DIFFERENT ROLES OF FLOWERING TIME GENES IN THE ACTIVATION OF FLORAL INITIATION GENES IN ARABIDOPSIS

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Plant development proceeds through the reiterated production of organ primordia by the shoot apical meristem which can give rise to vegetative (leaves and lateral branches) or reproductive (flowers) organs. The meristematic transition from the vegetative to reproductive developmental patterns, which we will refer to as the floral transition, is determined by the genotype of the plant and enviromental conditions like light and temperature that are sensed by different organs of the plant. Litle is known about the molecular mechanisms that regulate the time of this transition. However, the combined used of genetic and molecular tools in a model plant species like *Arabidopsis thaliana* is opening the way to the identification of the genetic determinants involved and their molecular characterization.

Arabidopsis belongs to a group of species that grow as rosettes during their vegetative development and which floral transition is followed by the elongation of the stem (bolting) to give rise to an inflorescence shoot. Before the floral transition, the Arabidopsis shoot apical meristem gives rise to primordia from which leaves with axillary shoot meristems are developed. After the floral transition, leaf development is inhibited and axillary shoot meristems acquire the identity of floral meristems and develop into flowers (Haughn et al., 1995). Mutant analyses in this species, have allowed the identification of more than 30 loci that are involved in the control of flowering time (Martínez-Zapater et al., 1994). Mutations at more than 10 of these loci produce a delay in the floral transition under flowering inductive conditions, indicating that the corresponding genes are required for the promotion of this transiton (Koornneef et al., 1991). Furthermore, phenotypic analyses of these mutants and their double mutants under different environmental conditions indicate that the corresponding genes are involved in at least two developmental pathways that are dependent or independent from enviromental conditions (Martínez-Zapater et al., 1994). As an example, mutants at loci like *FCA*, *FVE* or *FPA*, show a late flowering phenotype under inductive and non-inductive photoperiodic conditions that can be rescued by vernalization while mutants at either *FT* or *FWA* loci are unable to respond to inductive conditions like long photoperiods or vernalization.

With respect to the process of floral initiation (FLIP), at least five genes regulating this process have been identified and characterized at the molecular level: *LEAFY* (*LFY*), *APETALA1* (*AP1*), *CAULIFLOWER* (*CAL*), APETALA2 (AP2) and UNUSUAL FLORAL ORGANS (UFO) (Haughn et al., 1995). Among these genes, *LFY* and *AP1*, have been shown to be not only neccesary but sufficient to promote the initiation of flowers at the lateral meristems, being their gene products mutually enhancing each other's activity (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995). Moreover, mutations at the *TERMINAL FLOWER 1* (*TFL1*) locus cause an early flowering phenotype and the differentiation of the shoot apical meristem into a terminal flower, suggesting that this locus could be involved in repressing the floral transition at the shoot apical meristem.

Since flowering time genes should ultimately determine the time of expression of FLIP genes and because late flowering mutations had already being asigned to, at least, two different developmental pathways, we decided to analyze if late flowering mutations interacted differentially with FLIP genes. With this purpose, we have constructed and characterized double mutants that combined mutations at one of the late flowering loci *FPA*, *FVE*, *FT* or *FWA*, with mutations in either *LFY*, *AP1*, or *TFL1*.loci

Double mutants between *fve*, *fpa*, *ft* or *fwa* and *tfl1* showed a flowering time phenotype close to the phenotype of their late flowering parents. Furthermore, they all showed the presence of a terminal flower after an extended period of growth. Plants Fve Tfl1 and Fpa Tfl1 were as late as their late flowering parents suggesting that *TFL1* could be required downstream in their developmental pathway to repress their floral promotive effect. On the other hand, plants Ft Tfl1 and Fwa Tfl1 were consistently earlier than the corresponding late flowering parents, suggesting that these late flowering genes and TFL1 could be involved in different pathways.

Double mutants produced by the combination of strong mutant alleles at *LFY* or *AP1* with mutant alleles at *FVE*, *FPA*, *FT* or *FWA* bolted at the same time as their late flowering parents, with similar number of leaves; but

showed altered inflorescence phenotypes. Both *fve* and *fpa* mutations enhanced the number of lateral branches in the double mutant inflorescences in a similar way as the effect of short photoperiods on Lfy or Ap1 plants, suggesting that *FVE* and *FPA* are required to accelerate the establishment of FLIP genes in the lateral meristems.

Double mutants *fwa ap1* and *ft ap1* showed a more dramatic delay of floral initiation at the lateral meristems of the inflorescence giving rise to inflorescence structures reminiscent of the *ap1 cal* double mutants grown under short day. Scanning electron micrographs of the apical inflorescence meristems of these double mutants showed that during the initial stages of the inflorescence development, the primary inflorescence meristem only produced lateral inflorescence meristems (Figure 1). Only very late in development, it was possible to detect the differentiation of floral meristems giving rise to typical Ap1 flowers.

The combination of either *ft* or *fwa* with a strong *lfy* mutant allele produced the most extreme inflorescence phenotype, double mutants being unable to produce any flower-like structure and resulting in highly branched plants. This phenotype was even stronger than that described for the *lfy ap1* double mutants (Bowman et al., 1993). SEM micrographs showed that the shoot apical meristems of double mutants inflorescences continue producing leaf primordia with a spiral phyllotaxis (Figure 1). The virtual lack of floral initiation in these plants indicates that, in the absence of LFY function, both *FWA* and *FT* are required for the activity of the other FLIP genes, like *AP1* and *CAL*, that should be responsible for the development of of the flower-like structures found in the Lfy mutants (Madueño et al., 1996).

When RNA blot hybridization was used to analyze the levels of *AP1* and *LFY* mRNAs in the inflorescence apices of single and double mutants, the *AP1* mRNA could not be detected in the apices corresponding to double mutants *ft lfy* or *fwa lfy* while it was present at wild-type levels in Lfy plants. These results suggest that the role of *FT* and *FWA* in the activity of *AP1* is likely at the level of gene expression. Furthermore, the presence in these double mutants of wild-type alleles at loci like *FPA* or *FVE* indicates that in the absence of LFY and FWA or FT functions, the other late loci are not able to promote the floral transition. Taken together these results point to the existence of at least two different ways of interaction between the flowering time loci and the FLIP genes required for flower meristem identity

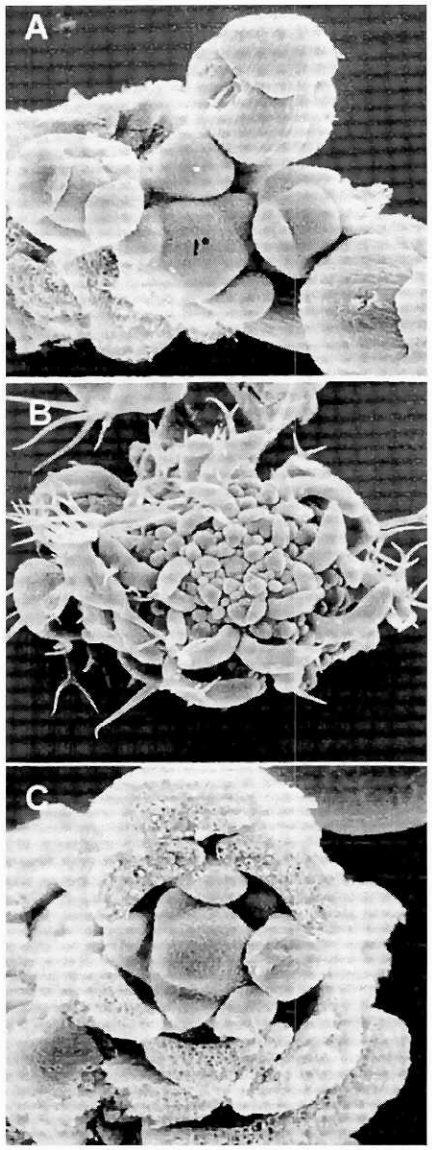

Figure 1. Scanning electron micrographs showing the morphology of the shoot apex. A.- Wild type plant. B.- Double mutant *ft ap1*, young apex. C.- Double mutant *ft lfy*, old apex.

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