10888–10902 *Nucleic Acids Research, 2014, Vol. 42, No. 17* *Published online 04 September 2014*
doi: 10.1093/nar/gku784

SURVEY AND SUMMARY

The yeast *PHO5* promoter: from single locus to systems biology of a paradigm for gene regulation through chromatin

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Received May 31, 2014; Revised August 13, 2014; Accepted August 19, 2014

ABSTRACT

Chromatin dynamics crucially contributes to gene regulation. Studies of the yeast *PHO5* promoter were key to establish this nowadays accepted view and continuously provide mechanistic insight in chromatin remodeling and promoter regulation, both on single locus as well as on systems level. The *PHO5* promoter is a context independent chromatin switch module where in the repressed state positioned nucleosomes occlude transcription factor sites such that nucleosome remodeling is a prerequisite for and not consequence of induced gene transcription. This massive chromatin transition from positioned nucleosomes to an extensive hypersensitive site, together with respective transitions at the co-regulated *PHO8* and *PHO84* promoters, became a prime model for dissecting how remodelers, histone modifiers and chaperones co-operate in nucleosome remodeling upon gene induction. This revealed a surprisingly complex cofactor network at the *PHO5* promoter, including five remodeler ATPases (SWI/SNF, RSC, INO80, Isw1, Chd1), and demonstrated for the first time histone eviction *in trans* as remodeling mode *in vivo*. Recently, the *PHO5* promoter and the whole *PHO* regulon were harnessed for quantitative analyses and computational modeling of remodeling, transcription factor binding and promoter input-output relations such that this rewarding single-locus model becomes a paradigm also for theoretical and systems approaches to gene regulatory networks.

Chromatin structure and dynamics contribute importantly to the regulation of gene expression in eukaryotes (1). This view is nowadays broadly accepted and inspires an ever growing number of studies, in part also as chromatin is seen as a major mechanism of epigenetic information (2–4). However, such consensus was not always the case, and many molecular mechanisms, even most basic ones like the determinants for nucleosome positioning (5–7), are still controversial. Nonetheless, our understanding of the relationship between chromatin and gene expression matured to an amazing extent during the last four decades. We review here how this development was pioneered and continues to be inspired by studies of the budding yeast *PHO5* promoter (8).

CLASSICAL STUDIES: PAST AND PRESENT

The *PHO* regulon and *PHO* induction

PHO5 is one of ∼20 *PHO* regulon genes in *Saccharomyces cerevisiae* that are regulated by the availability of phosphate and were first identified genetically by the Oshima group (9). *PHO* regulated genes are repressed if inorganic phosphate (P_i) is abundant and induced upon phosphate starvation. Importantly, this refers to intracellular P_i levels. Just removal of extracellular phosphate is not sufficient for *PHO* induction (10) as yeast cells have ample phosphate stores, especially in the form of polyphosphate in the vacuole (11–14). Only if this storage is used up, e.g. by DNA and phospholipid synthesis during proliferation or cell growth, without repletion from extracellular sources, physiological *PHO* induction will occur. So the classical induction protocol is incubation of replicating yeast cells in phosphate-depleted medium. Induction kinetics depends on the amount of residual extracellular phosphate—this is why phosphate-free medium is preferred to obtain faster and better de-

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fined induction conditions—and the extent of intracellular storage, which can be impaired by mutating enzymes of the polyphosphate storage pathway (14). The actual sensor of intracellular phosphate is still unclear, but signal transduction eventually leads to activation of the principal *PHO* regulator, the transactivator Pho4 (9,15). Pho4 is a basic helix-turn-helix protein (16) that specifically binds to E-box (CACGTG) based sequence elements upstream of Pho4-regulated genes (UASp = Upstream Activating Sequence phosphate) (17–19). Many UASp elements are close to binding sites of the pleiotropic homeobox-type transcription cofactor Pho2, which binds co-operatively with Pho4 at the *PHO5* promoter and can increase both the binding affinity as well as the transactivation potential of Pho4 (19–21). Pho4 itself is constitutively expressed (22), but regulated by phosphorylation through the cyclin/cyclin-dependent-kinase pair Pho80/Pho85, which is under negative control of its inhibitor Pho81 (Figure 1; (11,23–28)). Pho81 binds constitutively to Pho80/Pho85, but is turned into an inhibitor only during phosphate starvation 27. This is mediated by binding of a certain inositol polyphosphate (4/6-IP_7), which is increasingly produced upon phosphate starvation (29,30). Inositol polyphosphates also have a role in *PHO5* repression (31). Pho4 Phosphorylation expedites nuclear export, blocks nuclear import and inhibits the Pho4–Pho2 interaction and consequently decreases Pho4 binding to the promoter. Therefore, interference with this phosphorylation allows circumvention of the physiological signal transduction pathway and *PHO* induction despite the presence of phosphate. For example, the deletion (32), chemically (33) or temperature-induced (10,32) inactivation of Pho80 or Pho85, or simply the out-titration of the Pho80/Pho85 activity by overexpression of *PHO4* (34) will induce *PHO* genes, although not always to full induction levels. As intracellular phosphate and polyphosphate levels are transiently depleted during the cell cycle, also *PHO5* expression oscillates with the cell cycle (35). Accordingly, medium with intermediate phosphate levels, e.g. standard yeast extract-peptone-dextrose (YPD) medium, may allow weak induction of *PHO* genes, particularly during mitosis. The Kladde group found that this mitotic induction is weaker than that upon phosphate starvation and is regulated not only through Pho4 and Pho2, but also by Mcm1 and Fkh2 (14,36).

Choosing the *PHO5* promoter as model due to its massive chromatin transition upon activation

PHO5 encodes a secreted and glycosylated acidic phosphatase that scavenges phosphate from diverse extracellular substrates. As the Pho5 enzyme is located in the periplasm between the plasma membrane and cell wall in soluble form, its activity can be easily monitored with whole cells (37–39). It is non-essential and mutations do not matter under standard laboratory growth conditions making it amenable to extensive mutational analysis. The *PHO5* promoter stood out due to extremely high induction levels (~20–40 fold on mRNA and Pho5 enzyme activity level (40)) and was therefore even considered as a prospective biotechnology tool for recombinant protein production. However, the main interest in *PHO5* was kindled after analysis of the *PHO5* promoter chromatin structure in the repressed versus induced state (Figure 2a) (41–44). In the repressed state, there are five nucleosomes upstream of the transcription start site (TSS), numbered -1 to -5, with a short hypersensitive site of about 80 bp length between nucleosomes -2 and -3. This long linker was missed at first (44) but is of key importance as it harbors the low affinity UASp1 element 41and one Pho2 binding site (20,21). These nucleosomes are classic examples of well-positioned nucleosomes in yeast and were used as benchmark for genome-wide nucleosome mapping (45–47). As generally true, even these are not perfectly positioned but represent Gaussian distributions in ~10 bp increments around an average midpoint (48,49) which probably explains why restriction enzyme accessibility in their linker regions reaches only ~50% (41). Nonetheless, nucleosome positioning is precise enough such that the TATA box/TSS is covered by nucleosome -1 and a high affinity UASp2 element and two Pho2 sites by nucleosome -2. This ordered organization, especially nucleosomes -1 to -4, is remodeled into an extensive hypersensitive site (~600 bp) upon promoter activation. Such a massive chromatin transition is rather exceptional. For most yeast genes promoter chromatin perturbations upon expression changes are less extensive, maybe involving just one nucleosome (46,48,50–51). Of note, hypersensitive sites have been recently renamed in the course of genome-wide nucleosome mapping as 'nucleosome-free regions' (NFR) or 'nucleosome-depleted regions' (NDR) (e.g. (45,52)).

Overarching mechanistic questions

Since the discovery of the nucleosome (53–56) and the development of nucleosome mapping, especially via DNaseI indirect endlabeling (57,58), it became clear that nuclease hypersensitive sites were linked to functional DNA elements, like enhancers, promoters and replication origins, and that their appearance changed with biological conditions, e.g. during gene induction or differentiation (59). So there was a keen interest in the following mechanistic questions: is chromatin regulative, i.e. are chromatin changes cause or consequence of changes in DNA templated processes like transcription or replication? What is a hypersensitive site in molecular terms ('anatomy of a hypersensitive site' (60,61))? How are hypersensitive sites generated? For all these questions, the *PHO5* promoter turned out to be one of the most instructive models.

Early basics on *PHO5* promoter chromatin remodeling: chromatin regulates

Mainly Hörz and colleagues established the first basics of *PHO5* promoter chromatin and its transition. The principal transcription regulator Pho4 turned out to be also the principal inducer of *PHO5* promoter chromatin opening (34). Lack of Pho2 was compensated by overexpression of *PHO4* and even the whole physiological context of *PHO* induction was irrelevant as the full chromatin transition was observed also at usually repressive high phosphate conditions if *PHO4* was overexpressed (34). Further, the *PHO5* promoter could be moved to a plasmid, i.e. to a different nuclear location, and even truncated until only nucleosomes

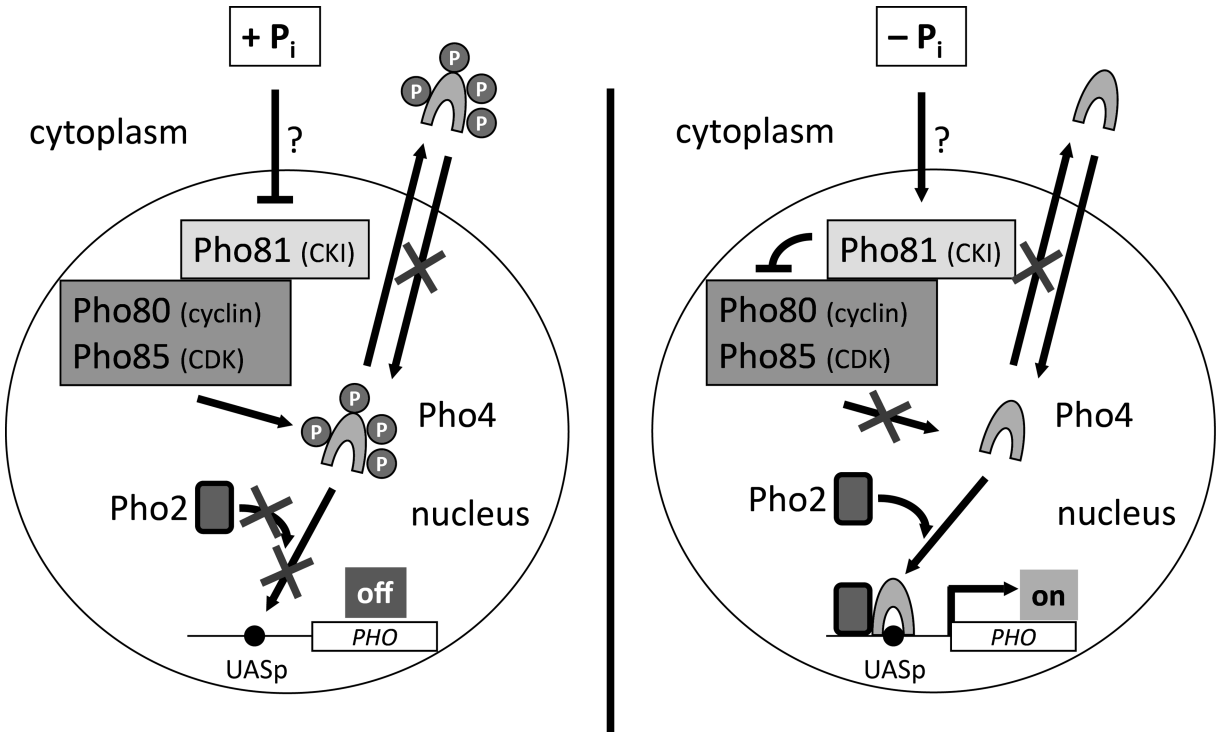

Figure 1. Schematics of *PHO* regulon signal transduction. For details see the main text (The *PHO* regulon and *PHO* induction).

-3 to -1 were left, flanked by prokaryotic sequences (vector backbone upstream, lacZ reporter downstream), without losing the positioned nucleosomes of the repressed state and the Pho4-triggered chromatin transition (43,62–63). So the *PHO5* promoter is a compact chromatin switch module, independent of the physiological, nuclear and DNA sequence context that is essentially triggered by a single factor, Pho4. This allows mechanistic studies without confounding peripherals.

In the beginning, the massive chromatin transition seemed likely to be a consequence of the strongly activated *PHO5* transcription. However, a series of studies from the Grunstein group showed that reducing histone levels *in vivo* led to up-regulation of many genes, including prominently *PHO5*, under otherwise repressive conditions (64–66). This suggested that nucleosomes are repressive and need to be removed prior to transcription initiation. Indeed, both at the *SUC2* (67) and at the *PHO5* promoter (62) a full chromatin transition was still possible upon induction even if gene transcription was abolished by deletion of the TATA box. Conversely, there is never substantial Pho5 activity without *PHO5* promoter chromatin opening. This clearly established that chromatin remodeling is a prerequisite and not consequence of gene transcription. Just recently this basic finding was confirmed again for the *PHO5* promoter by elaborate single molecule *in vivo* analysis and computational modeling (68).

Consistent with the pioneering Grunstein studies, impairment of histone reassembly by ablation of the histone chaperone Spt6 (69), which leads to reduced histone occupancy at the *PHO5* locus, sustained elevated *PHO5* transcription even after return to repressive high phosphate conditions (70). Similarly, a whole range of mutations that interfere with proper nucleosome assembly over coding regions, either affecting histone occupancy, modification, turnover or nucleosome positioning, alleviate the repressive effect of chromatin and allow transcription initiation from otherwise silent, so called 'cryptic' promoters (71,72).

Excluding other early concepts of nucleosome remodeling: not replication, not factor binding competition

In the late 1980s and early 1990s, it was unclear how nucleosomes could be remodeled *in vivo*. Until chromatin remodeling enzymes were recognized, polymerases were prominent candidates for nucleosome disruption and remodeling. Besides the process of transcription, it was proposed that DNA polymerase passage established a window of opportunity for DNA binding factors, like transcription factors, to occupy their binding sites, prevent nucleosome re-assembly and thereby generate a hypersensitive site (73). As physiological *PHO* induction required at least one round of replication—to deplete the intracellular phosphate stores—*PHO5* promoter opening may have seemed as an example for this mechanism. However, by use of a temperature-sensitive *pho80*^{ts} allele the Hörz group showed *PHO5* promoter chromatin switching between the closed and open state in the absence of DNA replication (10).

Another prominent hypothesis was that factors with high affinity to DNA sites could compete away nucleosomes by sheer mass action. There was evidence from *in vitro* (74) and *in vivo* (75) studies where positioned nucleosomes containing one or multiple binding sites for the transactivator Gal4 were disassembled upon binding of Gal4, even only by binding the Gal4-DNA binding domain. However, the anal-

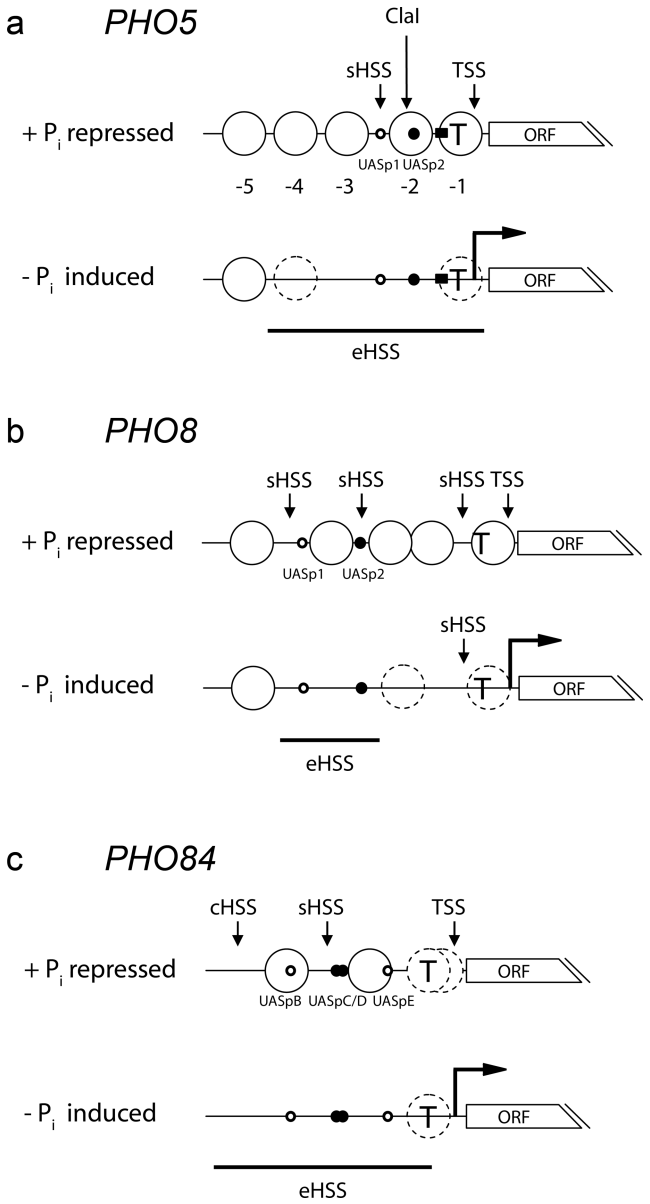

Figure 2. Nucleosome organization schematics for the repressed (+P_i) and induced (−P_i) states of *PHO* promoters. (**a**) *PHO5* promoter organization. Canonical and well-positioned nucleosomes that largely inhibit nuclease access are symbolized by large solid circles and regions of intermediate nuclease accessibility or unclear nucleosome positioning with large stippled circles. Pho4 binding sites (UASp elements) of low and high affinity are represented by open or closed small circles, respectively. Closed squares denote Mcm1 and Fkh2 binding sites and broken arrows induced transcription. sHSS, eHSS and cHSS stand for short, extended and constitutive hypersensitive site, respectively. TSS: transcription start site; ORF: open reading frame; T: TATA box; ClaI: ClaI restriction site. All relative positions approximately to scale. (**b**), (**c**) as (a) but for the *PHO8* and *PHO84* promoter, respectively. See main text for references.

ogous experiment did not work for the *PHO5* promoter. At this physiological example, in contrast to the engineered Gal4 site containing nucleosomes, it was rigorously shown that nucleosome -2 prevents Pho4 binding (76), and that this nucleosome was not displaced by mere binding competition even if the Pho4 DNA binding domain was overexpressed. The Pho4 activation domain was absolutely required to trigger chromatin opening (77). Nonetheless, this was not specific solely to the Pho4 activation domain. *PHO5* promoter opening also worked with the VP16 (77) or the glucocorticoid receptor (78) activation domain fused to the Pho4 DNA binding domain, or if the Pho4 DNA binding domain was fused to the mediator subunit Gal11/Med15 (79), even though mediator has only a minor role otherwise (80) or with a *PHO5* promoter variant activated by Gal4 instead of Pho4 (81,82). Attempts to dissect the Pho4 activation domain into parts responsible for either chromatin remodeling or for transactivation of transcription failed (83). Even though activation domains are known to interact with a surprising variety of cofactors it is still the common view that they cannot be subdivided into different parts that would target different interaction partners, probably because activation domains and their targets form so called 'fuzzy complexes' (84).

It should be noted that this series of clear negative evidence regarding the *PHO5* promoter chromatin remodeling mechanism—not signaling/nuclear/chromosomal context, not transcription, not replication, not factor binding competition, not specific activator—paved the way to the recognition of the actual chromatin remodeling enzymes (reviewed for yeast in (8)). "It was Wolfram [Hörz] who kept pointing out to us that all our mechanisms did not work for his *PHO5* promoter." (Jerry Workman, 2008, personal communication to PK) The ATP-dependent nucleosome remodeling activity of the yeast SWI/SNF complex (85,86) and its activation domain dependent targeting (87), explicitly shown also for Pho4, could explain in principle the transcription-, replication- and context-independent *PHO5* promoter chromatin remodeling. Soon many homologs to the SWI/SNF ATPase subunit Snf2 were recognized in eukaryotic genomes, already 17 in yeast (88), and all of these were tested with regard to *PHO5* promoter opening (14,79,89–96).

What remodels *PHO5* promoter chromatin? The hunt for involved chromatin cofactors

After identification of numerous Snf2-type remodeler ATPases and especially the characterization of the *bona fide* transcription factor Gcn5 as histone acetyl transferase (97–99) the field of transcription regulation finally acknowledged nucleosome remodeling and modification as an important level of gene regulation. Now the stage was set to call on the cofactors responsible for a given chromatin transition. Again the *PHO5* promoter was a most useful model and whichever chromatin cofactor mutant available was tested for effects on *PHO5* promoter opening, also in the context of genetic screens (93,100). At first this seemingly straight forward approach was somewhat frustrating as no cofactor's absence seemed to prevent *PHO5* promoter chromatin remodeling. Especially the first candidate mutations, *snf2* and *gcn5*, still showed full remodeling upon full induction (79,101). This was even more surprising as at the same time opening of the *PHO8* promoter, which is coregulated by Pho4 and also displays a substantial chromatin transition upon induction (18), was completely abolished

in *snf2* and confined to a minor local alteration in *gcn5* cells (102).

Nonetheless, lack of Gcn5 did impair *PHO5* promoter chromatin remodeling but only under sub-optimal induction conditions at high phosphate in *pho80* cells (101) or at early time points of induction kinetics (82). Especially the latter so called 'kinetic effect' made the important point that chromatin effects are often negligible under steady state conditions but very pronounced during the transition from one biological state to the other, e.g. from repression to induction. Just recently this concept was highlighted in a comprehensive study of many chromatin cofactor mutants and their effects during oxidative stress dynamics in yeast (103).

Consequently, cofactors involved in *PHO5* promoter opening were rather identified at suboptimal induction, e.g. at high phosphate conditions upon inactivation of Pho80/Pho85, or overexpression of *PHO4*, or early during induction kinetics upon phosphate starvation (14,82,91,93–94,100–101,104–105). As in all these cases mutation effects become stronger with decreasing induction strength (94) there were controversies about how important or even essential a cofactor is for *PHO5* promoter opening. For example, under suboptimal induction conditions an *asf1* or *isw1 chd1* mutant is unable to remodel *PHO5* promoter chromatin making these cofactors seem essential or others, like RSC, unimportant (89,106). However, upon full induction these mutants show full remodeling (95,104) demonstrating that other cofactors can compensate and are involved, too. Moreover, even if a remodeler is essential, e.g. SWI/SNF for opening the *PHO8* promoter (102), this does not preclude that yet others contribute, too, like INO80 is also involved at the *PHO8* promoter (91). We advocate the view that all cofactors with significant effects at weak or strong induction are part of the physiological remodeling mechanism, but only if lack of a cofactor prevents remodeling under fully inducing conditions, e.g. laboratory conditions of no phosphate, this factor is truly essential for chromatin opening. This is a purely mechanistic view and does not deny that it is the rapid adaptation to changed conditions which is ultimately important from a physiological point of view and in this sense 'essential' for yeast survival in the wild.

It is crucial to show that effects due to mutations *in vivo* are direct and not indirect. For example, effects on promoter chromatin remodeling may be exerted via effects on signal transduction and therefore induction strength. For the *PHO5* promoter, especially the use of the Gal4-driven promoter variant was very instructive as this variant recapitulated the same chromatin cofactor dependencies even though the entire physiological context of promoter induction, including the transactivator, were altered leaving the promoter nucleosomes as common denominator and therefore likely direct target of the identified cofactors (82,91,95,104). In addition, for SWI/SNF, RSC, INO80, SAGA and Rpd3 binding to the *PHO5* promoter was shown by chromatin immunoprecipitation (ChIP) (92,94,107–110) or mass spectrometry of isolated promoter minicircles (111) supporting their direct roles, but not necessarily excluding indirect effects. We caution that the assay monitoring the nuclear localization of Pho4-GFP (26) saturates soon and may not sufficiently discriminate weak from strong induction conditions.

Starting point for promoter opening: the dynamics of the repressed state

The collective efforts of several groups identified a surprisingly complex network of chromatin cofactors involved in the mechanism of *PHO5* promoter chromatin opening (Table 1 and Figure 3). At this point we consider first the repressed state and switch from a largely chronological review to a summary of present day knowledge.

The determinants for nucleosome positioning at the repressed *PHO5* promoter (Figure 2a) are still unknown, despite considerable progress in the identification of factors responsible for genome-wide nucleosome patterns (5–7). The genes encoding the principal factors binding at the *PHO5* promoter, i.e. Pho2 and Pho4 or Cbf1, which can bind to Pho4 sites (116), and even the long linker between nucleosomes -2 and -3, which would be a prime candidate region for binding sites of some organizing factors, can be deleted without losing proper nucleosome positioning ((34,62) and our own unpublished data regarding the *cbf1* mutant).

In general, positioned nucleosomes at yeast promoters are not set in stone but their histones undergo a rather rapid turnover (117–119). Such histone dynamics can be influenced by histone acetylation levels and determines basal promoter activity. In the case of *PHO5*, histone turnover is reduced by histone deacetylation through Rpd3, which is recruited via histone H3 K4 methylation by Set1 (110). This explains increased basal *PHO5* transcription in *set1, rpd3* and also *rpd3 pho4* cells (110,120–122). The latter Pho4-independence is again consistent with the early Grunstein studies showing elevated transcription just because of reduced nucleosome occupancy. The NuA4 complex bearing the histone acetyltransferase Esa1 is constitutively recruited via Pho2 bound close to the constitutively accessible UASp1 and seems important for efficient Pho4 binding during activation (114). In addition, the histone variant H2A.Z (Htz1 in budding yeast), which is preferentially found at promoters (49,123–124) and may facilitate promoter activation by increasing histone turnover (125), is acetylated by Gcn5 and Esa1 and was implicated in nucleosome reassembly at the *PHO5* promoter (126). Nonetheless, promoter opening is hardly affected by the absence of Htz1 (105), and lack of Pho2 or NuA4 can be compensated by overexpression of Pho4 (34,114). It was proposed that a certain level of basal promoter nucleosome dynamics is important for timely *PHO5* promoter opening and ensured by antisense transcription through the *PHO5* promoter (127). While the role of antisense transcription may need further validation, it is often true that mutations affecting cofactors involved in *PHO5* promoter opening, e.g. *gcn5* or *snf2* (Table 1 and references therein), also show reduced levels of basal transcription and promoter accessibility. This means that at least some of these cofactors constitutively keep *PHO5* promoter nucleosomes dynamic, it supports the Svejstrup notion (127) that such dynamics are important for efficient and timely remodeling, and shows that the molecular machinery for basal nucleosome dynamics is linked to that for promoter opening upon induction.

Table 1. Chromatin cofactors involved in chromatin remodeling at the *PHO5* promoter

Cofactor type	Role in *PHO5* promoter opening	References
Remodeling enzyme (name of ATPase subunit)	SWI/SNF (Snf2), RSC (Sth1), INO80 (Ino80), Isw1, Chd1	(14,79,89–96)
Histone acetylase (name of acetylase subunit)	SAGA (Gcn5), Rtt109, NuA4 (Esa1)	(82,93–94,101,105,114–115)
Histone deacetylase	Rpd3	(110)
Histone chaperone	Asf1, HIR, Spt6	(70,104,106)
Histone methylase	Set1	(110)

Molecular mechanism for generating an extended hypersensitive site: a surprisingly complex chromatin cofactor network remodels *PHO5* promoter chromatin

Upon induction, Pho4 translocates into the nucleus, binds co-operatively with Pho2 at UASp1 (20–21,128) and recruits via its activation domain the SAGA complex, which leads to hyperacetylation of promoter histones (90), and recruitment of TATA box binding protein (TBP) via its Spt3 subunit (109). Hyperacetylated histones stabilize recruitment of remodelers with bromodomains, like SWI/SNF (112). SWI/SNF is also directly targeted by the Pho4 activation domain (87). The accessible UASp1 may serve as 'pioneer' site from where Pho4-recruited cofactors can induce remodeling of the -2 nucleosome and generate access to UASp2 (62). A ΔUASp1 *PHO5* promoter variant is usually not opened at all (62,128), but *PHO4* overexpression restores full remodeling (76). In contrast, a ΔUASp2 variant is not fully remodeled (128), not even with *PHO4* overexpression (129). This makes Pho4 binding to UASp2 not only necessary, as recently confirmed (68), but also sufficient for promoter opening and induction. Consequently, there is the circular condition that nucleosome remodeling is required for Pho4 binding (76) and Pho4 binding for nucleosome remodeling. However, as mentioned above, the rapid turnover of promoter nucleosomes (117–119) and an almost equally rapid accessibility of both nucleosomal and non-nucleosomal regions, explicitly at the *PHO5* promoter, to photolyase DNA repair (130) show that nucleosomes do not pose a rigid barrier *in vivo* but that there are always windows of opportunity for factors to access the DNA. The question is if nucleosome disassembly as triggered by a given factor can eventually overcome nucleosome re-assembly. In the case of Pho4 this is not a matter of binding competition but only possible if an activation domain is involved 77, presumably as it recruits chromatin remodeling and modifying factors that tip the balance toward nucleosome disassembly and stable Pho4 binding (68). Even though binding competition to an intranucleosomal UASp element alone is not sufficient for remodeling, it does help the remodeling process (129).

Recently, the interplay of nucleosomes, Pho4, Pho2 and TBP at the *PHO5* promoter was systematically recapitulated (128) by the chromatin endogenous cleavage (ChEC) method (131) where MNase was fused to Pho4 and chromatin accessibility monitored after MNase activation by calcium. These and earlier (76) studies as well as X-ray crystallographical data on DNA binding by Pho4 (132) leave no doubt that Pho4 cannot bind to UASp2 if this site is within a canonical nucleosome. Nonetheless, there are probably non-canonical nucleosome intermediates during histone turnover and on the pathway to nucleosome disassembly that allow Pho4 binding. Such intermediates could explain why Pho4 binding kinetics need not become slower in the same way as histone eviction kinetics in an *asf1* and other mutants (106–107,133).

While Pho4 binding is the essential trigger, it is the co-operation between chromatin cofactors that actually removes the nucleosomes. The main lesson from *PHO5* here is the surprising complexity of this cofactor network (Table 1). This network is redundant (91) in the sense that chromatin can be opened even in the absence of many members of this network under full induction conditions. Nonetheless, the complete set is required to achieve full opening under sub-optimal conditions and wt kinetics upon strong induction. It was a recent surprise that five remodelers from four major remodeler families are involved in *PHO5* promoter opening, including the Remodels the Structure of Chromatin (RSC) complex (95). RSC was a prime candidate for *the* remodeler at the *PHO5* promoter since all other Snf2-type ATPases could be deleted without preventing *PHO5* promoter opening (91,134). RSC is the only remodeler essential for viability in budding yeast and was therefore difficult to test *in vivo* due to indirect effects in growth arrested or dying cells. A role for the RSC complex was initially suggested, at least indirectly (135,136), then dismissed based on *in vitro* studies (89,137), but now clearly demonstrated *in vivo* (95). Of note, the combined absence of RSC and Snf2 or RSC and Isw1/Chd1 represent the first *in vivo* cases where *PHO5* promoter opening was not possible even under strong induction conditions. Whether RSC is not only a major remodeler but maybe even truly essential in our sense (see 'What remodels *PHO5* promoter chromatin? The hunt for involved chromatin cofactors' section) remains to be established. To our knowledge, the *PHO5* promoter chromatin transition is the only one so far where the full complement of remodeler ATPases encoded in a genome was tested for involvement.

We expect that the co-operation of many remodelers for a given chromatin remodeling process will be the rule rather than the exception. Indeed, a recent study in mouse showed that several remodelers are present and involved at many loci undergoing chromatin remodeling (138). Potentially, even more remodeler ATPases could be involved at the *PHO5* promoter given the example of Isw1 and Chd1. Both remodelers at first seemed not involved as *isw1* and *chd1* single mutations did not have effects on *PHO5* induction, neither on the final level nor on the kinetics (91–93,134,139). However, the combined *isw1 chd1* double mutation showed a clear effect on *PHO5* induction (89) and promoter opening (95). Therefore these remodelers have a truly redundant

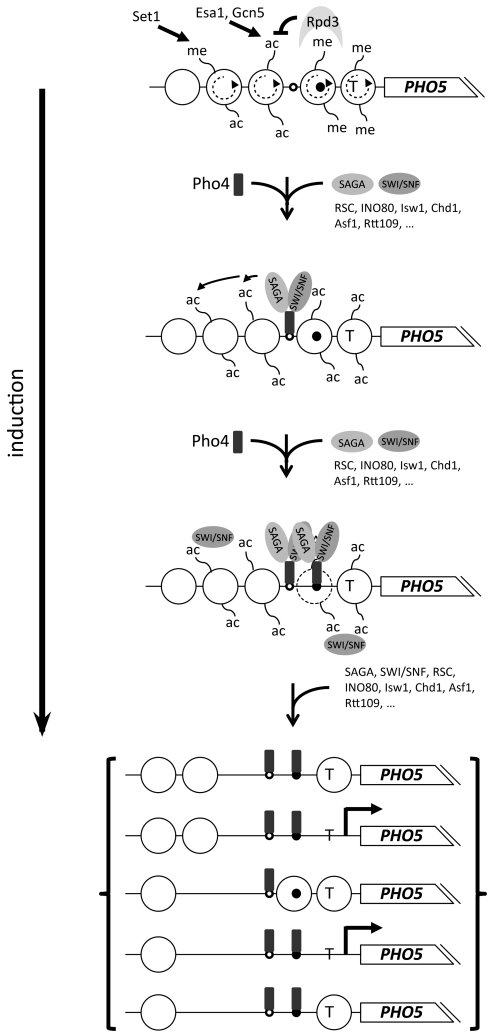

Figure 3. Mechanistic model for *PHO5* promoter chromatin opening upon induction. Top panel: the *PHO5* promoter nucleosomes of the repressed state (see also Figure 2a and corresponding symbols) are methylated (me) by Set1 at H3K4 (only two histone tails per nucleosome as proxy for all possible tails are shown), which recruits the histone deacetylase Rpd3 (gray arc). This curbs acetylation (ac) set by Esa1 and Gcn5 and keeps histone turnover (circular stippled arrows) low. Second panel: upon induction, Pho4 (rounded rectangle) becomes nuclear, binds first the constitutively accessible UASp1 element (small open circle) and recruits the SAGA and SWI/SNF complex. Only the direct recruitment for SAGA and SWI/SNF, for which there are clear Pho4 interaction data, is shown, but the other listed cofactors, and presumably even more, are involved too (see Table 1). The RSC complex, e.g. is present already under $+P_i$ conditions (108). SAGA and possibly other acetylases hyperacetylate promoter nucleosome histones. Third panel: SWI/SNF and/or other remodelers remodel nucleosome -2 such that UASp2 (small closed circle) becomes accessible for Pho4. At first this need not require complete nucleosome disassembly (stippled outline of nucleosome circle), although complete removal of nucleosome -2 is a hallmark of the activated *PHO5* promoter. Bracketed panels: continued recruitment of cofactors through bound Pho4 and/or acetylated histones (e.g. SWI/SNF through bromodomains 112) leads to increased nucleosome disassembly and an ensemble of nucleosome configurations with different combinations of nucleosomes absent or present. Bold brackets include just five examples out of up to 16 possible configurations (68,113). Recruited cofactors and histone tail modifications are not shown for simplicity. Transitions between states are possible by nucleosome assembly, disassembly and sliding. Only configurations without nucleosome -2 are conducive for promoter activation and only configurations with accessible TATA box (T) allow bursts of *PHO5* transcription (bold broken arrow). See main text for further references.

role that is only detected in their combined absence. As not all combinations of remodeler ATPase gene deletions were tested so far, or can even be tested with view of yeast viability, there remains some uncertainty as to how many remodeler ATPases can be involved in *PHO5* promoter opening.

'Anatomy of a hypersensitive site': *PHO5* promoter remodeling via histone eviction *in trans*

From the very beginning, it was clear that the molecular make up of hypersensitive sites does not correspond to canonical nucleosomes. Since the discovery of the nucleosome (53,56) the protection of ~140–150 bp of DNA from limited MNase digestion and relative inaccessibility to other endonucleases, like DNaseI and restriction enzymes, is still a valid operational definition of a canonical nucleosome. Accordingly, the first detailed study on *PHO5* promoter opening (41) demonstrated by several nuclease assays involving DNaseI, DNaseII, MNase and restriction enzymes that the extended hypersensitive site at the induced *PHO5* promoter cannot harbor the same full complement of canonical nucleosomes as in the repressed state. Nonetheless, there was the possibility that few nucleosomes remained, possibly delocalized, and/or that nucleosomes were remodeled into a non-canonical state (140–142) that allows nuclease cleavage and Pho4 binding but keeps histones bound to the DNA. The latter possibility necessitates the often subsumed or simply overlooked, but mechanistically important distinction if remodeling results merely in 'nucleosome eviction' or also in 'histone eviction'.

Chromatin remodeling at the *PHO5* promoter was the first demonstrated case of histone eviction *in vivo* (90,143). Up to now, the only assay for monitoring histone-DNA contacts *in vivo* is anti-histone ChIP. All other assays (nuclease accessibility, topology, methyltransferase accessibility, hydroxyl radical cleavage, psoralen crosslinking) can only monitor the presence of a canonical nucleosome but cannot or not strictly distinguish non-canonical nucleosomes from histone-free DNA. Similar to Pho4 binding and histone eviction, nucleosome remodeling as monitored by increased nuclease accessibility can be uncoupled from histone eviction. Steady-state high phosphate conditions in *gcn5 pho80* cells lead to altered nuclease accessibility due to randomized nucleosome positions at the *PHO5* promoter (101) but not to nucleosome loss (134). Further, histone loss kinetics during induction at the *PHO84* promoter is much delayed in *gcn5* cells while chromatin remodeling kinetics monitored by HhaI restriction enzyme accessibility is not (105). The *RNR3*, *CHA1* and other promoters are further examples where loss of cofactors inhibits histone eviction more than nuclease sensitivity (80,144).

Histone eviction is now firmly established as a global phenomenon across the genome and across species (1,145–146), and the test for histone eviction, i.e. monitoring histone occupancy as such (usually an anti-H3-C-terminus ChIP, which was first used at the *PHO5* promoter (90)), has become an essential normalization procedure for assays monitoring histone modifications. Indeed, histone acetylation *de*creases over the *PHO5* promoter during induction, but this observation is deceiving due to overall histone loss. If remodeling was slowed down in an *snf2* mutant, a tran-

sient peak of histone hyperacetylation could be observed (90). As opening of the *PHO8* promoter essentially depends on Snf2, this promoter becomes locked in the hyperacetylated state upon shift of *snf2* cells to inducing conditions (147,148). Given that such promoter chromatin is still fully closed, this was an important demonstration that hyperacetylation *per se* does not lead to increased DNA accessibility. This is in contrast to the still somewhat common notion that chromatin opening in the sense of chromatin fiber unfolding would be the same as chromatin opening in the sense of increased accessibility to DNA that was part of a nucleosome core. While charge neutralization by lysine acetylation, especially at histone H4 lysine 16, does lead to fiber unfolding, it is mostly not sufficient to destabilize nucleosome cores (149).

Histone eviction raised the question if histones were lost from the *PHO5* promoter region *in cis* due to lateral nucleosome sliding (150,151) or *in trans* due to nucleosome disassembly. Topology and nuclease assays on excised chromatin mini-circles (134) or on tiny plasmids bearing the *PHO5* promoter (152) demonstrated histone eviction *in trans*. This does not exclude nucleosome sliding *in cis* at early remodeling stages as shown, e.g. for histone eviction by SWI/SNF *in vitro* (153). We note that this is to our knowledge the only physiological case where the *cis-trans* distinction is clearly demonstrated so far. It is therefore only by extrapolation from the *PHO5* promoter when an observation of histone eviction is interpreted as eviction *in trans*, i.e. 'nucleosome disassembly'. The *PHO5* promoter was also the first model where histone exchange using differentially tagged histone isoforms ('histone double tag strategy') was monitored (154). By this approach, it was clearly established that histones return from a source *in trans* upon *PHO5* promoter repression, and it prompted a fruitful series of histone exchange studies that shaped our view of global histone dynamics (117–119,155).

As histones outside the nucleosome structure are notoriously aggregation prone both *in vivo* as well as *in vitro*, histone eviction *in trans* calls for a histone acceptor. Histone chaperones are the prime candidates. Indeed, there is a role for the histone chaperones Asf1 and HIR observed at the *PHO5* promoter under suboptimal induction conditions (104,106). However, Asf1 is also essential for H3K36 acetylation by the Rtt109 histone acetyl transferase (156–158) that also affects *PHO5* promoter opening (105,115). It is therefore unresolved if Asf1 contributes to *PHO5* promoter opening truly as histone acceptor or mainly as cofactor for Rtt109.

The question if few canonical, maybe delocalized, nucleosomes may still be present after *PHO5* promoter chromatin remodeling received different answers over the years but appears to be resolved just recently. Nuclease digestion and topology analyses suggested that not all nucleosomes, especially the -1 and -4 nucleosomes, are completely remodeled, but that the open state corresponds to an heterogeneous ensemble of nucleosome configurations and that about one out of three nucleosomes remains at the promoter (41,134–135,143,152,159). The Kladde group developed nucleosome mapping *in vivo* based on accessibility to DNA methylases (160) that allows single molecule analysis due to a cloning step. First applied to *PHO5* promoter opening kinetics (161), this technique revealed that opening leads to a heterogeneous population with variable numbers of nucleosomes per *PHO5* promoter molecule. There was the general trend that nucleosomes -2 and -3 are remodeled first and more often, while nucleosomes -1 and -4 were often still present, supporting the idea that remodeling spreads bidirectionally from UASp1 (161). Some templates showed remodeling even of the -5 nucleosome, but only very few templates lacked all four *PHO5* promoter nucleosomes, at least up to the last kinetic time point measured. All these initial observations suggested that hardly ever all *PHO5* promoter nucleosomes were removed ('nucleosome retention hypothesis' (135)). This made the *PHO5* promoter a favorite *in vivo* example for the highly intriguing concept that remodelers of the SWI/SNF family, SWI/SNF and RSC in yeast, remodel nucleosomes by 'sliding mediated disassembly' (135,153,162–163). The remodeler is thought to 'ride on top' of a nucleosome and slide it into neighboring nucleosomes leading to their disassembly. Accordingly, always one nucleosome should remain at the promoter. The remodeling concept as such was recently substantiated by domain swaps between remodelers of the SWI/SNF and the CHD family (164) and incorporated earlier into elaborate theoretical modeling by the Boeger group (135). However, the Boeger group very recently revisited the issue for the *PHO5* promoter using single molecule analysis via psoralen crosslinking. They refuted their stable nucleosome retention hypothesis as they found the zero nucleosome state as the most abundant (~25%) of eight possible states of nucleosome removal combinations and adapted their model accordingly (68).

Collectively, the molecular anatomy of induced *PHO5* promoter chromatin does not correspond to a single well-defined state, but to a heterogeneous ensemble of states where a variable and relative to the repressed state increased number of nucleosomes is disassembled, even all of them, such that histones are evicted *in trans*. This allows Pho4 and the transcription machinery to bind more frequently to the critical and otherwise occluded UASp2 and TATA box elements, respectively, leading to more frequent transcription initiation than in the repressed state and therefore higher expression levels.

The coregulated *PHO8* and *PHO84* promoters: differential cofactor requirements due to different intrinsic nucleosome stabilities

The *PHO8* and *PHO84* promoters are two members of the *PHO* regulon, i.e. coregulated by Pho4, that were characterized in detail regarding their promoter chromatin alterations upon induction (Figure 2b and c; (18,105,165)). Both show pronounced chromatin transitions, including histone eviction (105,106), and critically depend on Pho4. Nonetheless, they demonstrated a different stringency of cofactor requirements for chromatin opening compared to the *PHO5* promoter. Neither of these two promoters requires the RSC complex for chromatin opening (95), even though RSC is necessary for generating proper nucleosome positioning at the repressed *PHO8* promoter (166). Complete remodeling at the *PHO8* promoter strictly requires SWI/SNF and Gcn5 (102), while the *PHO84* promoter nucleosomes ap-

pear like a hybrid between those of the *PHO5* and *PHO8* promoters. The nucleosome downstream of the hypersensitive site bearing UASpE (Figure 2c) has relaxed cofactor requirements reminiscent of the *PHO5* promoter but the upstream nucleosome containing UASpB strictly depends on SWI/SNF like those at the *PHO8* promoter (96,105). Especially the close proximity of these two neighboring nucleosomes at the *PHO84* promoter and their similar distance to the Pho4 binding sites make it unlikely that their differential cofactor requirements are due to differences in cofactor recruitment strength or mode. Instead, intrinsic differences in nucleosome stability correlate with the differential requirement as shown *in vitro* for both the *PHO8* (167) and *PHO84* promoters (105) by using a reconstitution system at limiting histone concentrations. The causality was also demonstrated *in vivo* by introducing destabilizing poly(dA:dT) stretches into the Snf2-dependent nucleosome at the *PHO84* promoter such that remodeling of this nucleosome became possible even in an *snf2* mutant (105). Notably, also at the *PHO8* and *PHO84* promoters not just one but at least two remodelers, SWI/SNF and INO80 (91,105), participate in chromatin opening confirming again our notion regarding the pervasiveness of complex remodeler networks.

NEW TRICKS FOR OLD DOGS: *PHO* PROMOTERS GO *IN VITRO*, *IN SILICO* AND TO SYSTEMS LEVEL

In vitro reconstitution of nucleosome positioning and remodeling at *PHO* promoters

The exceedingly rewarding *in vivo* approach to chromatin remodeling mechanisms at the *PHO* promoters called for a corresponding *in vitro* system in order to further address mechanistic questions biochemically. So far *PHO5* promoter chromatin was either isolated *ex vivo* (89,168) or reconstituted *de novo* (129,169–170), and then Pho4-induced generation of the hypersensitive site was aspired. In some cases Pho4-, ATP- and extract-dependent nucleosome remodeling was observed, but the extent of remodeling was considerably less than *in vivo*, maybe due to irreversibly aggregated templates during *in vitro* handling or due to inherent difficulties in reconstituting the proper time course of histone modifications and cofactor interactions. Curiously, reconstituted *PHO8* promoter chromatin could not be remodeled at all under the same conditions (129). For whichever reason, there is still no *in vitro* system established that adequately recapitulates the *in vivo* chromatin transition of any *PHO* promoter.

Nonetheless, it is quite amazing to what degree of similarity the nucleosome positioning pattern of the repressed states of the *PHO5* (167,170), *PHO8* (167) and *PHO84* (105) promoters can be reconstituted *de novo* using a combination of salt gradient dialysis and incubation with yeast extract and ATP (171). This enabled comparisons of intrinsic stability of promoter nucleosomes at their proper position ((105,167)) and identified a necessary, direct and specific role for the RSC complex in nucleosome positioning at the repressed *PHO8* promoter (166). Such *in vitro* reconstitution of *in vivo*-like nucleosome positioning was achieved also on the genome level (172,173) and should allow the biochemical dissection of global nucleosome positioning mechanisms.

Theoretical modeling for the *PHO* promoters

Especially the O'Shea and Boeger groups combined quantitative measurements, including single cell and single molecule techniques, with theoretical modeling of *PHO5* promoter properties. *PHO5* (also *PHO84*) is a prime example for stochasticity in gene expression, also called 'intrinsic noise' of expression (174). Two identical copies of *PHO5* promoters but each driving a different reporter gene, YFP and CFP, showed considerably different expression levels in the same cell. This intrinsic noise scaled inversely with expression level and was exacerbated if chromatin remodeling was compromised (*snf6*, *gcn5*, *arp8* or UASp mutations). Modeling of such data suggested that a slow and stochastic *PHO5* promoter activation step, presumably chromatin opening, precedes transcription activation. A series of studies from the Boeger group elaborated on this topic. Modeling of quantitative data on nucleosome occupancy at the induced *PHO5* promoter confirmed that nucleosome disassembly is rate limiting for transcription (135) although this dataset erroneously assumed stable retention of one nucleosome at all times. Modeling the correlation between an extensive range of Pho4 activation domain mutations and the corresponding degrees of promoter opening and *PHO5* expression revealed a dual role for Pho4 in both increasing nucleosome disassembly and transcription activation (159) and confirmed the earlier finding (83) that both roles are structurally inseparable. Nonetheless, equivalent studies at the *PHO8* promoter showed a role for Pho4 in mainly accelerating nucleosome disassembly (96). These conclusions are consistent, regarding *PHO8*, but not, regarding *PHO5*, with the maintenance of full transcription levels even in the absence of Pho4 and Pho2 upon return to repressive conditions if chromatin reassembly was inhibited by Spt6 ablation (70). Maybe such Spt6 ablation conditions represent unusually open chromatin and do not represent the physiological state of the activated *PHO5* promoter. The general view is that, whereas lack of nucleosomes is conducive for elevated transcription, a transactivator is still required for full activation (175).

The recent modeling of single molecule psoralen crosslinking nucleosome mapping at induced *PHO5* promoter molecules combined with intrinsic noise data (68) suggests the following mechanistic picture and explains bursty transcription. Nucleosome configurations at the *PHO5* promoter are intrinsically stochastic as nucleosomes continuously assemble, disassemble and slide. The mechanistic involvement of both (dis)assembly and sliding is consistent with a role for remodeling enzymes in *PHO5* promoter opening (Table 1) of both the SWI/SNF family, i.e. SWI/SNF and RSC, as well as the ISWI and CHD families, i.e. Isw1 and Chd1, that rather mediate the former and latter mode of remodeling, respectively (176) (even though also nucleosome disassembly by SWI/SNF involves initial sliding (153)). Pho4 accelerates nucleosome removal via chromatin cofactor recruitment but does not inhibit nucleosome reassembly by mere steric exclusion. Removal of the -1 nucleosome requires disassembly of

nucleosome -2, consistent with earlier population (41) and single molecule (161) measurements. As nucleosomes are occlusive to transcription factor binding (76,128), and as the stochastic movement of nucleosomes always yields configurations both conducive and non-conducive for transcription activation, transcription occurs in bursts. Importantly, the model poses that increased transcription burst frequency rather than longer burst duration underlies the increased expression level.

While this Survey and Summary article was under review, another single molecule *PHO5* promoter study was published (113). The authors again monitored promoter nucleosome occupancy by protection from DNA methylation, but single molecule resolution was achieved by truly working with single cells rather than through a cloning step (161). This demonstrated in unprecedented resolution the variability of nucleosome positions, especially for nucleosome -3, that underlies the canonical patterns of the repressed and induced state (Figure 2a). Using a GFP reporter, cells with activated versus inactive *PHO5* promoter were sorted and their respective promoter nucleosome configurations were directly measured for the first time. This directly confirms all earlier conclusions regarding that promoter activation requires remodeling of the -1 and -2 nucleosomes and directly visualizes the chromatin basis for cell-to-cell expression differences (bursty expression). In contrast to the Boeger study (68), the authors did not detect completely nucleosome-free promoters under equivalent inducing conditions. Such nucleosome-free promoters were only detected upon introduction of poly(dA:dT) elements in the *PHO5* promoter. These shifted the distribution of nucleosome configurations toward open promoters and caused derepression under high phosphate conditions. Conversely, point mutations predicted to increase the intrinsic stability of the -3 nucleosome could even prevent *PHO5* promoter opening upon induction consistent with our conclusion that intrinsic nucleosome stability determines remodeling cofactor requirements (105).

Systems biology with the *PHO* regulon

The *PHO5* promoter and the *PHO* regulon were harnessed to derive quantitative and predictive models that are required for a systems approach to gene regulation. Importantly, such models explicitly accommodate the regulatory input of chromatin. Lam *et al.* (165) correlated the position of high and low affinity UASp elements relative to nucleosomes, i.e. intra- versus internucleosomal, of seven Pho4 regulated genes and eight *PHO5* promoter variants with the corresponding transcription induction kinetics during phosphate starvation. Consistent with the classical knowledge that nucleosomes are occlusive and necessitate remodeling prior to Pho4 binding (76–77,128), this study comprehensively showed that the affinity of the constitutively accessible UASp elements determines early or late onset of gene induction (threshold), but that all UASp elements that become accessible after promoter chromatin remodeling contribute in the end to the full expression level (dynamic range). So nucleosome positioning fine tunes the input-output correlation (gene regulatory function) of a promoter and allows independent regulation of threshold and dynamic range. This was followed up and modeled accordingly using an elegant system where Pho4 levels were controlled by a doxycycline inducible promoter and Pho4 levels as well as *PHO5* promoter output monitored via YFP tag and CFP reporter, respectively (177).

Nucleosome positioning also importantly contributes to the selection of functional transcription factor binding sites. The number of potential transcription factor binding sites encoded in eukaryotic genomes is often larger than that of biological function. Yeast Pho4 or Leu3 transactivator binding sites are good examples. A comparison of genome-wide Leu3 binding *in vivo* and to free DNA *in vitro* with *in vivo* nucleosome positioning (178) suggested that non-functional sites are mainly covered by nucleosomes. However, just the intra- versus internucleosomal position is clearly not sufficient to distinguish functional and non-functional Pho4 binding sites. UASp2 at the *PHO5* promoter is intranucleosomal and highly functional (41,76) while the constitutively accessible UASp1 at the *PHO8* and UASpA at the *PHO84* promoter are not (17,105,179). Genome-wide analysis of this issue (180) suggested that the competition between Pho4 and the alternative UASp binding factor Cbf1 (116) co-determines binding to internucleosomal, while the co-operation between Pho4 and its binding helper Pho2 (20,21) contributes to the decision about functionality of intranucleosomal UASp elements.

Just recently, an extensive set of 209 *PHO5* promoter variants bearing mutations of the UASp elements was tested both for Pho4 binding affinity *in vitro* (181) as well as *PHO5* expression output *in vivo* (182). This latter study underscored the importance of just the binding affinity in predicting promoter output but also found several examples, including the wt promoter, where nucleosome remodeling had to be included in the model to match the data. In contrast to the conclusions by Lam *et al.* (165), also intranucleosomal binding sites contributed to setting the threshold of induction, but the overall notion was confirmed that the wt *PHO5* promoter is optimized with regard to limiting activation at low while providing maximal output at full induction conditions. Such a *PHO5* promoter library or the set of Pho4 activation domain mutations (159) or the doxycycline inducible Pho4 system (177) together with a sizeable number of Pho4 regulated genes and detailed knowledge of nucleosome positioning and remodeling mechanisms provide a rich tool box for further studies of the *PHO* regulon as model for eukaryotic transcriptional networks.

RETROSPECTIVE AND OUTLOOK

Confirmation of classical findings with new methodology

It is highly affirmative how robust a model system the *PHO* promoters are. Despite the long history of research on these models, none of the classical conclusions was refuted, but all of them independently confirmed in several groups and with new techniques as noted above throughout our review. This includes, e.g. the nucleosome organization in the repressed states, that Pho4 is the trigger, that binding to UASp2 is critical but impeded by nucleosome -2 making nucleosome remodeling rate-limiting, that remodeling is mediated by activation domain dependent recruitment of chromatin cofac-

tors and not through steric exclusion, and that histones are evicted *in trans*.

Open questions

Besides confirming classical tenets, it is quite amazing how the *PHO* promoters keep turning up new insights and there are still numerous, even very basic questions unanswered regarding chromatin remodeling, for which *PHO* promoters continue to be promising models. Just to name a few: what determines nucleosome positioning in the repressed state? What determines the extent of remodeling in the induced state, i.e. why nucleosomes -1 to -4 at the *PHO5* and the respective ones at the *PHO8*, *PHO84* or other promoters? Why is remodeling of the TATA-box occluding nucleosome comparatively incomplete, even though this should be the most important one to remove? What is so repressive (179) and intrinsically stable (129,167) about *PHO8* promoter nucleosomes? What causes the strict dependencies on chromatin cofactors, e.g. the Snf2-dependency of *PHO8* and *PHO84* promoter nucleosomes? What is the role of H2A.Z at promoters in yeast? What is necessary for the maintenance of the open promoter state? Indeed, the whole process of promoter closing is relatively understudied, besides the demonstration of histone re-assembly from a source *in trans* (154) and a role for Spt6 (70) hardly anything is known about its mechanism.

A special case turned into a general paradigm

In retrospective, it is maybe surprising how the *PHO5* promoter became such a general paradigm even though it is a rather special case in many ways. For yeast standards, its extent of chromatin remodeling and induction is extremely high and its promoter chromatin organization is very much the exception rather than the rule. Genome-wide studies in yeast revealed that the vast majority of promoters harbor a broad hypersensitive site (NFR) just upstream of the TSS, drive constitutive expression of little intrinsic noise, are not much dependent on chromatin cofactors and do not rely on a canonical TATA box but on TATA-like elements (183–185). The *PHO5* promoter does not fit this stereotype but has its own individualistic architecture. Nonetheless, this is due to the unusual biology of yeast in having a mostly active genome most of the time. Larger eukaryotes require switching genes on and off to a much larger extent, e.g. during differentiation. In this regard, the *PHO5* promoter is a paradigm for a highly regulated promoter. With its large dynamic changes in chromatin structure and gene induction it therefore was and continues to be an experimentally very well accessible model for regulated chromatin transitions.

ACKNOWLEDGEMENTS

We thank Peter Becker for his support of this work and Corinna Lieleg for critical reading of the manuscript.
This article is dedicated to the memory of Wolfram Hörz (1944–2005), who would have turned 70 this year and who was a major promoter of chromatin research in yeast and beyond by his focus on *PHO* promoters (186).

FUNDING

The authors are funded through their positions at their respective institutions. Personal exchange between the authors was greatly facilitated through visiting researcher funds from the German Research Community (DFG, CRC1064). Funding for open access charge: German Research Community [DFG, CRC1064].
Conflict of interest statement. None declared.

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