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Floral Induction: : A Switch from Vegetative to Reproductive Phase in Flowering Plants [Version 2]

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Abstract

Reproductive success in flowering plants has remained dependent on the evolution of elaborate mechanisms that guarantee the flowering to occur at optimal time. For the floral transition to occur at optimal time, these regulatory mechanisms integrate varied environmental cues with the endogenous physiological ones. To understand the underlying mechanisms of floral transition, genetic models have been developed based on the extensive physiological experiments done in the last century and the current molecular studies. Physiological experiments have been performed with diverse species whereas current genetic models are mostly based on studies with *Arabidopsis thaliana*, a small flowering plant.

This review will focus on the four floral inductive pathways which operate in Arabidopsis: photoperiodic pathway, autonomous pathway, gibberellin promotion pathway and vernalization pathway. It will be discussed as how in this network of pathways; different nodes signify a site of signal integration and how the pathways are integrated leading to a coordinated initiation of flowering.

Keywords

Photoperiodic Pathway; Autonomous Pathway; Gibberellin Promotion Pathway; Vernalization Pathway

Developmental Phase Changes Manifest Floral Transition

Since the discovery of photoperiodic induction of flowering by Garner and Allard [1], lot of physiological studies have been carried on with the floral transition. The physiological analysis and dissection of floral induction was made possible by the introduction of an experimental system based on the understanding that external controllable stimulus when applied to certain plant can cause flowering. The subsequent studies considered that higher plants share two important features of floral transition that are; floral stimulus originates in leaves where from it is sent to the SAM, shoot apical meristem and SAM, which is the target of floral stimulus, must be competent to receive the floral stimulus [2,3]. After receiving the floral stimulus, SAM undergoes transition from vegetative to reproductive stage and this transition is well manifested by developmental progression in SAM morphology accompanied by a fundamental shift in gene expression [4]. The SAM occurs in two states, incompetent state when it is unable to perceive incoming floral inductive signals and competent state when it can interpret floral inductive signals and leads transition to flowering. Thus it is essential for the SAM to pass the developmental check point between incompetent and competent states [5]. This transition is marked by changes in organ production and gene expression [6-9]. Passing the first developmental check point makes the SAM competent to flower and floral transition can now be induced by the floral inductive signals generated by external and internal stimuli. Lot of work is being done to understand the determination of floral competence in SAM. The connection between meristem architecture and their ability to respond to inductive stimuli was suggested by the finding that when the function of two paralogous BEL1-like (BELL) homeobox genes PENNYWISE (PNY) and POUND-FOOLISH (PNF) was eliminated in *Arabidopsis*, it resulted in defective SAM that were unable to respond to floral inductive signals and remained in a vegetative state [4]. microRNAs like miR156 and miR172 – and their corresponding targets have been found to be the key regulators of the phase changes in the floral transition [10].

Floral Induction is Mediated Through Multiple Pathways

The shoot apical meristem produces leaves and vegetative shoots whereas floral meristem produces inflorescences and flowers. The transformation of shoot apical meristem into floral meristem is induced by floral inductive signals. Lot of work has been done to understand the role of external stimuli in floral transition for last past 100 years. Tournois [11] demonstrated that flowering time of plants can be affected by growing them in varying day lengths like shortening day length by shading or increasing the day length by incandescent light bulbs. Garner and Allard [1] put forward the concept of photoperiodism after examining a tobacco mutant that required short day lengths to induce flowering. A Maryland Mammoth variety of short-day (SD) tobacco was derived from a normally day-neutral tobacco (plants that flower at its own particular time irrespective of day length). In their night break experiments (interruption of long night period by brief exposure of light) they established that floral induction in SD plants depends on the duration of the night and not on the length of day. Knott [12] demonstrated by localized shading and lighting of both the SAM and leaves of spinach that leaves are the organs where inductive signals originate. Chailakhyan [13] revealed that in chrysanthemum and *Perilla frutescens* if induced leaves (donor) are grafted onto non-induced plants (recipient), it results in the early flowering of the recipient. So florigen theory was put forward by Chailakhyan in which it was supposed that florigen is a universal floral inducing substance or substances, produced in leaves and then transported to the shoot apex, leading to floral transition. It is now known that both the quantity (length of light exposure) and quality of light are essential signals for flowering. Plants possess specific receptors which not only sense the duration of light exposure but also differentiate between different wavelengths of light. It was established in the early twentieth century that flowering is also effected by temperature. It was shown that certain wheat and rye varieties need to over-winter for flower induction. Gassner [14] showed that in winter variety flowering is accelerated if during germination in pots it is exposed to cold temperatures and subsequently transferred to soil under normal temperatures. Such acceleration of flowering was not found in spring variety. Lysenko [15] coined the term vernalization and found that exposure to cold temperatures must be followed by increases in day length. It was thus suggested that there are different developmental checkpoints that a plant must pass to flower. The endogenous cues that communicate information about growth status like plant size, nutrient flow, hormones etc. can influence the timing of flowering [16]. The existence of an endogenous floral inductive pathway is evidenced by the studies of certain varieties of tabacco and maize which flower only after producing a predictable number of leaves [17,18].

Photoperiodic Control of Floral Induction

The plants coordinate their flowering by utilizing a reliable indicator of changes in the environment,"changes in day length". The change in day length can foretell the environmental changes like onset of cold period or the beginning of a rainy season thus allowing plants to adjust for flowering time. Flowering in SD plants occurs when day length shortens whereas in long-day plants (LD) flowering occurs with the increase in day length and day-neutral plants (DN) flower regardless of changes in day length [19]. The plants can be further categorized as obligate and facultative within the day length responses. Absolute inductive photoperiods are mandatory for obligate plants and such plants will remain in vegetative state if this requirement is not fulfilled. On the other hand facultative plants show accelerated flowering under inductive conditions and undergo normal flowering even in non-inductive photoperiod. It has now be-

come possible to identify the loci and genes that are involved in inductive day length determination by comparing the plant species and different varieties with different day length requirements for flowering. Genomic loci involved in flowering, or quantitative trait loci (QTL) have been identified in species like rice and maize [20-22]. Identification and isolation of specific flowering time genes in rice has been made possible by dissection and analysis of QTL [23-25]. OsCOL10, CONSTANS-like gene has been found to repress flowering by reducing expression of the FT-like genes RFT1 and Hd3a through Ehd1. OsCOL10 acts downstream of Ghd7, a key LD-specific flowering repressor by reducing expression of Ehd1 [26].

Light Signals are Transduced by Photoreceptors

There are two important classes of photoreceptors, phytochromes and cryptochromes, in higher plants that are involved in flowering. Phytochromes perceive red and far-red light while cryptochromes perceive UV-A and blue light. The day length response in *Arabidopsis* involves phytochrome A and B and cryptochrome 1 and 2 [27]. The light signal perception and flowering time in plants is found to be altered if the genes encoding these photoreceptors do not express correctly or are mutated. In *Arabidopsis*, far-red and blue light promote flowering whereas flowering is inhibited by red light [28]. The photoreceptors interact with their corresponding interacting proteins in a complex network to transduce the light signals. The photoperiodic perception acts as important transducers of plant's environment. Phytochromes and cryptochromes employ mechanism that encompasses entrainment of the circadian rhythm to communicate the photoperiodic activity. Circadian rhythm is a self-reinforcing endogenous clock that permits light/dark coordinated gene expression. The flowering time is also affected by additional inputs from changes in temperature, light quality and quantity through elements of the endogenous clock [29,30]. Additional role is supposed for light in terms of its quality perceived by plants [31,32]. An important component of a possible "light quality pathway", PHYTOCHROME AND FLOWERING TIME 1(PFT1) gene has been discovered in *Arabidopsis* [33]. PFT1 has also been implicated in disease resistance providing an example of the possible crosstalk between different environmental factors affecting plant development [34]. It has been shown that the 'shade avoidance response' is activated by the perception of low red/far-red ratio. The 'shade avoidance response' acts as a possible indicator of competition from neighbouring plants and a signal to the early flowering.

Self-Reinforcing Endogenous Clock / The Circadian Clock

Plants use a predictable mechanism that utilizes day length variation for detecting seasonal changes. The endogenous clock is enabled to detect the day length changes by comparing the light cycle through its entrainment and consequent setting of periodicity. An endogenous mechanism called as 'circadian rhythm' track the changes in light/dark cycles and results in self-reinforcing rhythmic gene expression patterns [35,36]. The blueprint of the *Arabidopsis* clock serves as a reference for clocks in other plants [37,38]. The endogenous rhythm is altered by the changes in periodicity of the light/dark cycles that leads to the floral induction by photoperiodic pathway in day length responsive species. Negative feedback loops have been suspected to underlie the circadian clock. It is suggested in a simple auto regulatory negative feedback loop, the clock gene is transcribed and the transcript is translated into a protein that accumulates in the nucleus to inhibit further transcription. Degradation of both mRNA and protein relieves this inhibition, and the cycle renews. Three transcription factors which interact to form a negative feedback loop in *Arabidopsis* have been found, which are: CIRCADIAN CLOCK ASSOCIATED1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY) and TIMING OF CAB EXPRESSION1 (TOC1) [39-41]. TOC1 inhibition by CCA1 and LHY moreover involves a co-repressor complex of CONSTITUTIVE PHOTOMORPHOGENIC10 (COP10), DE-ETIOLATED1, and DDB1 [42]. TOC1 belongs to a five-member family of ARABIDOPSIS PSEUDO-RESPONSE REGULAT-ORS (APRR) whose expression is regulated by circadian rhythms. A similar five-member PRR family is found in rice that shows rhythmic expression under circadian control, suggesting conservation of function amongst monocots and dicots. The working of circadian clock results in change of expression of downstream genes that coincide with favorable photoperiodic conditions for flowering. More recently positive elements of circardian rhythm have been discovered. FAR-RED IMPAIRED RESPONSE1 (FAR1), FAR-RED ELONGATED HYPOCOTYL3 (FHY3), and HY5 activate ELF4 during the day and ELF4 is repressed at dawn by CCA1 and LHY through direct interaction with these activators [43]. It is being suggested by the increasing number of reciprocal interactions that intricate web of connections operate the clock [44].

Key Genes Integrate Photoperiodic Induction

In *Arabidopsis*, circadian rhythm controls the expression of one of the important genes involved in floral inductive pathway, CONSTANS (CO), a zinc finger protein functioning as transcription factor [45]. The co mutant was originally identified as late flowering in long days but flowering at the normal time in short days [46]. CO was cloned using a map-based approach [45]. Because the co mutant plants retain their ability to flower, CO is precisely having role in photoperiodic induction under LD conditions. Overexpression of CO results in early flowering and plants remain insensitive to changes in day length [47]. CO expression is regulated by the endogenous circadian clock on a 24-hour cycle that peaks during the night. So, in short days CO expression does not overlap with the period of daylight, but in longer days there is an overlap of CO expression and daylight in the evening [48]. The findings from previous physiological

experiments that in LD plants the day length is important in producing a floral inductive signal is supported by the evidence that CO expression levels are increased under LD conditions [48,49]. Further the timing of CO expression is linked to circadian clock is evidenced by the finding that light stabilizes CO expression whereas CO protein undergoes proteasome-mediated degradation during night [50]. In the morning the repression of CO transcription is done by CYCLING DOF FACTOR (CDF) proteins. The regulation of levels of CDF1 and CDF2 is carried out by proteolytic degradation through a light-dependent complex of the clock proteins GI and FKF1 [51]. The ability of GI to bind CO promoter is restricted during night as ELF4 regulates its access to chromatin by sequestering it from nucleoplasm into subnuclear bodies [52].

Two regions have been found to be vital for the proper function of CO protein: **a zinc-finger motif** and a **CCT region**. Zinc- finger motif is similar to protein–protein interacting zinc-fingers found in animals whereas CCT region is found in CO, CO-LIKE and TOC1 genes and is required for the nuclear localization of these proteins [53]. The expression of CO occurs in several cell types but the expression specific to phloem companion cells results in early flowering and that if its expression is restricted to SAM, early flowering does not occur [54]. Under the control of galactinol synthase promoter when the CO expression was confined to minor vein companion cells, early flowering resulted under non-inductive SD conditions. Also flowering was accelerated in co-mutant receptor plants when the leaves expressing CO in minor veins were grafted to them [55]. Thus, CO is supposed to mediate the photoperiod-induced floral stimulus generated in leaves and its transport through phloem to SAM. CO and FLOWERING LOCUS T (FT) expression is promoted by PHYTOCHROME AND FLOWERING TIME (PFT1)/ MED25 (a subunit of mediator complex which bridges transcriptional factors with RNA polymerase II), in a CO-independent manner [56]. The function of PFT1is governed by photoperiod and length of short tandem repeat region that encodes 90 amino acid region rich in Q [57].

FLOWERING LOCUS T (FT) is a direct downstream target of CO. FT is a RAF-kinase inhibitor-like protein and late flowering ft mutants were isolated during a genetic screen of early flowering CO overexpressing lines [58]. As the over-expression phenotype is repressed by FT, its position can be suggested as downstream of CO and also specifies that another target gene exists that acts in parallel with CO because flowering still occurred sooner than in co knockouts. The early flowering phenotype in CO over-expressing lines was also found to be suppressed by mutation in SUPRESSOR OF OVEREXPRESSION OF CO 1(SOC1), a MADS-box transcription factor, another direct target of CO [58]. soc1 mutants show delayed transition to flowering same as ft mutants and as with ft, flowering is only delayed slightly relative to co mutants. But eliminating the function of both FT and SOC1 by mutation, CO over-expressing plants are delayed to nearly the same degree as co mutants. So, it can be inferred that FT and SOC1 are the two key downstream targets of CO which function in parallel partially redundant inductive pathways. LEAFY (LFY) and APETALA1, two floral meristem identity genes are up-regulated by FT and SOC1.

The homologues for CO and FT have been found in both dicot and monocot species suggesting their role in flower transition in other plants. The loss of co function in *Arabidopsis* mutants was complemented by the CO from *Brassica napus* and *Pharbitis nil* [59,60]. The QTL that is associated with flowering time mutants in rice, called *Heading date*, encode genes related to CO and FT. A CO homologue, *Heading Date 1(Hd1)*, effects the early transition to flowering in inductive SD conditions and inhibits flowering under LD conditions [61]. *Hd1* displays diurnal pattern of expression with peak levels at dawn and during the night under LD conditions, coinciding with circadian clock expression pattern [62]. The hd1 mutants exhibiting late flowering phenotype show lower expression levels of an essential flower timing gene *Heading date 3a (Hd3a). Hd3a* has high sequence similarity to the *Arabidopsis* FT gene [63]. *hd3a* mutants show delayed flowering under short day conditions than wild type plants. Thus, in rice, the CO/FT interaction seems to be conserved even though flowering in rice is induced by SD and not by LD conditions. It is suggested that Hd1 inhibits Hd3a expression under long day conditions and induces it under SD conditions as under SD conditions the level of expression of Hd3a is highest during day and with no expression under LD treatments [62]. As the CO-like family genes in both dicots and monocots are regulated by circadian clock, the mechanism for photoperiodic induction can be said to be evolutionarily conserved. The day-length induced changes in circadian rhythms from non-inductive to inductive period results in the activation of CO-LIKE genes which in turn regulate the FT-LIKE genes in a linear cascade and induce floral transition. Even though CO and FT may be conserved in various species, other independent parallel mechanisms are suggested to have evolved to regulate photoperiodic floral transition

CO-Independent Pathways Of Photoperiodic Induction

Different species show photoperiodic floral induction through non-CO pathways and it is suggested that these pathways have evolved independently [64]. In rice, an alternate pathway exists wherein flowering is initiated in an Hd1-independent manner by a rice flowering time locus, EARLY HEADING DATE 1 (EHD1) [64]. A B-type response regulator is encoded by Ehd1. Ehd1 does not have any functional orthologue in *Arabidopsis* and is supposed to represent a unique evolutionary adaptation as this gene has been co-opted to induce photoperiodic flowering regardless of the presence of an already conserved linear pathway. In hd1 mutants, Ehd1 is enough to induce early flowering under short day conditions but Ehd1 unlike Hd1 cannot repress flowering under long day conditions. It can be established from the presence of CO-independent pathway that

adaptation to local environments, i.e. different day lengths, can bring about changes to an already existing pathway and new gene incorporation to already existing regulatory network signifies the fine tuning to local environmental conditions.

Autonomous Pathway

The flowering in most plants occur even when the external inductive signals are absent and plants that exhibit obligate requirement of such external signals are rare. Thus, additional factors that are intrinsic to plant growth are supposed to provide signals for floral induction. Such constitutive or autonomous signals are derivative of plant's physiological outputs that determine the readiness to flower like plant age, size or number of leaves. These endogenous signals are possibly related directly to the amount of resources that are accumulated in the plant [65]. Several mutants in *Arabidopsis* flower late irrespective of inductive and non-inductive photoperiods and thus represent the autonomous pathway, different from photoperiodic pathway. But many such mutant genes respond to elements of the vernalization pathway suggesting the intersection of these two pathways at certain points. Many assumptions have been made about the nature of specific physiological elements that together create an autonomous inductive signal. The general assumption is that autonomous signal consists of combination of nutrients, particularly sucrose and phytohormones like cytokinins or gibberellins which move from leaves (and possibly roots) to SAM to induce flowering [65]. Other factors that can be part of the signal might be small proteins and RNA found in phloem sap. However, the exact nature of these components has not yet been revealed. But the studies of autonomous pathway genes in *Arabidopsis* have shown that these genes encode factors that are involved in processing of RNA and in maintaining the epigenetic state of key regulatory genes [66]. FLOWERING LOCUS C (FLC) gene in *Arabidopsis*, is common component of both autonomous and vernalization pathways and is suggested to act as essential node for signal integration. The functional equivalents of FLC have not yet been identified in other species so the universal function of FLC as global flowering repressor remains undefined. But it offers a valuable model to understand the molecular nature of floral inductive signals.

Different Floral Inductive Pathways are Integrated by FLOWERING LOCUS C

The identification of genes that are involved in the autonomous pathway in *Arabidopsis* were first recognized as mutants that flowered late regardless of day length; i.e. in both SD and LD conditions [67,68]. The first such flowering time gene isolated is, LUMINIDEPENDENS (LD) LD encodes a homeodomain protein that may bind DNA or possibly RNA. The molecular function of LD is unknown but the simplest role that can be hypothesized for LD from its sequence is a role in transcriptional repression of FLC [69]. Other genes identified were found to function through altering the expression levels of a known floral inhibitor FLOWERING LOCUS C (FLC). FLC has many positive and negative regulators that affect its transcription rate and it acts in a dosage dependent manner [70,71]. A MADS box protein is encoded by FLC that functions to maintain vegetative state by repressing several floral induction genes like SOC1 and FT [58,72,73]. Both SOC1 and FT are maintained by FLC in repressed state in a way that if FLC function is eliminated or inducers of SOC1 and FT are up-regulated, it results in floral transition. FCA and FY are the two other genes that function in autonomous pathway. These two genes interact with each other and repress FLC expression [74]. FCA has an RNA-binding domain that is supposed to interact directly with FY. This RNA binding domain shares homology to known 3-end RNA-processing proteins from *Saccharomyces cerevisiae*. The FCA–FY complex is thought to play a role in premature polyadenylation of the FLC mRNA during intron splicing and hence resulting in premature termination of the FLC transcript [66].

The flowering in autonomous pathway can also be induced by genes involved through epigenetic control. The FLC expression is repressed by FLD and FVE genes through transcriptional silencing. FLD is found to have homology to components of histone deacetylase complexes (HDAC) of animal systems [75]. It is well known that histone deacetylation is an important regulator of euchromatic gene silencing and is involved in altered histone packaging. The FVE protein contains six WD40 repeat domains, and shares sequence and structural elements with proteins from yeast and mammals that are present in complexes involved in chromatin modification. Like FLD, FVE has been shown to be involved in histone deacetylation of FLC chromatin, presumably modifying FLC transcript level in the process [76]. The FLD and FVE interfere with the transcriptional initiation of FLC and thus lower FLC expression but low level of FLC expression still occurs. FVE, FLD, FY and FCA are found to act in parallel for inactivating FLC through activities like histone methylation and polyadenylation [66]. Keeping in view that multiple mechanisms act in autonomous regulation of FLC levels, FLC can be said to function as a node in floral induction in *Arabidopsis*. Also, autonomous pathway acts through FLC-independent responses as is evidenced by the late flowering in fpa fve double mutants with flc-null background [77].

Vernalization Pathway

Temperature and particularly vernalization, exposure to prolonged period of cold, is used by plants to regulate the onset of flowering. Vernalization, thus can be viewed as a checkpoint which several plants must pass to undergo flowering [78]. It has been shown that it is the SAM that perceives the signal of vernalization as the flowering time was not accelerated when leaves from vernalization induced plants were grafted into vernalization non-induced plants [16]. Floral induction in several plants have been found to depend on subsequent photoperiodic response after their prolonged exposure to cold temperature. In this way, the precocious flowering is prevented due to a short warm period before the onset of winter conditions. Vernalization includes two separate processes, perception of prolonged

period of cold temperature and 'remembering' that perception in order to induce flowering later in the spring or summer. There are only very few plants that immediately flower on the release from the cold period, so it is clear that the 'memory' of winter is retained over many weeks and months.

The vernalization is somatically heritable state. The state induced by extended cold exposure is retained by the daughter cells from vernalization induced SAM cells [78]. In plants that need photoperiodic floral induction after vernalization, non-inductive photoperiods following vernalization leads to continuation of vegetative growth while inductive photoperiods lead to flowering of plants after an extended period post vernalization event. Vernalization results in accelerated flowering in some varieties of wheat and *Arabidopsis*, but it is supposed that genes that are involved in mediating vernalization may be different for each species [79].

Vernalization in Arabidopsis is Mediated by FLC Repression

Prolonged exposure to cold leads to decrease in the FLC transcript level in plants and this decreased expression of FLC is maintained even after the plants are transferred to warmer conditions. It has been shown that if periods of cold exposure are increased it results in increasing downregulation of FLC. It can be viewed as a quantitative effect that seems to be related to the level of initial downregulation of FLC and not to the security of maintenance of the repressed state [80]. Thus, based on maintenance of repressed state of FLC and also temporal separation between the timing of cold treatment and the onset of flowering, it has been hypothesized that the vernalization functions through epigenetic control of FLC repression.

The vernalization in *Arabidopsis* encompasses several steps that ultimately lead to stable repression of FLC. To distinguish between short cold periods and prolonged cold periods, winter plants have developed different mechanisms to respond to these, with cold acclimation to short periods and vernalization to longer periods. It has been shown that extended cold periods result in inactivation of FLC locus in *Arabidopsis* and this inactivation is mitotically heritable [81]. In non-vernalized plants, FLC chromatin is in an active conformation; i.e., the chromatin is enriched in modifications, such as acetylation of lys 9 and 14, of histone 3 (H3K9 and H3K14) and methylation of lys 4 of histone 3 (H3K4), that are hallmarks of active genes [82]. During the vernalization process, the levels of these "active" modifications are reduced, and FLC chromatin becomes enriched in methylation of lys 27 of histone 3 (H3K27) and H3K9 [83]. Methylation of H3K9 and H3K27 is a hallmark of repressed regions of chromatin, and in other eukaryotic systems these modifications lead to mitotically stable repressed states of target genes through the recruitment of repressor complexes [84]. These complexes most likely establish autoregulatory loops that propagate the silenced state throughout rounds of DNA replication. In every generation, the epigenetic state is reset ensuring the requirement of vernalization remains for each plant generation [22,85].

It is now revealed that epigenetic silencing of FLC is brought about by a multistep process. In the initial step at the FLC locus histones are hypoacetylated by VIN3, a plant homeodomain (PHD) protein [78]. It should be noted that PHD proteins function in chromatin remodeling, most probably through protein-protein interactions [86]. It has been found that mRNA of VIN3 can be detected only after extended exposure to cold temperatures and levels of VIN3 expression decreases after plants are returned to warm conditions. The histone acetylation patterns in FLC promoter does not change in vin3 mutant plants during cold exposure e.g., histone 3 acetylation remains high and there is no observable increase in the repressive chromatin modifications, H3K9 and H3K27 methylation and thus FLC expression remains unaffected. As the VIN3 is expressed only during cold periods, VIN3 is said to play role in initial inactivation of FLC but not in the repression following removal from cold conditions.

The repression of FLC is stabilized through the action of two other genes, VERNALIZATION 1 and 2 (VRN1andVRN2). These two genes are said to function in maintaining the repressed state of FLC expression and also in propagating the 'memory' of cold exposure, because vrn1 and vrn2 mutants are unable to maintain vernalization induced repressed state of FLC after returning to warm temperatures [87,88]. VRN1 specifies a nuclear localized DNA binding protein that binds DNA in non-sequence specific manner [88]. Many developing tissues show expression of VRN1 before, during and after vernalization. VRN1 acts through vernalization-dependent and independent pathway as has been evidenced by over-expression analysis. Non-vernalized over-expression lines show an accelerated floral transition without any changes in FLC levels. Over-expression of VRN1 activates FT that initiates the floral transition [88]. VRN2 specifies a zinc-finger protein, homolog of the Polycomb group protein *Suppressor of Zeste 12* (Su (z) 12). Polycomb genes play role in the stable repression of genes in *Drosophila*, but not in the initiation of the repressed state, as is the case with VRN2 [89]. Polycomb gene products in *Arabidopsis* are found to interact with each other and form multimeric complexes that function as a histone methyltransferase. A complex called, Polycomb Repression Complex 2 (PRC2)-like complex has been identified in *Arabidopsis* that contains VRN2, CURLY LEAF (an Enhance of zeste homolog), SWINGER (an E (z) homolog) and FERTILIZATION-INDEPENDENT ENDOSPERM. Also, this complex is said to interact with VIN3 and it is supposed to be involved in the initial stages of vernalization-mediated FLC repression [90].

In *Drosophila*, a second complex known as Polycomb Repression Complex 1 (PRC1) is required to maintain the repression that is initiated by PRC2. PRC1 binds to targets methylated at K27 by PRC2 via the chromodomain of one of its components, Polycomb protein (Pc) [91,92]. PRC1 components do not have any homolog in *Arabidopsis* genome [93]. Besides, in *Dro-*

sophila the Polycomb repression is linked to the methylation of H3K27, whereas epigenetic silencing of FLC by vernalization requires methylation of H3K27 and H3K9, and methylation of H3K27 alone is not associated with repression in vrn1 mutants [78,83].

It has been revealed through reverse genetics approach that LIKE HETERO-CHROMATIN PROTEIN 1 (LHP1) is required for maintaining the repressed state of FLC after cold exposure [94,95] so LHP1 is supposed to play a role in plants related to that of the PRC1 complex in animals. In *Drosophila* and mammals, the LHP1 homolog, HETEROCHROMATIN PROTEIN 1 (HP1), is not involved in Polycomb repression and immunocytochemistry reveals that HP1 is most strongly associated with constitutive heterochromatin regions of the genome that constitutively remain in a condensed state, such as the pericentromeric regions flanking centromeres. However, there was accumulating evidence that LHP1 was not involved in the maintenance of regions of the plant genome silenced from generation to generation such as pericentric regions or stably silenced epi-alleles of genes such as SUPERMAN [96] and LHP1 in plants does not localize to pericentromeric regions [97]. It has also been shown that during cold exposure LHP1 association with FLC chromatin increases, and the association is maintained in warm conditions. Also, LHP1 is not required for the initiation of H3K9 methylation, but is essential for its maintenance (and the silenced state of FLC) after plants are returned to warm [94]. Now it is shown that LHP1 in plants and HP1 in animals are both involved in maintaining gene silencing, but in plants LHP1 may be used primarily for silencing euchromatic genes, perhaps serving a role similar to that of PRC1 in animals. Besides, in plants both H3K27 and H3K9 methylation may contribute to the ability of LHP1 to maintain silencing. Interestingly, VRN1, like LHP1, is required for the maintenance of FLC silencing and is localized throughout euchromatin. Thus, these proteins may form a complex to propagate the silenced state of genes throughout cell divisions. The mathematical models elucidate the quantitative aspect of vernalization such as how plants remember the duration of cold and also how vernalization is distinguished from other developmental switches [98].

Association of Hormones and Other Factors in Floral Induction

The induction of flowering in many plant species have been found to be associated with the transmission of hormones, nutrients and other endogenous compounds to the SAM during floral transition [99,100]. But not any hormone or compound alone has been found to induce the flowering in all higher plants. The putative florigenic substance is supposed to be universal in all species and many models have been put forward to integrate physiological and genetic data but such understanding is yet in infancy [65].

Nutrient Diversion Hypothesis of Floral Induction

During floral transition, the nutrient content at the SAM changes and such changes are induced by changes in transport of sucrose and other sugars to the apex [101]. The sucrose concentration during floral transition is the base on which nutrient diversion hypothesis of floral transition depends. An increase in sucrose levels at the SAM was shown following floral inductive treatments in species like *Sinapis alba* [99,101-103]. Nutrient diversion hypothesis also associates the changes in the relative ratio of sugar and nitrogen transport to the apex [104]. Though this model is not supported by any genetic data till date but it has been shown by analysis of carbohydrate to nitrogen ratios prior to and during floral transition that the ratio is consistent under vegetative conditions and nitrogen transport becomes proportionally greater to the apex during flowering [105]. In *Fuchsia hybida*, the sucrose levels at the apex, however, change during flowering time irrespective of whether a photoperiod occurred or not [106]. It can also be possible that variations in concentrations of sucrose or other nutrients can be the outcome of floral transition and such variations are not acting as signals for inducing flowering. The concentration of sucrose at the apex does not change when LD inductive signals are applied at low levels but the plants flowered in response to the changes in day length [106] suggesting that sucrose indicates the photosynthetic activity and photo induction pathway does not simply require photosynthesis to induce flowering. Though it becomes clear from these results that changes in the nutrient concentrations at the apex is not essential for floral evocation but it is still to be unraveled if such changes do have any role in floral induction.

Gibberellin Promotion Pathway

It is now well known that gibberellic acid (GA) affects the flowering time in *Arabidopsis*. The GA application is found to result in early flowering in SD and LD and bypass the late flowering phenotype of many mutants from the other three pathways [100]. The biosynthesis, turnover and signal transduction of GA is regulated by developmental and environmental cues, of which GA concentration is most important [107]. The generation of a signal by GA1, which is one of the first step in GA pathway, converts geranylgeranyl pyrophosphate to copalyl pyrophosphate, but the signal that induce GA1 are unknown. The role of GA in floral induction is established by the GA biosynthesis deficient mutants that do not flower under SD conditions due to the absence of active GA [108]. It has been shown from mutational analysis of genes, involved in GA pathway, that it is not the GA itself but the generation of the signal that leads to floral induction [109]. The molecular targets for GA were first defined in barley and it was shown that GAMYB gene is the integral part of GA signal transduction. The effects of GA application are mimicked by over-expression of GAMYB [110,111]. GAMYB binds to GA-response element (GARE), specific DNA

sequences in promoter regions of genes that are up-regulated by GA application. Three genes similar to barley GAMYB have been found in *Arabidopsis* and among these AtMYB33 shows highest degree of similarity [112]. AtMYB33 is supposed to be involved in flowering in *Arabidopsis* and is found to bind in vitro to GARE sequence upstream of floral meristem identity gene, LEAFY [113,114]. For regulating floral induction, AtMYB33 is supposed to act in concert with other inducers of LFY like SOC1.

Two floral transition repressors, RGA (Repressor of ga1-3) and GIBBERELLIC ACID INSENSITIVE (GAI), both of which are DELLA class proteins, are affected by GA [115]. DELLA proteins possess an N-terminal DELLA domain, which is supposed to act as the target for GA-directed ubiquitination followed by consequent proteasome-mediated destruction. The importance of GAI and RGA in inhibiting the flowering is evidenced by the finding that GAI represses the expression of SOC1 but there has not been found such direct effect of GAI on LFY [116]. Both GAI and RGA function to repress the expression of the microRNA miR159, a 21-bp sequence with significant identity to the AtMYB genes [112,117]. It should be noted that microRNAs play important role in transcriptional silencing of target genes, either by binding mRNA transcripts and initiating the RNA interference response, or by binding to mRNA and preventing translation [118]. When miR159 is overexpressed, the levels of AtMYB33 decreases in leaves and results in significant delay in flowering. The lower levels of AtMYB33 are possibly the result of miRNA-mediated destruction. miR159 levels seem to vary with changes in the GA signaling pathway, as well as in response to other hormones like auxin and ethylene, which can affect DELLA protein levels [116]. AtMYB33, which contains a putative GARE motif, positively regulates miR159, proposing the probability of a negative feedback loop that prevents over-expression, and therefore premature flowering. GA is supposed to overcome SOC1 repression in *Arabidopsis* by targeting the DELLA proteins for ubiquitination, leading to protein degradation in the proteasome [116]. Floral repression is alleviated and LFY is induced through promotion of SOC1 and AtMYB33 when the GAI and RGA are degraded. It is suggested that a conserved regulatory pathway has been utilized for different developmental purposes as the GA mediated destruction of DELLA proteins and microRNA regulation and pathway of DELLA protein repression, has been found in other developmental pathways as well as other plant species.

The floral evocation can be affected by the changes in concentrations of hormones or nutrients [105]. While GA has a major influence on floral transition in *Arabidopsis*, with a complex balance of gene products that promote and repress flowering, other hormones may also have a role in flowering. The mutants for ethylene and ABA signaling do show defects in flowering time. Even though the GA promotes flowering in species different from *Arabidopsis* like *Lolium*, the flower inducing effect of GA is not universal to all plants [119]. With the presence of evidence for floral inductive effect of cytokinins in many species, an intricate model has been proposed that postulates that flowering is induced by the movement of a combination of cytokinins with sucrose to the apex [65].

Long-Distance Floral Inductive Signals

Floral inductive signals were discovered to be transferred from leaves to the shoot apex and a proposition was made by Chailakhyan (1936) that the floral inductive signal was a universal hormone because at the same time it was discovered that different features of plant growth were regulated by certain simple compounds, phytohormones that were common to all plants. The hypothetical flowering inducing substance was called, florigen and it was supposed to have specific features: (a) florigen is produced in leaves and is sent to shoot apex (b) its transport occurs only through phloem and it cannot pass through non-living tissues (c) the rate of its movement can be measured (d) it has same function in all plants i.e. it induces floral transition. The phloem-specific activity of the *Arabidopsis* CO gene provided beginning to make headway to unravel the nature of signal. The insight into the long-distance floral inductive signals was also provided by the genetic study of maize. INDETERMINATE1 gene ID1 of maize that play an important role in the regulation of flowering time, shows exclusive expression in developing leaves [120]. ID1 is the only flowering time regulator discovered so far that accumulates exclusively in leaf tissue. Severe id1 mutants remain in a prolonged vegetative state and eventually produce inflorescences with vegetative characteristics. The involvement of ID1 in generation or transmission of a leaf-derived floral inductive signal is suggested by its unique spatial and temporal expression pattern [120,121]. The ID1 like other flowering time genes from different plants encodes a zinc-finger protein that has DNA-binding activity [122]. Though genes with high level of similarity to maize ID1 are found in all higher plants, none has been found to play role in flowering. The biochemical and molecular nature of floral stimulus can be understood by identifying the targets genes of ID1. It is also suggested that microRNAs isolated from phloem sap may be involved in transmitting the information from one part of the plant to another [123] which opens up the interesting assumption on the nature of florigen [124]. miRNA-based floral stimulus also might fit most of the criteria for florigen described earlier. FT protein has emerged as the candidate for a universal florigen however additional or alternate signals cannot be ruled out. There are convincing evidences which support gibberellins acting as mobile florigen signals in some circumstances. Sucrose and cytokinins are also suggested to be associated with some components of the inductive process [125].

Integration and Instigation of the Floral Transition

Notwithstanding the origin of the signal, the ultimate purpose of floral induction is to convert the SAM from a vegetative to a reproductive state [126]. It is thought that the flowering time pathways converge on a set of genes called,

flowering-time integrators. One can think of pathways converging on the activity of the floral repressor, FLC, but FLC is situated immediately upstream of the point of convergence. As FLC activity is moderated by some pathways and bypassed by others, the true point of convergence can be suggested at the genes which FLC down regulates itself. Many floral pathways which perceive different developmental and environmental stimuli have been found to converge to few integrators of flowering like FLOWERING LOCUS T (FT), AGAMOUS-LIKE 24 (AGL24), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1) in *Arabidopsis*. [127-129,131]. In autonomous pathway, FLC is downregulated through RNA binding and chromatin remodeling and thus flowering-time integrators are activated. In vernalization promotion pathway, FLC is downregulated by VIN3 and is maintained in non-transcribed state by VRN1 and VRN2; thus flowering-time integrators are activated. FLC is bypassed by photoperiodic promotion pathway and flowering time-integrators are activated through the circadian clock-regulated CONSTANS (CO) expression. Gibberellin also bypass the FLC and directly activate the flowering-time integrators. Thus, it can be said that all pathways converge on the activity of a small subset of flowering-time integrator genes, either directly or through FLC. At a given time, flowering-time genes can be found to face some degree of inhibition due to FLC activity and some degree of activation due to CO activity and/or gibberellin-induced signals. So, flowering-time integrators will express and floral transition will occur only when activation signals are stronger than FLC-based inhibition.

Role of Flowering-Time Integrators

The flowering-time regulators act either directly or indirectly as transcriptional regulators and activate expression of subset of genes called, floral meristem identity genes (FMI), that operate at the SAM to make it determined to produce flowers. The activity of each of the flowering-time integrators is based around the expression of these 'floral meristem identity genes' (FMI genes). Not only is the regulatory activity of each flowering-time integrator slightly different, but the output pathways that lead into them vary slightly as well [130].

FLOWERING LOCUS T (FT)

Koornneef et al. [68] identified one of the original late flowering lines, the *ft* mutant, which showed normal expression of LEAFY (LFY), but lfy and ft double mutant exhibited significantly reduced expression of the floral meristem identity gene APETALA1 (AP1). In 1999 isolation of FT was carried out by two independent research groups. Kardailsky et al. [131] in 1999 developed activation tagged lines of plants wherein an enhancer of transcription was transformed randomly into their genome. They found an early flowering lines having an insertion mapping to the same place as the FT locus and used the insertion for isolating the gene. The other research team headed by Kobayashi et al.[132] in 1999 isolated FT locus by using T-DNA insertion line. The protein encoded by FT locus shows similarity to the membrane bound mammalian proteins that bind hydrophobic ligands. Thus, this protein is believed to be involved in signal transduction. The expression of FT occurs mainly in the leaf and is then transported to shoot apical meristem (SAM) where it induces the floral transition by interacting with FLOWERING LOCUS D (FD). FT expression has been found to be regulated by several transcription factors in response to different stimuli. CRYPTOCHROME-INTERACTING BASIC–HELIX–LOOP–HELIX1 (CIB1), CONSTANS (CO), Morf-related Gene 2 (MRG2), WRKY71 and PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) directly activate the transcription of FT [133-137]. Direct repression of FT is carried by TEMPRANILLO (TEM) 1 and 2, SHORT VEGETATIVE PHASE (SVP), TARGET OF EAT (TOE) 1 and 2, EARLY-FLOWERING MYB PROTEIN (EFM), CYCLING DOF FACTOR1 (CDF1), SCHNARCHZAPFEN (SNZ) and SCHLAFMUTZE (SMZ) [138-141]. So, FT acts as an important member in controlling the flowering time in *Arabidopsis*.

The expression of FT is induced by photoperiodic induction pathway while FLC represses its expression. The autonomous promotion pathway and vernalization induce FLC expression indirectly while there is no indication of operation of gibberellin promotion pathway through FT. Kobayashi et al. [132] provided the first evidence of FT induction by photoperiodic pathway. This group of researchers fused a rat glucocorticoid receptor to CO and ectopically expressed the construct in *Arabidopsis*. The expression of FT showed rapid induction when CO activity was induced by treatment of steroid. CO is found to induce FT expression only during daylight which suggests photoperiodic control to the system [50]. The inhibitory signals from FLC are received by FT at the same time. The FT transcripts are not found in the transgenic plants in which FLC is ectopically expressed [73]. The FT transcription is believed to be inhibited when the FLC protein binds to the first intron of FLC as multi-protein complex [142]. The FT protein has been found to induce flowering when it is transferred from induced leaves to shoot apical meristem.

LEAFY and APETALA1

The severe lfy and ap1 mutants of *Arabidopsis* show a phenotype where floral organs are replaced by vegetative characteristics [143-145]. All higher plants have a unique transcriptional factor, LFY, which directly targets floral meristem identity genes such as, AP1 and CAULIFLOWER (CAL). LFY plays an important role in integration of floral inductive signals from different pathways and activation of floral meristem identity genes [146]. LFY is regulator of AP1 and a direct positive feed-back interaction of LFY and AP1 determines the commitment to flower. The transcription of AP1 leads to the activation and regulation of expression of specific floral meristem identity, assuring the floral transition [147]. The floral transition is influenced by gibberellins through the regulation of SOC1 [148]. From multiple pathways signals are integrated and outcome transmitted by

SOC1 to LFY [149]. SOC1 is suggested to function at least partly through a positive feedback loop wherein AGAMOUS-LIKE 24 (AGL24) is supposed to be involved upon dimerizing with SOC1 [150].

Conclusion and Future Perspectives

Lot of research has been done to understand the underlying mechanism of the floral induction in different flowering plants. First the focus was laid on the physiological experiments to understand the major cues which lead to the floral induction and then attention was shifted to the molecular approaches to know the key genetic players involved and their possible role in this process. External stimuli have been found to be important for the plants to undergo flowering, but in most plants flowering can occur in absence of such stimuli, suggesting intrinsic factors to plant growth provide the signals for the floral induction. Multiple complex pathways regulate the process of floral induction and many key genetic players have been discovered which are either specific to a floral induction pathway or are playing their role in other floral induction pathways also; suggesting the possible intersection of different floral induction pathways. The regulation of these pathways occurs at genetic as well as the protein level. microRNAs (microRNAs) regulate the key genes involved in floral induction both at transcriptional and post transcriptional levels. The pathways involved in floral induction have several nodes which signify the site of signal transduction and the pathways are integrated that result in a coordinated initiation of flowering. Despite lot of research going on, the full mechanism operative in floral induction is not yet clear. It is not fully understood that how the known floral regulators are integrated into flowering gene network and what are their target genes and mechanism of activity. Also, how the floral induction is controlled by the collective action of transcription factors, epigenetic regulators, hormones and small RNAs, is yet to be fully unraveled. The future research can target such aspects by employing novel molecular approaches like genomics, transcriptomics, proteomics and targeted genome engineering approaches like CRISPR/CAS system, to establish and perfect the molecular mechanism of floral induction.

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