Comparison of Nucleosome Remodeling by the Yeast Transcription Factor Pho4 and the Glucocorticoid Receptor*

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Chromatin reorganization of the PHO5 and murine mammary tumor virus (MMTV) promoters is triggered by binding of either Pho4 or the glucocorticoid receptor (GR), respectively. In order to compare the ability of Pho4 and GR to remodel chromatin and activate transcription, hybrid promoter constructs were created by insertion of the MMTV B nucleosome sequence into the PHO5 promoter and then transformed into a yeast strain expressing GR. Activation of either Pho4 (by phosphate depletion) or GR (by hormone addition) resulted in only slight induction of hybrid promoter activity. However, simultaneous activation of both Pho4 and GR resulted in synergistic activation to levels exceeding that of the wild type PHO5 promoter. Under these conditions, Pho4 completely disrupted the nucleosome containing its binding site. In contrast, GR had little effect on the stability of the MMTV B nucleosome. A minimal transactivation domain of the GR fused to the Pho4 DNA-binding domain is capable of efficiently disrupting the nucleosome with a Pho4-binding site, whereas the complementary hybrid protein (Pho4 activation domain, GR DNA-binding domain) does not labilize the B nucleosome. Therefore, we conclude that significant activation by Pho4 requires nucleosome disruption, whereas equivalent transcripational activation by GR is not accompanied by overt perturbation of nucleosome structure. Our results show that the DNA-binding domains of the two factors play critical roles in determining how chromatin structure is modified during promoter activation.

The chromatin organization of a gene is an important determinant of transcriptional regulation. Different genes employ different strategies to regulate access of the transcriptional apparatus to the promoter. One class of genes, termed remodeling genes (2), undergoes a transition at the promoter following activation. In this case, a transactivator first establishes a foothold either by binding to an accessible site in the promoter and then subsequently exposing other binding sites. Alternatively, a transactivator may recognize and bind to a site even when the site is present in a nucleosome.

The two best characterized promoters in the remodeling class are the yeast PHO5 promoter (3) and the MMTV LTR (2, 4). Phosphate starvation activates the PHO5 gene through binding of a basic-helix-loop-helix protein, Pho4, to two sites within the PHO5 promoter region. A second factor, Pho2, interacts with Pho4 and increases the affinity of Pho4 for its binding sites (5, 6). It is largely dispensable when Pho4 is overexpressed (7). Activation of the PHO5 promoter is accompanied by the disruption of four positioned nucleosomes, and many requirements for this remodeling have been defined (3).

Both the glucocorticoid receptor (GR) and nuclear factor I (NF1) are essential for activation of the MMTV LTR in response to glucocorticoids. It has become apparent that activation of the promoter proceeds in two steps (8, 9). The first step involves the binding of GR to sites contained within the second nucleosome upstream of the site of transcriptional initiation (the B nucleosome). A localized change in chromatin structure then allows binding of NF1 to its site within this nucleosome. Affinity of GR for its binding site within a nucleosome is only marginally lower (about 3-fold) than its affinity for a non-nucleosomal binding site (10, 11). In contrast, NF1 is not capable of binding to its target when contained in a nucleosome (12). This simple model, the facilitation of NF1 binding by GR-mediated nucleosome disruption, was subsequently modified, when Truss et al. (13) showed that the B nucleosome persists following receptor binding in vivo. This observation was consistent with similar results obtained in earlier in vitro studies, showing that GR can bind to a nucleosome without disrupting it (14), but the precise composition of the B nucleosome in the activated MMTV promoter remains undefined (15). Nonetheless, the B nucleosome appears to be subtly modified during receptor binding, as demonstrated by a localized increased accessibility to restriction nucleases (13). The altered nucleosome allows simultaneous binding of GR and NF1, something never observed with free DNA (10). Therefore, the model of MMTV activation has evolved from requiring nucleosome removal to tolerating the presence of a conformationally changed nucleosome, and finally to requiring the persistence of the nucleosome as an essential component of the activation mechanism.

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Nucleosome Remodeling by Pho4 and Glucocorticoid Receptor

Much of the MMTV LTR regulation has been reconstructed in yeast (16, 17). Originally, constitutively active derivatives of GR (lacking the hormone-binding domain) were shown to activate transcription in yeast (18). It was later shown that hormone-dependent gene activation could be obtained in yeast cells expressing the intact GR or derivatives containing the ligand-binding domain (19–21). Further refinement of the yeast experiments has made it possible to demonstrate a synergy between GR and NFI activation, which was dependent on the presence of a modified nucleosome in the MMTV promoter (16, 17).

In the present work, we have attempted to compare directly the chromatin remodeling inherent in the PHO and the MMTV system. To that end, we have generated hybrid PHO5 MMTV promoter constructs that include the nucleosomes remodeled in PHO5 and the B nucleosome of the MMTV LTR. The purpose of this study was to perform a side by side comparison of how these nucleosomes respond to binding of either GR or Pho4. Our results suggest that nucleosome stability and the DNA-binding domain of the transactivator both play important roles in determining the mechanism of chromatin modification.

**EXPERIMENTAL PROCEDURES**

**Yeast Strains and Media**—The Saccharomyces cerevisiae strains used in this study were AH220 (a, trp1, his3-11, leu2-3, leu2-112, pho5, pho3) (22) and AH240, a pho4::URA3 derivative of AH220 and YS33 (23). Yeast strains were grown in YNB medium supplemented with the required amino acids (high phosphate conditions = repressible) or in phosphate-free synthetic medium (= activating) (24). Glucocorticoid induction was accomplished by addition of 10 μM deoxycorticosterone (DOC) to the medium. Combined induction by hormone and phosphate depletion was achieved by adding DOC to cultures growing in YNB for at least 7 h and then transferring the cells to phosphate-deficient medium supplemented with DOC. After 10–15 h, cells were analyzed for activity or their chromatin organization.

**Plasmids**—The construction of the PHO5-lacZ reporter plasmid (pP5-GRE-Z) was described previously (25). The wild type PHO5 promoter and variants thereof containing the MMTV B nucleosome or a GRE are schematically depicted in Fig. 1. DNA corresponding to the PHO5 promoter was generated as a 176-base pair PCR fragment derived from position 232 to 46 of pMVT-v-ATH (26) using primers A (5′-TTTACTGACCTGGTCCGTTCTCC-3′) and B (5′-ATAAACATGAGCATAGACCTTGAT-3′). pP5-M1 was generated by inserting the MMTV B nucleosome as an XhoI/NcoI fragment into the PHO5 promoter construct already generated (23). pP5-M2 was generated by inserting a double-stranded DNA oligonucleotide (GGAGCTGGAGGCGAGCACTAAG) after cleavage with XhoI and NcoI sites generated by inserting the following sequence between positions 169 and 168 of the PHO5 promoter (XhoI and NcoI sites underlined): TCGAGNNNNNCCATGTCGAGAACTGTAC. The same vector was also used to create pG5-GRE-Z by inserting a double-stranded DNA oligonucleotide (AGAGCTGGAGGCGAGCACTAAG) after cleavage with XhoI and NcoI.

pG7-N795, a GR expression plasmid, was constructed from pG7-N795 (20), a gift of D. Picard, by replacing the TRP1 with the HIS3 marker. pRS424-NFI, an NFI expression plasmid, was constructed from pBSSK (+) (27), a gift of S. Gasser, by inserting the NFI cDNA sequence into pRS424 using EcoRI and NcoI sites. The Pho4 DNA-binding domain was inserted into the YEplac424 vector expressing yeplac424-tau1 as constructed from YEplac424-tau1 (designated YEplac424 in Ref. 23) by inserting a PCR fragment from the human glucocorticoid receptor cDNA with the tau1 domain (amino acids 187–244) (29) and a 5′ EcoRI site as well as a 3′ BamHI site into BamHI and EcoRI digested YEplac424. Pho4 derivatives containing the GR DNA-binding domain were created from YCpPho4 and YCpPho4-act (designated YCpPho4 and YCpPho4-act, respectively, in Ref. 23) by replacing the Pho4 basic-helix-loop-helix domain by the GR DNA-binding domain. The two primers GTACTCGAG TCGATTGTTTTCAATTGG and TTACGCTAATTTTGGATGGT were used to generate a PCR fragment from pG7-N795 as template. The fragment contains the codons for amino acids 407–536 of GR and an adjacent in frame stop codon and was inserted into the XhoI- and Cln1-digested X vector of YCpPho4 and YCpPho4-act to yield YCpPho4-GR and YCpPho4-GR

**RESULTS**

**Synergistic Activation of Hybrid PHO5-MMTV Promoter Constructs by Pho4 and GR**—Hybrid promoter constructs were created to compare directly aspects of chromatin modulation by GR and Pho4. In one construct, nucleosome −2 of the PHO5 promoter was replaced with the MMTV B nucleosome (pP5-M1), and in another, the B nucleosome was inserted into the full-length PHO5 promoter (pP5-M2, see Fig. 1). The hybrid promoter constructs were introduced as lacZ fusions into yeast strains that also contained a plasmid expressing the intact GR (20). Activation of PHO5 UAS elements and glucocorticoid response elements (GRE) contained within the B nucleosome could be achieved by growth in medium lacking phosphate and by addition of hormone, respectively.

We first tested the substitution construct in which the B nucleosome replaces nucleosome −2 (pP5-M1), which removes one of the PHO5 UAS elements. The resulting promoter responds weakly to hormone addition and is almost refractory to activation by phosphate starvation (Fig. 2A). However, the combination of the two conditions leads to substantial activity that is comparable to that of the wild type promoter. In contrast, a promoter construct in which nucleosome −2 was replaced by only a GRE not contained in a nucleosome (pG5-GRE, see Fig. 1) is more strongly induced by low phosphate conditions, activity increases from 1.2 to 8% (Fig. 2B). The further induction resulting from the addition of hormone is only 4–5-fold, however. This contrasts with the 60-fold induction by hormone if the hormone-responsive elements are contained in a nucleosome (Fig. 2A).

The PHO5 promoter variant with an added B nucleosome that does not replace nucleosome −2 (pP5-M2) allows side by side comparison of chromatin remodeling of the PHO5 nucleosome −2 and the MMTV B nucleosome (see below). Interestingly, the addition of the B nucleosome almost completely prevents activation of the promoter by phosphate depletion in the absence of hormone (Fig. 3). However, with both signals present, added hormone and phosphate depletion, activation is almost 50% greater than that of the wild type PHO5 promoter (Fig. 3). These results demonstrate very strong synergistic activation by Pho4 and GR with multiple promoter constructs.

**Chromatin Transitions at a Hybrid PHO5-MMTV Promoter**—In order to investigate the chromatin transition of the hybrid PHO5-MMTV promoter, we analyzed nucleosome accessibility of the PHO5 promoter variant with an additional B nucleosome. We first monitored the effects of phosphate depletion in the absence of hormone. As shown in Fig. 4, the accessibility of some restriction sites within and around the B nucleosome increases somewhat as a consequence of activation through the two Pho4-dependent UAS elements. Opening is limited, however, as shown by the SacI site, the accessibility of which increases from <5% to 20%. This is consistent with the low activity of the promoter when only the phosphate depletion signal is active (5% of fully induced level, see Fig. 2). In con-
Fig. 1. Hybrid PHO5-MMTV promoter variants. The chromatin structure at the wild type (wt) PHO5 promoter under noninduced (+Pi) and induced (−Pi) conditions is shown at the top. Nucleosomes −1, −2,−3, and −4 are disrupted upon activation. The small circles mark UASp1 (open) and UASp2 (solid), which are Pho4-binding sites found by in vitro and in vivo footprinting experiments. The positions are listed relative to the coding sequence (solid black). T denotes the TATA box. The three PHO5-MMTV promoter variants analyzed here are shown underneath. The hatched circle denotes the MMTV B nucleosome, either replacing the PHO5 nucleosome −2 (pP5-M1) or inserted as an extra nucleosome between PHO5 nucleosomes −1 and −2 (pP5-M2) as described under “Experimental Procedures.” In pP5-GRE, the PHO5 nucleosome −2 was replaced by a GRE.

Fig. 2. Activation of PHO5 promoter variants containing the MMTV B nucleosome or a GRE. The activities of pP5-M1 (A) and pP5-GRE (B) as lacZ fusions in the yeast strain AH220 before and after DOC addition and/or phosphate depletion are shown. Solid bars indicate high phosphate, and gray bars indicate no phosphate conditions. 100% activity is defined as the activity of the wild type PHO5 promoter induced by phosphate starvation. The promoter structure is shown schematically underneath in both cases (compare Fig. 1).

In contrast, the PHO5 nucleosome −2 is fully disrupted as illustrated by a ClaI accessibility of more than 90% (see below).

The combined effect of phosphate depletion and glucocorticoid addition on the chromatin structure of the hybrid promoter is depicted in Fig. 5. The ClaI site, which had been fully accessible even in the absence of hormone, remains 90% accessible. Unexpectedly, however, there is almost no effect by the added hormone on sites within and around the B nucleosome. This contrasts with the dramatic effect of hormone on transcriptional activation, which increases approximately 20-fold under these conditions. Apparently, the receptor hormone complex is capable of activating transcription through the GREs in...
the B nucleosome without disrupting its structure as monitored by our protection assay. The chromatin structure of the hybrid promoter was also analyzed by DNase I digestion experiments (Fig. 6). The pattern confirms the disruption of the PHO5 nucleosomes upstream of the B nucleosome and again demonstrates the relative stability of the B nucleosome, even after hormone addition. DNase I digestion of free DNA (right panel in Fig. 6) shows that in the chromatin digests, protection of the DNA contained in the B nucleosome is not a property of just the DNA itself but of its chromatin organization.

Is the Relative Stability of the B Nucleosome Due to the Absence of Nuclear Factor I?—NFI plays an important role in the activation of the MMTV LTR both in mammalian cells (33, 34) and when it is reconstituted into yeast (6). There is strong synergy between NFI and GR in the activation of chromatin templates. In order to find out if the stability of the B nucleosome in the context of the PHO5 promoter was due to the absence of NFI in our system, we introduced an NFI expression plasmid into our strains and analyzed the effects on transcriptional activation and chromatin structure.

NFI augmented glucocorticoid-dependent promoter activity, especially at high phosphate conditions. Activity increased from 8 to 25% at high phosphate and from 144 to 150% at low phosphate conditions when NFI was expressed (compare Fig. 3). However, examination of the stability of the B nucleosome under these conditions revealed no effect due to expression of NFI. The B nucleosome remained as stable in the presence of NFI as in its absence when assayed by restriction accessibility measurements (Fig. 7). We conclude therefore that the stability of nucleosome B in the hybrid promoter is not due to the absence of NFI.

Does the Glucocorticoid Receptor Lack a Chromatin Opening Domain?—Previously, we have shown that the ability of Pho4 to disrupt chromatin at the PHO5 promoter requires its transcriptional activation domain (23), and we have so far been unable to separate functionally transcriptional activation from chromatin disruption (35). We had also shown that other activation domains like VP16 are capable of remodeling PHO5 chromatin when they are fused to the Pho4 DNA-binding domain (23). Because GR transactivates without substantially remodeling a GRE-containing nucleosome, it was conceivable that its activation domains cannot disrupt chromatin. In order to test this possibility, we generated a hybrid protein containing the Pho4 DNA-binding domain (Pho4Δact) and the minimal N-terminal activation domain of GR, the tau1 core domain (29) to give Pho4Δact-tau1 (see Fig. 8).

The hybrid activator was introduced into a yeast strain lacking endogenous Pho4, and its ability to transactivate and modulate chromatin structure was monitored. The Pho4 GR hybrid protein gave strong transactivation, which reached levels similar to those attained by native Pho4. Furthermore, the hybrid protein was fully capable of disrupting nucleosome −2 (Fig. 8). The effect of the tau1 domain is not simply due to a stabilizing effect on Pho4Δact and thereby increases a potential chromatin opening activity of the Pho4 DNA-binding domain. This possibility was ruled out by Western analysis that demonstrated that Pho4Δact and Pho4Δact-tau1 are expressed to very similar levels (Fig. 9). Therefore, the absence of disruption of the B nucleosome by GR is not due to the absence of an activity in GR capable of disrupting chromatin.

The Differential Ability of Pho4 and GR to Disrupt Chromatin Does Not Reside in Their Activation Domains—After analyzing a transactivator containing the Pho4 DNA-binding domain and the GR tau1 core transactivation domain, we created the reciprocal construct by replacing the Pho4 DNA-binding domain with the corresponding domain from GR (YCpPho4-GRDBD). In addition, we generated a truncated hybrid protein lacking the Pho4 activation domain (YCpPho4Δact-GRDBD). Expression of Pho4-GRDBD resulted in strong activation of the hybrid PHO5-MMTV promoter with the extra B nucleosome (130% at −P1 conditions, Table I), which is almost identical to the activity obtained with native GR (144%, see Fig. 3). As expected, this activation depended entirely on the integrity of the Pho4 activation domain. In its absence, only background activity of the reporter was obtained (Table I).

Although Pho4-GRDBD contains the Pho4 activation domain, its effect on the B nucleosome is indistinguishable from that of either native GR or Pho4Δact-GRDBD. Limited opening was obtained with both proteins (Table I) very similar to what had been obtained with GR as shown in Figs. 5 and 6. Given that the two proteins can activate to similar levels yet differ strikingly in their ability to open up chromatin demonstrates that the inability of GR to disrupt the B nucleosome does not reside in its activation domain.

DISCUSSION

The most important outcome of the present study is the finding that Pho4 and GR differ very much in the way they affect nucleosome structure during activation. The two factors have quite similar effects on transcriptional activation. However, Pho4 disrupts nucleosome structure in a much more profound fashion than GR. Upon activation, the endogenous nucleosomes of the PHO5 promoter became completely disrupted as shown by their accessibility to restriction nucleases and DNase I. In contrast, the B nucleosome is virtually unaffected by the presence of the receptor-hormone complex as judged by the same criteria.

The relative stability of the B nucleosome is not unique to the PHO5 context. In mammalian cells, the B nucleosome also undergoes only a subtle change in structure following activation. Truss et al. (13) have shown that the nucleosome persists upon hormone-dependent activation of the promoter, albeit in...
an altered form that allows the simultaneous binding of GR and NFI. They have proposed that an important role of the nucleosome is to actually enable simultaneous binding of NFI and GR, because the two factors cannot bind to free DNA together (36). Transposition of the entire MMTV LTR into yeast has been shown to reconstruct many of the properties of the system. Again only subtle perturbations of the chromatin structure occur upon activation (16, 17).

The differential effects exerted by Pho4 and GR on chromatin structure are apparently dictated by differences in how these two factors interact with their cognate sites. Pho4 binds to its site throughout two turns of DNA (37), suggesting that binding would not be possible when the DNA is tightly complexed to a histone octamer. Accordingly, Pho4 has a much lower affinity for its binding site within a nucleosome as compared with free DNA. We have shown this to be the case in vivo (23), and in our in vitro footprint experiments, we have never detected complexes between Pho4 and nucleosomes. In contrast, GR binds quite well to nucleosomes, and the difference in its affinity for target sites in free DNA compared with a nucleosome is only about 3-fold (10, 11). GR binds to two distinct motifs in the DNA exposed on the same helical surface and therefore can access its target site within a nucleosome.

There is considerable evidence that transactivators can alter the equilibria between nucleosomal and nucleosome-free states in chromatin (38, 39). A factor like Pho4, which can stably bind only to free sites, would be expected to shift the equilibria toward the nucleosome-free state. In this case, extensive disruption might facilitate binding of Pho2, the other transcription factor required for PHO5 activation, and also interactions between proteins bound to the upstream and downstream ele-
ments of the promoter. In contrast, such equilibria would presumably be only marginally shifted by GR, which binds nucleosomal target sites almost as well as nucleosome-free target sites. Furthermore, the persistence of an altered nucleosome plays a positive role in facilitating synergy between GR and NFI (40).

Although binding of Pho4 and GR has very different consequences in our experiments, we could demonstrate that GR does possess an activation domain that is capable of disrupting nucleosomes when it is fused to the Pho4 DNA-binding domain. It is possible that this domain within GR is utilized in other GR target promoters to cause chromatin disruption. However, this result also indicates that the DNA-binding domain makes an independent contribution to chromatin disruption. This conclusion was confirmed by the observation that the Pho4 activation domain has no effect on B nucleosome stability when fused to the DNA-binding domain of GR.

One of the most notable aspects of the hybrid PHO5-MMTV...
Pho4 is indicated at the top of the activation domain in full-length binding domain of Pho4, and the location notes the basic-helix-loop-helix DNA-binding domain of GR.

The GR tau1 domain is capable of disrupting the wild type PHO5 promoter when recruited to the Pho4-binding sites. The yeast strain AH240 (pho4), harboring the wild type PHO5 promoter on the pPHO5-Z plasmid and expressing either Pho4act or Pho4act-tau1, was grown in the absence of phosphate. Isolated nuclei were digested with Clal and analyzed as in Fig. 3. The construction of Pho4act-tau1 from the human glucocorticoid receptor (hGR) cDNA and Pho4act is shown schematically at the top of the figure. T1 and T2 indicate the two transactivation domains; DNA indicates the DNA-binding domain, and Steroid indicates the hormone-binding domain of GR. bHLH denotes the basic-helix-loop-helix DNA-binding domain of Pho4, and the location of the activation domain in full-length Pho4 is indicated at the bottom.

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![Diagram](image.png)

Fig. 8. The GR tau1 domain is capable of disrupting the wild type PHO5 promoter when recruited to the Pho4-binding sites. The yeast strain AH240 (pho4), harboring the wild type PHO5 promoter on the pPHO5-Z plasmid and expressing either Pho4act or Pho4act-tau1, was grown in the absence of phosphate. Isolated nuclei were digested with Clal and analyzed as in Fig. 3. The construction of Pho4act-tau1 from the human glucocorticoid receptor (hGR) cDNA and Pho4act is shown schematically at the top of the figure. T1 and T2 indicate the two transactivation domains; DNA indicates the DNA-binding domain, and Steroid indicates the hormone-binding domain of GR. bHLH denotes the basic-helix-loop-helix DNA-binding domain of Pho4, and the location of the activation domain in full-length Pho4 is indicated at the bottom.

![Diagram](image.png)

Fig. 9. Analysis of Pho4act-tau1 and Pho4act expression levels by Western blotting. Whole cell extracts from AH240 (pho4) cells, expressing either Pho4act or Pho4act-tau1 that were used in Fig. 8, were analyzed on 12.5% SDS-polyacrylamide gels. Proteins were blotted and detected with antibodies against the Pho4 DNA-binding domain as described under "Experimental Procedures."

TABLE I

| Pho4-GR<sub>DBD</sub> | Activity | Accessibility at Clal | SacI |
|-----------------------|----------|----------------------|------|
| +P<sub>1</sub>         | -P<sub>1</sub> | 5–130                 | 90   | 40   |
| Pho4act-GR<sub>DBD</sub> | 4–10     | 90                    | 35   |

promoters is the profound synergy observed upon activation of both Pho4 and GR. It is striking that activation by either pathway alone gave very little activity, especially since the hybrid constructs retain many binding sites for both Pho4 and GR. One general feature of the nucleosomal organization of promoters seems to be to keep the repressed levels very low with very little detrimental effect on the activated levels. This flexibility of the nucleosome has been documented both in vivo and also by in vitro transcription systems using chromatin templates (41). Our results suggest that the repressed B nucleosome that is not bound by GR may exert a generally repressive effect on adjacent promoter regions. Conversely, binding of GR to multiple sites on the B nucleosome cannot adequately activate transcription when this nucleosome is surrounded by the nucleosome-repressed PHO5 promoter.

Several important characteristics of the two regulated gene systems, PHO5 and MMTV LTR, have become apparent from their direct comparison within the PHO5 promoter context. Our results indicate that modification of chromatin structure during promoter activation has evolved as a complex interplay between nucleosome stability, transactivation domains, and DNA-binding domains. The variations on these themes exhibited by the PHO5 and MMTV promoters indicate that nucleosomes are flexible structures that mediate both repression and synergistic activation and have established these systems as valuable paradigms for the analysis of how chromatin structure participates in the regulation of eukaryotic promoters.

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