‘Florigen’ enters the molecular age: long-distance signals that cause plants to flower

Joseph Colasanti and Venkatesan Sundaresan

The transition from vegetative to reproductive growth is a critical event in the life cycle of plants. Previous physiological studies have deduced that hormone-like substances mediate this important transition but the biochemical nature of the putative signaling molecules has remained elusive. Recent molecular and genetic studies of key flowering-time genes offer new approaches to understanding the mechanisms underlying the initiation of flowering.

The principal functions of the SAM are: (1) to initiate the formation of lateral organs and structures, such as leaves and flowers; and (2) to perpetuate itself by maintaining a population of undifferentiated cells that remain uncommitted to a specific program. This ability of the cells in the SAM to remain uncommitted to flowering at a particular time endows the plant with a great deal of developmental flexibility. The transition from vegetative growth to flowering is a clear illustration of the importance of this. However, signals that originate outside the SAM are essential for directing the conversion to reproductive development. In this article, we describe recent progress in discerning the nature of the external signals that are responsible for the transition to flowering.

J. Colasanti is at the Plant Gene Expression Center and the Department of Plant and Microbial Biology, University of California, Berkeley, 800 Buchanan St, Albany, CA 94710, USA; and V. Sundaresan is at the Institute of Molecular Agrobiology, 1 Research Link, The National University of Singapore, Singapore 117604. Email: colasanti@ucmerced.edu; director@ima.org.sg

The transition to flowering is a flexible process

The life of higher plants is broadly divided into a vegetative phase and a reproductive phase. The SAM initiates structures such as leaves during vegetative growth and inflorescences and flowers during reproductive growth. The transition to flowering, when a plant switches from vegetative to reproductive growth, is a pivotal event in the life of a plant. Developmental signals that cause this transition to flowering originate outside the SAM (Fig. 1), and so the SAM remains uncommitted to flowering prior to its perception of external signals. Floral induction causes a cascade of processes within the SAM that result in its restructuring, accompanied by changes in the rate and pattern of cell division, and the formation of floral structures instead of leaves.

Regulation by photoperiod and the search for ‘florigen’

The ability of signals that originate outside the SAM to direct the development of the SAM allows plants to coordinate flowering time with environmental conditions that favor successful pollination and seed production. Studies of the biochemical nature of these external signals followed an important discovery by Garner and Allard of a mutant variety of tobacco, Maryland Mammoth, that flowers only when exposed to a particular day length4. The wild-type tobacco used in their experiment normally flowers after attaining a particular size, regardless of day length, whereas Maryland Mammoth requires a minimal length of night (short-day conditions) to induce flowering.

This discovery led to higher plants being classified as long-day, short-day or day-neutral plants according to their response to particular photoperiods. Obligate long-day and short-day plants have an absolute requirement for photoperiod signals to cause flowering, whereas day-neutral plants (also called autonomously flowering plants) will flower after making a particular number of leaves; that is, after they attain a particular size. Most plants use a combination of environmental signals and autonomous developmental signals to induce flowering at the appropriate time (Fig. 1). It is important to emphasize that, in both environmentally responsive and autonomous plants, the signals that regulate the transition to flowering originate outside the SAM; in both cases, they probably originate in the leaves5,6.

An important consequence of the discovery of photoperiodism was that the transition to flowering could be precisely controlled. Many experiments aimed at characterizing the leaf-generated floral stimulus were performed by using either long-day or short-day plants that responded to a single stimulus. This research was inspired by the concurrent findings that most plant-growth regulators, such as auxins, cytokinins and ethylene, are relatively simple chemical compounds that are freely mobile within all plants7. The quest for a biochemically defined floral stimulus was spearheaded by M.K. Chailakhyan, who coined the term ‘florigen’ to describe a hypothetical substance that induces flowering in all higher plants.

Most experiments involving grafting leaves from plants exposed to flowering-inducing photoperiods onto plants that had not been induced. It was found that a signal of measurable mobility moved from leaf to SAM to cause the transition to flowering. In this way, the ‘velocity’ of signal movement was found to be 2.4±3.5 mm h⁻¹, depending on the species5. These experiments showed that the floral stimulus had the properties of a mobile growth regulator but a biochemically defined substance with florigenic activity has never been isolated. Consequently, it has been proposed that florigen might be not a single molecule but a combination of different signals or signaling pathways that can vary among plant species8.
A genetic approach to the ‘florigen’ problem

Although early physiological studies failed to identify flower-inducing compounds explicitly, they laid the groundwork for a molecular and genetic approach to understanding the mechanisms underlying the transition to flowering. Studies by Murfet and co-workers combined grafting with genetic experiments to bridge physiological and genetic studies. Working with the pea plant, they identified several genes that are required to mediate the transition to flowering. Some of these genes act in leaves to produce a floral stimulus (or to inhibit a promoter of vegetative growth), whereas other genes function at the SAM to mediate responsiveness to external signals. Genes that might produce floral inhibitors or 'antiflorigenes' were also identified. The identification and characterization of several loci that control flowering in pea has revealed a complex picture of interconnected pathways but there is, to date, no molecular information on the relevant genes and their expression.

*Arabidopsis*. More-recent insights about the regulatory networks controlling flowering have come from genetic and molecular studies of the model plant *Arabidopsis*. As flowering-time genes in *Arabidopsis* have been reviewed exhaustively in recent articles, only a brief summary will be presented here. Mutants in the flowering-time genes have defined three main pathways that regulate the flowering transition in *Arabidopsis*: a day-length response pathway, an autonomous promotion pathway, and a gibberellin (GA) phytohormone pathway.

Flowering time in *Arabidopsis* is accelerated significantly by exposure to long days. There are two components to this day-length response pathway – light quality and the circadian clock. The plant’s perception of these are mediated by phytochromes and cryptochromes. Phytochrome regulation of flowering has been studied extensively, although it is only recently that components of the clock regulation of flowering have been identified. These include the cloning and characterization of the GI-GANTEA gene, which provides a key link between the clock genes and the flowering genes. It appears that the gene CONSTANS (CO) is the downstream target of both the light and the clock signals. CO encodes a zinc-finger transcription factor, and ectopic expression of CO causes early flowering under short days.

The second pathway is the autonomous promotion pathway, which is required for flowering in response to internal developmental signals (e.g., the decision to flower after making a fixed number of leaves). Several genes in this pathway have been described and reviewed elsewhere. The autonomous promotion pathway can be substituted for by vernalization (the exposure of germinated seeds to low temperatures). It appears that the autonomous pathway and vernalization operate through the same downstream regulatory gene, FLC, which encodes a protein similar to the MADS-domain class of regulatory factors. The third pathway that promotes flowering in *Arabidopsis* involves the phytohormone GA, which is required for early flowering in *Arabidopsis* but not in all plant species. It has been shown that no other flowering pathways operate in *Arabidopsis* but that the three pathways have been knocked out; the resultant plants are completely unable to flower (G. Coupland, pers. commun.).

An important regulator of the final stages of flowering is the LEAFY gene, which encodes a transcription factor and is itself a presumed target for regulation.
of the flowering-time regulators\(^1\), acts at the SAM and surrounding leaf primordia to activate \textit{APETELA1} and floral homoeotic genes required for flower development\(^2\,3\). Expression studies suggest that \textit{LEAFY} plays a central role in integrating external floral-inductive signals received at the SAM with the developmental events of flower formation\(^4\,5\).

The day-length pathway operates through signals that are perceived in the leaves (photoperiod) but affect the SAM, implying that signal transmission must be an important part of its action. However, there is no evidence that any of the genes from this pathway that have been characterized to date play a specific role in generating or transmitting the signal. For example, the key \textit{CO} gene, the downstream target of the photoperiod and clock-perception genes, is expressed uniformly in both leaves and in the SAM\(^6\,7\). This is consistent with a role for \textit{CO} at any step from the production of a leaf-generated signal to reception of the signal at the SAM. The action of the autonomous promotion pathway might not require the existence of leaf-to-SAM signals, and there is currently no evidence for such a signaling mechanism in \textit{Arabidopsis}. Nevertheless, it is interesting that the \textit{FCA} gene, which encodes a regulatory RNA-binding protein\(^8\), has been shown to act non-autonomously over cell layers, which raises the possibility that it regulates a diffusible flowering signal\(^9\). However, \textit{FCA}, like \textit{CO}, is expressed ubiquitously, making it difficult to determine its site of action.

\textit{GA} is diffusible and thus would be a potential candidate for a flowering signal. However, because it is synthesized throughout the plant, it appears unlikely to serve as the long-distance flowering signal. Moreover, the fact that \textit{Arabidopsis} \textit{GA} mutants can still flower in response to photoperiod implies that the day-length pathway must use a signal other than \textit{GA}. Thus, despite the impressive amount of knowledge obtained on the genetic control of flowering in \textit{Arabidopsis}, there is very little information about the identities of the genes and signals involved in communicating from the leaves to the SAM to initiate the flowering transition.

\textbf{Maize}. A key flowering-time gene recently isolated from maize could provide the first clues about the molecular nature of floral inductive signals\(^2\). Maize plants that have mutations in the \textit{id1} gene (\textit{id1}) are unable to undergo a normal transition to flowering; rather, the SAM of \textit{id1} mutants continues to initiate leaves long after normal plants have flowered (Fig. 2).

Eventually, \textit{id1} mutants do undergo the transition to flowering but they produce aberrant floral structures with vegetative characteristics\(^2\,7\). The \textit{id1} gene was found to encode a putative transcriptional regulator\(^2\), similar to what has been found in \textit{Arabidopsis}, in which many of the late-flowering genes that have been isolated have possible regulatory functions.

However, the expression pattern of \textit{id1} is unlike the expression patterns of any of the flowering-time genes found in \textit{Arabidopsis}. The \textit{id1} mRNA is restricted to a specific domain; \textit{id1} transcript is detected only in young leaves\(^2\) (Fig. 3). The presence of \textit{id1} in leaves and its absence in the SAM suggests that the \textit{id1} gene does not act at the SAM to mediate the transition to flowering. Rather, \textit{id1} could have a role in regulating the production of a floral stimulus or, perhaps, in repressing the activity of a floral inhibitor. The specific function of \textit{id1} is not known; the predicted protein sequence reveals that \textit{ID1} contains zinc-finger motifs related to transcriptional regulators found in animals\(^7\). Therefore, \textit{id1} probably regulates the activity of other genes. Establishing the function of \textit{id1} awaits the identification of downstream target genes.

The finding that \textit{ID1} might act in leaves reinforces studies by Irah and co-workers, who studied the effects of physically separating the SAM from the rest of the plant\(^2\,5\). Maize plants from a particular inbred line make a fixed number of leaves and then flower. By surgically removing...
and rerooting the SAMs of growing plants, they showed that the cells of the apex were not committed to produce a particular number of leaves. SAMs that were excised made more leaves before flowering than SAMs left connected to the rest of the plant. However, additional leaves were not made if a critical number of leaves were excised along with the SAM. There are two important conclusions from these studies. First, the maize SAM can be reprogrammed to generate different numbers of leaves prior to flowering. Second, leaves must be capable of signaling the SAM to switch to reproductive growth at a specific developmental stage; that is, leaves are the likely source of the flowering stimulus.

There are many notable differences between maize and Arabidopsis with respect to flowering time. Flowering in maize is nearly autonomous, with the developmental stage being a more significant determinant of flowering time than environmental conditions. By contrast, flowering in Arabidopsis is greatly accelerated by long-day photoperiods. Furthermore, the phytohormone GA has little effect on flowering in maize, whereas sinks are non-photo- synthetic tissues such as roots, immature leaves that fix carbon by photosynthesis, whereas sinks are non-photosynthetic tissues such as roots, immature leaves and floral organs that rely on source tissues to supply fixed carbon. Another possible mechanism that could be invoked to explain the transmission of long-distance signals is gene silencing. Studies with tobacco have found that epigenetic states of genes can be transferred from one part of the plant to another via plasmodesmata and phloem channels, for example, an ectopically applied marker gene could be silenced the expression of an endogenous gene. An interesting similarity to the mobile floral stimulus is that the endogenous gene was silenced first at the site of the ectopic gene’s application in the leaf and then the effect slowly migrated throughout the plant. Although gene silencing is thought to be important for subduing invading viral genes, an intriguing possibility is that mobile gene silencing, from leaf to apex, could provide the developmental signals that cause the transition to flowering. If this were the case, it would explain the difficulty in identifying fucinc gene compounds, because such a signal could not be isolated biochemically.

**Outlook**

Early attempts to isolate and to characterize flower-inducing compounds by biochemical means have not been successful. Molecular characterization of the genes that control the transition to flowering...

**Figure 3**

(a) The id1-mRNA localization pattern of a maize seedling that is approaching the transition to flowering, showing where this diagram is in relation to the whole plant (b). The SAM is at the bottom center, surrounded by developing leaves, with leaves initiated by the SAM elongating and growing outwards from this point. The id1 mRNA (red) is only detected within immature leaves of the growing plant. The apical portions of the leaves are more mature than the basal regions and outer leaves are more mature than inner leaves. A log scale (in mm) shows the vertical distance from the SAM to the ends of the leaves. A black dotted line indicates the approximate point at which the leaves emerge from the shoot; this is correlated with the time in leaf development at which leaves change from sink to source tissues.

unpublished) (Fig. 3). That is, as leaves emerge and become photosynthetically active (i.e. become source tissues), id1-mRNA levels decrease. This correlation suggests a possible mechanism by which, instead of regulating the production of the floral stimulus, the ID1 protein could control the transmission of the signal by regulating its flow in the developing leaves. Further studies of ID1 function are needed to investigate this possibility.

At least one study has shown that changes in the flow of small molecules into the SAM occur during the transition to flowering in Arabidopsis. Using fluorescently labeled tracers, Geel and co-workers showed that the movement of molecules greater than a particular size were excluded from the Arabidopsis SAM just before the transition to flowering. Whether controlling the movement of assimilates and other molecules from one part of the plant to another is an important regulator of flowering time will be determined by further experiments.

The transmission of long-distance flowering signals: a plumber’s perspective

Until now the discussion of the transition to flowering has centered around two components of the system – the leaves of the plant, where the flower inducing signals are produced, and the SAM, the ultimate target of the signals. However, another component that should be considered in the signal-transport system (Fig. 1). Early physiological studies showed that mobile signals travel in the xylem, the sapwood of the plant, specifically, through the phloem, which also transports nutrients and other molecules from source tissues to sink tissues. Source tissues include expanded, mature leaves that fix carbon by photosynthesis, whereas sinks are non-photosynthetic tissues such as roots, immature leaves and floral organs that rely on source tissues to supply fixed carbon. It is interesting to note that the id1-mRNA expression pattern in young leaves corresponds to the transition in the apical portions of the leaves from sink to source tissues (J. Colasanti, unpublished) (Fig. 3). That is, as leaves emerge and become photosynthetically active (i.e. become source tissues), id1-mRNA levels decrease. This correlation suggests a possible mechanism by which, instead of regulating the production of the floral stimulus, the ID1 protein could control the transmission of the signal by regulating its flow in the developing leaves. Further studies of ID1 function are needed to investigate this possibility.

At least one study has shown that changes in the flow of small molecules into the SAM occur during the transition to flowering in Arabidopsis. Using fluorescently labeled tracers, Geel and co-workers showed that the movement of molecules greater than a particular size were excluded from the Arabidopsis SAM just before the transition to flowering. Whether controlling the movement of assimilates and other molecules from one part of the plant to another is an important regulator of flowering time will be determined by further experiments.

Another possible mechanism that could be invoked to explain the transmission of long-distance signals is gene silencing. Studies with tobacco have found that epigenetic states of genes can be transferred from one part of the plant to another via plasmodesmata and phloem channels; for example, an ectopically applied marker gene was able to silence the expression of an endogenous gene. An interesting similarity to the mobile floral stimulus is that the endogenous gene was silenced first at the site of the ectopic gene’s application in the leaf and then the effect slowly migrated throughout the plant. Although gene silencing is thought to be important for subduing invading viral genes, an intriguing possibility is that mobile gene silencing, from leaf to apex, could provide the developmental signals that cause the transition to flowering. If this were the case, it would explain the difficulty in identifying fucinc gene compounds, because such a signal could not be isolated biochemically.

**Outlook**

Early attempts to isolate and to characterize flower-inducing compounds by biochemical means have not been successful. Molecular characterization of the genes that control the transition to flowering...

unpublished) (Fig. 3). That is, as leaves emerge and become photosynthetically active (i.e. become source tissues), id1-mRNA levels decrease. This correlation suggests a possible mechanism by which, instead of regulating the production of the floral stimulus, the ID1 protein could control the transmission of the signal by regulating its flow in the developing leaves. Further studies of ID1 function are needed to investigate this possibility.

At least one study has shown that changes in the flow of small molecules into the SAM occur during the transition to flowering in Arabidopsis. Using fluorescently labeled tracers, Geel and co-workers showed that the movement of molecules greater than a particular size were excluded from the Arabidopsis SAM just before the transition to flowering. Whether controlling the movement of assimilates and other molecules from one part of the plant to another is an important regulator of flowering time will be determined by further experiments.

Another possible mechanism that could be invoked to explain the transmission of long-distance signals is gene silencing. Studies with tobacco have found that epigenetic states of genes can be transferred from one part of the plant to another via plasmodesmata and phloem channels; for example, an ectopically applied marker gene was able to silence the expression of an endogenous gene. An interesting similarity to the mobile floral stimulus is that the endogenous gene was silenced first at the site of the ectopic gene’s application in the leaf and then the effect slowly migrated throughout the plant. Although gene silencing is thought to be important for subduing invading viral genes, an intriguing possibility is that mobile gene silencing, from leaf to apex, could provide the developmental signals that cause the transition to flowering. If this were the case, it would explain the difficulty in identifying fucinc gene compounds, because such a signal could not be isolated biochemically.

**Outlook**

Early attempts to isolate and to characterize flower-inducing compounds by biochemical means have not been successful. Molecular characterization of the genes that control the transition to flowering...

unpublished) (Fig. 3). That is, as leaves emerge and become photosynthetically active (i.e. become source tissues), id1-mRNA levels decrease. This correlation suggests a possible mechanism by which, instead of regulating the production of the floral stimulus, the ID1 protein could control the transmission of the signal by regulating its flow in the developing leaves. Further studies of ID1 function are needed to investigate this possibility.

At least one study has shown that changes in the flow of small molecules into the SAM occur during the transition to flowering in Arabidopsis. Using fluorescently labeled tracers, Geel and co-workers showed that the movement of molecules greater than a particular size were excluded from the Arabidopsis SAM just before the transition to flowering. Whether controlling the movement of assimilates and other molecules from one part of the plant to another is an important regulator of flowering time will be determined by further experiments.

Another possible mechanism that could be invoked to explain the transmission of long-distance signals is gene silencing. Studies with tobacco have found that epigenetic states of genes can be transferred from one part of the plant to another via plasmodesmata and phloem channels; for example, an ectopically applied marker gene was able to silence the expression of an endogenous gene. An interesting similarity to the mobile floral stimulus is that the endogenous gene was silenced first at the site of the ectopic gene’s application in the leaf and then the effect slowly migrated throughout the plant. Although gene silencing is thought to be important for subduing invading viral genes, an intriguing possibility is that mobile gene silencing, from leaf to apex, could provide the developmental signals that cause the transition to flowering. If this were the case, it would explain the difficulty in identifying fucinc gene compounds, because such a signal could not be isolated biochemically.

**Outlook**

Early attempts to isolate and to characterize flower-inducing compounds by biochemical means have not been successful. Molecular characterization of the genes that control the transition to flowering...
Although at an early stage, the genetic networks that interact to control flowering are being elucidated by analysing flowering-time genes from *Arabidopsis*, maize and other species. As more genes are isolated, their function will be defined in terms of where in the floral induction pathway and whether they mediate environmental signals or control autonomous signals. A new perspective on this problem is to consider the plant as a multicomponent system comprising the SAM (the target of the signal), the leaf (the floral-signal generator) and the plasmodesmata and vascular (the signal conduit) (Fig. 1).

Many important questions remain to be answered; for example, how universal are flower-inducing signals? Which genes have been conserved among species to control flowering and which are unique to a particular species? Early attempts to isolate florigen were based on the assumption that a universal flowering hormone existed. The physiological studies aimed at searching for a single flower-inducing hormone might have been confounded by the ‘lure of universality’. The identification of the SAM (the signal conduit) (Fig. 1).

Plant growth and reproduction in plants. J. Agric. Res. 18, 506–553.


References


