Floral induction and determination: where is flowering controlled?

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Flowering is controlled by a variety of interrelated mechanisms. In many plants, the environment controls the production of a floral stimulus, which moves from the leaves to the shoot apex. Apices can become committed to the continuous production of flowers after the receipt of sufficient amounts of floral stimulus. However, in some plants, the commitment to continued flower production is evidently caused by a plant’s commitment to perpetually produce floral stimulus in the leaves. Ultimately, the induction of flowering leads to the specification of flowers at the shoot apex. In Arabidopsis, floral specification and inflorescence patterning are regulated largely by the interactions between the genes TERMINAL FLOWER, LEAFY and APETALA1/CAULIFLOWER.

However, both types of determination assay have limitations, and it is important to note that different determination assays might yield alternative conclusions for the same primordia (the caveats associated with determination experiments are discussed in Ref. 11). A third type of assay has been used to test leaf commitment to the continued production of floral stimulus: in this assay, photoinduced leaves are removed from the plant following an inductive treatment12.

In this review we discuss firstly a variety of experiments that indicate the site(s) that control flowering. Secondly, we review recent studies that indicate how a few key molecular players regulate the specification of flower primordia in Arabidopsis.

Floral induction is the process by which stimuli originating outside the shoot apex induce the formation of flower primordia (Fig. 1). The photoperiodic induction of flowering was discovered 86 years ago by Julien Tornois in hops 1. Shortly afterwards, additional experiments suggested that the photoperiodic control of flowering was a general phenomenon, which controlled flowering in most plants 2. Later, focused-light experiments showed that leaves perceive photoperiodic signals 3. These studies, and numerous grafting experiments, indicate that the production of the photoperiod-induced floral stimulus occurs in the leaves of a wide variety of flowering plants 4–7.

In contrast with floral induction, floral determination can be defined as the assignment of flowering fate, which is persistent even when the flower-inducing conditions no longer exist 8,9. Assays for floral determination include:

- Changing environmental conditions (from inductive to non-inductive)
- Microsurgical removal of shoot apices, and the placement of those apices into neutral environments8,10.

In the past two decades, microsurgery experiments have been used to test for a commitment to persistent floral stimulus production in the leaves. In red-flowered Impatiens balsamina, it has been shown that a continuous supply of a leaf-derived floral stimulus is needed to maintain floral identity in developing flower primordia. Without a persistent supply of floral stimulus, the inner whorls of red-flowered Impatiens flowers revert to vegetative growth. Although red-flowered Impatiens flowers revert when returned to non-inductive conditions, other lines of Impatiens do not revert (i.e., once flowers begin to form, the plant is irreversibly committed to produce complete flowers).

Recent leaf-removal experiments on a non-reverting purple-flowered Impatiens indicate that the irreversible commitment to flowering is because of the continued production of floral stimulus by leaves that unfolded during the photoperiodic induction treatment. In these experiments, when the leaves that unfolded during the photoperiodic induction treatment were removed, floral shoots reverted to vegetative growth. If the same leaves were not removed, the plants did not revert after they were placed in non-inductive conditions. This indicates that commitment to flowering did not occur because the meristem became determined to produce flowers, but because the leaves became committed to continually produce floral stimulus. Additionally, the observation that commitment and reversion are often incomplete in Impatiens suggests that above-threshold levels of floral stimulus are required for the full specification of flower primordia.

This recent work on Impatiens fits well with the initial concept of photoperiodic induction put forward by V.N. Lubimenko and O.A. Sceglova. They proposed that photoperiodic induction treatments cause stable changes that affect the whole plant, and not just the ephemeral production and movement of a 'florigen' that acts at the shoot apex. Of course, in the case of purple-flowered Impatiens, changes at the shoot apex are stable only if the communication with the leaves is maintained. The evidence for a leaf-based commitment to flowering is also consistent with classical grafting experiments in which leaves detached from photoperiodically induced plants were able to induce flowering when grafted onto non-induced plants growing in non-inductive photoperiods.

**Floral induction, determination and specification in Arabidopsis**

**Determination experiments**

The types of leaf-removal experiments described here have not been reported for Arabidopsis. Indeed, the growth habit and architecture of Arabidopsis would make such experiments difficult. However, regions of the plant are removed and grafted to rootstocks. Grafted apices that have not been determined to flower produce approximately the same number of vegetative nodes as a normal plant. However, grafted apices that have been determined to flower produce flowers much more rapidly, indicating that they were committed to flower before they were removed from the original plant. Because meristems cannot be removed and grafted without the inclusion of a few young leaves or leaf primordia, one potential limitation of a grafting experiment is that it does not conclusively indicate whether meristems have been determined. Interestingly, the expression of two genes involved in the control of flowering, *CONSTANS* in maize and *CONSTANS* in Arabidopsis, occurs in young leaves. The expression of these flower-promoting genes in young leaves suggests that leaves that are removed along with the short meristem might be able to affect the determination status of the shoot apex. It is possible that, in some cases, these leaves can be induced to produce sufficient floral stimulus to affect the determination status of the shoot apex (Fig. 1).

**Assays for leaves that are committed to the persistent production of floral stimulus**

Leaf removal experiments can be used to test for a commitment to persistent floral stimulus production in the leaves. In red-flowered Impatiens balsamina, it has been shown that a continuous supply of a leaf-derived floral stimulus is needed to maintain floral identity in developing flower primordia. Without a persistent supply of floral stimulus, the inner whorls of red-flowered Impatiens flowers revert to vegetative growth. Although red-flowered Impatiens flowers revert when returned to non-inductive conditions, other lines of Impatiens do not revert (i.e., once flowers begin to form, the plant is irreversibly committed to produce complete flowers).
the results of simple photoperiodic determination assays have been reported. It is evident from these studies that the early-flowering ecotypes of Arabidopsis can be irreversibly committed to flower within one day of the start of photoinduction, and that low red:far-red light ratios strongly promote the commitment to flowering. This is not surprising, as high red:far-red ratios, acting through phytochromes (particularly PHY B), are known to prolong vegetative growth in Arabidopsis. Additional determination assays indicate that plants grown in long-day photoperiods are determined to flower after approximately seven days, when the first two leaves are approximately the same size as the cotyledons.

Photoinduction experiments also indicate that flowers can be formed from undetermined primordia that are present already at the shoot apex at the start of strong photoinduction treatments. These experiments, and additional experiments in which a partial reversion of flowering occurred, suggest that the determination of primordium identity in Arabidopsis is not instantaneous. Thus, developing primordia evidently respond to floral induction signals over a period of time.

It is not known whether Arabidopsis meristems are florally determined, or if the leaves are committed to a perpetual production of floral stimulus. Although it might be tempting to assume that the Arabidopsis shoot meristem is determined to flower because Arabidopsis apical meristems cannot be reverted to vegetative growth, there is no evidence to indicate that this is the case. Furthermore, data on the commitment to flowering in Arabidopsis is consistent with the hypothesis that the irreversible commitment to flowering is controlled outside the shoot meristem.

The mechanism(s) by which Arabidopsis primordia are committed to flower should, in part, be elucidated by the molecular and biochemical analyses of flowering-time genes and mutants over the next few years. Within the catalog of genes that correspond to early and late-flowering genes (reviewed in Refs 29,30), we should find genes involved in floral stimulus transport and floral stimulus perception within the shoot apex. It will be interesting to see if we also find genes for meristem determination, and/or genes for the irreversible commitment to floral stimulus production in the leaves.
Control of flower primordium specification by LEAFY and APETALA1/CAL/FLower

Although we know little about the mechanisms involved in the production of floral stimuli in the leaves of Arabidopsis, we have a much better idea of the mechanisms by which flowers are specified on the flanks of the shoot apex. For example, the replacement of flowers with indeterminate shoots in in leaf primordia only after they have become distinct from the meristem, whereas the ectopic expression of LEAFY induces the ectopic expression of AP1 in leaf primordia and in axillary flower primordia.

In wild-type plants, LEAFY is expressed throughout flower primordia early in their ontogeny, AP1 and CAL expression also occurs throughout flower primordia, although the expression of these two highly similar and largely redundant genes occurs in primordia only after they have become distinct from the meristem.

The conversion of shoots to meristem specification was reviewed recently, we will limit our discussion primarily to the molecular interactions between LEAFY and the MADS-Box genes AP1/CAL and AGAMOUS (AG).

In Arabidopsis, we have uncovered much about the
initial specification of flowers, and many key genes involved in floral meristem identity have been identified. One of these genes, LFY, promises to be critically important for the further unveiling of floral induction mechanisms. LFY is of particular interest because the transition to flowering in Arabidopsis is modulated by levels of LFY activity in the meristem. Thus, analyses of LFY promoter activation might well identify molecules that make up the floral stimulus. The makeup of the floral stimulus remains one of the great mysteries of plant science. It potentially includes proteins or peptides, sugars, plant hormones and/or other small diffusible molecules. Will these molecules, or their second messengers, interact with the emerging primordia (at, for example, the LFY promoter) or with the shoot meristem? Will we find molecular evidence for irreversible changes that lead to the perpetual production of floral stimulus within the leaves, and so, within which leaves? Time will tell.

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