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Regulation of Chromatin Remodeling by Inositol Polyphosphates

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Abstract

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 relation 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 unphosphorylated 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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Supporting Online Material www.sciencemag.org/cgi/content/full/1078062/DC1 SOM Text Figs. S1 to S6 References and Notes

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 (IP_3) kinase activity and the production of inositol hexakisphosphate (IP_6) are required for efficient export of mRNA from the nucleus (14,15). The relation between the role of Arg82 in transcriptional control and its IP_3 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 $Asp^{131} \rightarrow Ala^{131}$ in Arg82 ($Arg82^{D131A}$), an inositol polyphosphate kinase-deficient mutant (12). The amount of *PHO5* mRNA in the $arg82^{D131A}$ strain was reduced under inducing conditions (Fig. 1B) as compared to that in the wild type. We assayed chromatin remodeling in the $arg82^{D131A}$ 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 $arg82^{D131A}$ strain (Fig. 1C) as compared with that of the wild-type strain, suggesting that the IP_3 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 *plc1*△ and *arg82*△ strains but not in the *ipk1*△ and *kcs1*△ strains (Fig. 1D) (SOM Text). Therefore, the defect in *PHO5* transcription in the *arg82*△ strain is not likely to result from a lack of IP_6 production or a defect in mRNA export, because all these strains are impaired for the production of IP_6 and mRNA export (14), whereas only the *plc1*△ and *arg82*△ strains are severely defective for *PHO5* induction. Also, PP-IP_4 is unlikely to play an important role in *PHO5* transcription, because *kcs1*△ strains have only a modest defect in *PHO5* activation. Because *plc1*△ cells do not produce IP_3, it is likely the lack of IP_4 and/or IP_5 production rather than the increased production of IP_3 in *arg82*△ 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 $Phe^{82} \rightarrow Gly^{82}$($Pho85^{F82G}$)] 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 *snf6*△ and *arp8*△ strains but not in the *isw1*△, *isw2*△, or *chd1*△ strains (Fig. 2A) as compared to the wild-type strain. *PHO5* chromatin remodeling was also defective in the *snf6*△ and *arp8*△ 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 *pho4*△ 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 IP_4 and/or IP_5.

To determine if Pho4 is still able to bind to phosphate-responsive promoters in *arg82*△ 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 *arg82*△ strains, though less Pho4 was bound in the *arg82*△ strain (Fig. 3A and fig. S4B). This result is consistent with the observation that when *PHO5* promoter chromatin cannot be remodeled, Pho4 can bind the accessible site in the upstream activation sequence 1 in the *PHO5* promoter (UASp1) but not UASp2, which is incorporated into a nucleosome (23). Indeed, in *arp8*△ and *snf6*△ strains where chromatin remodeling is defective, the amount of Pho4 bound at the *PHO5* promoter was less than that in the wild-type strain and similar to that in the *arg82*△ strain (Fig. 3B).

To test the hypothesis that Pho4 binds primarily to UASp1 in strains defective for chromatin remodeling, we performed ChIP in strains carrying substitutions in the Pho4-binding sites in UASp1 and UASp2 (fig. S5). The mutation of UASp1 severely impaired binding of Pho4 to the *PHO5* promoter in all strain backgrounds (Fig. 3B). In contrast, mutation of UASp2 resulted in ∼50% reduction of Pho4 binding in the wild-type strain but had no significant effect on binding of Pho4 in *arg82*δ, *snf6*δ, and *arp8*δ strains (Fig. 3B). Thus, when Pho4 is nuclear but *PHO5* promoter chromatin is not efficiently remodeled, most if not all of the Pho4 binding to the *PHO5* promoter occurs at UASp1. We conclude that the defect in recruitment of SWI/SNF and INO80 in the *arg82*δ strain occurs at a step after Pho4 binding (fig. S6).

We have identified a role for inositol polyphosphates in the regulation of chromatin remodeling and transcription. Our findings suggest that the production of IP_4 and/or IP_5 modulates the ability of the SWI/SNF and INO80 chromatin remodeling complexes to induce transcription of some phosphate-responsive genes, perhaps by affecting the ability of these complexes to interact with Pho4, Pho2, and/or chromatin. These observations are consistent with results of Wu and colleagues, who demonstrated that the nucleosome-sliding activity of SWI/SNF is stimulated by IP_4 and IP_5 in vitro and that *INO1* transcription is influenced by inositol polyphosphate amounts in vivo (24). It is possible that the amounts or ratios of inositol polyphosphates are altered under certain physiological conditions and that this change may be used by the cell as a signal for global regulation of mRNA export and transcription.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References and Notes

1. Jenuwein T, Allis CD. Science 2001;293:1074. [PubMed: 11498575]
2. Narlikar GJ, Fan HY, Kingston RE. Cell 2002;108:475. [PubMed: 11909519]
3. Oshima Y. Genes. Genet. Syst 1997;72:323. [PubMed: 9544531]
4. Kaffman A, Herskowitz I, Tjian R, O'Shea EK. Science 1994;263:1153. [PubMed: 8108735]
5. Komeili A, O'Shea EK. Science 1999;284:977. [PubMed: 10320381]
6. Almer A, Rudolph H, Hinnen A, Hörz W. EMBO J 1986;5:2689. [PubMed: 3536481]

7. Fascher KD, Schmitz J, Hörz W. EMBO J 1990;9:2523. [PubMed: 2196175]
8. Vogelauer M, Wu J, Suka N, Grunstein M. Nature 2000;408:495. [PubMed: 11100734]
9. Barbaric S, Walker J, Schmid A, Svejstrup JQ, Hörz W. EMBO J 2001;20:4944. [PubMed: 11532958]
10. Kaffman A, Rank NM, O'Shea EK. Genes Dev 1998;12:2673. [PubMed: 9732266]
11. El Bakkoury M, Dubois E, Messenguy F. Mol. Microbiol 2000;35:15. [PubMed: 10632874]
12. Odom AR, Stahlberg A, Wente SR, York JD. Science 2000;287:2026. [PubMed: 10720331]
13. Saiardi A, Caffrey JJ, Snyder SH, Shears SB. J. Biol. Chem 2000;275:24686. [PubMed: 10827188]
14. York JD, Odom AR, Murphy R, Ives EB, Wente SR. Science 1999;285:96. [PubMed: 10390371]
15. Saiardi A, Caffrey JJ, Snyder SH, Shears SB. FEBS Lett 2000;468:28. [PubMed: 10683435]
16. Dubois E, Dewaste V, Erneux C, Messenguy F. FEBS Lett 2000;486:300. [PubMed: 11119723]
17. Peterson CL, Dingwall A, Scott MP. Proc. Natl. Acad. Sci. U.S.A 1994;91:2905. [PubMed: 8159677]
18. Shen X, Mizuguchi G, Hamiche A, Wu C. Nature 2000;406:541. [PubMed: 10952318]
19. Tsukiyama T, Palmer J, Landel CC, Shiloach J, Wu C. Genes Dev 1999;13:686. [PubMed: 10090725]
20. Tran HG, Steger DJ, Iyer VR, Johnson AD. EMBO J 2000;19:2323. [PubMed: 10811623]
21. Yudkovsky N, Logie C, Hahn S, Peterson CL. Genes Dev 1999;13:2369. [PubMed: 10500094]
22. Neely KE, et al. Mol. Cell 1999;4:649. [PubMed: 10549297]
23. Svaren J, Schmidtz J, Hörz W. EMBO J 1994;13:4856. [PubMed: 7957055]
24. Shen X, Xiao H, Ranallo R, Wu W-H, Wu C. Science 2003;299:112. [PubMed: 12434013]
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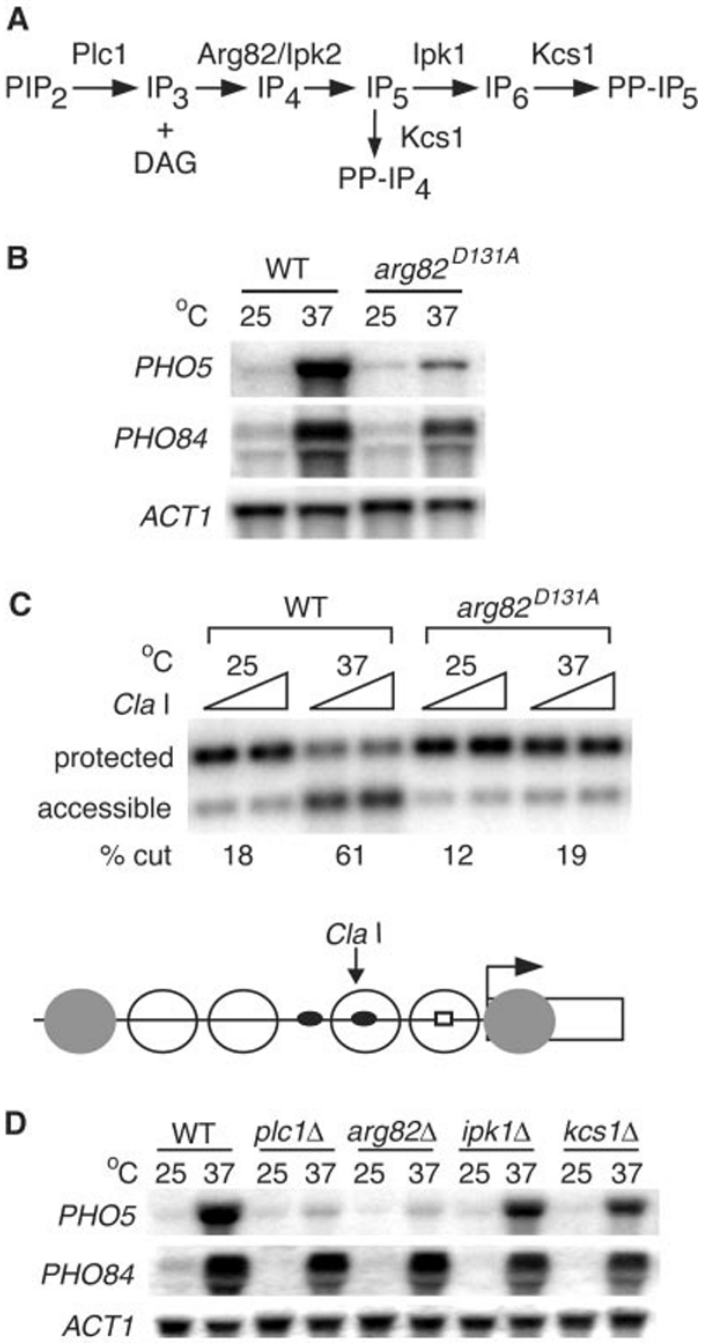

Fig 1.

Arg82 kinase activity and IP_4 and/or IP_5 are important for *PHO5* transcription and chromatin remodeling. (**A**) Pathway for the synthesis of soluble inositol polyphosphates (13,14). (**B**) Northern analysis or (**C**) Cla I restriction enzyme accessibility assay of *pho81*δ *pho80*^{ts}wild-type (WT) and *arg82*^{D131A} *pho81*δ pho80^{ts} strains grown under repressing (25°C) or shifted to inducing (37°C) conditions for 1 hour. *PHO5* and *PHO84* mRNA amounts in the *arg82*^{D131A} strain were reduced fivefold and 1.6-fold under inducing conditions. % cut indicates the amount of accessible fragment divided by the sum of accessible and protected fragments. A schematic model of the *PHO5* promoter region is shown (bottom), indicating the nucleosomes that are perturbed during induction as open circles, the Pho4 and Pho2 binding

sites as dark ovals, the TATA box as an open square, and the Cla I site. (**D**) Northern analysis of *pho81*δ *pho80*^{ts} strains with the relevant genotypes indicated. *PHO5* and *PHO84* mRNA amounts in the *plc1*, *arg82*, *ipk1*, and *kcs1* mutants were 6% and 77%, 4% and 76%, 61% and 64%, and 45% and 50%, respectively, of the wild-type amount under inducing conditions. See fig. S1 for methods.

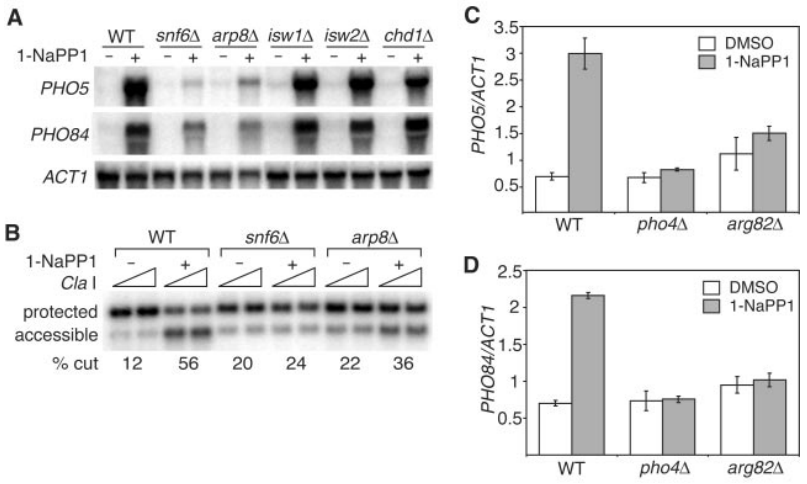

Fig 2.

The functions of INO80 and SWI/SNF at the *PHO5* and *PHO84* promoters are regulated by Arg82. (**A**) Northern analysis of $Pho85^{F82G}$-expressing strains with the relevant genotypes indicated. Strains were grown under repressing conditions (-) or shifted to inducing conditions (+) by adding 10 μM 1-NaPP1 for one hour. (**B**) Cla I restriction enzyme accessibility assay for the $PHO85^{F82G}$, *arp8*δ $PHO85^{F82G}$, and *snf6*δ $PHO85^{F82G}$ strains, grown under repressing conditions (-) or shifted to inducing conditions (+) by adding 10 m μM 1-NaPP1 for 30 min. % cut, as in Fig. 1C. ChIP using a monoclonal antibody to (**C**) Ino80-HA or (**D**) Snf2-Myc in $Pho85^{F82G}$-expressing strains with the relevant genotypes indicated. Immunoprecipitate efficiency for *PHO5* or *PHO84* DNA relative to *ACT1* DNA is represented by (immunoprecipitated DNA/input DNA)/(*ACT1* immunoprecipitated DNA/*ACT1* input DNA). Values are the averages of three independent experiments; error bars represent standard error of the mean. DMSO, dimethyl sulfoxide. See fig. S3 for ChIP method.

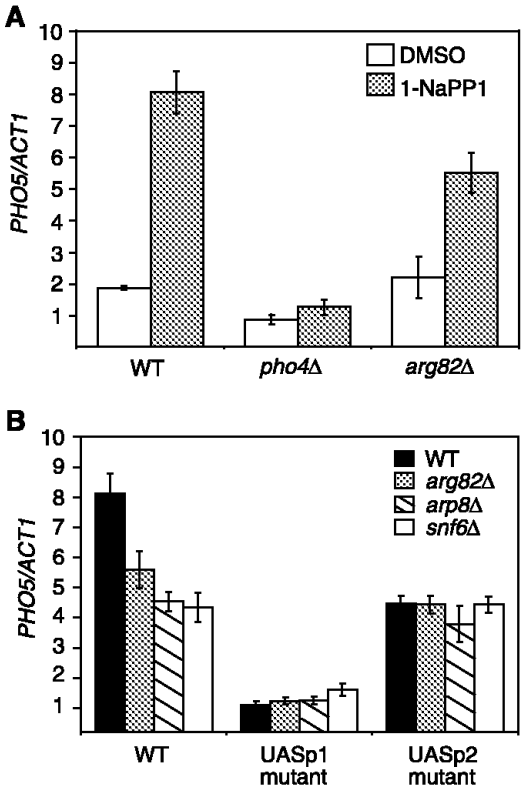

Fig 3.

Pho4 binds to UASp1 in mutants impaired for *PHO5* chromatin remodeling. *PHO5/ACT1*, as in Fig. 2C. (**A**) ChIP using affinity-purified polyclonal antibodies to Pho4 in Pho85^{F82G}-expressing strains with the relevant genotypes indicated. DMSO, as in Fig. 2. (**B**) ChIP of Pho4 in *PHO85*^{F82G}, *arg82*δ *PHO85*^{F82G}, *arp8*δ *PHO85*^{F82G}, and *snf6*δ *PHO85*^{F82G} strains carrying substitutions in the Pho4-binding sites of UASp1 and UASp2 in the *PHO5* promoter grown under inducing conditions.