Molecular Cell
Article

Pin1At Encoding a Peptidyl-Prolyl *cis/trans* Isomerase Regulates Flowering Time in *Arabidopsis*

Yu Wang,[1,2] Chang Liu,[1,2] Daiwen Yang,[1] Hao Yu,[1,2,*] and Yih-Cherng Liou[1,*]
[1]Department of Biological Sciences, Faculty of Science
[2]Temasek Life Sciences Laboratory, 1 Research Link
National University of Singapore, Singapore
*Correspondence: dbsyuhao@nus.edu.sg (H.Y.), dbslyc@nus.edu.sg (Y.-C.L.)
DOI 10.1016/j.molcel.2009.12.020

SUMMARY

Floral transition in plants is regulated by an integrated network of flowering genetic pathways. We show that an *Arabidopsis* PIN1-type parvulin 1, Pin1At, controls floral transition by accelerating *cis/trans* isomerization of the phosphorylated Ser/Thr-Pro motifs in two MADS-domain transcription factors, SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1) and AGAMOUS-LIKE 24 (AGL24). Pin1At regulates flowering, which is genetically mediated by *AGL24* and *SOC1*. Pin1At interacts with the phosphorylated AGL24 and SOC1 in vitro and with AGL24 and SOC1 in vivo and accelerates the *cis/trans* conformational change of phosphorylated Ser/Thr-Pro motifs of AGL24 and SOC1. We further demonstrate that these Ser/Thr-Pro motifs are important for Pin1At function in promoting flowering through AGL24 and SOC1 and that the interaction between Pin1At and AGL24 mediates the AGL24 stability in the nucleus. Taken together, we propose that phosphorylation-dependent prolyl *cis/trans* isomerization of key transcription factors is an important flowering regulatory mechanism.

INTRODUCTION

Floral transition in *Arabidopsis* is coordinately controlled by multiple genetic pathways in response to various developmental and environmental cues (Koornneef et al., 1998; Levy and Dean, 1998; Mouradov et al., 2002). The autonomous and gibberellic acid (GA) pathways are responsive to endogenous developmental and physiological state, while the photoperiod and vernalization pathways monitor the alteration of environmental signals such as day length and temperature. These genetic pathways ultimately converge at the floral pathway integrators, which in turn regulate the activity of flower meristem identity genes to initiate the transition from vegetative to reproductive growth. Regulatory genes acting at the convergence point of the multiple floral induction pathways include two MADS-box transcription factors, *SOC1* and *AGL24* (Lee et al., 2000; Liu et al., 2008; Michaels et al., 2003; Samach et al., 2000; Yu et al., 2002).

Peptidyl-prolyl *cis/trans* isomerases (PPIases) are enzymes that accelerate energetically unfavorable *cis/trans* isomerization of the peptide bond preceding a proline (Hunter, 1998; Kiefhaber et al., 1990). PPIases include four structurally distinct subfamilies: cyclophilins, FK506-binding proteins, parvulins, and PP2A phosphatase activator (Lu et al., 2007). Members from the parvulin subfamily, such as PIN1 from human and ESS/PTF1 from *Saccharomyces cerevisiae*, have been shown to be essential for cell cycle and growth (Hanes et al., 1989; Hani et al., 1995; Lu et al., 1996). In the parvulin subfamily, PIN1-like PPIases are the only type of PPIases that specifically recognize phosphorylated Ser/Thr residues preceding proline (pSer/Thr-Pro) and catalyze the conformational change of the phosphorylated substrates, thereby controlling their function (Hsu et al., 2001; Huang et al., 2001; Liou et al., 2002, 2003; Lu et al., 1996, 1999; Pastorino et al., 2006; Ranganathan et al., 1997; Stukenberg and Kirschner, 2001; Yaffe et al., 1997).

Pin1At was early-identified as the only PIN1-type PPIase from *Arabidopsis* (He et al., 2004; Landrieu et al., 2000). Sequence comparison showed that PIN1 homologs from human, yeast, and *Drosophila* consist of two domains, an N-terminal WW regulatory domain and a C-terminal PPIase catalytic domain, whereas plant PIN1s, including Pin1At, contain only a PPIase domain with four additional amino acids (Figure S1) (Yao et al., 2001). Although several Pin1 homologs have been identified in plants (Landrieu et al., 2000; Metzner et al., 2001; Yao et al., 2001), their substrates and biological function in plants are so far unknown. Here, we show that Pin1At affects floral transition in *Arabidopsis* and that phosphorylation-dependent prolyl *cis/trans* isomerization of key transcription factors such as SOC1 and AGL24 by Pin1At emerges as an important flowering regulatory mechanism.

RESULTS

Pin1At Promotes Flowering

To investigate the biological function of *Pin1At*, we first examined the effect of modulating *Pin1At* expression in *Arabidopsis*. Since insertion mutants were not available in public resources, transgenic plants with antisense suppression of *Pin1At* were generated. Among 56 transgenic lines containing *Pin1At* N-terminal antisense fragment driven by a double cauliflower mosaic virus 35S promoter (*Pin1At-AS*), 30 plants showed late flowering under long days (LDs) (Figures 1A and 1B). Under

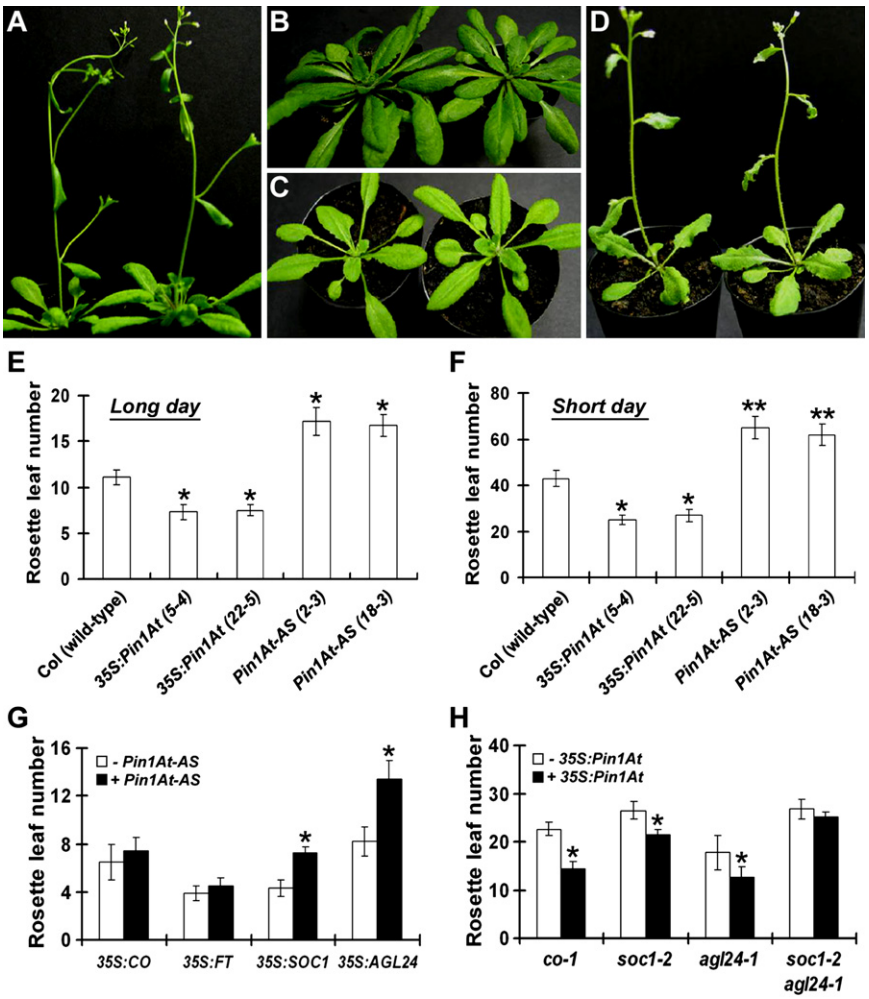

Figure 1. *Pin1At* Regulates Flowering Time in *Arabidopsis*

(A and B) Wild-type plants (A) show earlier flowering than representative *Pin1At* antisense plants (line 18-3) (B) at 35 days after germination under long days.

(C and D) Wild-type plants (C) show later flowering than representative *35S:Pin1At* plants (line 22-5) (D) at 28 days after germination under long days.

(E and F) Flowering time of *Pin1At* transgenic plants under long days (E) and short days (F). The number of rosette leaves on the main shoot represents flowering time. Values presenting the mean ± standard deviation were scored from at least 15 plants of each genotype. Significant differences in comparison with wild-type plants are indicated with asterisks: *$p < 0.05$; **$p < 0.01$, by Student's t test.

(G) Effect of antisense suppression of *Pin1At* (line 18-3) on overexpression of various flowering promoters under long days. Error bars indicate standard deviation. Significant differences between with and without antisense suppression of *Pin1At* are indicated with asterisks: *$p < 0.05$, by Student's t test.

(H) Effect of overexpression of *Pin1At* (line 22-5) on various flowering mutants under long days. Error bars indicate standard deviation. Significant differences between with and without overexpression of *Pin1At* are indicated with asterisks: *$p < 0.05$, by Student's t test.

both long and short days (SDs), representative *Pin1At-AS* plants with reduced expression of *Pin1At* flowered much later than wild-type plants (Figures 1E, 1F, and S2A). On the contrary, among 39 transgenic plants containing *Pin1At* cDNA under the control of 35S promoter (*35S:Pin1At*), 21 showed early flowering under LDs (Figures 1C and 1D). Representative *35S:Pin1At* lines with overexpression of *Pin1At* showed early flowering under both LDs and SDs (Figures 1E, 1F, and S2B). In addition, these plants also exhibited serrated rosette and cauline leaves (Figure 1D). These observations suggest that *Pin1At* at least affects flowering and leaf development.

The Photoperiod and Vernalization Pathways Affect *Pin1At* Expression

To examine the flowering pathways that regulate *Pin1At*, we examined its expression in various environmental conditions and flowering mutants. In wild-type Col plants under LDs, *Pin1At* expression gradually increased during floral transition occurring from 9 to 13 days after germination (Figure 2A) and decreased afterwards. On the contrary, its expression was not significantly changed under SDs within 21 days after germination. These results suggest that the photoperiod pathway affects *Pin1At* expression. Further examination of *Pin1At* expression revealed that its expression was affected by *CONSTANS* (*CO*), but not *FLOWERING LOCUS T* (*FT*) (Figure S3). *Pin1At* expression was also affected by vernalization (Figure 2B), but not by autonomous pathway mutants and GA treatment (Figures S4 and S5). Notably, dramatic downregulation of a flowering repressor, *FLOWERING LOCUS C* (*FLC*), in *FRI FLC*, wherein the dominant allele of *FRIGIDA* (*FRI*) causes high expression of *FLC* by vernalization, did not enhance the upregulation of *Pin1At* (Figure 2B), indicating that vernalization regulates *Pin1At* in an *FLC*-independent manner. Regulation of *Pin1At* by the vernalization pathway partly explains that manipulation of *Pin1At* expression affects flowering time in SDs, under which the LD photoperiod pathway is not activated.

Pin1At Regulates Flowering Time through *AGL24* and *SOC1*

Since the photoperiod and vernalization pathways converge on the regulation of *Pin1At* expression, we speculated that *Pin1At* function may be relevant to flowering regulators located downstream of several genetic pathways, such as *SOC1*, *FT*, and *AGL24* (Kardailsky et al., 1999; Kobayashi et al., 1999; Lee et al., 2000; Michaels et al., 2003; Samach et al., 2000; Yu et al., 2002). Thus, we performed genetic crossing to identify potential interacting regulators of *Pin1At*. While downregulation of *Pin1At* in *Pin1At-AS* caused late flowering, it did not