Isolation of an activator-dependent, promoter-specific chromatin remodeling factor

Andreas H. Ehrensberger¹ and Roger D. Kornberg²

Department of Structural Biology, Stanford University School of Medicine, Stanford, CA 94305

Edited by Michael Grunstein, David Geffen School of Medicine at University of California, Los Angeles, CA, and approved April 11, 2011 (received for review January 27, 2011)

Repressed *PHO5* gene chromatin, isolated from yeast in the native state, was remodeled by yeast extract in a gene activator-dependent, ATP-dependent manner. The product of the reaction bore the hallmark of the process in vivo, the selective removal of promoter nucleosomes, without effect on open reading frame nucleosomes. Fractionation of the extract identified a single protein, chromodomain helicase DNA binding protein 1 (Chd1), capable of the remodeling activity. Deletion of the *CHD1* gene in an *isw1Δ pho80Δ* strain abolished *PHO5* gene expression, demonstrating the relevance of the remodeling reaction in vitro to the process in vivo.

Chromatin structure plays a regulatory role in transcription and other DNA transactions. Classical studies of the *PHO5* gene of yeast demonstrated a change in chromatin structure upon transcriptional activation (1), which is now known to reflect the removal of promoter nucleosomes (2). We have sought to reconstitute this process with purified components, including chromatin assembled in vivo at its native chromosomal locus (native chromatin).

A procedure has been developed for the isolation of native chromatin from any chromosomal region in yeast (Fig. 1A): The region of interest is excised as a closed circle by a sequence-specific recombinase (3) and isolated by cell lysis, differential centrifugation, and affinity purification (4). When this procedure was applied to the *PHO5* gene in the transcriptionally repressed or activated state, the structure of the purified chromatin circles proved to be indistinguishable from that of the gene at the chromosomal locus: In the repressed state, the promoter was occupied by three nucleosomes, denoted N-1, N-2, and N-3 (Fig. 1B), whereas in the activated state, only one nucleosome remained. The chromatin structure of the ORF was the same under repressing and activating conditions (2). Removal of promoter nucleosomes is presumed to relieve the repression brought about through their interference with the transcription machinery (5, 6) and may be a general feature of eukaryotic genes (7–10).

Activation of the *PHO5* gene, which encodes an acid phosphatase, is provoked by removal of phosphate from the growth medium (11). The Pho4 activator enters the nucleus (12) and binds two sites in the promoter: UASp1, which is located between nucleosomes N-2 and N-3, and UASp2, which lies within N-2 (Fig. 1B).

Although no single chromatin remodeling complex appears to be essential for activation of *PHO5* (13, 14), genetic studies have implicated several factors at this locus, including the chromatin remodeling complexes INO80 (15) and SWI/SNF (16), the histone acetyltransferases SAGA (17) and NuA4 (18), and the histone chaperone Asf1 (19, 20). The question arises whether the actions of these factors are direct or indirect and whether additional and possibly essential factors could be revealed through unbiased biochemical fractionation of yeast extract.

With the goal of identifying an enzyme capable of directly remodeling native promoter chromatin under conditions of gene activation (presence of Pho4), we reconstituted the process in vitro using repressed *PHO5* chromatin circles. A reaction that met these criteria served as a basis for the fractionation and

Fig. 1. Purification and structure of *PHO5* chromatin circles. (A) Purification of chromatin circles. The region of interest is flanked by two recognition sites for the R recombinase (boxes with black triangles). Excision of the *PHO5* locus occurs after expression of recombinase is induced with galactose. The resulting closed chromatin circles are purified by differential centrifugation and by affinity purification with the use of a LexA binding site (gray rectangle) and a TAP-tagged LexA adaptor protein expressed from an exogenous plasmid. Schematics of *PHO5* chromatin circles containing nucleosomes of both promoter (beige ovals) and ORF (dark gray ovals) are shown. The remainder of the chromosome (black ovals) remains intact after recombination. (B) Structure of *PHO5* promoter. The minimal *PHO5* promoter contains three nucleosomes, labeled N-1, N-2, and N-3, two of which are removed upon gene activation (14). The two Pho4-binding sites UASp1 and UASp2 are shown as black circles, and the transcription start site as a black square. The two Clal sites are indicated, one of which, in N-1, was inserted in place of the TATA box, and which allows simultaneous monitoring of nucleosomes N-2 and N-1.

subsequent isolation of the cellular machinery involved, whose relevance was subsequently validated in vivo.

Results

Activator- and ATP-Dependent Remodeling of *PHO5* Promoter Nucleosomes in Yeast Extract. Chromatin circles containing both *PHO5* promoter and ORF (Fig. 1A) were purified from *pho4Δ* cells grown under repressing conditions. The circles were treated with *pho4Δ* yeast extract and ATP, with or without the addition of recombinant Pho4. Removal of promoter nucleosomes was monitored by the accessibility of two restriction sites: a naturally

Author contributions: A.H.E. and R.D.K. designed research; A.H.E. performed research; A.H.E. analyzed data; and A.H.E. and R.D.K. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹Present address: London Research Institute, Cancer Research UK, Clare Hall Laboratories, Blanche Lane, South Mimms, Herts EN6 3LD, United Kingdom.

²To whom correspondence should be addressed. E-mail: kornberg@stanford.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1101449108/-DCSupplemental.

occurring *Cl*I site at nucleosome N-2 and a second *Cl*I site inserted at N-1 (Fig. 1*B*). Including Pho4 in the reaction increased the accessibility of the two sites from 28% to 37% (N-2, 9% activity) and from 26% to 37% (N-1, 11% activity; Fig. 2*A*).

Both effects were dependent on yeast extract (Fig. 2*A*) and ATP (Fig. 2*B*). The inclusion of carrier DNA or chromatin proved to be essential, because no reaction was observed in its absence (Fig. S1*A–C*). The carrier may remove nonspecific inhibitory activities or serve as a histone acceptor, as has previously been observed for the chromatin remodeling complex RSC (21).

The reactions at N-1 and N-2 could be distinguished by truncation of Pho4. A typical transcriptional activator, Pho4 comprises a DNA-binding domain (DBD) and an activation domain (AD), both required *in vivo* for activation of *PHO5* (22, 23). In our assay, a truncated Pho4 protein lacking the AD (Pho4Δ4) failed to support removal of nucleosome N-1 but still brought about removal of N-2 (Fig. 2*A*). The reaction at N-1, with its requirement for the AD, likely reveals the remodeling activity relevant *in vivo*, and was therefore used to track the activity during purification. In contrast, the reaction at N-2 may reflect nonspecific nucleosome sliding activities or destabilization of N-2 due to Pho4 DBD binding to UASp2 (Fig. 1*B*).

The promoter specificity of the reaction was initially investigated by restriction enzyme digestion. The accessibility of a *Bst*EII site in the promoter increased upon addition of Pho4, whereas that of a second *Bst*EII site inside the ORF was unchanged (Fig. 2*C*). The accessibility of *Eco*RV and *Pfi*MI sites in the ORF was similarly unaffected, confirming that the reaction specifically targeted the promoter.

Fig. 2. *PHO5* promoter remodeling in yeast extract. *PHO5* chromatin circles containing promoter and ORF were treated with yeast extract and ATP, with and without Pho4. The reaction product was digested with *Cl*I (A and B) or with other restriction enzymes (C), and DNA was extracted and analyzed by blot hybridization with a 900 bp *PHO5* *Eco*RV probe. (A) Promoter nucleosomes N-1 and N-2 are remodeled in the presence of extract and the activator Pho4. Activity is defined as the difference between the accessibility of a given nucleosome to *Cl*I in the presence and in the absence of Pho4. When a truncation of Pho4 lacking the activation domain (Pho4Δ4) was used instead of Pho4, only N-2 but not N-1 was remodeled. Neither Pho4 nor Pho4Δ4 supported remodeling of nucleosomes in the absence of extract. N/A, not applicable. (B) ATP is required for chromatin remodeling. Pho4-dependent remodeling of N-1 and N-2 does not occur in the absence of ATP or when apyrase is added to remove ATP from the reaction. (C) The promoter but not the ORF is remodeled in a Pho4-dependent manner. The chromatin remodeling assay was performed with extract followed by incubation with restriction enzymes that cut *PHO5* either in the promoter (*Cl*I), in both promoter and ORF (*Bst*EII), or only in the ORF (*Eco*RV and *Pfi*MI). DNA was detected using the 900 bp *Eco*RV ORF probe (green line). Numbers show Pho4-dependent chromatin remodeling of a given nucleosome with quantitation performed as in A.

Fractionation and Purification of Remodeling Activity. The activator-dependent, promoter-specific chromatin remodeling activity was purified in five chromatographic steps (Fig. 3*A*). Single peaks of activity were observed at every stage of purification (as in Fig. 3*B* and *C*). Specificity for the promoter was evaluated both by digestion with restriction endonucleases, as described above, and by digestion with micrococcal nuclease (MNase). Because MNase digests naked but not nucleosomal DNA, it only generates a ladder of bands in Southern blot analysis when the region covered by the probe is protected by nucleosomes. With a probe covering the promoter, we observed a nucleosomal ladder for reactions performed in the absence of Pho4, but not for reactions performed in its presence. When the same membrane was stripped of the promoter probe and rehybridized with a probe covering the ORF, a ladder was observed both in the presence and absence of Pho4, indicating promoter-specific nucleosome removal (Fig. 3*D*). Peak DEAE and hydroxylapatite fractions catalyzed promoter-specific removal of 41 ± 3% and 35 ± 1% of nucleosomes, respectively, compared to the removal of 44–63% of promoter nucleosomes *in vivo* (2) (Fig. 3*D*).

Activator-Dependent Remodeling of Promoter Nucleosomes by chromodomain helicase DNA binding protein 1 (Chd1). SDS-PAGE and mass spectrometry of the fractions from the final gel filtration step revealed three coeluting sets of polypeptides (Fig. 4*A*), corresponding to the chromatin remodeling complex RSC (bands due to Sth1 and Rsc2, fractions 23–28), DNA polymerase ε (bands due to Pol2 and Dpb2, fractions 30–33), and the chromatin remodeling ATPase Chd1 with Sub1 (fractions 32–35). MNase analysis of *PHO5* chromatin remodeling revealed a peak of Pho4-dependent, promoter-specific activity in fractions 32–35 (Fig. 4*B* and Fig. S2*A*).

Because Chd1 was the most abundant protein in the active fractions and is known to have ATP-dependent chromatin remodeling activity (24), we assayed it in recombinant form. Chd1 was TAP-tagged at its C terminus and purified by IgG-affinity chromatography and gel filtration (Fig. S3*A*). Sub1, which comigrated with Chd1 in the original purification, was not found to be associated with Chd1, in agreement with previous reports (24). When fractions containing pure Chd1 were assayed, a Pho4-dependent, promoter-specific chromatin remodeling activity comigrated with Chd1 (Fig. 4*C* and Fig. S2*B*). The specific activity of pure Chd1 was about 10-fold lower than that of Chd1 fractionated over five columns. The diminished activity may be due to inhibition by the TAP-tag, to the high salt concentration required to keep Chd1-TAP in solution, or to loss of additional unknown stimulatory factors.

Because the chromatin remodeling complex RSC coeluted with Chd1 until the final gel filtration step of the original purification, we investigated whether it is also active in our assay. Gel filtration fractions 23–28, containing the peak of RSC protein, yielded a diffuse MNase pattern both with and without Pho4 and on both the promoter and the ORF (Fig. S4*A*). Such a pattern may be indicative of disorganized nucleosome arrays and is markedly different from the pattern observed *in vivo* (2). The same pattern was obtained with RSC purified via a TAP-tag on the Rsc2 subunit (Fig. S4*B*), consistent with other studies (25).

Activation of *PHO5* in *chd1Δ* Cells. To assess the requirement for Chd1 *in vivo*, we induced *PHO5* expression in *chd1Δ* strains. Induction was accomplished by deletion of *PHO80*, a repressor in the signaling cascade for *PHO5* activation (12, 26). *PHO5* expression, as measured by acid phosphatase activity and mRNA analysis, was only slightly reduced in the absence of Chd1, in agreement with previous reports (13, 14), indicating that additional factors may be capable of remodeling *PHO5* promoter chromatin *in vivo* (Fig. 5*A* and *B*). A candidate for this role is the Isw1 chromatin remodeler, which has been shown to act in

Fig. 3. Fractionation of remodeling activity. (A) Purification scheme. Extract from 1 kg of yeast cells was fractionated by step-elution from Bio-Rex 70 and DEAE, gradient elution from hydroxylapatite (HAP) and Uno-S and gel filtration (Superose 6). (B) Chromatin remodeling assays were performed with Uno-S fractions as described in Fig. 2A and *Materials and Methods*. "Load" corresponds to the active pool of HAP fractions loaded on Uno-S. Note that the peak of activity resides in fractions 100–109. (C) Activity profile corresponding to B. The red line represents the activity and the blue line corresponds to the total protein concentration. (D) Limit digestion with MNase confirms promoter specificity of the activity during fractionation. Lanes 1–12: Chromatin remodeling assays were performed as before with peak fractions from the columns indicated. The product of two identical reactions was digested with MNase (1.5 and 4.5 units/reaction) and the DNA was extracted and analyzed by blot hybridization with a probe covering the *PHO5* promoter (PRO), followed by stripping and rehybridizing with a probe covering the ORF (ORF). Numbers under the blots show the mean Pho4-dependent and promoter-specific activity from the average of both MNase concentrations, calculated as described in *Materials and Methods*. Lanes 13 and 14: Nuclei from cells repressed (rep, strain yM2.1) or activated (act, strain yM8.14) at *PHO5* were digested with 4 units of MNase as described in Boeger et al. (2). DNA was analyzed and promoter-specific chromatin remodeling quantified as for lanes 1–12.

similar pathways as Chd1 (27–29). Deletion of only *ISW1* had little effect on acid phosphatase activity, but double deletion of *ISW1* and *CHD1* completely abolished *PHO5* gene expression (Fig. 5A and B). Induction by phosphate starvation rather than *PHO80* deletion in *chd1Δ isw1Δ* cells significantly delayed but did not completely abolish production of acid phosphatase, presumably because of the weak *PHO5* mitotic induction pathway (Fig. S5) (30). We conclude that Chd1 directly participates in the activation of *PHO5* and in the remodeling of promoter nucleosomes in vivo.

Discussion

We report here a fully defined system for promoter chromatin remodeling. The system is reconstituted from a promoter in the repressed state, an activator protein, a remodeling ATPase, and ATP, and faithfully reproduces the reaction observed in vivo on the basis of four criteria: The substrate is native chromatin, purified to near homogeneity, indistinguishable in structure from the same gene region at its chromosomal locus; the process depends on the activation domain of the activator protein, as it does in vivo; promoter nucleosomes are removed in the process, whereas ORF nucleosomes are unaffected, again as found in vivo; and the remodeling ATPase involved is capable of remodeling the same promoter in vivo, as shown by its requirement for the process in a suitable mutant background.

Results from the reconstituted system and mutational analysis identify a factor capable of remodeling the *PHO5* promoter during gene activation. The results not only establish a role for Chd1 but also show that it can act directly, because the process takes place in the absence of other genes and of the complex regulatory circuitry found in living cells. The RSC remodeling complex, suspected of playing a role at *PHO5* (13), was shown not to be involved: Purified RSC perturbed the distribution of

both promoter and ORF nucleosomes in both the presence and absence of activator; and remodeling did not occur in a *chd1Δ isw1Δ* mutant background in vivo, despite the continued presence of RSC.

The mechanism by which Chd1 removes nucleosomes in vitro is likely to be nucleosome disassembly, as has been observed in vivo (14). The alternative of nucleosome sliding would result either in smearing of the nucleosomal ladder, as observed for RSC (Fig. S4A and B), or in a gain of signal in the ORF, due to capture of nucleosomes from the promoter. Neither effect was observed for Chd1 in our system.

The chromatin remodeler Isw1 is evidently able to substitute for Chd1 at *PHO5* in vivo. The form of Isw1 that acts at *PHO5* is likely to be the monomer, because deletion (in a *chd1Δ* background) of the other subunits of Isw1 complexes (31), Ioc3 (present in ISW1a), and Ioc2 and Ioc4 (present in ISW1b), failed to phenocopy the *chd1Δ isw1Δ* mutation. The loss of Isw1 activity during fractionation could be explained by a low specific activity, and consequent lack of detection in our assay, or by the presence of inhibitors, as previously reported for the Isw1-containing Bio-Rex 70 flow-through fraction (31, 32). A role for Isw2, which has partly overlapping functions with Chd1 (27–29), was excluded because Isw2 alone could not maintain activation of *PHO5* in *chd1Δ isw1Δ* cells.

Chd1 is one of seven chromatin remodelers and the only member of the CHD family of remodelers found in yeast. It has homologues in all eukaryotes and has been implicated in various nuclear functions, including transcription initiation (29), elongation (33), and termination (27), and RNA splicing (34). It associates with active genes (33, 35), and its murine homologue, Chd1, is required in embryonic stem cells for the retention of open chromatin and maintenance of pluripotency (36). Like other members of the family, Chd1 has two chromodomains, a Swi2/Snf2 ATPase

in vivo Svaren et al. (23). Recombinant proteins were purified from BL21 *Escherichia coli* cells as previously described (4).

Chromatin Circles. Circles were purified as described in Griesenbeck et al. (4), with four modifications to the protocol: The R-recombinase and the LexA-TAP adaptor protein were expressed from one plasmid, pR2; histone deacetylase inhibitors were included in all buffers (100 nM trichostatin A and 5 mM sodium butyrate); only calmodulin, but not IgG chromatography was used for affinity purification; and circles were equilibrated to 50 mM potassium acetate on the calmodulin resin, to be eluted in the same salt concentration.

Whole-Cell Extract, Fractionation, and Purification of Chd1-TAP. Whole-cell extract was prepared by an adaptation of a protocol provided by J. Svejstrup (Cancer Research UK), which itself is an adaptation of the Wootner and Jaehning (43) protocol. Whole-cell extract for fractionation was prepared with a slightly different protocol adapted from ref. 32. Chd1-TAP was prepared with an adaptation of the standard TAP-tag protocol (44). For detailed protocols, see *SI Materials and Methods*.

Carrier Chromatin. Carrier chromatin was prepared from rat livers as in ref. 45. Final yields were about 3 mg with a concentration of 0.1–1 mg/mL and a DNA fragment size of 3–5 kbp. The material was highly pure, as confirmed by SDS-PAGE (Fig. S1A).

Chromatin Remodeling Assay. The following reagents are added to each tube for each 30 μ L reaction (in order of addition): 10 μ L (9 μ L circles at 5–10 amol/ μ L and 1 μ L carrier chromatin at approximately 0.1 mg/mL); 3 μ L Pho4 mix (1 μ L Pho4 buffer, 1 μ L 2 μ M Pho2, and 1 μ L 2 μ M Pho4 or 1 μ L Pho4 buffer); 9 μ L RM/H₂O (6 μ L 5 $\times$ RM, 3 μ L water, 0.3 μ L 100 $\times$ protease inhibitors); 5 μ L extract or fractions; 3 μ L 10 $\times$ energy mix. The corresponding buffers are as follows: 5 $\times$ RM [125 mM potassium acetate, 125 mM Hepes (pH 7.4), 12.5 mM DTT, 0.5 mg/mL BSA, 25 mM sodium butyrate, and 40 mM magnesium acetate, and 5 $\times$ protease inhibitors; ref. 46]; Pho4 buffer [100 mM sodium chloride, 50 mM Hepes (pH 7.5), 10% glycerol, and 5 mM β -mercaptoethanol]; and 10 $\times$ energy mix (50 mM ATP, 10 mM acetyl-CoA, 10 mM S-adenosyl methionine, 8 mg/mL creatine kinase, 40 mM phosphoenol pyruvate, and 150 mM phosphocreatine). Final reaction mixtures contain 50–100 amol of purified chromatin circles, 0.1 μ g carrier chromatin, 2 pmol of Pho2 $\pm$ 2 pmol of Pho4, 5 μ L extract or fractions, 5 mM ATP, 25 mM potassium acetate, 25 mM Hepes (pH 7.4), 8 mM magnesium acetate, 2.5 mM DTT, 0.1 mg/mL BSA, 1 mM acetyl-CoA, 1 mM S-adenosyl methionine, 0.8 mg/mL creatine kinase, 4 mM phosphoenol pyruvate, 15 mM phosphocreatine, and 1 $\times$ protease inhibitors. After all reagents have been added, tubes are incubated at room temperature for 90 min. Chromatin remodeling is stopped by adding 1 μ L apyrase at 50 units/ μ L (New England Biolabs, NEB) and incubating for 10 min at room temperature. Nuclease digestion of circles is then performed either with a restriction enzyme like ClaI to monitor loss of individual nucleosomes or with MNase to monitor longer regions of chromatin. For ClaI, add 70 μ L ClaI mix [56 μ L water, 10 μ L 10 $\times$ NEBuffer 4 (NEB), 1 μ L 10 mg/mL BSA, and 3 μ L ClaI at 5 U/ μ L] and incubate for 30 min at 37 $^{\circ}$ C. Reactions are stopped by adding 100 μ L stop buffer (87 μ L IRN, 10 μ L 10% SDS, and 3 μ L proteinase K at 22 mg/mL) and incubating for 30 min at 65 $^{\circ}$ C. For MNase, add 70 μ L freshly prepared MN digestion buffer [57 μ L water, 10 μ L 10 $\times$ MNase buffer, 1 μ L 10 mg/mL BSA, 1 μ L 1% salmon sperm DNA (boiled and sheared), and 1 μ L micrococcal nuclease at the indicated concentrations] and incubate for 5 min at 37 $^{\circ}$ C. 10 $\times$ MNase buffer is 130 mM Tris (pH 8.0), 500 mM sodium chloride, 64 mM calcium chloride, 2 mM EDTA, and 2 mM EGTA; IRN is 20 mM EDTA, 50 mM Tris (pH 8.0), 500 mM NaCl. Digestions are stopped by adding 100 μ L MN stop buffer (90 μ L IRN/20 mM EGTA, 10 μ L 10% SDS and 3 μ L proteinase K at 22 mg/mL) and incubating for 30 min at 65 $^{\circ}$ C. From now on, proceed identically for ClaI and MNase reactions: Add 30 μ g glycogen (Roche) to each reaction. Remove all proteins through phenol-chloroform extraction and precipitate DNA with ethanol by standard procedures. For ClaI reactions, add 25 μ L fresh NcoI mix [21 μ L water, 3 μ L 10 $\times$ NEBuffer 4 (NEB), and 1 μ L NcoI at 10 units/ μ L] directly to the pellets and incubate for 30 min at 37 $^{\circ}$ C (NcoI cuts circles outside of the *PHO5* gene immediately downstream of the LexA binding site and is added to linearize uncut circles). Then add 5 μ L 6 $\times$ DNA loading buffer. For MNase reactions, add 30 μ L 1 $\times$ DNA loading buffer directly to pellets and flick tubes to resuspend the DNA. DNA was separated on a 1.5% agarose gel, transferred to a nylon membrane by capillary transfer, and hybridized to a radiolabeled probe using standard Southern blotting procedures (47). The probes used were all fragments of the *PHO5* gene: Promoter (BamHI/DraI) and ORF (700 bp EcoRV). For restriction enzyme analysis, we used the ORF probe, whereas for

MNase analysis we used the promoter probe followed by stripping and rehybridizing with the ORF probe.

Quantitation of Activator-Dependent and Promoter-Specific Chromatin Remodeling ClaI Analysis. The degree to which nucleosome N-1 is remodeled in a given reaction is proportional to the accessibility of N-1 to ClaI, α_{N-1} :

$$\alpha_{N-1} = \frac{I_{N-1}}{I_{\text{uncut}} + I_{N-2} + I_{N-1}},$$

where the intensity I of each band is measured directly from the blot. The Pho4-dependent activity is the degree to which $N-1$ is disassembled in response to Pho4 and corresponds to the difference in the accessibilities of $N-1$ to ClaI between a reaction performed with Pho4 and one performed without Pho4:

$$\text{activity}(N-1, \%) = 100 \times (\alpha_{N-1}^{\text{Pho4}} - \alpha_{N-1}^{\text{NoPho4}}).$$

A similar analysis can be performed with other restriction enzymes as well.

MNase Analysis. MNase exhibits strong preference for naked over nucleosomal DNA and will digest DNA from which nucleosomes have been removed more readily than DNA that is still nucleosomal. The percent nucleosomes left on the promoter after addition of Pho4 is given by P :

$$P = \left(\frac{I^{\text{Pho4}}}{I^{\text{NoPho4}}} \right),$$

where I^{Pho4} is the intensity of a whole lane corresponding to a single reaction performed with Pho4, and I^{NoPho4} is the equivalent value for a reaction performed without Pho4. The intensity of a given lane is proportional to the average amount of nucleosomes present on the region covered by the probe. After stripping and rehybridizing the membrane with a probe against the ORF, we obtain the percent nucleosomes left on the ORF after addition of Pho4:

$$O = \left(\frac{I^{\text{Pho4}}}{I^{\text{NoPho4}}} \right).$$

The activity is defined as the percent nucleosomes lost specifically from the promoter upon addition of Pho4:

$$\text{activity}(\text{MNase}, \%) = 100 \times \left(1 - \frac{P}{O} \right).$$

Acid Phosphatase Assay. The acid phosphatase assay measures the activity of secreted acid phosphatase through colorimetric monitoring of the hydrolysis of *p*-nitrophenyl phosphate to *p*-nitrophenolate. Because Pho5 is the predominant secreted acid phosphatase in yeast, this assay can reliably be used to determine Pho5 levels (26): Saturated cell cultures grown in YPD were diluted 1:30 in 3 mL YPD and grown for 3–5 h at 30 $^{\circ}$ C. Two million cells were placed in an Eppendorf tube, pelleted, and residual media removed. Cell pellets were then resuspended in 250 μ L 0.1 M sodium acetate (pH 4.2) and 250 μ L fresh *p*-nitrophenyl phosphate at 9 mg/mL, vortexed, and incubated at 37 $^{\circ}$ C for 9 min. Reactions were stopped by addition of 800 μ L 1.4 M sodium carbonate, vortexed, centrifuged for 3 min at 22,000 $\times g$, and the A_{420} measured.

RT-PCR. Reactions were performed according to the manufacturer's instructions (StepOne, Applied Biosystems), using the following primers: PHO5-RT, TCGTGAAGGTGTCATTGCGAGGTCGACATCTTAGAATTAG; PHO5-5': GCTCGTGACTTCTTGGCTCA; PHO5-3', TCGTGAAGGTGTCATTGCGAGGTC; ACT1-RT, TGTCAGCGGATACGGGATGGTCATAGTCAGTCAAACTCTCA; ACT1-5', TCCATC-CAAGCCGTTTTGTCC; ACT1-3', TGTCAGCGGATACGGGATGGTC.

ACKNOWLEDGMENTS. We thank J. Griesenbeck for helpful discussions and advice on preparing chromatin circles, R. Siller and S. Nagai for help preparing strains, B. Davis (Stanford University) for providing TAP-tagged RSC, and H. Boeger, K. Eagen, J. Griesenbeck, S. Nagai and J. Svejstrup for critical comments on the manuscript. This research was supported by National Institutes of Health Grant GM36659. A.H.E. was supported by a predoctoral fellowship from the Boehringer Ingelheim Fonds and by a Stanford Graduate Fellowship.

1. Almer A, Rudolph H, Hinnen A, Horz W (1986) Removal of positioned nucleosomes from the yeast PHO5 promoter upon PHO5 induction releases additional upstream activating DNA elements. *EMBO J* 5:2689–2696.
2. Boeger H, Griesenbeck J, Strattan JS, Kornberg RD (2003) Nucleosomes unfold completely at a transcriptionally active promoter. *Mol Cell* 11:1587–1598.
3. Gartenberg MR (1999) Formation of extrachromosomal DNA rings in *Saccharomyces cerevisiae* using site-specific recombination. *Methods Mol Biol* 94:125–133.
4. Griesenbeck J, Boeger H, Strattan JS, Kornberg RD (2003) Affinity purification of specific chromatin segments from chromosomal loci in yeast. *Mol Cell Biol* 23:9275–9282.
5. Lorch Y, LaPointe JW, Kornberg RD (1987) Nucleosomes inhibit the initiation of transcription but allow chain elongation with the displacement of histones. *Cell* 49:203–210.
6. Han M, Grunstein M (1988) Nucleosome loss activates yeast downstream promoters in vivo. *Cell* 55:1137–1145.
7. Hirschhorn JN, Brown SA, Clark CD, Winston F (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev* 6:2288–2298.
8. Beato M (1996) Chromatin structure and the regulation of gene expression: Remodeling at the MMTV promoter. *J Mol Med* 74:711–724.
9. Verdin E, Paras P, Jr, Van Lint C (1993) Chromatin disruption in the promoter of human immunodeficiency virus type 1 during transcriptional activation. *EMBO J* 12:3249–3259.
10. Levings PP, Bungert J (2002) The human beta-globin locus control region. *Eur J Biochem* 269:1589–1599.
11. Lohr D (1997) Nucleosome transactions on the promoters of the yeast GAL and PHO genes. *J Biol Chem* 272:26795–26798.
12. O'Neill EM, Kaffman A, Jolly ER, O'Shea EK (1996) Regulation of PHO4 nuclear localization by the PHO80-PHO85 cyclin-CDK complex. *Science* 271:209–212.
13. Barbaric S, et al. (2007) Redundancy of chromatin remodeling pathways for the induction of the yeast PHO5 promoter in vivo. *J Biol Chem* 282:27610–27621.
14. Boeger H, Griesenbeck J, Strattan JS, Kornberg RD (2004) Removal of promoter nucleosomes by disassembly rather than sliding in vivo. *Mol Cell* 14:667–673.
15. Steger DJ, Haswell ES, Miller AL, Wente SR, O'Shea EK (2003) Regulation of chromatin remodeling by inositol polyphosphates. *Science* 299:114–116.
16. Gaudreau L, Schmid A, Blaschke D, Ptashne M, Horz W (1997) RNA polymerase II holoenzyme recruitment is sufficient to remodel chromatin at the yeast PHO5 promoter. *Cell* 89:55–62.
17. Barbaric S, Reinke H, Horz W (2003) Multiple mechanistically distinct functions of SAGA at the PHO5 promoter. *Mol Cell Biol* 23:3468–3476.
18. Nourani A, Utley RT, Allard S, Cote J (2004) Recruitment of the NuA4 complex poises the PHO5 promoter for chromatin remodeling and activation. *EMBO J* 23:2597–2607.
19. Adkins MW, Howar SR, Tyler JK (2004) Chromatin disassembly mediated by the histone chaperone Asf1 is essential for transcriptional activation of the yeast PHO5 and PHO8 genes. *Mol Cell* 14:657–666.
20. Korber P, et al. (2006) The histone chaperone Asf1 increases the rate of histone eviction at the yeast PHO5 and PHO8 promoters. *J Biol Chem* 281:5539–5545.
21. Lorch Y, Zhang M, Kornberg RD (1999) Histone octamer transfer by a chromatin-remodeling complex. *Cell* 96:389–392.
22. Ogawa N, Oshima Y (1990) Functional domains of a positive regulatory protein, PHO4, for transcriptional control of the phosphatase regulon in *Saccharomyces cerevisiae*. *Mol Cell Biol* 10:2224–2236.
23. Svaren J, Schmitz J, Horz W (1994) The transactivation domain of Pho4 is required for nucleosome disruption at the PHO5 promoter. *EMBO J* 13:4856–4862.
24. Tran HG, Steger DJ, Iyer VR, Johnson AD (2000) The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. *EMBO J* 19:2323–2331.
25. Lorch Y, et al. Selective Removal of Promoter Nucleosomes by the RSC Chromatin Remodeling Complex. *Nat Struct Mol Biol*, in press.
26. To EA, Ueda Y, Kakimoto SI, Oshima Y (1973) Isolation and characterization of acid phosphatase mutants in *Saccharomyces cerevisiae*. *J Bacteriol* 113:727–738.
27. Alen C, et al. (2002) A role for chromatin remodeling in transcriptional termination by RNA polymerase II. *Mol Cell* 10:1441–1452.
28. Tsukiyama T, Palmer J, Landel CC, Shiloach J, Wu C (1999) Characterization of the imitation switch subfamily of ATP-dependent chromatin-remodeling factors in *Saccharomyces cerevisiae*. *Genes Dev* 13:686–697.
29. Xella B, Goding C, Agricola E, Di Mauro E, Caserta M (2006) The ISWI and CHD1 chromatin remodelling activities influence ADH2 expression and chromatin organization. *Mol Microbiol* 59:1531–1541.
30. Neef DW, Kladde MP (2003) Polyphosphate loss promotes SNF/SWI- and Gcn5-dependent mitotic induction of PHO5. *Mol Cell Biol* 23:3788–3797.
31. Vary JC, Jr, et al. (2003) Yeast Isw1p forms two separable complexes in vivo. *Mol Cell Biol* 23:80–91.
32. Sayre MH, Tschochner H, Kornberg RD (1992) Reconstitution of transcription with five purified initiation factors and RNA polymerase II from *Saccharomyces cerevisiae*. *J Biol Chem* 267:23376–23382.
33. Simic R, et al. (2003) Chromatin remodeling protein Chd1 interacts with transcription elongation factors and localizes to transcribed genes. *EMBO J* 22:1846–1856.
34. Sims RJ, 3rd, et al. (2007) Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing. *Mol Cell* 28:665–676.
35. Stokes DG, Tartof KD, Perry RP (1996) CHD1 is concentrated in interbands and puffed regions of *Drosophila* polytene chromosomes. *Proc Natl Acad Sci USA* 93:7137–7142.
36. Gaspar-Maia A, et al. (2009) Chd1 regulates open chromatin and pluripotency of embryonic stem cells. *Nature* 460:863–868.
37. Stockdale C, Flaus A, Ferreira H, Owen-Hughes T (2006) Analysis of nucleosome repositioning by yeast ISWI and Chd1 chromatin remodeling complexes. *J Biol Chem* 281:16279–16288.
38. Walfridsson J, Khosroutina O, Matikainen P, Gustafsson CM, Ekwall K (2007) A genome-wide role for CHD remodelling factors and Nap1 in nucleosome disassembly. *EMBO J* 26:2868–2879.
39. Sengstag C, Hinnen A (1987) The sequence of the *Saccharomyces cerevisiae* gene PHO2 codes for a regulatory protein with unusual amino acid composition. *Nucleic Acids Res* 15:233–246.
40. Gietz RD, Woods RA (2002) Transformation of yeast by lithium acetate/single-stranded carrier DNA/polyethylene glycol method. *Methods Enzymol* 350:87–96.
41. Knop M, et al. (1999) Epitope tagging of yeast genes using a PCR-based strategy: More tags and improved practical routines. *Yeast* 15:963–972.
42. Janke C, et al. (2004) A versatile toolbox for PCR-based tagging of yeast genes: New fluorescent proteins, more markers and promoter substitution cassettes. *Yeast* 21:947–962.
43. Woontner M, Jaehning JA (1990) Accurate initiation by RNA polymerase II in a whole cell extract from *Saccharomyces cerevisiae*. *J Biol Chem* 265:8979–8982.
44. Puig O, et al. (2001) The tandem affinity purification (TAP) method: A general procedure of protein complex purification. *Methods* 24:218–229.
45. Kornberg RD, LaPointe JW, Lorch Y (1989) Preparation of nucleosomes and chromatin. *Methods Enzymol* 170:3–14.
46. Flanagan PM, et al. (1990) Resolution of factors required for the initiation of transcription by yeast RNA polymerase II. *J Biol Chem* 265:11105–11107.
47. Sambrook J, Fritsch EF, Maniatis T (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Plainview, NY), 2nd Ed., pp 6-33–6-59.
48. Lenburg ME, O'Shea EK (1996) Signaling phosphate starvation. *Trends Biochem Sci* 21:383–387.