

Florigen Goes Molecular: Seventy Years of the Hormonal Theory of Flowering Regulation

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Abstract—As early as in 1936, the comprehensive studies of flowering led M.Kh. Chailakhyan to the concept of florigen, a hormonal floral stimulus, and let him establish several characteristics of this stimulus. These studies set up for many years the main avenues for research into the processes that control plant flowering, and the notion of florigen became universally accepted by scientists worldwide. The present-day evidence of genetic control of plant flowering supports the idea that florigen participates in floral signal transduction. The recent study of arabidopsis plants led the authors to conclusion that the immediate products of the gene *FLOWERING LOCUS I*, its mRNA and/or protein, move from an induced leaf into the shoot apex and evoke flowering therein.

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In 2006, it is 70 years since the theory of hormonal regulation of flowering has been forward by Mikhail Khristoforovich Chailakhyan in 1936 in three consecutive publications *On the Mechanism of Photoperiodic Reaction* [1], *On the Hormonal Theory of Plant Development* [2], and *New Facts in Support of the Hormonal Theory of Plant Development* [3]. These papers described the data obtained in the experiments that were carried out first in 1932–1934 in the Department of Plant Physiology, All-Union Institute of Plant Industry, Pushkin, near Leningrad, and in the Laboratory of Plant Biochemistry and Physiology, Leningrad, and later, in 1935–1936, in the Institute of Plant Physiology, Moscow.

To investigate plant transition to flowering, one should acquire the means to manipulate this process in experiment. Plant photoperiodic response discovered in 1920 by Garner and Allard [4] turned out both feasible and reliable method to control the process of flowering. In this way, by changing day length, Chailakhyan managed to accelerate or slow down plant transition to flower. Therefore his very first experiments employed plant species of vivid photoperiodic response: the short-day species with their flowering promoted by a short 10-h light period and dramatically delayed in 16–18-h day, and the long-day species manifesting the opposite pattern of photoperiodic induction of flowering. By using diverse plant models, Chailakhyan compared flowering regulation in plant species that differed in their ontogenetic adaptation responses and in this way revealed the basic regularities governing higher

plant transition from vegetative to reproductive development.

The publications of 1936 singled out plant organs that responded to day length and in this way distinguished between the photoperiod effects on leaves and shoots (shoot apices) by differential illumination of leaf blades and partly defoliated shoots. The plants flowered only in the case when leaves were treated to the favorable day length. These experiments led the author to conclusion that “the processes that are evoked by the change in the day length and induce plant flowering take place in leaf tissues. Later, the effects of these processes are transmitted from leaves into shoot apices, and the latter are affected to proceed to flower formation.” The author emphasized that “the transmittance of the day-length effect depended on some substances that could move for considerable distances along the stem.” These substances inducing flowering in shoots, or in other words, the floral stimulus, were given a short name of florigen, that is, a flower formation agent [3].

These papers together with the book *The Hormonal Theory of Plant Development* [5] based on these papers describe some characteristics of florigen. It was shown to display activity already in low quantity: in many plant species, the transition to flower occurred when a single leaf or even small portion of the single leaf was exposed to favorable day length. Plant girdling and excision of induced leaves following various time intervals after light induction demonstrated that the floral stimulus is transmitted, both acropetally and basipetally, along the stem cortex, most probably via the

phloem elements. The stimulus can pass through a graft from flowering to non-induced components and promote flowering of the latter under unfavorable photoperiod. The grafts were also obtained between plants belonging to different species and diverse photoperiodic groups. Thus the transition to flowering in the vegetative apices of red Jerusalem artichoke, the short-day plant, grafted on the flowering day-neutral sunflower plants occurred on the long day unfavorable for Jerusalem artichoke due to the effect of rootstock leaves. This evidence led the author to an important postulate: the floral stimulus was not species-specific; rather, it was of common nature at least within the related plant species. As a whole, upon the data from these experiments Chailakhyan concluded that the floral stimulus florigen was of hormonal nature. The leaf phase of plant response to an external stimulus was called the induction (initiation) of flowering, and the subsequent response of the shoot apex, the evocation of flowering. When florigen activity was compared to the activity of auxin, the already established plant growth hormone, the researchers found that florigen was not auxin: two substances dramatically differed in their regulatory functions, the conditions and place of synthesis.

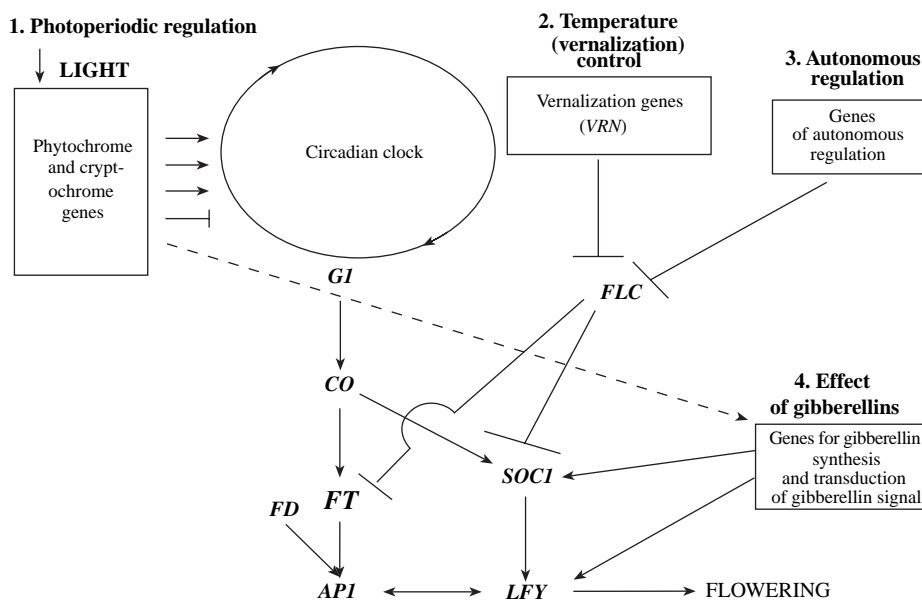
The hormonal theory of flowering developed by Chailakhyan immediately aroused worldwide interest and ready response of the scientific community. This theory presented new ideas and general conclusions and opened wide vistas for fundamental research into plant development. Therefore it carved a long-standing trend in the worldwide studies of plant flowering control. The references to the pivotal Chailakhyan's data and conclusions of 1930s can be so far found in the major international journals and in the current textbooks on plant physiology [6–10]. The references to basic Chailakhyan's publications and his portrait with a short biography were included to the fundamental volume on general history of biology published in Germany [11], and the term florigen coined by Chailakhyan is among the firmly grounded notions of the present-day plant physiology.

Through the subsequent decades, Chailakhyan headed the studies that expounded, step by step, the concept of florigen and rallied further experimental support to this concept. The detailed study of the activities of such phytohormones as gibberellins, cytokinins, auxins, and ethylene on plant flowering in diverse photoperiodic groups led to the notion of biphasic flowering process and two components of florigen comprising two complementary groups of phytohormones, gibberellins and anthesins [12]. In these experiments, gibberellin deficiency was shown to prevent flowering in several long-day species [13]; these phytohormones were reported to play an important role in floral transition in plants belonging to various biotypes [14]. As to the second component of florigen, anthesins, Chailakhyan hypothesized, already in 1960, that anthesins would comprise specific nitrogenous compounds, including the metabolites of nucleic acids. He wrote: "Floral tran-

sition in short-day species relies on enhanced nitrogen metabolism and primarily on such highly specialized products of nitrogen metabolism as the nucleic acid metabolites and probably other nitrogenous compounds" [15]. The extracts from flowering tobacco plants were found to produce an anthesin-like physiological effect: they made short-day plant species shift to flowering under the long-day conditions [16, 17]. However, the attempts to decipher the chemical constituents of anthesins failed both in the Chailakhyan's laboratory and elsewhere. This early disappointment was apparently caused by the macromolecular structure of anthesins which probably comprised some regulatory proteins or even mRNAs [18]; it was only lately that the adequate methods have been developed for analyzing these compounds. The most recent studies in this field [19–21] produced a sound support to such idea.

The Chailakhyan's laboratory also investigated various physiological aspects of flowering control in the plant species that belonged to diverse developmental types: long-day, short-day, long-short-day, short-long-day, neutral, winter, spring, early-maturing, with extended juvenility phase, tuber-forming, etc. These studies were the basis of the concept of autonomous and induced mechanisms to control the transition to flowering [22] that evolved in the course of plant speciation under the particular environmental conditions and were further inherited. The induced mechanism is best illustrated by the shift to the reproductive development following the induction with light (photoperiodic induction) or temperature (vernalization) factor; the case of self-imposed control is best exemplified by the neutral plant species wherein the transition to flowering is produced by internal age-dependent changes. The mechanisms of autonomous and induced regulation of flowering are essential elements of all current models of the genetic control of plant reproductive development [23].

Summing up his research in his last book [24], Chailakhyan particularly emphasized the importance and prospects of the gene-focused studies of plant flowering regulation. Wrote he: "The outstanding discoveries in molecular biochemistry and genetics have demonstrated the great potential of investigating the hormonal control of the transition from vegetative to floral morphogenesis in the terms of the gene control... In this aspect, I would suggest an analogy; though lacking the strength of direct support, it can considerably clarify the pattern of the interlaced effects of environmental factors, hormonal effectors, and genetic factors. Imagine the piano keyboard, and the masterful fingers of piano player who moves the particular keys and their combinations and in this way gives birth to a beautiful melody. Likewise in the plant genome, the external factors and then internal hormonal effectors set going the particular genes and their combinations, and plants perform their life symphony [24, p. 496].



Genetic network that controls the transition to flowering in arabidopsis (a simplified model). *API*—*APETALA 1*; *CO*—*CONSTANS*; *FLC*—*FLOWERING LOCUS C*; *FT*—*FLOWERING LOCUS T*; *GI*—*GIGANTEA*; *LFY*—*LEAFY*; *SOC1*—*SUPEREXPRESSION OF OVEREXPRESSION OF CONSTANS 1*. — stimulation, —| blockage.

Current research into the regulation of flowering is focused on progressive characterization of the genes participating in the control networks and clarification of gene interactions and their relationship with external inducers. Such approach employs the methods of molecular genetics and genetic engineering and involves a few model species. On the other hand, this approach is based on the fundamental concepts established earlier by physiological methods including the studies by Chailakhyan of florigen formation in leaves, its long-distance transport, and the florigen-evoked initiation of floral morphogenesis in shoot apices. Such association of molecular-genetic data with the major conclusions of the florigen theory has been attempted earlier [18]. Here we will discuss in short new evidence obtained with *Arabidopsis thaliana* L., a model plant of geneticists, matching the basic idea of the florigen theory: the existence of long-distance transmissible floral signals.

Presently the studies of molecular-genetic mechanisms that control flowering are among the most rapidly evolving issues of biological research. Dozens of genes have been characterized that control the transition to flowering in arabidopsis and regulate the particular steps of floral morphogenesis. The responses of these genes to the external and internal factors were described together with the specific details of the complex interactions in this multigenic network [25]. Several models have been recently developed that reflect the present knowledge of the genes engaged in flowering control, and probably these models will be soon upgraded to keep pace with the rapid progress of experimental research. The models somewhat differ as to the

position of particular genes that promote or repress flowering. The figure presents a simplified scheme based on already published data [23–26]. Similar and more intricate models can be found in recent reviews on the genetic control of flowering [23, 25].

The figure illustrates four major pathways involved in the transition of arabidopsis plants to flowering; these pathways are initiated by favorable photoperiod, favorable temperature, autonomous age-related changes, and gibberellins. When integrated, these four pathways affect in the shoot apex the genes of floral meristem identity *APETALA 1* (*API*) and *LEAFY* (*LFY*) and the latter genes, in their turn, control the genes for floral organ development. We will describe in short some key links in these regulatory chains.

Arabidopsis is a long-day plant with the quantitative-type response to photoperiodic induction of flowering. The long-day signal is perceived in arabidopsis leaves, with phytochromes and cryptochromes for mediators, and transduced along the signal system to the photoreceptor mechanism of the endogenous circadian clock and the gene *GIGANTEA* (*GI*) [27]. The expression of *CONSTANS* (*CO*) in the leaf under the light and circadian clock control and the expression of *FLOWERING LOCUS 1* (*FT*) localized in the leaf and shoot apex vascular tissues are currently seen as the key steps in the photoperiodic response [28, 29] that produce florigen under favorable illumination conditions [19–21]. Under short-day conditions, arabidopsis plants also commence flowering, although at much slower rate; in this case the transition to flowering is run by the autonomous mechanism. The genes of the auton-

omous pathway (see figure) promote flowering by repressing the expression of the floral repressor *FLOWERING LOCUS C (FLC)*. The vernalization pathway of flowering control involving several *VERNALIZATION* genes) also involves the repression of *FLC* [26]. The treatment with gibberellin under short-day conditions dramatically enhances flowering in arabidopsis and other long-day rosette plants. In this case, gibberellin effect is mediated by several genes involved in the transmission of the gibberellin signal [25]. The effect of long day is realized, at least in some cases, by promoting gibberellin biosynthesis in leaves [13, 14]. Apparently gibberellin promotes flowering by activating the gene *SUPEREXPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)*. The signals from four regulation pathways converge at several intersections of the regulatory network and jointly determine the timing of transition to floral morphogenesis. The genes *FT*, *SOC1*, and *LFY* are considered the major loci for the integration of diverse signals that control the evocation of flowering [25, 26].

The notion of florigen as a long-distance floral stimulus is basic for most studies dealing with the flowering genes. The biochemists and molecular geneticists in many countries, such as United States, Japan, Russia, Sweden, Norway, United Kingdom, Belgium, Australia, etc., have been working on the search and characterization of florigen. The focus of these studies is on the function of *CO* and *FT* in arabidopsis leaves, the relation of these genes to the synthesis and chemical composition of the floral stimulus, the mechanism of its translocation into the shoot apex, and its effect on the expression of the genes of floral meristem identity in shoot buds.

Present-day researchers employ, once again, the grafting technologies worked out long ago by Chailakhyan. His studies [2, 3] demonstrated that the transmission from the flowering component of the graft to the vegetative one is the characteristic property of the floral stimulus. Recently a new micrografting technology was worked out for arabidopsis plants [30], and it was immediately employed in the floral stimulus studies. In particular, Turnbull and Justin [31] used Y-grafts (two shoots on a single root system) that combined various arabidopsis genotypes, including the mutants with the impaired genes *GI*, *CO*, and *FT* and also the wild-type plants. Under the long-day conditions, flowering was delayed in the mutants *gi*, *co*, and *ft*; this delay also affected the transition to flowering in the second shoot. Meanwhile under short day, the superexpression of *CO* in the shoots of transgenic *35S::CO* plants promoted flowering in the wild-type shoots. In other study [32] arabidopsis *CO* was put under the control of the galactinol synthase (*GAS*) promoter from melon; *GAS* is specifically expressed in the companion cells of minor veins in mature leaves; however, the gene is expressed neither in larger vascular bundles, nor in apices. The artificially promoted *CO* expression in the companion cells of the phloem sufficed to accelerate flowering in

the apical meristem of arabidopsis plants under short day. The grafts combining the vegetating arabidopsis plants with transgenic plants that differed in time to flower demonstrated that the expression of *CO* in minor veins in the leaves may sometimes mimic the effect of the photoperiod-related floral signal. As a whole, the grafting experiments confirmed the considerable role of *CO* expression in arabidopsis leaf veins in the photoperiodic control of flowering in this plant species.

In August 2005, the journal *Science* published at once three papers [19–21] dealing with the role of the gene *FT* in formation, translocation, and functioning of the floral stimulus, florigen. Huang et al. [19] studied transgenic arabidopsis plants with the gene *FT* put under a heat-shock inducible promoter *Hsp* from soybean. Heating a single leaf of the transgenic plant at 37°C induced strong expression of *Hsp::FT* in the leaf followed by rapid plant transition to flowering even under the short-day photoperiod. Six to twelve hours after heating, *FT* transcripts were transported from the leaf into the shoot apex; in its turn, mRNA of *FT* induced *FT* expression in the shoot apex followed by the induction of other genes involved in flowering evocation, such as *SOC1*, *LFY*, and *API*. This evidence let the authors to suggest that mRNA of *FT* is florigen or at least its important component. Indeed, *FT* transcript manifested several properties of the classical long-distance floral stimulus. It was highly active at low concentrations: local heating of a single leaf induced flowering in the *Hsp::FT* transgenic plants even when the leaf was detached five hours later. The velocity of movement of the *FT* transcript from the leaf into the shoot apex was estimated as 1.2–3.5 mm/h. In the light of recent experimental evidence, the possibility of long-distance movement of individual plant mRNAs, which had been already discussed in detail [18], does not look any more out of the ordinary.

Two other studies [20, 21] focused on the mechanism of *FT*-induced floral morphogenesis in the shoot apex following florigen arrival from the leaf. The authors investigated the role of the product of *FT*, a small (20 kD) protein homologous to phosphatidylethanolamine-binding factor, or the inhibitor of RAF kinase. Transition to flowering in the shoot apex required the expression of the gene *FD* encoding a bZIP transcription factor. FD protein was found in the nuclei of the shoot apical cells and was absent from leaves. The synthesis of this factor in the shoot apex preceded the photoperiodic induction and when flowering stimulus arrived to the apex, two agents evoked flowering. In cell nuclei, proteins FT and FD produced the complex which apparently activated *API*, the gene for floral meristem identity.

These authors also presume that the products of *FT* (mRNA or protein) could be a signal or part of the signal produced in the leaf phloem in response to the photoperiodic induction and transmitted into the shoot

apex. At the molecular–biochemical level, these events are crucial for transition to flowering.

Undoubtedly, much should be done before the florigen structure and activity are deciphered completely. The best formula for the current state of the florigen problem postulated by Chailakhyan as early as in 1936 is found in one of the papers dedicated to the long-distance (interorgan) control of flowering. Its title runs as following: “Florigen” Enters the Molecular Age: Long-Distance Signals That Cause Plants to Flower [33].

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