

Upstream Regions Required for Expression Control of the *Arabidopsis* floral homeotic gene *PISTILLATA*

Takashi Honma

PISTILLATA is a B-class floral organ identity gene required for the normal development of petals and stamens in *Arabidopsis thaliana*. Its expression is induced in the stage 3 flowers (early expression) and is maintained until anthesis (late expression). To explore in more detail the developmentally regulated gene expression of *PISTILLATA*, the author dissected its upstream DNA region followed by analyzing the promoter activity with transgenic plants carrying various mutations in meristem and organ identity genes. The results indicate that *LEAFY* and *UNUSUAL FLORAL ORGANS* induce *PISTILLATA* expression in a flower-independent manner through a distal promoter, and that *PISTILLATA* together with *APETALA3* maintain *PISTILLATA* expression in the later stages of flower development through a proximal promoter.

Keywords: *Arabidopsis thaliana*/ Flower development/ Homeotic gene/ Transcriptional control

The *Arabidopsis* flower consists of the four type organs: sepals, petals, stamens and carpels. These organs develop by proliferation of cells in meristem. The developmental fate of meristem is controlled by the floral homeotic genes. When the activity of a homeotic gene is lost, conversions of one organ type to other occur. Their functional difference allow us to classify these genes into (i) the meristem identity genes which establish the floral meristem and (ii) the organ identity genes which are required for appropriate organ development in their respective place in the flower.

The organ identity genes *PISTILLATA* (*PI*) and *APETALA3* (*AP3*) are necessary for petal and stamen de-

velopment. The loss of either gene function result in similar phenotypes, with petals being transformed into sepals and stamens into carpels [1]. Both genes encode the MADS-box transcription factors that are capable of forming a heterodimer and bind to DNA containing the consensus sequence CC(A/T)₆GG [2, 3]. Therefore, the PI/AP3 complex appears to have a role as the regulator essential for petal and stamen development.

PI transcripts become first detectable after the sepal primordia begin to form at stage 3 [2]. During the petal and stamen primordia emerge, *PI* transcripts continue to be detected in both petals and stamens throughout the flower development. *PI* expression is regulated in two

MOLECULAR BIOLOGY AND INFORMATION — Molecular Biology —

Scope of research

Attempts have been made to elucidate structure-function relationships of genetic materials and various gene products. The major subjects are mechanisms involved in signal transduction and regulation of gene expression responsive to environmental stimuli, differentiation and development of plant organs, and plant-microbe interaction. As of December 2000, study is being concentrated on the roles of homeodomain proteins and DDK response regulators of higher plants in developmental and signal transduction processes.



Prof
OKA, Atsuhiko
(D Sc)



Assoc Prof
AOYAMA, Takashi
(D Sc)



Assoc Instr
HONMA, Takashi

Students:

OHGISHI, Maki (DC)
SAKAI, Hiroe (DC)
LIANG, Yajie (DC)
OHASHI, Yohei (DC)
MURAMOTO, Takuya (RF, D Sc)
IWAKOSHI, Shintaro (RS)

steps; the establishment of initial expression and the maintenance of their expression by PI and AP3 proteins (autoregulation). Genetic analysis has proposed that the initial expression is induced by combinations of the meristem identity genes, *LEAFY* (*LFY*) and *UNUSUAL FLORAL ORGANS* (*UFO*), and after once established, *PI* expression in the petals and stamens is maintained by the activities of PI and AP3 proteins [2, 4, 5].

In order to define the regulatory elements in the *PI* promoter region, various deletions in the 1.5-kb region upstream of *PI* were generated and then connected to a reporter gene that had been made by an in-frame translational fusion of *PI* with the *uidA* gene coding for the β -glucuronidase (GUS). The author introduced these constructs (*PI::GUS* series) into the *Arabidopsis* genome, and flowers of the resulting transgenic plants were stained for GUS enzyme activity. *PI::GUS* transgenic lines carrying a promoter DNA region to nt -498 (or to more) from the *PI* translation initiation site showed petal- and stamen-specific GUS expression pattern, similar to the localization of *PI* transcript. An additional deletion to nt -399 led to alteration of GUS expression in the stage 3 flowers (early expression), suggesting that this -498 to -399 region contains a *cis* element(s) required for early expression occurring in response to induction signals (the distal region). In *PI::GUS* transgenic lines containing up to nt -266, the level of GUS expression in petals and stamens (late expression) was reduced. Further deletions up to nt -233 entirely abolished GUS expression. These results suggest that the deletions to nt -266 and to nt -233 remove *cis* elements essential for the late expression (the proximal region), partially and entirely, respectively.

In order to define *cis* elements responsive to the PI/AP3 complex in the *PI* promoter region, each of the above deletion derivatives of *PI::GUS* was introduced into the loss- and gain-of-function alleles of *PI* and *AP3*. In the loss-function alleles of *PI* or *AP3*, the early expression mediated by the distal region remained but the late expression mediated by the proximal region disappeared. These results indicate that the early *PI* expression is independent of *PI* and *AP3* and that the late expression requires the functional *PI* and *AP3* gene products. In the gain-function alleles of *PI* and *AP3* (35S-*PI* and 35S-*AP3*), strong GUS activity mediated by the proximal region was observed in any of the flower or-

gans. It was thus concluded that the proximal promoter contains the *cis* elements that respond to the autoregulatory signals of the PI/AP3 complex.

To test whether the early and late expression of *PI* are influenced by *LFY* and *UFO*, the deletion derivatives of *PI::GUS* were similarly introduced into the loss- and gain-of-function alleles of *LFY* and *UFO*. In plants deficient in *LFY*, GUS expression mediated by the distal region in the stage 3 flowers was completely eliminated. In contrast, defects in *UFO* did not influence GUS expression promoted by either of the distal and proximal regions. These results suggest that *LFY* affects the early but not the late expression of *PI*, and that *PI* expression is not highly influenced by the *UFO* mutations. In the gain-of-function allele of *LFY* (35S-*LFY*), partial ectopic GUS expression was observed, depending on the distal *PI* promoter. Furthermore, 35S-*LFY* plants carrying simultaneously introduced 35S-*UFO* showed GUS expression mediated by the distal region in the whole plant body; however, GUS expression directed by the proximal region was restricted within the flower organs. These results indicate that the coexistence of *LFY* and *UFO* is sufficient to induce *PI* expression in a flower-independent manner and that the *cis*-acting elements responsive to *LFY/UFO* are located in the distal region of the *PI* promoter.

In summary, I have demonstrated that the *PI* promoter consists of discrete *cis*-acting elements; one in the distal region is responsive to induction signals mediated by the meristem identity genes *LFY* and *UFO*, and the other element in the proximal region is responsive to autoregulatory signals produced by the PI/AP3 complex [6].

References

1. Bowman, J. L., Smyth, D. R. and Meyerowitz, E. M. *Plant Cell*, **1**, 37-52 (1989).
2. Goto, K. and Meyerowitz, E. M. *Genes Dev.*, **8**, 1548-1560 (1994).
3. Jack, T., Brockman, L. L. and Meyerowitz, E. M. *Cell*, **68**, 683-697 (1992).
4. Weigel, D. and Meyerowitz, E. M. *Science*, **261**, 1723-1726 (1993).
5. Levin, J. Z. and Meyerowitz, E. M. *Plant Cell*, **7**, 529-548 (1995).
6. Honma, T. and Goto, K. *Development*, **127**, 2021-2030 (2000).