of INO1. We observed a reduction to 41% of INO1 mRNA in the ipkΔ mutant defective for IP₃ production, which is rescued by introduction of wild-type IPK1 (Fig. 3B) (19). Moreover, SNF2 and IPK2 are synthetically lethal, and INO80 and IPK1 display synthetic phenotypes (Fig. S3) (19).

Proper expression of INO1 likely involves the integration of contributions from INO80, SNF2, and ISW2, which act as positive or negative regulators of transcription (14, 28). Given that INO80 and SNF2 regulate INO1 positively (13, 15, 16) and that ISW2 regulates INO1 negatively (14), cellular levels of IP₃, IP₄, and IP₅ could modulate the balance between synergistic and antagonistic chromatin-remodeling activities (Fig. S4). The observed stimulation of SWF/SNF-induced nucleosome mobilization by IP₅ and IP₆ is consistent with findings of O’Shea and colleagues, who showed that transcription and chromatin remodeling of PHO5 in vivo, mediated by SNF2 and INO80, is dependent on production of IP₅ or IP₆ (29).

The mechanism(s) by which inositol polyphosphates modulate the activities of ATP-dependent chromatin-remodeling complexes are unknown. Recombinant NURF and ISWI protein can bind to IP₃ (30), which suggests that inositol polyphosphates might alter their activities by effects on protein conformation (31). IP₃ or IP₄ might affect the interaction between SWI/SNF and chromatin, as has been seen for PIP₂ (32). Knowledge of physiological conditions affecting intracellular levels of soluble inositol polyphosphates, as well as corresponding studies of chromatin remodeling and gene expression, will be essential to define the signaling pathway to chromatin.

**References and Notes**

19. Materials and methods are available as supporting material on Science Online.
23. X. Shen et al., unpublished observations.
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33. We thank E. O’Shea for communicating results before publication; T. Tsukiyama, Y. Zhang, and R. J. Fack for reagents; S. Wente, M. Lichten, C. Klee, G. Storz, and members of our laboratory for helpful discussions. This work was supported by the Intramural Research Program of the National Cancer Institute and a fellowship from the American Cancer Society to S.S.

**Supporting Online Material**

www.sciencemag.org/cgi/content/full/1078068/DC1
Materials and Methods. Figs. S1 to S4
Table S1

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**Regulation of Chromatin Remodeling by Inositol Polyphosphates**

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Chromatin remodeling is required for efficient transcription of eukaryotic genes. In a genetic selection for budding yeast mutants that were defective in induction of the phosphate-responsive PHO5 gene, we identified mutations in ARG82/IPK2, which encodes a nuclear inositol polyphosphate kinase. In arg82 mutant strains, remodeling of PHO5 promoter chromatin is impaired, and the adenosine triphosphate-dependent chromatin-remodeling complexes SWI/SNF and INO80 are not efficiently recruited to phosphate-responsive promoters. These results suggest a role for the small molecule inositol polyphosphate in the regulation of chromatin remodeling and transcription.

DNA in the eukaryotic nucleus is packaged into chromatin, which forms a repressive structure that tends to limit the access of DNA-binding proteins to DNA. Cellular activities have been identified that function to counteract chromatin-mediated repression through acetylation, methylation, or phosphorylation of histones (1). Additionally, complexes such as SWI/SNF alter the association of histones with DNA by using the energy from adenosine triphosphate (ATP) hydrolysis (2). Though many chromatin-modifying activities have been characterized mechanistically, little is known about their regulation.

The budding yeast PHO5 promoter and gene compose a useful system to investigate the relationship between chromatin structure and gene expression. Transcription of PHO5 is regulated in response to phosphate availability by the transcription factors Pho4 and Pho2 (3). When yeast cells are grown in a phosphate-rich medium, Pho4 is phosphorylated by the cyclin-CDK (cyclin-dependent kinase) complex Pho80–Pho85 (4) and inactivated (5). In addition, four positioned nucleosomes reside over the PHO5 promoter, and PHO5 transcription is repressed (6). Upon phosphate starvation, Pho4 is dephosphorylated and active (5), the positioned nucleosomes are no longer detectable (6), and PHO5 is induced. Remodeling of PHO5 chromatin structure requires Pho4 and Pho2 (7) and is facilitated by the histone acetyltransferase Gcn5, which acetylates histones in the promoter region (8, 9).

To identify additional factors important for remodeling chromatin at the PHO5 promoter, we designed a genetic selection to identify mutants defective in PHO5 transcription [Supporting Online Material (SOM) Text]. This selection identified mutations in PSE1, which encodes the import receptor for Pho4 (10), and a mutation in ARG82/IPK2 (denoted arg82-153) (SOM Text). Under inducing conditions, PHO5 transcription and chromatin remodeling are reduced in the arg82-153 mutant (fig. S1).

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Arg82 functions in at least two different cellular processes: (i) It regulates transcription of arginine-responsive genes (11), and (ii) with Plc1 and Ipk1, Arg82 functions in a pathway leading to the production of soluble inositol polyphosphates in the nucleus (12, 13) (Fig. 1A). Arg82 inositol trisphosphate (IP3) kinase activity and the production of inositol hexakisphosphate (IP6) are required for efficient export of mRNA from the nucleus (14, 15). The relation between the role of Arg82 in transcriptional control and its IP3 kinase activity is unclear, because Arg82 kinase activity is dispensable for transcriptional regulation of some arginine-responsive genes (16).

To determine whether PHO5 induction requires Arg82 kinase activity, we examined PHO5 transcription and chromatin remodeling in a strain expressing the point mutation Asp131 → Ala131 in Arg82 (Arg82D131A), an inositol polyphosphate kinase-deficient mutant (12). The amount of PHO5 mRNA in the arg82D131A strain was reduced under inducing conditions (Fig. 1B) as compared to that in the wild type. We assayed chromatin remodeling in the arg82D131A strain by monitoring a Cla I restriction site in the PHO5 promoter that undergoes an increase in accessibility when wild-type cells are shifted to inducing conditions (Fig. 1C) (6). Little change in Cla I accessibility was observed in the arg82D131A strain (Fig. 1C) as compared with that of the wild-type strain, suggesting that the IP3 kinase activity of Arg82 is important for PHO5 transcription and chromatin remodeling (see also fig. S2).

To better define the role of nuclear inositol polyphosphates in PHO5 transcription, we examined PHO5 induction in plc1, ipk1, and kcs1 mutants. Under inducing conditions, amounts of PHO5 mRNA decreased dramatically in the plc1Δ and arg82Δ strains but not in the ipkΔ and kcs1Δ strains (Fig. 1D) (SOM Text). Therefore, the defect in PHO5 transcription in the arg82Δ strain is not likely to result from a lack of IP6 production or a defect in mRNA export, because all these strains are impaired for the production of IP6 and mRNA export (14), whereas only the plc1Δ and arg82Δ strains are severely defective for PHO5 induction. Also, PP-IP4 is unlikely to play an important role in PHO5 transcription, because kcs1Δ strains have only a modest defect in PHO5 activation. Because plc1Δ cells do not produce IP3, it is likely the lack of IP4 and/or IP5 production rather than the increased production of IP7 in arg82Δ cells that affects PHO5 transcription.

To better understand the requirements for PHO5 promoter chromatin remodeling and transcription, we assayed PHO5 induction in strains with mutations in the following chromatin-remodeling complex components: SNF6, encoding a component of the SWI/SNF complex (SOM Text) (17); ARP8, encoding a component of the INO80 complex (SOM Text) (18); ISW1 and ISW2 (19); and CHD1 (20). For these and subsequent experiments, we induced PHO5 transcription with the use of a strain in which the wild-type CDK Pho85 is replaced with a mutant [Pho85 with the mutation Phe82 → Gly82 (Pho85F82G)] that can be selectively inhibited by addition of a cell-permeable kinase inhibitor, 1-NaPP1 (SOM Text). In activating conditions, PHO5 mRNA (and, to a lesser extent, PHO84) was reduced in the snfΔ and arg82 Δ strains but not in the iswΔ, isw2Δ, or chd1Δ strains (Fig. 2A) as compared to the wild-type strain. PHO5 chromatin remodeling was also defective in the snfΔ and arg82Δ strains (Fig. 2B), suggesting that SWI/SNF and INO80 are required for efficient remodeling of PHO5 promoter chromatin structure.

To determine whether SWI/SNF and INO80 act directly on the PHO5 and PHO84 promoters, we performed chromatin immunoprecipitation (ChIP) experiments. Upon a shift to activating conditions, we observed increased association of Ino80 with PHO5 (Fig. 2C) and PHO84 (fig. S4A) promoters. Recruitment of Ino80 to both promoters was reduced in a pho4Δ strain. For Snf2, we observed Pho4-dependent recruitment to the PHO84 promoter under inducing conditions but were unable to detect substantial (> twofold) recruitment to the PHO5 promoter (Fig. 2D). The requirement of Pho4 to efficiently recruit INO80 and SWI/SNF is in agreement with earlier studies demonstrating activator-dependent recruitment of SWI/SNF to promoters on which it acts (21, 22).

To investigate the connection between inositol polyphosphate metabolism and PHO5 promoter chromatin remodeling, we asked whether the functions of Snf2- or Ino80-containing complexes were influenced by mutations in ARG82. Deletion of ARG82 decreased recruitment of Ino80 to PHO5 (Fig. 2C) and PHO84 (fig. S4A) promoters and Snf2 to the PHO84 promoter (Fig. 2D), suggesting that the function of INO80 and SWI/SNF may be regulated by the production of inositol polyphosphates IP6 and/or IP7.

To determine if Pho4 is still able to bind to phospho-responsive promoters in arg82Δ cells, we characterized Pho4 binding with the use of ChIP. In activating conditions, Pho4 is bound to PHO5 and PHO84 promoters in wild-type and arg82Δ strains, though less Pho4 was bound in the arg82Δ strain (Fig. 3A and fig. S4B). This result is consistent with the observation that when PHO5 pro-
**References and Notes**

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SOM Text

Figs. 51 to 56

References and Notes

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