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minireview

The Yeast PHO Promoters as Paradigm for Transcriptional Regulation by Chromatin Remodelling: Current State of the Art

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Summary

It has been widely acknowledged that modulation of chromatin structure at the promoter region influences the usage of factor binding sites and thus provides first, important level of transcriptional regulation. Chromatin-remodelling complexes utilize the energy of ATP hydrolysis to disassemble nucleosomes, and their functions are prominently correlated with promoter activation and also repression. Mechanistic details of individual steps and their orchestration in complex remodelling events, as well as regulatory mechanisms controlling remodeller activity, are subjects of current and future studies. The yeast *PHO5* promoter was the first and still is one of the best characterized examples of a massive chromatin transition associated with transcriptional activation. Studies with this promoter provided several breakthrough findings and established basic principles of chromatin-remodelling process. Recent studies have revealed a network of five remodellers from all four remodeller subfamilies involved in this chromatin transition. Importantly, requirement for chromatin remodellers at the *PHO8* as well as at *PHO84* promoter, activated by the same transactivator as *PHO5*, are rather different. All these findings point out that chromatin remodelling process is in general even more complex than presumed, and it could be expected that further studies with the well-established PHO promoter system will be rather valuable for its further understanding.

Key words: chromatin remodelling, *PHO* genes, transcriptional regulation, *Saccharomyces cerevisiae*

Transcriptional Regulation by Promoter Chromatin Structure Remodelling

It is today fully acknowledged that chromatin structure of eukaryotic genes generally represses gene transcription by inhibiting the binding and consequently the function of transcription factors and components of the general transcriptional apparatus, and that modulation of nucleosome occupancy in the promoter region provides an important level of transcriptional regulation (*1*).

As recently revealed from genome-wide studies, promoters in *Saccharomyces cerevisiae* may be broadly divided into two classes with respect to architecture of their promoter chromatin structures: so-called open and covered promoters (*2*). Chromatin architecture of open promoters contains an approx. 150-bp nuclease-hypersensitive region or nucleosome-free region, located immediately upstream of the transcriptional start site, allowing assembly of the preinitiation complex (so-called 'open door policy', *3*). Covered promoters, on the other hand, have more regular nucleosome arrangements, where site for preinitiation complex assembly and other transcription factor binding sites are covered by precisely positioned nucleosomes. Such promoters are typical for inducible or stress-acti-

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This paper is dedicated to Pavao Mildner, professor emeritus at the University of Zagreb, my teacher and mentor, with whom I successfully and enjoyably worked for a long time

vated genes and their activation ultimately depends on the activity of chromatin modifying and remodelling factors which govern nucleosome remodelling process. Modulation of nucleosome occupancy at covered promoters regulates the availability of transcription factor binding sites and thus represents the first level of transcriptional regulation.

There are two groups of protein complexes that modify chromatin structure upon promoter induction. The first class involves a variety of enzymes that covalently modify nucleosomes. One of the most studied enzymes in yeast is the histone acetyltransferase (HAT) Gcn5, a subunit of the yeast SAGA complex (*4*, for review see *5*,*6*). The second class, ATP-dependent chromatin-remodelling complexes, uses the energy of ATP hydrolysis to remodel chromatin structure by different mechanisms: to slide nucleosomes along the DNA, to alter the nucleosome structure, or to disassemble nucleosomes and evict the histones from the promoter DNA (*7*,*8*). They are recruited to promoters by specific transactivators (*9*) and their functions are concomitant with promoter induction (*10*), but in several cases their roles in transcriptional repression were also reported (*10*,*11*). Remodelling complexes often contain a number of subunits involved in the regulation of their intrinsic catalytic activities, but these regulatory mechanisms are presently mostly unclear (*12*). Often, chromatin modifiers and remodellers collaborate in the process of chromatin structure remodelling (*13*,*14*).

The strategies for gene transcriptional regulation by promoter chromatin structure remodelling have been revealed to some degree (*2*), but mechanistic details and the sequence of individual steps and their orchestration in complex remodelling events are the subject of current and future studies.

Chromatin Remodelling at the Yeast PHO Promoters

PHO gene family of yeast *S. cerevisiae* includes genes whose expression products are involved in phosphate uptake and metabolism, and expression of these genes is regulated at the level of transcription in response to phosphate availability in the cell. In a phosphate-containing medium transcription of these genes is repressed, whereas phosphate starvation results in more or less strong induction. Extensive genetic studies of Oshima revealed positive and negative transcription factors in this regulation (for review see *15*). One of the strongly regulated PHO genes is *PHO5*, which codes for the major extracellular nonspecific acid phosphatase isoenzyme (*16*), whose physiological role is to provide cell with phosphate in the conditions of inorganic phosphate starvation by hydrolyzing extracellular phosphomonoesters. Physicochemical and enzymatic properties of this periplasmic enzyme were extensively studied in the early 1980s in Mildner's laboratory (*17–19*). The pioneering work of Wolfram Hörz revealed that massive transition of chromatin structure at the *PHO5* promoter was concomitant with the gene induction and thus provided early evidence about the correlation between the promoter chromatin structure and transcription. Using DNaseI indirect end-labelling method (*20*), they mapped a large hypersensitive region at the induced promoter, located just upstream of the coding region. At a repressed promoter, this region encompassed four positioned nucleosomes (*21*,*22*). The strong inducibility of the *PHO5* gene, simplicity of induction level monitoring by measuring the activity of acid phosphatase (the *PHO5* expression product) with whole cells and a rather simple quantitative restriction enzyme accessibility assay developed for probing chromatin structure opening (*23*) made the *PHO5* promoter rather attractive model for studies of transcriptional regulation through promoter chromatin structure modulation.

Extensive studies of mechanisms of *PHO5* promoter opening performed in the laboratory of W. Hörz, for more than 20 years, and during the past decade in the laboratories of R. Kornberg, M. Kladde, J. Tyler, E. O'Shea and P. Korber, have provided several breakthrough findings and established the basic principles of transcriptional regulation by chromatin remodelling (for review see *24*,*25*). For instance, it was clearly established that the chromatin transition is a prerequisite for the subsequent promoter activation (*26*) and that nucleosome disruption upon induction occurred in the absence of replication (*27*). The *PHO5* promoter was the first example where histone eviction *in trans* was confirmed as remodelling mechanism *in vivo* (*13*,*28–30*).

The search for the chromatin remodelling and modifying complexes involved in chromatin transition at the *PHO5* promoter was, however, unsuccessful for a while. Namely, chromatin opening was found to be largely independent of both Gcn5 and Snf2 (*31*,*32*). Nonetheless, when the role of Gcn5 was later re-examined, strongly delayed kinetics of chromatin remodelling process was observed in its absence, demonstrating an important contribution of Gcn5 in increasing the rate of remodelling, rather than in affecting the final steady-state level (*33*). With this 'kinetic effect' approach, we focused in the past on a comprehensive search for remodeller(s) involved in or even essential for *PHO5* promoter opening. This included all 15 viable chromatin-remodeller gene deletion mutants. Among these, only the *snf2* and *ino80* mutants showed a strong delay in chromatin remodelling kinetics (*34*), but no mutant lost the ability to ultimately open the *PHO5* promoter upon full induction conditions. Moreover, the *snf2 ino80* double mutation had a synthetic kinetic effect, but eventually a high level of *PHO5* induction was achieved, too.

It has more recently been reported that combined absence of Isw1 and Chd1 strongly affected but did not abolish the activation of the *PHO5* promoter under physiological inducing conditions and only under weaker, semi-inducing conditions, the activation of *PHO5* transcription was prevented. On the basis of these and additional results obtained by *in vitro* experiments, supporting the main role of Chd1 in chromatin remodelling at the *PHO5* promoter, the authors concluded that Chd1 is essential for this remodelling process (*35*). We, however, later found that chromatin remodelling step was indeed significantly delayed in the double *isw1chd1* mutant, similarly as previously found for individual or combined absence of Snf2 and Ino80 (*34*), but clearly not prevented (*36*). These apparently contradictory conclusions about the essential role of Chd1 at the *PHO5* promoter could be explained by the fact that the effect on *PHO5* tran-

scription in *isw1chd1* mutant was examined in the Kornberg group under weak, semi-inducing conditions, where remodeller requirement stringency is much higher, as we and others demonstrated for several chromatin cofactors (*34*,*37*,*38*). In addition, the results obtained by *in vitro* experiments cannot be used straightforward as conclusive argument for *in vivo* situation.

Transcriptional regulation of the two other PHO family genes, *PHO8* and *PHO84*, which are activated by the same transactivator as the *PHO5* gene (*39*,*40*), also includes large remodelling of chromatin structure at their promoters (*41*,*42*). In contrast to the *PHO5* promoter, where several remodellers are involved in the process of chromatin structure remodelling, but none of them being essential, chromatin remodelling at the *PHO8* promoter was essentially dependent on SWI/SNF2 complex activity (*43*) and this is also true for one of the two nucleosomes at the *PHO84* promoter that undergoes remodelling upon induction (*42*). Remodelling kinetics of another nucleosome at the *PHO84* promoter is just slightly affected by the absence of Snf2. We showed that such striking difference between three coregulated promoters concerning stringency of remodeller requirement could be in part due to difference in intrinsic nucleosome stability (*42*).

The RSC (Remodels the Structure of Chromatin) is the only remodeller in yeast essential for cell survival (*44*). The RSC catalytic subunit Sth1 has a high degree of homology with Snf2, a catalytic subunit of SWI/SNF complex and two complexes belong to the same SWI/SNF remodeller subfamily. Remodelling activity of RSC is well documented *in vitro* (*44–46*), however, there are only a few studies with single promoters demonstrating its activity in transcriptional regulation *in vivo* (*47–49*).

A role of RSC in chromatin remodelling particularly at the *PHO5* promoter was addressed by *in vitro* experiments but non consistent results from two studies were reported (*35*,*46*), leaving the issue of possible RSC involvement at the *PHO5* promoter fully unclear. By carefully controlled *in vivo* experiments using a temperature-sensitive degron mutant of the RSC catalytic subunit, $Sth1^{td}$ (*48*), we have recently demonstrated a nonessential role of RSC in *PHO5* promoter opening under strong physiological induction, just affecting kinetics of remodelling process. Requirement for RSC activity became, however, stronger under weaker semi-induction conditions (*36*). It cannot be, however, excluded that RSC ablation through this particular $sth1^{td}$ allele was incomplete and that complete inactivation of RSC would fully prevent *PHO5* promoter opening also at the strong induction. Importantly, RSC became essential in the absence of the Snf2 or both Isw1 and Chd1 remodellers, indicating a major role of RSC in *PHO5* promoter opening. Interestingly, RSC activity was dispensable for chromatin opening at the *PHO8* and *PHO84* promoters even under weak induction. Moreover, remodelling of one of *PHO84* nucleosomes was practically not affected even in $isw1chd1sth1^{td}$ triple mutant and was only delayed in $snf2sth1^{td}$ mutant, while in the same cells remodelling at the *PHO5* promoter was almost fully prevented (*36*). This is a rather surprising finding since presence of RSC at all three PHO promoters under repressed conditions was reported (*50*). Furthermore, a role of RSC in maintaining the architecture of repressed chromatin structure at the *PHO8* promoter was also reported (*51*), raising the possibility that the RSC and SWI/SNF remodellers antagonize each other here in the sense that RSC closes and SWI/SNF (together with INO80 (*34*)) opens the *PHO8* promoter.

Taken together, search for a remodeller essential for *PHO5* promoter opening resulted in surprisingly large set of involved remodellers but none of them individually seems to be essentially required (and the *PHO5* promoter is likely the first case where all chromatin remodellers encoded in the yeast genome were examined). It is even more interesting that the identified set of remodellers included factors from all four major subfamilies of yeast ATP-dependent chromatin-remodelling complex (*34–36*,*52*). Knowing that remodellers from these subfamilies employ a different mechanism for chromatin structure opening (*53*), the mechanism of chromatin structure remodelling at the *PHO5* promoter is apparently a more complex process than it was previously presumed.

Concluding Remarks and Perspectives

Recent studies of chromatin remodelling process at the PHO promoters further confirmed those promoters as suitable and valuable model system for elucidation of basic principles and mechanisms of chromatin structure remodelling. Search for remodeller(s) responsible for chromatin remodelling at the *PHO5* promoter clearly showed that such studies should be generally approached by measuring the effect of certain remodeller on kinetics of chromatin opening process rather than only the effect on final steady-state level. This 'kinetic effect' approach revealed a network of five remodellers involved at this promoter, while their individual absence had no effect on the final level of chromatin opening (*34–36*). In agreement with this, a recent genome-wide study showed that chromatin regulators had far greater effects on gene induction kinetics than on a steady-state mRNA level (*54*). Furthermore, since some remodellers can be fully replaceable by each other, as found for Isw1 and Chd1 at the *PHO5* promoter, number of remodellers involved at the *PHO5* promoter could be even higher than presently revealed. So generally, final negative conclusion about the involvement of a certain chromatin cofactor cannot be simply based on the lack of effect in a single mutant.

Our recent work with the *PHO5* promoter has brought about a surprising finding that whole set of remodellers, including members from all four subfamilies in yeast cells, was involved in chromatin remodelling process at this promoter (*36*). Very recently, it has also been found for mouse cells that multiple remodellers cooperate at given loci to achieve chromatin structure remodelling (*55*). Therefore, research on the yeast PHO system pioneered again a basic principle that proved generally valid also in multicellular eukaryotes. Intriguingly, none of the many remodellers involved in *PHO5* promoter opening seemed to be essentially required. The fact that no essential remodeller is involved in remodelling process at the *PHO5* promoter suggests that remodelling process could be accomplished through more than one mechanistically different alternative pathway. Proposed major role of RSC, based on the finding that inactivation of RSC combined with inactivation of either Snf2 or both Isw1 and Chd1 prevents chromatin remodelling (*36*),

suggested that RSC-involving remodelling pathway is the most efficient one. Alternatively, apparent major role of RSC could simply reflect the largest contribution of RSC remodelling activity to the total sum of nonspecific and therefore replaceable remodelling activities, due to its far greater abundance than other remodellers (*56*). So the question of remodeller specificity, which is of general interest for understanding chromatin remodelling process, remains to be further elucidated *in vivo* and PHO promoters would be rather suitable model system.

New findings concerning the involvement of RSC complex at three coregulated PHO promoters (*36*) emphasize previous observations of differential cofactor requirements for nucleosome remodelling at these promoters (*42*). The obtained results further support a general concept that remodeller requirements at a particular promoter, as well as stringency of requirement for particular remodeller are not, or not strictly, determined by recruitment specificity of the transactivator (*36*,*42*,*57*), but it is rather determined by specific promoter chromatin structure and other aspects of promoter architecture which influence nucleosome stability. Comparative studies with three PHO promoters could likely be very helpful to unravel the causal relationship between specific architecture of promoter chromatin structure and specific remodeller requirements at a particular promoter.

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