, a rather abundant chromatin-remodelling complex in yeast belonging to the SWI/SNF family (*58*), has recently been shown to completely disassemble nucleosomes in the *in vitro* experiments (*59*). It is therefore possible that this complex plays a role, or even that it is a dedicated remodeller at the *PHO5* promoter. However, since the RSC activity is essential for the cell growth and, on the other hand, the induction of *PHO5* in a phosphate-free medium requires some rounds of replication (*37*), a possible critical role of the RSC complex in nucleosome disassembly at the *PHO5* promoter is not straightforward to examine *in vivo.*

Although the search for essential chromatin cofactor at the *PHO5* promoter under conditions of full induction failed, we showed that under submaximal induction conditions, achieved by the overexpression of Pho4 under otherwise repressive conditions, chromatin structure at the promoter was largely open in wt cells, but practically no opening in the absence of Snf2 or Ino80 was noticed (*53*) (Table 1). Such submaximal induction condition probably corresponds to early time points of induction kinetics in our measurements, or *PHO5* induction in the low-phosphate medium. Namely, Dhasarathy and Kladde (*60*) showed that more stringent cofactor requirements were observed using low-phosphate rather than phosphate-free medium. At low nuclear Pho4 concentration that occurred under low-phosphate conditions, SWI/SNF and Gcn5 were absolutely required for chromatin remodelling, while high nuclear Pho4 concentrations that occurred under phosphate-free conditions bypassed the need for both cofactors. Taken together, all these studies strongly suggested that the extent of induction led to more or less pronounced chromatin cofactor requirements at the *PHO5* promoter.

Chromatin Remodelling at the *PHO5* Promoter Results in Nucleosome Disassembly and Histone Eviction in *trans*

Early studies of elucidation of chromatin remodelling mechanisms at the *PHO5* promoter were focused on identification of chromatin-modifying and -remodelling activities involved in chromatin modulation and on the interplay between transcriptional activators and chromatin cofactors during remodelling process. A fundamentally different question, concerning the molecular nature of a remodelled chromatin structure, emerged with time, *i.e.* what the broadly defined term 'chromatin remodelling' meant. Namely, it was unclear if at active promoters 'persistently altered nucleosomes' were present, or if remodelled promoter regions represented histone-free DNA. By using different approaches, it was simultaneously shown in Hoerz's and Kornberg's laboratories that chromatin remodelling at the *PHO5* promoter resulted in complete unfolding of nucleosomes and the loss of histones from the remodelled region (*23*,*61*), by an eviction mechanism in *trans* (*62*,*63*). However, even in the fully induced state on an average one nucleosome always remained at the promoter region that underwent remodelling (*62*–*64*). It was also recently suggested that sliding-mediated nucleosome disassembly mechanism might be involved at the *PHO5* promoter (*65*).

The finding that chromatin remodelling process at the *PHO5* promoter brought about disassembly of nucleosomes was rather suggestive for the involvement of histone chaperones, which could act as histone acceptor in *trans*. Indeed, we showed that the rate of histone eviction upon promoter induction was significantly delayed in the absence of histone H3/H4 chaperone Asf1 (*56*), while under submaximal induction conditions, in the low-phosphate medium, Asf1 function was essential for chromatin disassembly and *PHO5* activation (*56*,*57*). All these findings are in a good agreement with the existence of dynamic interplay between nucleosome assembly and disassembly at the activated *PHO5* promoter, resulting in net nucleosome depletion in the remodelled promoter region.

The Two Promoters Coregulated with the *PHO5* Promoter, *PHO8* and *PHO84*, Show Differential Cofactor Requirements for Nucleosome Disassembly

The *PHO8* promoter is coregulated by the same specific transactivator as *PHO5*, but it is a weaker promoter, *i.e.* transcriptional activity upon full induction is much lower (*32*). The two Pho4 binding sites at the *PHO8* promoter were mapped *in vitro*, but only the high affinity site, UASp2, was found to be functional *in vivo* (*29*), which could be an explanation for the lower strength of the *PHO8* promoter. The *PHO8* promoter is also regulated by remodelling of its chromatin structure. Under repressive conditions, the *PHO8* promoter is organized into an array of nucleosomes with UASp2 site localized in a short hypersensitive region between nucleosomes –3 and –4, while TATA element is covered by a stable, positioned nucleosome. Upon the promoter activation, a massive perturbation of the repressed chromatin structure was observed. In contrast to the *PHO5* promoter, only partial accessibility to nucleases and restriction enzymes was demonstrated at certain promoter regions, including the region that is under repressive conditions covered by nucleosome –1. This finding suggested the presence of incompletely remodelled or destabilized nucleosomes at the active promoter (*32*).

A search for cofactors required for modulation of chromatin architecture at the *PHO8* promoter revealed, as at the *PHO5* promoter, that the SWI/SNF and Ino80 remodelling complexes, the Gcn5 HAT activity and the histone chaperone Asf1 were involved (*33*,*55*,*56*) (Table 1). Interestingly, while at the *PHO5* promoter there is redundancy of chromatin remodelling pathways and no essential chromatin cofactor has been indentified yet, the Snf2 subunit of the SWI/SNF complex and Gcn5 are critically required at the *PHO8* promoter (*55*). It is also rather surprising that besides Snf2, another remodeller, Ino80, contributes to remodelling process as well (*53*). By elegant *in vitro* studies, different intrinsic stabilities of the *PHO5* and *PHO8* promoter nucleosomes were demonstrated, suggesting that the higher stability of *PHO8* promoter nucleosomes could explain higher stringency of cofactor requirements at this promoter (*66*).

With the aim to get more clear insight into the interplay between promoter chromatin architecture and nu-

cleosome stabilities at one side, and the number, affinity and positions of activator binding sites in the promoter chromatin context on the other side, we have recently extended our study on the third, the strongest *PHO* promoter, *PHO84*. Since the three *PHO* promoters of different strength are regulated by the same specific transactivator, they are an excellent model for comparative studies of chromatin modulation mechanisms at three promoters, particularly of their requirements for chromatin cofactors, without complication due to comparison of different transactivation mechanisms at the same time.

It had been reported previously that chromatin structure at the *PHO84* promoter underwent transition upon induction, but the requirement for chromatin cofactors was not studied (*34*). We have performed comprehensive studies with the *PHO84* promoter, regarding its chromatin architecture of repressed and active states as well as the role of chromatin cofactors and *cis*-regulatory elements in chromatin structure transition. In the repressed state, the *PHO84* promoter contains a short NFR, flanked by two positioned nucleosomes. Two high-affinity Pho4 binding sites are located in this NFR, while the other two low-affinity Pho4 sites are covered by nucleosomes positioned upstream and downstream from NFR. Interestingly, in contrast to the *PHO5* and *PHO8* promoters, proximal promoter region around TATA box is only semiprotected at the repressed promoter, suggesting the increased plasticity of a chromatin structure in this region. Upon induction, two nucleosomes flanking the short NFR region undergo remodelling, resulting in a large hypersensitive region upstream of the TATA box. Accessibility of the promoter region around the TATA box also increased upon induction, but not to the same high level as at the upstream regulatory region. We further showed that chromatin remodelling at the *PHO84* promoter eventually led to histone eviction (*33*), as it had been shown previously at the *PHO5* and *PHO8* promoters. Therefore, at all three *PHO* promoters, nucleosome disassembly is a common mechanism involved in their transcriptional activation.

As in the case of other two coregulated promoters, chromatin transition at the *PHO84* promoter and the consequent promoter activation were also strongly affected in the absence of Snf2, Ino80 and Gcn5 and to a lesser degree in the absence of Asf1 (*33*). However, with respect to stringency of cofactor requirements, the *PHO84* promoter behaved differently from either the *PHO5* or *PHO8* promoter. Surprisingly, remodelling of the upstream nucleosome critically depends on Snf2, whereas remodelling of the downstream one does not. Even under submaximal induction conditions, which can enhance the requirement for chromatin cofactors as shown at the *PHO5* promoter (*53*), the downstream nucleosome at the *PHO84* promoter was fully remodelled in the absence of Snf2. Furthermore, remodelling of the Snf2-dependent nucleosome is more strongly dependent on Ino80 than the remodelling of the other nucleosome (*33*) (Table 1). To our knowledge, the *PHO84* promoter is the first such example of differential remodelling pathways involved in disassembly of two neighbouring nucleosomes at the same promoter. Actually, the *PHO84* promoter appeared as a hybrid of the *PHO5* and *PHO8* promoters, containing one strictly Snf2-dependent nucleosome, reminiscent of the *PHO8* nucleosomes, and the other less stable, redundantly remodelled nucleosome, similar to the *PHO5* nucleosomes.

Intrinsic Properties of Individual Promoter Nucleosomes Determine the Stringency of Remodelling Cofactor Dependency

Although there is a substantial knowledge about recruitment of chromatin cofactors to promoters, it is still rather unclear why the promoters exhibit differential requirements for chromatin modifiers and remodellers. Studies with the three *PHO* promoters, activated by the same transactivator but *via* distinct remodelling pathways, clearly show that the requirements for chromatin cofactors are not determined exclusively by a specific activator that triggers chromatin remodelling process at promoters. This is even more clearly shown at the *PHO84* promoter, where two neighbouring nucleosomes are remodelled by different remodelling pathways. Moreover, the *PHO5* promoter variant, which is under control of the Gal4 activator, demonstrated the same chromatin transition pattern upon activation and the same cofactor requirements as the wild type *PHO5* promoter (*51,53*), showing that the program of chromatin cofactor recruitment and stringency of cofactor dependence does not depend strictly on Pho4 as the trigger, but it is rather determined by the specific promoter chromatin structure.

As already mentioned, it was shown that the stringency of cofactor requirements for chromatin remodelling at the *PHO5* promoter was dependent on the amount of Pho4 recruited to the promoter (*53,56,60*) and this was also true for the upstream nucleosome at the *PHO84* promoter, which became critically dependent on Ino80 under submaximal induction conditions, *i.e.* when less Pho4 was bound to the promoter (*33*). This relationship between Pho4 occupancy at the promoter and the stringency of cofactor requirements could be a valid explanation for the difference in the stringency of cofactor requirements between the *PHO5* and *PHO8* promoters, since the *PHO8* promoter has only one Pho4 binding site and therefore less Pho4 could be recruited to this promoter than to the *PHO5* promoter, containing two cooperative Pho4 binding sites. However, this effect cannot explain the promoter-internal difference in cofactor requirements for remodelling of the two neighbouring nucleosomes at the *PHO84* promoter, since here both nucleosomes are simultaneously remodelled under the same level of Pho4 recruitment. An alternative explanation for differential stringency of cofactor dependence at individual nucleosome was offered by previous *in vitro* studies, demonstrating that the nucleosomes at the *PHO8* promoter were intrinsically more stable than those at the *PHO5* promoter, which raised a hypothesis that different stringency of cofactor requirements for nucleosome remodelling was due to their different intrinsic stabilities (*66*). Using the same methodology, we demonstrated that two nucleosomes at the *PHO84* promoter differed in their intrinsic stabilities, as predicted also *in silico* (*67*): the upstream nucleosome, remodelling of which was strictly Snf2-dependent, was more stable than the downstream one (*33*). Causal relationship between cofactor requirements and nucleosome stabilities at the *PHO84* promoter

was also confirmed *in vivo*. By introducing destabilizing mutations at the position of the upstream nucleosome, as confirmed by progressively lowered N-score (*67*) for this region and the lower nucleosome stability in the *in vitro* assay, progressively more remodelling of this nucleosome in the absence of Snf2 was observed. On the basis of these findings we concluded that the stringency of chromatin cofactor requirements for nucleosome disassembly at the *PHO84* promoter is determined, at least to a large degree, by intrinsic stabilities of individual promoter nucleosomes (*33*).

Concluding Remarks

Transcriptional regulation by remodelling promoter chromatin structures has been studied for decades and it is now widely accepted that this regulation involves dynamic competition between nucleosomes and transcription factors for regulatory sequences in the promoters. Under appropriate conditions, transcription factors collaborate with nucleosome-modifying and -remodelling factors in modulation of chromatin structure to expose regulatory sites and allow promoter activation. The general logic and outline of chromatin-remodelling strategies have been revealed to a large degree, but details and a sequence of individual steps and their orchestration in remodelling events are a subject of current and future studies.

The pioneer studies with the yeast *PHO5* promoter and the later studies with two other coregulated *PHO* promoters, *PHO8* and *PHO84*, have had an important impact on our present understanding of the general principle of transcriptional regulation by remodelling promoter chromatin structure. These three promoters represent an attractive model system for further elucidation of chromatin remodelling mechanisms. Regarding their overall chromatin structure at the repressed state, the *PHO5* and *PHO84* promoters could be considered as 'closed' or, as recently suggested, 'covered' promoter category (*68*) typical for highly regulated promoters. At promoters of this type, nucleosomes cover the transcription start site and proximal promoter elements as well as at least some of the transcriptional activator-binding sites and therefore these promoters show rather strong remodelling dependence for their activation. Although remodelling event at these promoters is triggered by the same specific activator, distinct chromatin transition patterns and differential cofactor requirements have been observed. Therefore, the three *PHO* promoters are a favourable system to address the question of causal dependence between promoter chromatin architectures, as well as intrinsic properties of their nucleosomes and a stringency of cofactor requirement for their remodelling, without complication of comparing different transactivation mechanisms, as would be the case if the promoters activated with different activators were compared.

It is of essential importance for further elucidation of chromatin remodelling mechanisms to directly connect mechanistic abilities of chromatin remodellers determined *in vitro* with remodelling events observed *in vivo*. In this regard, further studies with the *PHO* promoters, as an already well characterised and established model system *in vitro* and *in vivo*, are expected to reveal the important details and to further enhance our understanding of chromatin-remodelling mechanisms.

Acknowledgements

We would like to thank V. Fajdetić for technical assistance.

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