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similar to *Arabidopsis* CLV2 and an extracellular peptide similar to *Arabidopsis* CLV3 (1, 5). Other proteins involved in signal transduction have also been shown to interact with CLV1 in *Arabidopsis* (5).

Intriguingly, *GmNARK* is most similar to CLV1, whereas two receptor-like kinase genes in the regions on chromosomes 2 and 4 of *Arabidopsis* syntenic with the soybean *NTS-1* region are much more distantly related (Fig. 1D). There is no synteny between the *NTS-1* region of soybean and the vicinity of CLV1 (29). One possible explanation for this finding is that a localized gene recombination or conversion-like event may have occurred in evolution involving the CLV1 ortholog and another receptor-like kinase gene, such as to change the chromosomal location of CLV1 in either *Arabidopsis* or soybean.

Other receptor-like kinases have been shown to participate in environmental sensing—for example, in the perception of hormones, pathogens, symbionts, or cellular interactions (1–3, 7–8). The discovery of a divergent *Arabidopsis* CLV1 ortholog in soybean effecting long-distance nodulation control extends this spectrum of activities to cell division events in a distal organ that are first sensed, then homeostatically controlled.

Our findings suggest evolutionary mechanisms for the development of the root nodule symbiosis. Duplication of genes followed by divergence in function is a common theme in evolution (30). Ancestral duplication of a gene controlling stem cell proliferation in the SAM may have led to a variant mechanism in which shoot control of cell proliferation is extended to root tissue. Research in legumes into CLAVATA-related signaling will undoubtedly facilitate the understanding of key developmental processes such as nodulation that are absent in the model plant *Arabidopsis*.

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- Abbreviations for amino acid residues: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Modulation of ATP-Dependent Chromatin-Remodeling Complexes by Inositol Polyphosphates

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Eukaryotes use adenosine triphosphate (ATP)-dependent chromatin-remodeling complexes to regulate gene expression. Here, we show that inositol polyphosphates can modulate the activities of several chromatin-remodeling complexes in vitro. Inositol hexakisphosphate (IP₆) inhibits nucleosome mobilization by NURF, ISW2, and INO80 complexes. In contrast, nucleosome mobilization by the yeast SWI/SNF complex is stimulated by inositol tetrakisphosphate (IP₄) and inositol pentakisphosphate (IP₅). We demonstrate that mutations in genes encoding inositol polyphosphate kinases that produce IP₄, IP₅, and IP₆ impair transcription in vivo. These results provide a link between inositol polyphosphates, chromatin remodeling, and gene expression.

In eukaryotes, the SWI2/SNF2 family of ATP-dependent chromatin-remodeling complexes is widely used to regulate DNA accessibility for transcription. Four related classes of protein complexes (SWI2/SNF2, ISWI, Mi2, and INO80) use the energy of ATP hydrolysis to alter nucleosome architecture (1–3). Although there have been significant advances in understanding the mechanism and function of chromatin-remodeling complexes, the interaction of these complexes with cell signaling pathways has not been widely explored. One major mechanism for communicating environmental signals is the inositol signaling pathway. Activation of phosphatidylinositol-specific phospholipase C at the cell membrane leads to cleavage

of phosphatidylinositol 4,5-bisphosphate (PIP₂), generating secondary messengers inositol 1,4,5-trisphosphate (IP₃), a regulator of calcium release and diacylglycerol (DAG), an activator of protein kinase C (4, 5). IP₃ can undergo additional phosphorylation to IP₄, IP₅, or IP₆, and di-phosphorylated derivatives (6). Recent advances have revealed multiple and varied functions for IP₄, IP₅, and IP₆ in nucleic acid and viral metabolism (7–11).

The regulation of *INO1*, encoding inositol-1-phosphate synthase (12), by *SNF2*, *ISW2*, and *INO80* (13–16), encoding the core ATPases of three chromatin-remodeling complexes, prompted us to consider whether soluble inositol metabolites could influence ATP-dependent chromatin remodeling. We investigated this question by an in vitro nucleosome mobilization assay, which uses native gel electrophoresis to distinguish between nucleosomes at different locations on a DNA fragment. The *Drosophila* ISWI-containing complex NURF mobilizes reconstituted nucleosomes to favor one dominant position (N3) on *hsp70* promoter DNA (17–19). We found that IP₆ inhibits nucleosome mobili-

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zation by the NURF complex (Fig. 1A, see 40 μ M and 100 μ M), in the range of cellular IP₆ levels (20–22). Control compounds (6) inositol hexasulfate (IS₆), EDTA, EGTA, and IP₃ showed little effect (Fig. 1A). We also tested IP₄ and IP₅ (500 μ M) and found no effects for IP₄ and some inhibition for IP₅ (23).

The nucleosome-stimulated ATPase activity of NURF was correspondingly inhibited by IP₆ (19) (Fig. 1B), which suggests that inhibition by IP₆ occurs, at least in part, through modulation of the ISWI ATPase. IP₆ did not block the unrelated myosin ATPase or the ATPase activity of Fun30 (23), another SWI2/SNF2 family member (24). We tested the effect of IP₆ on the yeast ISWI-containing chromatin-remodeling complex ISW2 (25). Yeast ISW2 mobilizes *hsp70* nucleosomes from N4 to N3 positions. IP₆ inhibited nucleosome mobilization (Fig. 1C) and nucleosome-stimulated ATPase activities of ISW2 (fig. S2A).

Yeast SWI/SNF mobilizes N1, N2, and N3 *hsp70* nucleosomes to a novel position (N*) slightly above N2 (Fig. 2A, right). We found little effect of IP₆ on yeast SWI/SNF (Fig. 2A, left). However, when SWI/SNF levels were reduced, we found that IP₄ (1,4,5,6), the major IP₄ isomer in wild-type yeast (26), and IP₅ (500 μ M) consistently stimulated nucleosome mobilization, whereas another isomer, IP₄ (1,3,4,5) did not stimulate but was inhibitory (Fig. 2A, right). We also detected stimulation of SWI/SNF remodeling activity by IP₄ (1,4,5,6) and IP₅ (at 100 μ M), whereas IP₆ showed no stimulatory effects (23). The stimulatory concentrations are higher than steady-state estimates of IP₄ and IP₅ in yeast (26); this could be a limitation of the remodeling assay, or concentrations could vary locally or transiently in vivo. The ATPase activity of SWI/SNF was unaffected by IP₄ or IP₅ (Fig. 2B), which suggests that stimulation occurs through a different mechanism.

We next analyzed the INO80 chromatin-remodeling complex, using mononucleosomes reconstituted on 359-base pair (bp) *INO1* promoter DNA (27). The INO80 complex mobilizes nucleosomes mainly from N3 to N1 and N2 positions in an ATP-dependent manner (Fig. 3A). We observed neither inhibition (Fig. 3A) nor stimulation (23) of nucleosome mobilizing activity by IP₄ and IP₅. However, IP₆ inhibited INO80-induced nucleosome mobilization (Fig. 3A). The ATPase activity of INO80 was correspondingly inhibited by IP₆ but not by other inositol polyphosphates (fig. S2B).

The integrity of the inositol signaling pathway is required for the expression of *INO1* in vivo. *INO1* mRNA is reduced to 18% in the *ipk2* Δ mutant and is rescued by introduction of wild-type *IPK2*, but only partially by the mutant with an Asp¹³¹Ala substitution (*D131A*), which impairs kinase activity (Fig. 3B). Hence, production of IP₄ and IP₅ is required for expression

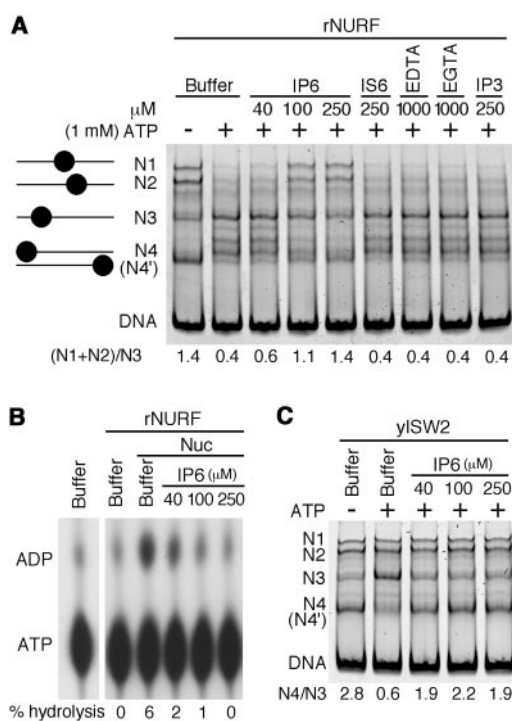


Fig. 1. IP₆ inhibits chromatin remodeling by NURF and ISW2. (A) Native polyacrylamide gel electrophoresis (PAGE) showing the effect of inositol polyphosphates on nucleosome mobilization. Positions of mononucleosomes (filled circles) are shown on the left. (N1+N2)/N3 ratios are given at the bottom; SD < 0.1. (B) Thin-layer chromatography analysis showing nucleosome (Nuc)-stimulated ATPase activity of rNURF. Percent ATP hydrolysis is shown at the bottom for all ATPase assays hereafter with standard deviation < 1%. (C) Nucleosome mobilization by ISW2 complex. N4/N3 ratios are given at the bottom; SD < 0.1.

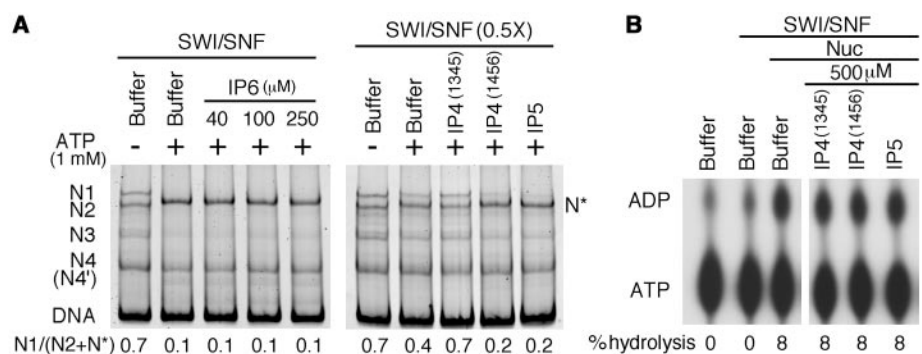


Fig. 2. IP₄ and IP₅ stimulate chromatin remodeling by SWI/SNF. (A) Native PAGE showing nucleosome mobilization. N1/(N2+N*) ratios are given at the bottom; SD < 0.05. (B) ATPase activities of SWI/SNF.

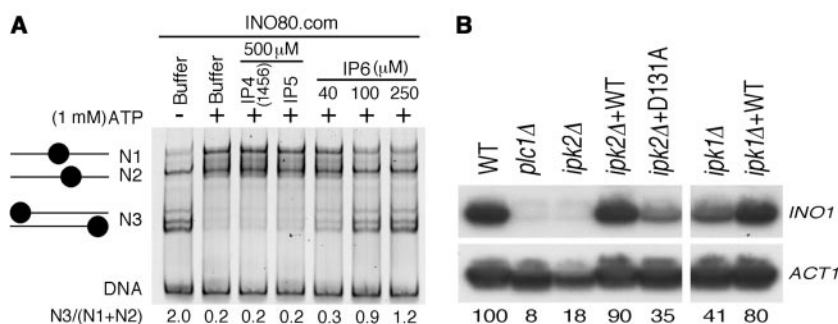


Fig. 3. IP₆ inhibits chromatin remodeling by INO80 complex. (A) Native PAGE showing nucleosome mobilization. Positions of mononucleosomes (filled circles) reconstituted on *INO1* promoter DNA are shown on the left. N3/(N1+N2) ratios are given at the bottom; SD < 0.1. (B) Northern analyses of *INO1* expression. Percent wild-type expression normalized using *ACT1* is given at the bottom; SD < 10%.

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of *INO1*. We observed a reduction to 41% of *INO1* mRNA in the *ipk1Δ* mutant defective for IP₆ 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 IP₄, IP₅, and IP₆ could modulate the balance between synergistic and antagonistic chromatin-remodeling activities (fig. S4). The observed stimulation of SWF/SNF-induced nucleosome mobilization by IP₄ and IP₅ 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 IP₄ or IP₅ (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 IP₆ (30), which suggests that inositol polyphosphates might alter their activities by effects on protein conformation (31). IP₄ or IP₅ 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.

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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 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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