PHOTOMORPHOGENESIS

Light is an important environmental factor which controls growth and development in plants. Besides photosynthesis in which light is harvested by green plants and is converted- into chemical energy, there are numerous other plant responses to light such as phototropism, germination of some light sensitive seeds e.g. lettuce, de-etiolation of monocot and dicot seedlings etc., which are quite independent of photosynthesis and in which light just acts as environmental signal to bring about the particular photo-response.

Most of these photo-responses control **genetically defined *structural development*** or morphogenesis (i.e., origin of form) of plants. The role of light in regulating morphogenesis is known as photo-morphogenesis. In plants, red and blue light are especially effective in inducing a photo-morphogenetic response.

The effect of light in controlling morphogenesis can best be demonstrated by comparing a monocot (maize) or dicot (bean) seedling grown in light with one grown in darkness both of which have been reared from genetically identical seeds. Abundant reserve food in seeds eliminates the need for photosynthesis for many days.

It can easily be noticed that **dark grown** seedling has become **etiolated** (i.e., pale and weak) while the one grown in **light** has **stockier and green appearance** with short stem and large leaf area. Since both etiolated and light grown seedlings were reared from genetically

identical seeds, light must have altered the gene expression during germination so that the appearance or form of etiolated and light grown seedlings looks different.

De-etiolation of light grown seedling can be done in very short period (hours) by placing it even in dim light. During de-etiolation, marked reduction in the rate of stem elongation, straightening of apical hook and development of green pigments can easily be noticed. The etiolated form of the seedling is thus gradually transformed to stockier green appearance and is the result of photo-morphogenesis. ***The development of seedling in darkness is called as skoto-morphogenesis*** (from Greek word Skotos = darkness).

Fig: It can easily be noticed that dark grown seedling has become etiolated while the one grown in light has stockier and green appearance with short stem and large leaf area. De-etiolation of light grown seedling can be done in very short period (hours) by placing it even in dim light. During de-etiolation, marked reduction in the rate of stem elongation, straightening of apical hook and development of green pigments can easily be noticed.

According to Hans Mohr (1983), there are two important stages of photo-morphogenesis:

(i) ***Pattern specification***, in which cells and tissues develop specific ability or competence to respond to light during certain developmental stage and

(ii) ***Pattern realization***, during which time the photo-response occurs.

There are two main categories of plant responses to light signals:

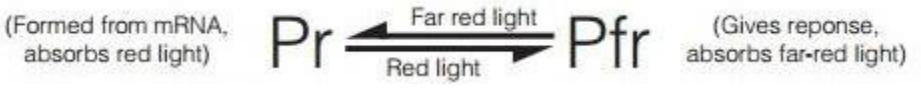
(i) **Phytochrome** mediated photoresponses and

(ii) Blue-light responses or **cryptochrome** mediated photo-responses.

PHYTOCHROME

Phytochrome is synthesized as a protein, **Pr**, able to absorb ***red light*** (666 nm). When it absorbs red light, it converts to a **Pfr** able to absorb ***far-red*** light at **730 nm** (that converts it back to Pr). Many phytochrome responses show '**red–far red reversibility**' – when a process has been activated by a short period of red light, it will be stopped or reversed by a subsequent pulse of far-red light. *Phytochrome is a protein made up of two identical sub-units, in total sized 250 kDa. Each monomer* (sub-unit) has a pigment (**chromophore**) molecule attached to it through an -S- (thioether) bond to the amino acid cysteine. When the chromophore absorbs red light, its structure alters slightly and this alters the conformation of the protein initiating events which ultimately results in altered gene expression.

A multi-gene family of phytochromes has been identified in ***Arabidopsis***, with five members, ***PHYA***, ***PHYB***, ***PHYC***, ***PHYD*** and ***PHYE***. These can be subdivided into two types of phytochrome: ***PHYA*** encodes **type 1 phytochrome**, which is the most abundant form in etiolated seedlings; ***PHYB–E*** encode the **type II phytochrome** which is synthesized at much lower rates.Transcription of the*PHYA* gene is regulated by **negative feedback** in red light(which causes the formation of Pfr); so when an etiolated seedling (with highlevels of type 1 phytochrome) is exposed to light, production of type 1 is greatlyreduced as one part of photomorphogenesis (*Fig. 3*). In addition, type 1 Pfrphytochrome is very sensitive to proteolysis, so the level of the protein quicklyreduces when it is not being newly synthesized. Transcription of the *PHYB–E* genes is not sensitive to light, and type II phytochrome is much less sensitive to proteolysis, so it remains more or less constant in the plant.



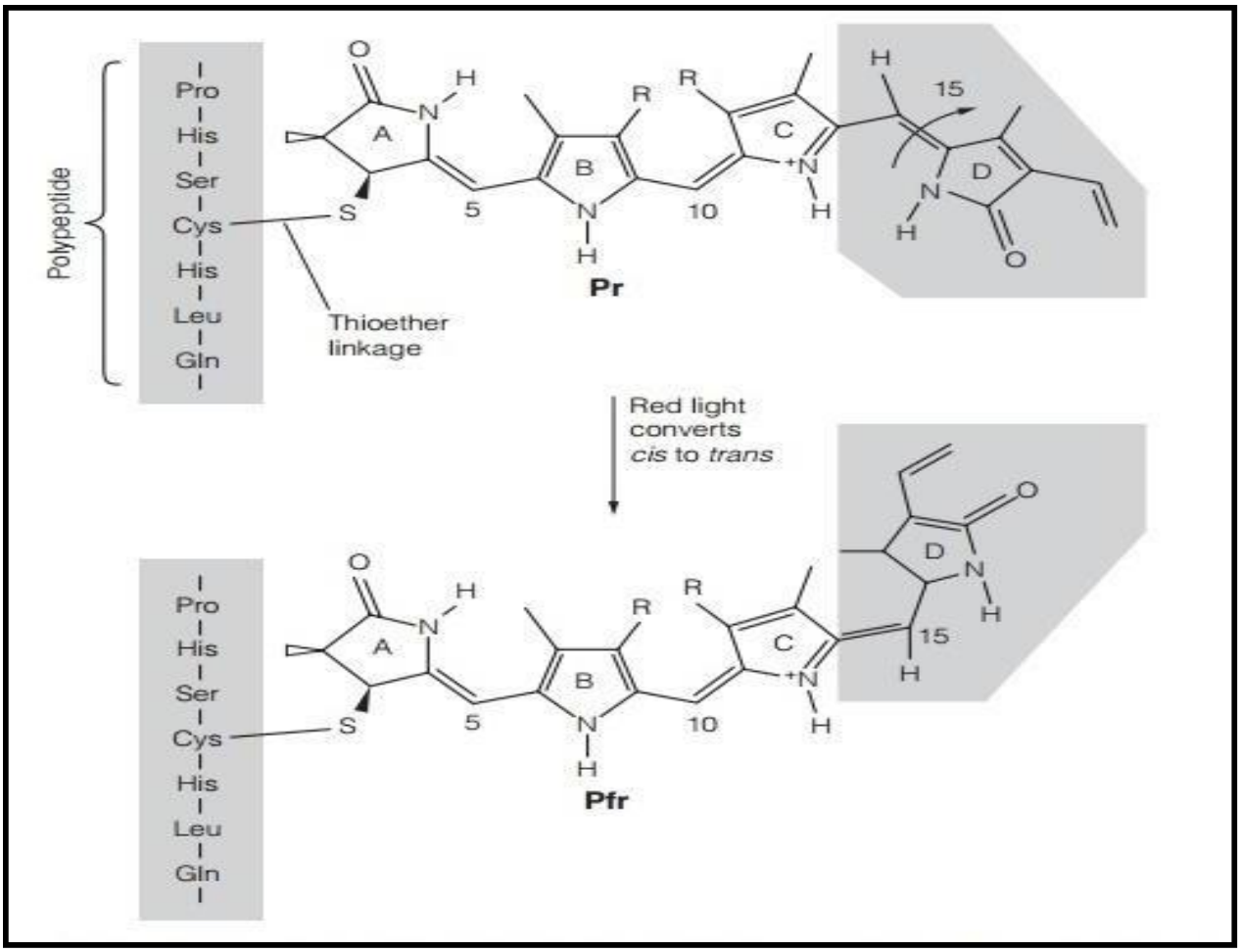


Fig: Structure of Pr and Pfr forms.

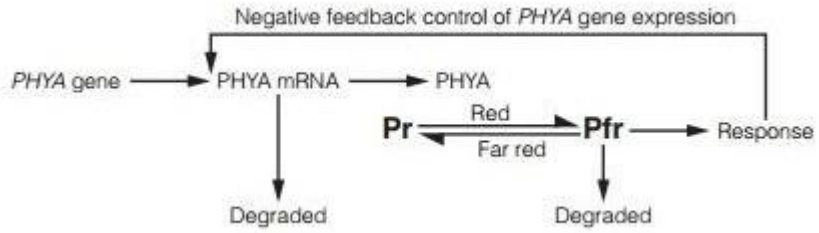


Fig: ***PHYA*** encodes **type 1 phytochrome**, which is the most abundant form in etiolated seedlings and very sensitive to proteolysis. ***PHYB to E*** encode the **type II phytochrome** which is synthesized at much lower rates and less sensitive to proteolyis.

Phytochrome responses: The **red:far red ratio** of light changes in different environments and through the day. Daylight, for instance, has an R:FR ratio of 1.19, while at sunset it is 0.96 and under a leaf canopy can be 0.1. Light intensity also varies throughout the day. Phytochrome is involved in a wide range of plant responses to light, including:

●**Etiolation**, in which a seedling or organ rapidly elongates without the production of chloroplasts until it receives red illumination, whereupon **deetiolation** occurs and functional chloroplasts are produced.

●**Circadian rhythms**. A number of plant processes, including metabolism and leaf positioning follow a periodic cycle of 24 h. The phytochrome response ensures synchrony of the rhythm with daylength.

●**Seed germination**. Many seeds are stimulated to germinate by light in a phytochrome-mediated response. This may require only brief irradiation or prolonged illumination, depending on species. Other seeds (such as wild oat) show germination inhibited by light, though this requires intense irradiation over long periods, and is unlikely to involve phytochrome.

CRYPTOCHROMES

Cryptochromes are receptors for blue and ultraviolet (UV-A) light that share sequence similarity to DNA photolyases, DNA-repair enzymes that use blue light to repair UV-induced DNA damage by removing pyrimidine dimers from DNA. There are two types of DNA photolyase, which repair different types of damage: CPD photolyases repair cyclobutane pyrimidine dimers (CPDs), and 6-4 photolyases repair 6-4 pyrimidine pyrimidone photoproducts. These photolyases together with the cryptochromes make up the photolyase/cryptochrome superfamily.

It was initially thought that only higher eukaryotes had cryptochromes and that prokaryotes had photolyases but not cryptochromes, but further searches of the more recently available genome databases revealed the presence of a cryptochrome gene in cyanobacteria (*Synechocystis*). This new type of cryptochrome was referred to as CRY-DASH, to underscore its relationship with cryptochromes found in *Drosophila*, *Arabidopsis*, *Synechocystis*, and *Homo* (although CRY-DASH itself is not found in *Drosophila* or humans). CRY-DASH proteins have been found not only in the photosynthetic cyanobacteria but also in non-photosynthetic bacteria, fungi, plants and animals, including *Arabidopsis*, *Neurospora*, zebrafish, and *Xenopus*. The biological function of CRY-DASH proteins remains unknown at present.

Cryptochromes show an overall structural similarity to DNA photolyases, despite the fact that cryptochromes possess no photolyase activity. Most cryptochromes, with the exception of CRY-

DASH proteins, are composed of two domains, an amino-terminal photolyase-related (PHR) region and a carboxy-terminal domain of varying size. The PHR region of cryptochromes appears to bind two chromophores, cofactors that absorb light; one chromophore is flavin adenine dinucleotide (FAD) and the other 5,10-methenyltetrahydrofolate (pterin or MTHF). Photolyases also have FAD, and the second chromophore can be either pterin or deazaflavin. The carboxy-terminal domain of cryptochromes is generally less conserved than the PHR region; it is longer in most plant cryptochromes than animal cryptochromes, and CRY-DASH proteins lack this domain.

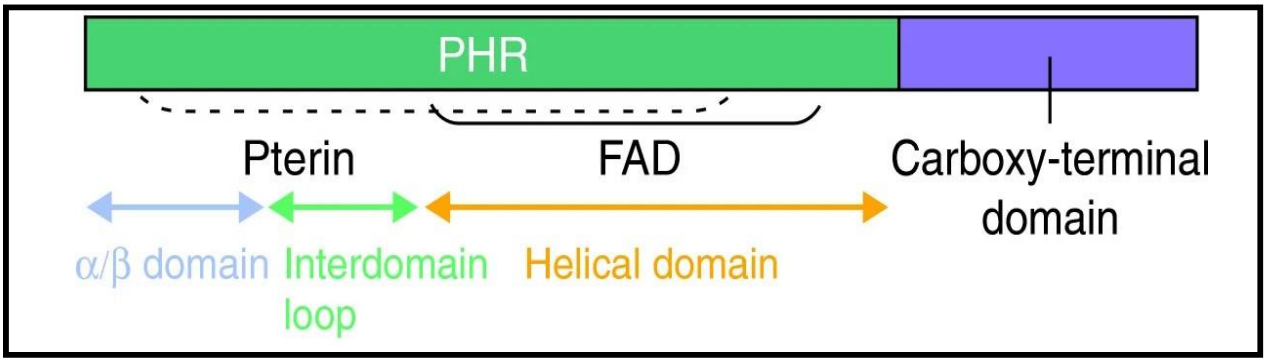


Fig: Structure of cryptochrome.

Function

Arabidopsis CRY1 and CRY2 are predominantly nuclear proteins that mediate regulation of gene expression and entrainment of the circadian clock in response to light. CRY1 and CRY2 play major roles in plant photomorphogenesis, such as inhibition of stem elongation by blue light, stimulation of leaf expansion by blue light, and regulation of floral initiation by day length. It appears that cryptochromes control developmental changes in plants via changes of gene expression in response to light. CRY1 and CRY2 together are responsible for blue-light-dependent changes in gene expression of up to 10-20% of the *Arabidopsis* genome.

There are at least two mechanisms by which cryptochromes may affect nuclear gene-expression changes in response to light. First, a cryptochrome molecule may interact with proteins associated with transcriptional machinery to affect transcription directly. *Arabidopsis* CRY2 binds to chromatin in a DNA sequence-independent manner and M. Maymon and C.L., unpublished observations), but it is unclear how a sequence-independent chromatin-interacting protein may regulate gene expression. Unlike the animal cryptochromes that have been shown to

regulate transcription via physical interactions with promoter-binding transcription regulators, no such interaction has been reported for plant cryptochromes. An alternative model is that plant cryptochromes may interact with proteins exerting other cellular functions to regulate the stability, modification, cellular trafficking of the transcriptional regulators. For example, plant cryptochromes have been found to interact with an E3 ubiquitin ligase, COP1, suggesting that plant cryptochromes may act in the way not yet discovered for the animal cryptochromes. Consistent with this view, it has also been found recently that *Arabidopsis* cryptochromes mediate suppression by blue light of the proteasome-dependent degradation of an important floral regulator, CONSTANS. Exactly how cryptochromes do this needs to be investigated further.

Mechanism

The catalytic mechanism of cryptochromes has not been fully elucidated, but some clues can be found in the mechanism of CPD (Cyclobutane pyrimidine dimer) photolyases, where FAD plays the main catalytic role. In a DNA-repair reaction, CPD photolyase binds to the pyrimidine dimer of DNA and 'flips' it out from within the DNA duplex into the FAD-access cavity of the enzyme, to form a stablecomplex. The other chromophore (pterin or deazaflavin), which is also called the 'antenna' chromophore, absorbs photons of blue or UV-A light, and it transfers the excitation energy to the flavin of FAD. Flavin in the excited state donates an electron to the pyrimidine dimer to split the cyclobutane ring. The electron is transferred back to flavin in this process, resulting in regeneration of groundstate flavin. The repaired dinucleotide no longer fits into the FAD-access cavity, so it disassociates from the photolyase. The exact role of FAD and the FAD-access cavityin the function of cryptochromes remains unclear, but it is conceivable that it may also be involved in electron-transfer reactions.

Although the PHR region that contains the chromophore(s) is the most conserved part of the proteins, the carboxy-terminal domain has been shown to have a role in the function or regulation of both animal and plant cryptochromes. Expression of the carboxy-terminal domains of *Arabidopsis* cryptochromes fused to the marker enzyme b-glucuronidase confers a constitutive growth response to light even in darkness in the absence of the PHR region. In contrast, the PHR regions of the *Drosophila* and *Xenopus* cryptochromes are physiologically active in the absence of the carboxy-terminal domain. The carboxy-terminal domain of *Drosophila* Cry is important

for protein stability, interaction with Tim, and sensitivity of the photoreceptor to circadian light signals, whereas the carboxy-terminal domain of *Xenopus* Cry is required for its nuclear localization.

Cryptochromes are regulated by phosphorylation. It has been shown that *Arabidopsis* cryptochromes are phosphorylated in response to blue light and that this is associated with the function and regulation of the photoreceptors. Moreover, when *Arabidopsis* CRY1 was expressed in insect cells, it was found to undergo ATP-dependent and blue-light-dependent autophosphorylation. It is not known whether animal cryptochromes also bind to ATP, although it has been shown that mouse cryptochromes are phosphorylated.

The interaction between the *Arabidopsis* CRY1 PHR region and ATP has a few interesting features reminiscent of the interaction between pyrimidine dimer and photolyase: the phosphate groups of ATP are exposed to solvent; the adenine and ribose moieties are buried deep within the FAD-access cavity; and ATP can have a water-mediated contact with FAD. The interaction of the *Arabidopsis* CRY1 pHR region with ATP also lacks several features commonly found in protein-ATP interactions, such as protein-to-phosphate interaction, protein-to-Mg^{2+} contact, and a nearby serine residue for phosphotransfer [15]. An examination of the topology of the CRY1 PHR region structure shows, however, that all these features could potentially be provided by the carboxy-terminal domain of the cryptochrome. The observation that the serine-rich carboxy-terminal domains of *Arabidopsis* cryptochromes fused to β-glucuronidase are constitutively phosphorylated *in vivo*, suggests that a phosphotransfer may occur from ATP bound to the FAD-access cavity to the nearby carboxy-terminal domain. It is also conceivable that photon-excited FAD may trigger electron transfer to the nucleotide and phosphotransfer from ATP to serine residues on the carboxy-terminal domain. Because the surface of the PHR region is predominantly negatively charged, especially in the place where the carboxy-terminal domain is likely to interact with it, the phosphorylated carboxy-terminal domain would then be repelled from the PHR region surface, resulting in a change of cryptochrome conformation. This conformational change would allow it to interact with other signaling proteins and to propagate the light signal. Alternatively, another molecule of cryptochrome binding to the FAD-access cavity may also provide the missing features needed for a productive ATP-cryptochrome interaction. Indeed, both CRY2-CRY2 interaction and CRY1-CRY2 interactions can be detected

in *Arabidopsis* (D. Shalitin, X. Yu, and C.L., unpublished observations). Formation of either a homo-oligomer or a hetero-oligomer of cryptochromes would provide a mechanism for intermolecular phosphotransfer, which may change the structure of the cryptochromes.

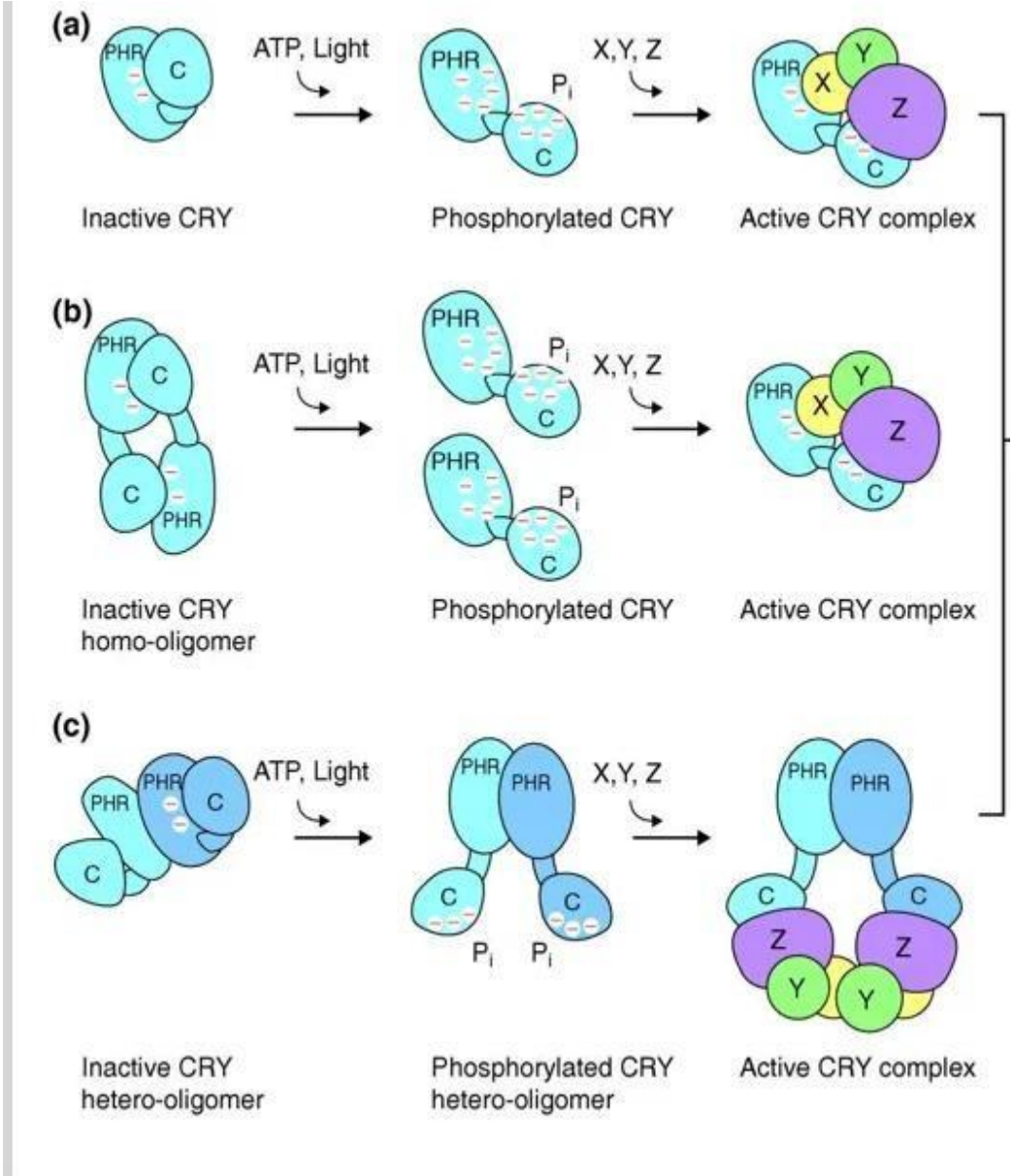


Fig: There are three possible ways for activation of CRY that finally leads to maintenance of Circadian biological clocks and initiation of flowering: **(a)** One model is that phosphorylation of the carboxy-terminal domain in response to light is performed by ATP bound to the PHR region; this leads to dissociation of the two domains. **(b)** A second possibility is that phosphotransfer in response to light involves the interaction of two cryptochromes encoded by the same gene. **(c)** Alternatively, intermolecular phosphotransfer could involve the interaction of different cryptochromes. All three scenarios may exist in plant cells, and the activity of a cryptochrome may be determined by the kinetics of the different reactions.

PHOTOTROPINS

Light is one of the most important environmental cues controlling plant development, and is achieved through a suite of photoreceptor proteins. Like photoreceptors associated with our vision, plant photosensors can detect the presence, intensity, direction and color of light, and in turn, utilize this information to direct their growth. To date, four different types of photoreceptors have been identified in plants. Among them is a small family of proteins known as the phototropins, which are activated specifically by UV/blue wavelengths of light. The photoactivation of these proteins stimulates a range of processes that ultimately optimize the photosynthetic efficiency of plants, including phototropism, after which they were named. For instance, phototropins direct the movement of chloroplasts (Greek for "green maker"), which represent the heart of the photosynthetic machinery as their position within the cell can greatly affect the efficiency of energy production. Likewise, chloroplasts reside in leaves, which can be viewed simply as solar panels. Leaf positioning and expansion is also directed by the phototropins. Additionally, phototropins control the opening of stomata pores in the leaf epidermis, which regulate gaseous exchange. Stomatal opening is important for energy production, as it allows CO2 uptake for photosynthesis. Collectively, these responses serve to enhance the photosynthetic performance of plants and maximize their growth potential. Many plant species are able to track the movement of the sun by a process known as heliotropism (Greek for "towards sun"). This photomovement response is also likely mediated by phototropins.

Despite extensive attempts, the molecular identity of the blue light-absorbing photoreceptor responsible for phototropism remained elusive until relatively recently, owing to the availability of genetic methods using the model plant *Arabidopsis thaliana. Arabidopsis* or thale cress, as it is more commonly known, is not the most exciting plant to look at. But its small size and short lifecycle, combined with its plentiful seed production, make it an ideal genetic tool for laboratory work. More importantly, *Arabidopsis* can be manipulated easily to generate mutants that show altered characteristics. It was the isolation of *Arabidopsis* mutants altered in phototropism that eventually led to the cloning and characterization of the first phototropin gene.

Phototropin Structure and Light Sensing

The structure of plant phototropins can be separated into two parts: a N-terminal photosensory input region coupled to a C-terminal effector or output region that contains a classic serine/threonine kinase motif. The N-terminal region comprises two so-called LOV domains, each of which binds the vitamin-B derived cofactor flavin mononucleotide (FMN) as a blue light-absorbing chromophore. LOV domains exhibit protein sequence homology to motifs found in a diverse range of eukaryotic and prokaryotic proteins involved in sensing Light, Oxygen, or Voltage, hence the acronym LOV.

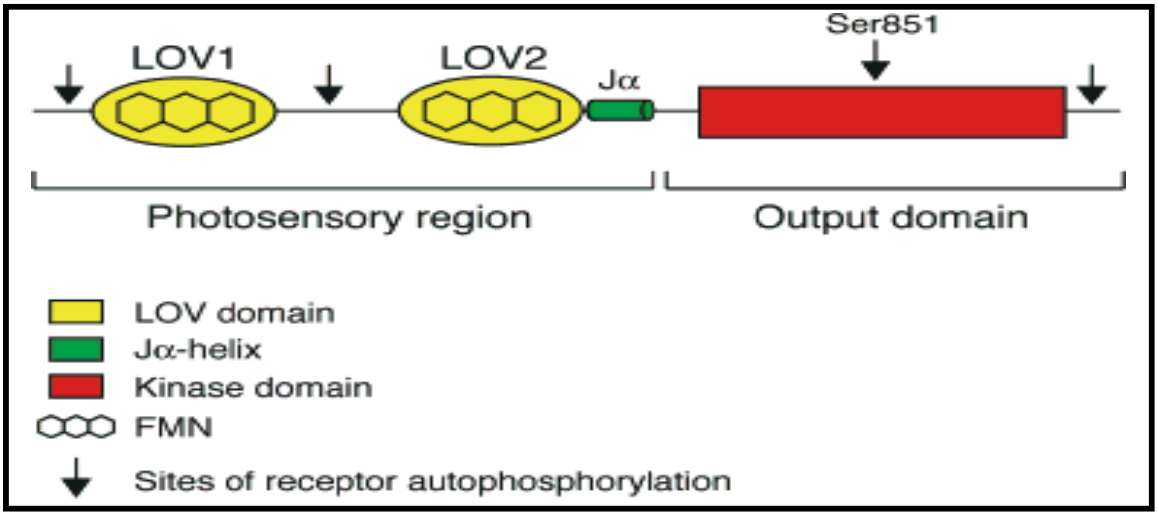


Fig: structure of phototropin blue light receptors

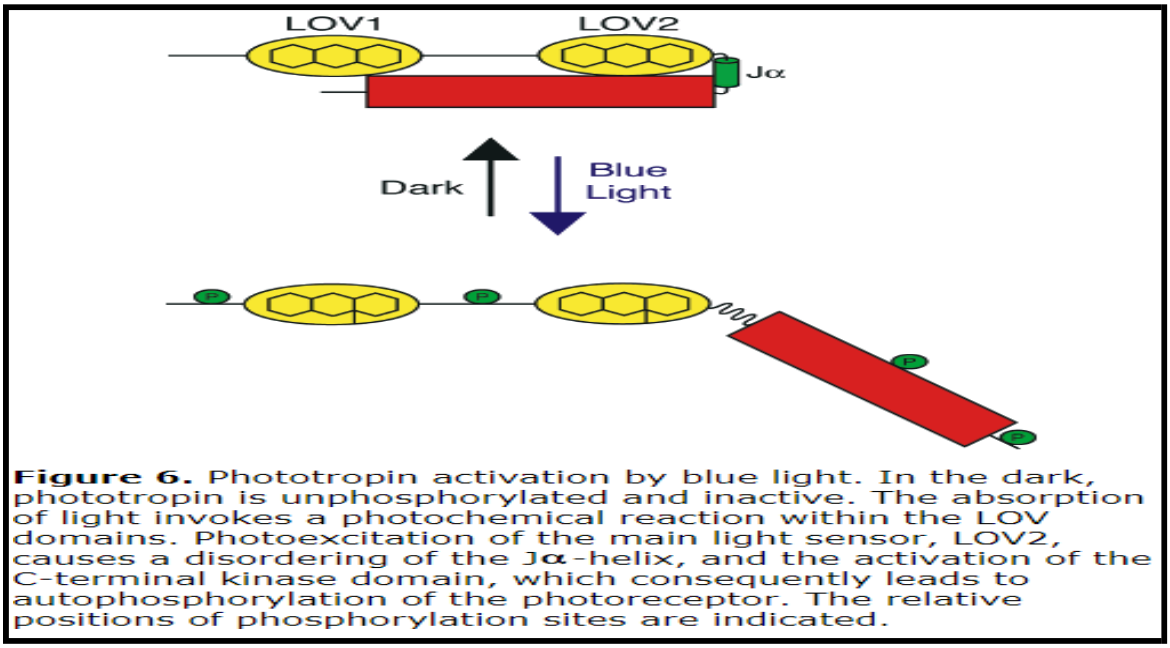


Figure 6. Phototropin activation by blue light. In the dark, phototropin is unphosphorylated and inactive. The absorption of light invokes a photochemical reaction within the LOV domains. Photoexcitation of the main light sensor, LOV2, causes a disordering of the Jα-helix, and the activation of the C-terminal kinase domain, which consequently leads to autophosphorylation of the photoreceptor. The relative positions of phosphorylation sites are indicated.

PHYSIOLOGY OF FLOWERING

About 90% of the ≈ 350,000 known plant species are the flowering plants. Flowering is the most enigmatic phase in the life of a plant. It provides a mechanism to plants for genetic outcrossing which provides a means of securing a greater variety of genetic recombination. Flowers are specialized structures which differ extensively from the vegetative plant body in form and cell types. Numerous physiological and biochemical changes take place within the shoot apex when it prepares itself for transition into floral bud. The precise time of flowering is important for reproductive success of the plant. Plants need to sense when to produce flowers so that fruit and seed development can be attained which will ensure its survival in the next season. Synchronous flowering is significant in outcrossing plants. Since long, people have wondered how plants are able to flower in a particular season. Plants possess the ability to anticipate and sense change of seasons. It has always been a fundamental question as to how environmental signals influence flowering and how these signals are perceived.

Photoperiodism: The Light-Dependent Pathway

Flowering is so predictable in plants that it is used as a floral calendar. As we know that roses bloom in summer and chrysanthemums in winter. It is generally the length of day that gives the most reliable indication of advancing season. An organism's capacity to measure day length is known as **photoperiodism.** Initial experiments on photoperiodism were conducted by a French scientist J. Tournois in 1912. He observed that *Cannabis* plants flower vigorously when planted early in the spring but remain vegetative if planted in late spring or summer. He concluded that shortening of day length was not as important for early flowering as lengthening of night. At about the same time, George Klebs from Germany demonstrated that *Sempervivum funkii* could be induced to flower in winter in greenhouse when exposed to artificial light although normal time is June. First clear-cut hypothesis of photoperiodism was given by W.W. Garner and H.A. Allard from the US Department of Agriculture (Beltsville, Maryland) in 1920. They observed that *Biloxi* soybean flowers around same time in September/October even if it is germinated over a 3-month period from May to July, i.e., irrespective of how long they have been growing, they flower around same time. Garner and Allard hypothesized a seasonal timing mechanism in soybean. They also observed flowering response of tobacco (Maryland strain) which normally flowers in summer. A mutant of the

plant called Maryland Mammoth was observed to grow up to the height of 3–5 m in summer without any flowering. The plants growing in green house under relatively short photoperiods flowered profusely in mid-December when the relative length of day was shorter than the length of the dark period. The mutants could be made to flower when exposed to short-day length next year in summer by placing the plants in darkness after placing the plants in light equivalent to that of winters. These observations lead to the discovery of the phenomenon as well as for the coining of the term "photoperiodism" by Garner and Allard. These observations also lead to the fact that plants vary considerably in their response to day length.

Critical Day Length

On the basis of photoperiodic requirement for floral induction, plants have been classified under different categories. **Short-day plants (SDPs)** will flower if the day length is shorter than a critical photoperiod. Hillman (1959) showed that SDPs are capable of flowering even if kept continuously in dark provided with adequate sucrose. This shows that the SDPs require light only for carrying on photosynthesis. Examples of SDPs are soybean, poinsettia, potato, sugarcane, cosmos, chrysanthemum, etc. **Long-day plants (LDPs)** require a photoperiod of more than a critical length which varies from 14 to 18 h. The best flowering usually occurs in continuous light. A flash of light during a long dark period can induce flowering even under short- day conditions. Since dark phase has inhibitory effect on flowering, these plants can also be called as *short-night plants*. Examples of this category are spinach, lettuce, radish, alfalfa, sugar beet, larkspur, etc. The critical value of the photoperiod requirement is not absolute rather varies according to species. In **day-neutral plants**, flowering is not affected by day length. For example, tomato, cucumber, cotton, pea, and sunflower. Within this category, there are obligate or facultative requirements for a particular photoperiod. Plants having absolute requirement for a particular photoperiod for flowering are called **qualitative photoperiod** types. For example, *Xanthium strumarium* does not flower unless it receives an appropriate short photoperiod. It is a qualitative SDP. In quantitative SDPs, flowering is accelerated by short days, e.g., *Cannabis sativa* (hemp) and *Helianthus annuus* (sunflower). Spring cereals, like *Triticum aestivum* (spring wheat) and *Secale cereale* (winter rye), are quantitative LDPs. They do flower under short days, but flowering is accelerated under long days. Qualitative LDPs include *Hyoscyamus niger* (black henbane) and *Arabidopsis thaliana*. Photoperiod requirement is often modified by external conditions like temperature. There are

also other response types in which plants respond to long and short days in some combination. Thus, *Bryophyllum* is a **long-short-day plant**. It flowers when a certain number of short days are preceded by a specific number of long days. *Trifolium repens* exhibits a reverse condition of **short-long-day plant**. Some plants, like winter cereals, require a low temperature treatment before they become responsive to photoperiod, while others may have a qualitative photoperiodic requirement at one temperature but a quantitative requirement at another temperature. Some plants are **intermediate-day length** plants. They flower in response to day length of intermediate range but remain vegetative when the day is too long or too short. Interestingly, flowering is delayed in *Madia elegans* under intermediate-day length (12–14 h) but occurs under day length of 8 or 18 h. It may be noted here that this classification is based on whether a particular plant will flower when subjected to photoperiod that exceeds or is less than a critical length.

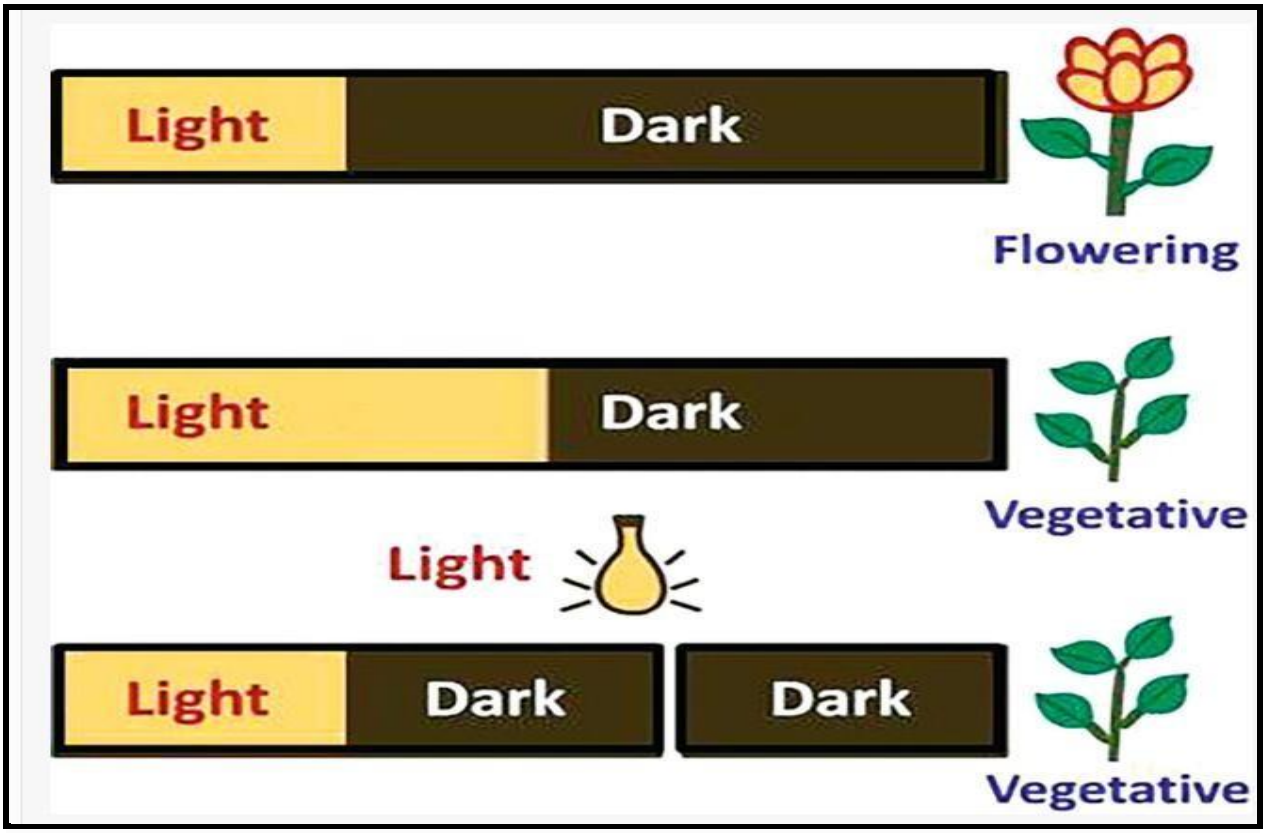


Fig: Photoperiodic control of flowering. Decrease in critical dark period leads to vegetative stage. Also a flash of light during dark period inhibits flowering.

Critical Role of Dark Period

Plants neither measure relative length of day and night nor the length of photoperiod. They measure the length of dark period. This was demonstrated by K.C. Hammer and J. Bonner (1938) in experiments conducted with *Xanthium*. In a 24 h cycle of light and dark periods, *Xanthium* flowers only when dark period exceeds 8.5 h but remains vegetative when provided with 16 h of light followed by 8 h of dark (Fig. 25.4). Similarly, long-day plants require a dark period shorter than some critical maximum. In LDPs, a flash of light in the middle of an otherwise noninductive long dark period will shorten the dark period requirement to less than the maximum and permit flowering to occur. Measuring the time of dark period is central to photoperiodic time keeping.

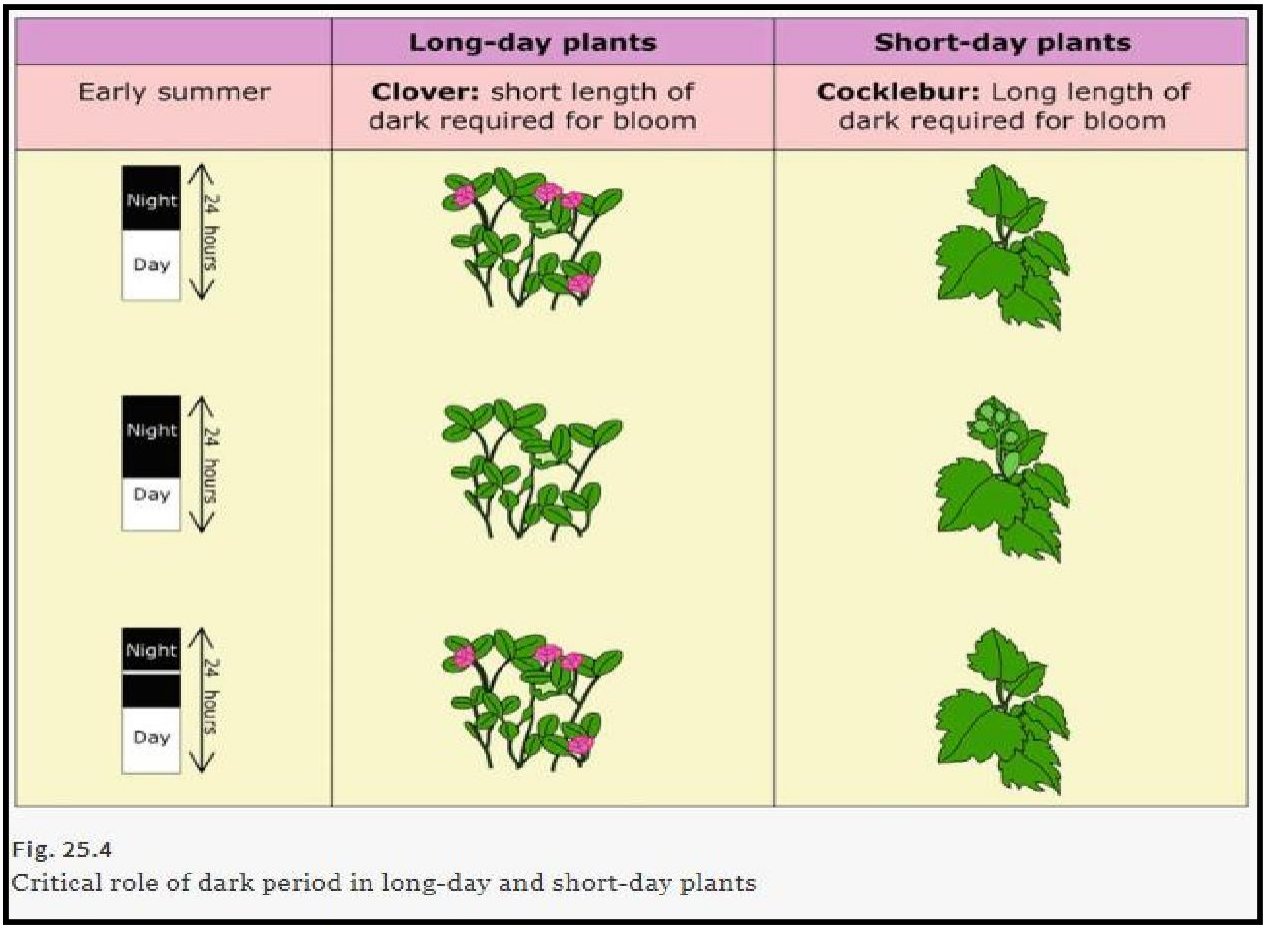


Fig. 25.4
Critical role of dark period in long-day and short-day plants

Fig. Critical role of dark period in long-day and short-day plants.

Photo-inductive Cycle

In nature, plants are exposed to photoperiodic cycles which consist of alternate periods of light and dark diurnally. Any photoperiodic cycle which induces flowering in a plant is called **photoinductive cycle**. On the contrary the photoperiodic cycle which does not induce flowering in a plant is non-photoinductive cycle. A photoperiodic cycle consisting of 16 h light and 8 h dark period generally induces flowering in LDPs, while a cycle consisting of 8 h light and 16 h dark period induces flowering in SDPs. The number of cycles required to induce flowering in a plant varies. One SD photoinductive cycle is sufficient to induce flowering in *Xanthium strumarium* and *Pharbitis nil*, while *Salvia occidentalis*, a SDP, may require at least 17 cycles. *Plantago lanceolata*, a LDP, requires 25 photoinductive cycles for maximum floral response. If the plant is returned to non-photoinductive cycle after ten cycles, it will not flower. However, if returned to photoinductive cycle, only 15 cycles are required. This indicates that some factor responsible for flowering response gets accumulated during inductive cycle.

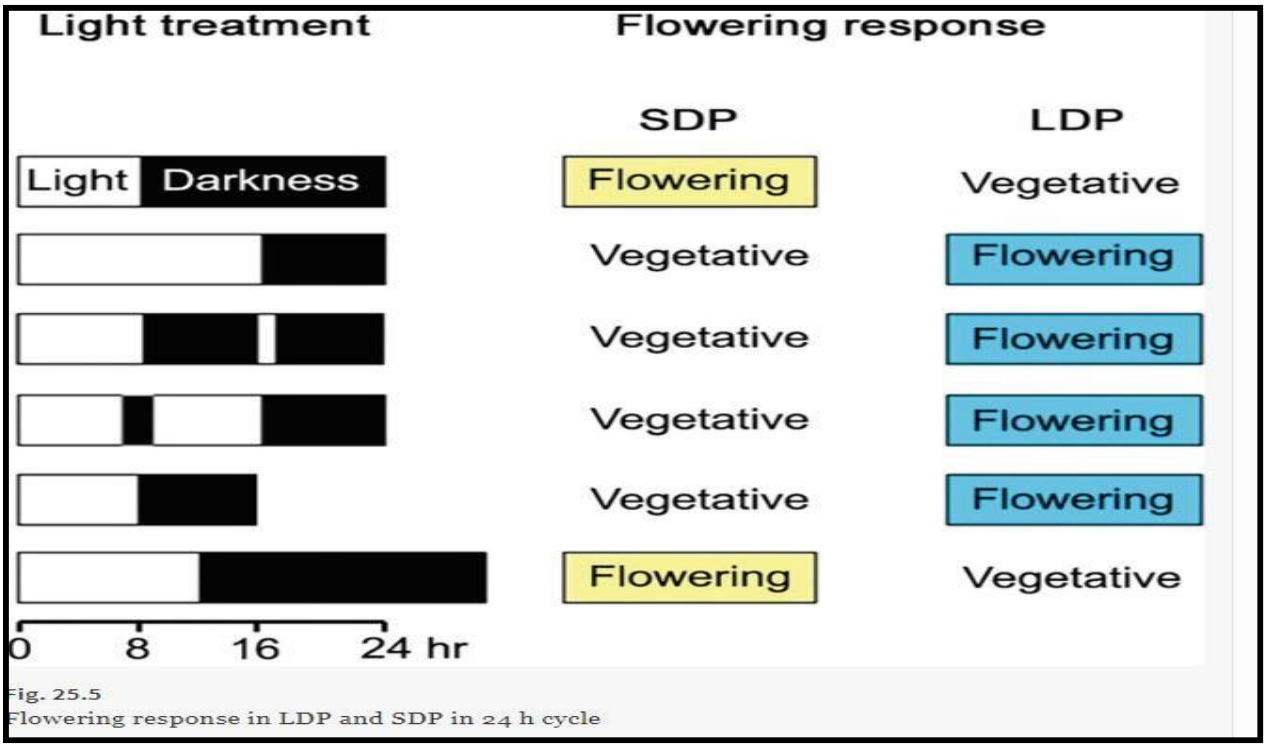


Fig: Flowering response in LDP and SDP in 24 h cycle

Perception of Photoperiodic Signal and Florigen

The photoperiodic signal for floral induction is perceived by the leaves and not by SAM. This was demonstrated in experiments conducted by the Russian plant physiologist M. Chailakhyan in 1937. He reported flowering in *Chrysanthemum morifolium*, a SDP, when a leafy portion of the plant was subjected to short days and the apical meristem and defoliated portion of the shoot were subjected to long days. However, the plants remained vegetative when conditions were reversed, i.e., the upper defoliated portion kept in short days and the leafy portion in long days In another set of experiments, SDPs *Perilla* and *Xanthium* could be induced to flower even when all the leaves had been removed except one leaf which was kept in SD conditions. When leaves taken from the induced plants were grafted to non-induced ones, it resulted in induction of flowering in the non-induced ones. Even the excised leaves of *Perilla frutescens* (SDP), when exposed to photoinductive cycle and grafted back to non-induced plants, induced flowering even when plants were maintained under noninductive long-day conditions. A rapidly expanding leaf is most sensitive to perceive the photoperiodic stimulus when it is half of its final size. Even when several *Xanthium* plants were joined to each other through grafts, all plants flowered even when only the first plant was exposed to short days. From these experiments Chailakhyan suggested that the floral stimulus might be a hormone which could diffuse through graft union. He called this stimulus as **florigen**. Grafting was also done in between the plants belonging to the same family but having different photoperiodic requirements, such as between SDP *Nicotiana tabacum* and LDP *Hyoscyamus niger*. *Hyoscyamus niger* flowers under short days if tobacco is kept under short days. Conversely, the grafted tobacco plants flower if *Hyoscyamus* is kept under long days. This experiment indicates that floral stimulus might be same in all photoperiodic classes. Chailakhyan proposed flowering stimulus to be a hormone, which he called **florigen**. He proposed **florigen** to be synthesized in leaves and transmitted to the shoot apex. Attempts to isolate and identify florigen remained unsuccessful until a protein encoded by **FLOWERING LOCUS T (FT)** was identified as a major component of the mobile signal in *Arabidopsis*. FT was found to contain phosphatidylethanolamine-binding domain which, in mammals, is involved in kinase signaling and mediates protein-protein interaction. In *Arabidopsis*, FTmRNA expression in the companion cells of the phloem in leaves triggers flowering when FT protein is transported to the apical meristem through phloem sieve elements where it interacts with bzip transcription factor encoded by **FLOWERING LOCUS D (FD)**, and it is

responsible for the regulation of genes involved in the change of vegetative meristem to produce flowers. The florigen model was replaced by **nutrient diversion hypothesis**. According to this hypothesis, an inductive treatment stimulates the flow of nutrients into the apical meristem. A high level of nutrients has been found to stimulate flowering. This hypothesis is based on the observation that induction of flowering in white mustard (*Sinapis alba*), a LD plant, gives rise to a rapid and transient increase in the export of sucrose from leaves to the shoot apex. The third hypothesis, the **multifactorial hypothesis**, proposes that flowering occurs when a number of factors, including promoters, hormones, and nutrients, are present in the apex at an appropriate time and in appropriate concentrations. This hypothesis points at multiple genes that control flowering.

Circadian Rhythm

In addition to photoperiodism, plants also display other time measuring systems. Endogenous rhythms persist even when plants are placed in constant environmental conditions. They are based on a cycle of approx. 24 h and are known as circadian rhythms (circa = about, diem = day). Circadian rhythms are synchronized with the daily day-night cycle, which is known as **entrainment**. Erwin Bunning (1936) proposed that daily rhythms consist of two phases, i.e., **photophil phase** (light-loving phase) and **skotophil phase** (dark-loving phase). Photophil phase is characterized by intensive photosynthesis and weak respiration (anabolic processes predominate). On the contrary, skotophil phase is characterized by intensive respiration. In this phase, hydrolytic activity increases, and decomposition of starch into sugars takes place (predominance of catabolic processes). According to *Bunning hypothesis*, the two phases alternate about every 12 h. Under constant environmental conditions, photophil phase would probably correspond to subjective day, while skotophil phase is equivalent to subjective night. The ability of light to promote or inhibit flowering depends on the phase in which light is given. When light signal is applied during light-sensitive phase of the rhythm, the effect is either to promote flowering in LDPs or to prevent flowering in SDPs. In an experiment, *Chenopodium rubrum* plants (a SDP in which exposure to single photoinductive cycle is sufficient to induce flowering) were shifted to 72 h. dark period after being exposed to a photoperiod. Two minutes of night break was given at different time intervals in the dark period before transferring the plant to continuous light. Inhibition of flowering was most effective if night break was given at 6, 33 or 60 h after the start of dark period. This is the time when the plant might have been in

darkness in a normal 24 h cycle, i.e., skotophilous phase. However, night breaks do not result in inhibition of flowering if the night breaks are given near 18 and 46 h after the start of dark period. This is the time when plant would have been in light in a 24 h cycle, i.e., photophil phase. This indicates interaction of photoinduction with endogenous rhythm of the plant. Flowering in both LDPs and SDPs is induced when light exposure is coincident with the appropriate phase of the rhythm. Some kind of regulating mechanism is present which is called circadian regulator. Bunning's hypothesis has evolved into **coincidence model**. According to this model, a key regulator accumulates in LDPs and reaches a maximum concentration during LDs. The regulator also requires light for its activation, i.e., the presence of light coincides with the accumulated regulator, followed by cascade of events leading to flowering. In *Arabidopsis* (a quantitative LDP), the genes which have been identified and characterized as key regulators of flowering include GIGANTEA (GI), CONSTANS (CO), and FLOWERING LOCUS (FT). Isolation of a mutant (*co*) of *Arabidopsis*, in which flowering was delayed under LD but without affecting the response under SD, leads to identification and isolation of CO gene. The gene has been found to be a key regulator in photoperiodic control of flowering. In *Arabidopsis*, mRNA for CO (which encodes a nuclear zinc-finger transcription factor) starts accumulating and reaches a peak in LD and is translated in light. CO protein is stabilized by exposure to blue and FR light which is absorbed via the pigments CRY2 (cryptochrome) and PHYA (phytochrome), respectively. CO expression and activation of FT gene occur in the companion cells. As a result, FT protein is transported to the shoot apex. Thus, flowering in *Arabidopsis* occurs only when transcription and translation of CO gene coincide with exposure to light, which occurs under LD. There is an overlapping (coincidence) between CO mRNA synthesis and day light so that light can permit active CO protein to accumulate to a level that promotes flowering. Thus, rhythmicity of accumulation of COSTANS mRNA in photoperiod and its light-dependent translation to CO protein provide the molecular basis for external coincidence model. Interestingly, FT is a target gene downstream of CO. FT is expressed in the companion cells. Thus, CO activity is mediated by the expression of FT. Movement of FT from the companion cells to the sieve elements requires ER-localized protein called FT INTERACTING PROTEIN (FTIP1). Once in floral meristem, FT protein enters the nucleus and forms a complex with bzip transcription regulator FD, which is encoded by the gene FLOWERING LOCUS D (FD). FT-FD activates expression of **floral meristem-identity** genes, the MADS box transcription factors, such as SUPPRESSOR

OF OVEREXPRESSION OF CONSTANS-1(SOC1) and AP1 (Fig. 25.9). These genes specify that the vegetative shoot meristem of the plant gets differentiated into floral meristem. Investigations have been undertaken on the flowering behavior of rice (SDP) plants. The major genes, i.e., CO and FT, which have regulatory function in *Arabidopsis*, are conserved in rice (SDP). However, their specific regulation has been altered by evolution to promote flowering under short days. The genes **Heading-date1 (Hd1)** and **Heading-date3a (Hd3a)** are homologous to *Arabidopsis* CO and FT, respectively. Similar to FT in *Arabidopsis*, overexpression of Hd3a in rice results in rapid flowering irrespective of photoperiod. Besides the expression of FT in *Arabidopsis* and that of Hd3a gene in rice, flowering is elevated during the inductive photoperiods, i.e., LD and SD, respectively. However, unlike in *Arabidopsis* (LDP), where coincidence of CO with light period promotes flowering in rice, coincidence of Hd1 expression with the light period suppresses flowering since Hd1 acts as the suppressor of Hd3a. The lack of coincidence between Hd1mRNA expression and day light prevents accumulation of Hd1 protein, which acts as a repressor of the gene encoding the transmissible floral stimulus, Hd3a, in rice. In the absence of the Hd1 protein repressor, Hd3a mRNA is expressed, and the protein it encodes is translocated to the apical meristem where it causes flowering. Under long days (sensed by phytochrome), the peak of Hd1 mRNA expression overlaps with the day, allowing the accumulation of the Hd1 repressor protein. As a result, HD3a mRNA is not expressed, and the plant remains vegetative.

Photoreceptors

Phytochrome and cryptochrome play important roles in photomorphogenesis of plants. One of the best studied SDPs in terms of effect of light on flowering is *Pharbitis nil*. It is a qualitative SDP in which 4–5-day-old cotyledonary photoresponsive tissue can receive the stimulus for floral induction when given a single photoinductive cycle. In experiments with this plant, night breaks given during photoinductive dark period, which prevent attainment of critical dark period, inhibit flowering. Night breaks were found to be most effective if red light was used. However, the effect was reversed if red light treatment was immediately followed by exposure to far-red light. The photoreversible effect of R/FR light suggested the role of phytochrome. Phytochrome comprises of nuclear encoded proteins. The *Arabidopsis* genome encodes five phytochromes (PHYA to PHYE) that are involved in floral induction. Late-flowering mutants

(phyA) are defective in genes that promote flowering, while early-flowering mutants (phyB) are defective in genes that ordinarily repress flowering.

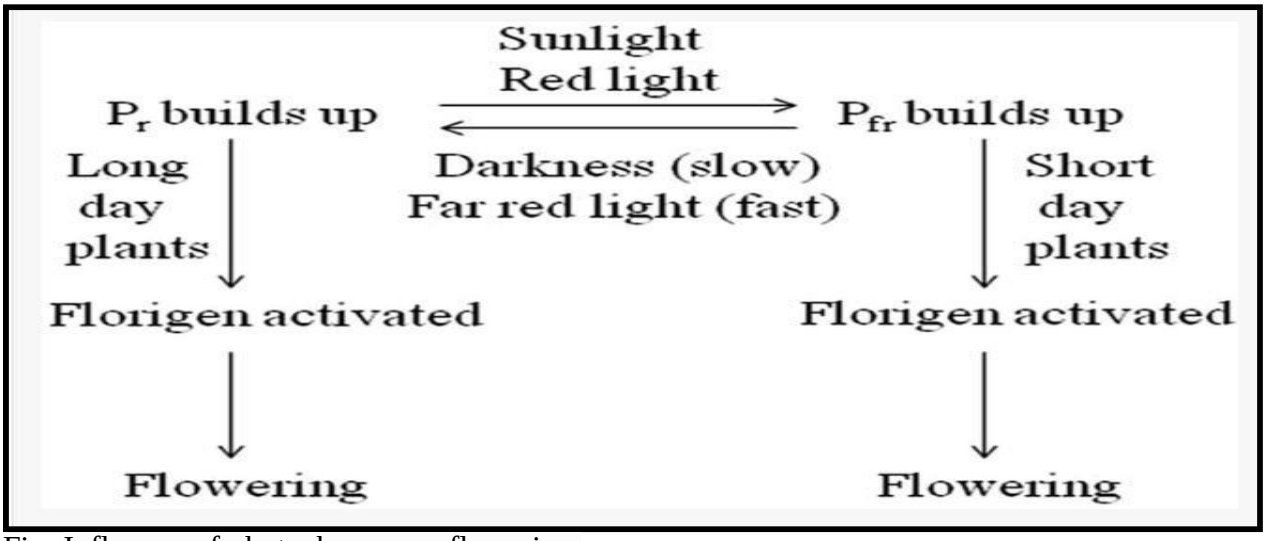


Fig: Influence of phytochrome on flowering.

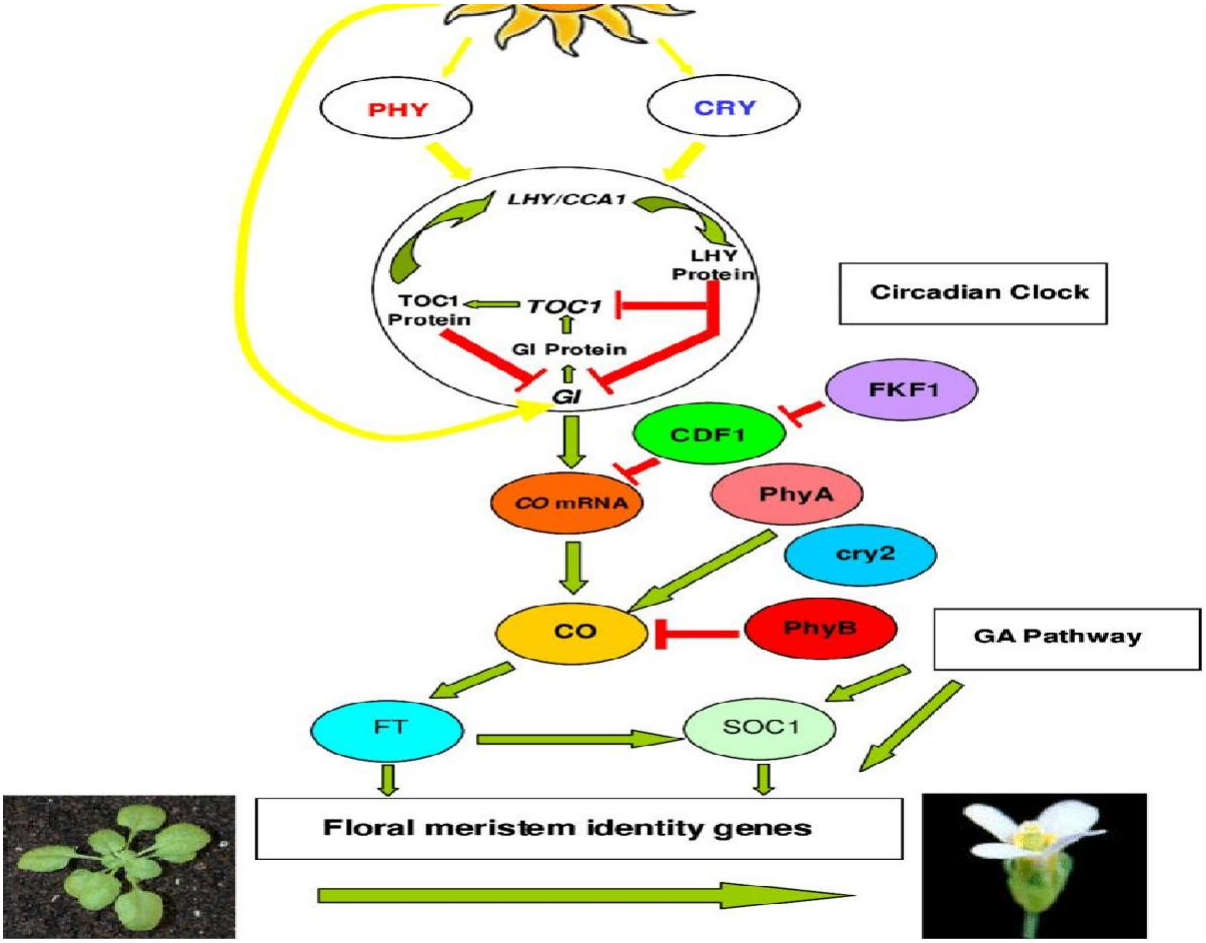


Fig: Light is perceived by the phytochromes and cryptochromes that entrain the circadian clock. The clock controls the expression of CO (coincidence mRNA) and GI (Gigantea mRNA). Activated CO protein then induces the expression of the floral integrators FT (Flowering Locus) and SOC1 (suppressor of overexpression of constans-1) which in turn control the expression of the floral meristem identity genes to induce flowering.

Blue light promotes effect on flowering in LDPs, especially in members of family Cruciferae. Two members of cryptochrome gene family (CRY1 and CRY2) are present in *Arabidopsis*. Cryptochromes are flavoproteins that act as the blue light receptors. Both CRY1 and CRY2 function in stabilizing CO protein along with PHYA toward the end of light period, whereas in other plants this role is taken up by PHYA alone. CRY2 mutants of *Arabidopsis* flower later than the wild type under inductive long days. Under continuous white light exposure, phy1 mutant (any type of phytochrome cannot be synthesized in them because of defective enzyme, which is required for synthesis of chromophore of the pigment) plants flower similar to the wild types. This indicates that in continuous white light exposure, no phytochrome is required, and the blue light receptor is involved. Mutation in one of the cryptochrome genes (CRY2) causes a delay in flowering.

According to the coincidence model, CO gene is expressed during light period. The effect of light on CO stability further depends on the photoreceptor involved. In morning hours (after dark), phyB signaling enhances CO degradation, whereas in the evening (when CO protein accumulates after long day), cryptochromes and phyA antagonize this degradation and allow CO protein to build up. CO, a transcriptional regulator, promotes flowering by stimulating the expression of a key floral signal, FLOWERING LOCUS T (FT).

Vernalization

In many long-day plants, exposure to low temperature is critical for the acquisition of competence to respond to photoinductive conditions for flowering. This cold temperature requirement is called **vernalization**, which acts as a time computing mechanism that measures the passage of winter and ensures that flowering does not begin until the favorable conditions of spring arrive. The concept was introduced by T. D. Lysenko (1920) who observed the ability of cold treatment to make the winter cereal behave as spring cereal. This could be of practical utility like: (1) crops can be harvested much earlier, (2) crops can be grown in regions where

they are not naturally productive, and (3) plant breeding experiments can be accelerated. Generally, it is the stem apex which perceives the cold temperature signal. The dividing cells in plants perceive vernalization stimulus. Period of chilling can vary from few days to weeks and from plant to plant, but longer exposure to low temperature will be more effective for early flowering. Response due to vernalization decreases if it is interrupted by heat treatment. In contrast to photoperiodic effect, which leads to flower initiation, vernalization prepares plants for flowering. G. Melchers and A. Lang (1948) demonstrated that the biennial LDP *Hyoscyamus niger* (which requires a low temperature season before flowering unlike the annual type which flower in one season) should be at least 10 days old before becoming responsive to the low temperature treatment. However, Gregory and Purvis in 1930s suggested that hydrated seeds of Petkus winter rye (*Secale cereale*) may be vernalized making them sensitive to LD photoperiod. The cold treatment of the seeds reduces the number of photoinductive period required for flowering since the Petkus winter rye does not have obligate requirement for vernalization. That vernalization is an energy-dependent process was demonstrated in an experiment in which excised embryos were supplemented with carbohydrates and oxygen. Melchers had demonstrated that vernalization stimulus could be transmitted through graft union. He was the first to coin the term vernalin for the hypothetical active factor required for vernalization. It was observed that once a plant has been vernalized, it remembers the cold treatment throughout its life. The memory is maintained in cell derived from the induced cell through mitotic division but not the one which are derived through meiotic division. Lang stated a direct connection between vernalin and florigen.

One of the pathways for flowering is through vernalization, where low temperature treatment leads to accumulation of vernalin which in turn stimulates the flowering stimulus florigen. Vernalization affects competence of a plant to flower by bringing about stable changes in the pattern of gene expression in the meristem after cold treatment. Such changes are termed as epigenetic changes. Requirement of vernalization is conferred by two genes, FRIGIDA (FRI) and FLOWERING LOCUS C (FLC). FRI acts in upregulation of FLC. FLC encodes MADS-domain DNA-binding protein that functions as a repressor of flowering. Levels of FLC are the primary determinant of vernalization requirement in *Arabidopsis*. It is highly expressed in the shoot apical meristem of non-vernalized plants. It represses flowering by repressing the

expression of **floral integrators**, such as FT, FD, and SOC1. Floral integrators are the genes that are involved in regulation of meristem-identity genes. These are so named because these integrate the floral stimulus which is due to some environmental cues and trigger the vegetative to reproductive transition. Binding of FLC with the promoters of SOC1, FD, and FT decreases the ability of the photoperiods to activate these integrators. During vernalization FLC is **epigenetically** switched off for the rest of plant's life cycle. These are stable changes in gene expression that do not involve alterations in DNA sequence and which can be passed on to descendent cells through mitosis or meiosis. This is achieved by repressive changes in FLC which includes **chromatin remodeling**. This includes histone methylation of lysine-27 and lysine-9 residues which are characteristics of heterochromatin, and acetyl groups are removed from lysine-9 and lysine-14 of H3 which otherwise are characteristics of euchromatin. Thus, low temperature induces conversion of FLC from active to inactive form. The importance of histone modification was further clarified after mutants of *Arabidopsis* have been identified which do not respond to vernalization. These mutants included *vernalization insensitive* (*vin*) and *vernalization* (*vrn*) mutants. These mutants prevent vernalization and alter histone modifications. Thus, photoperiod pathway, vernalization pathway, and autonomous pathway form a regulatory network which converges to modulate the activities of a set of genes that integrate the floral stimulus and trigger the transition from vegetative to reproductive phase.

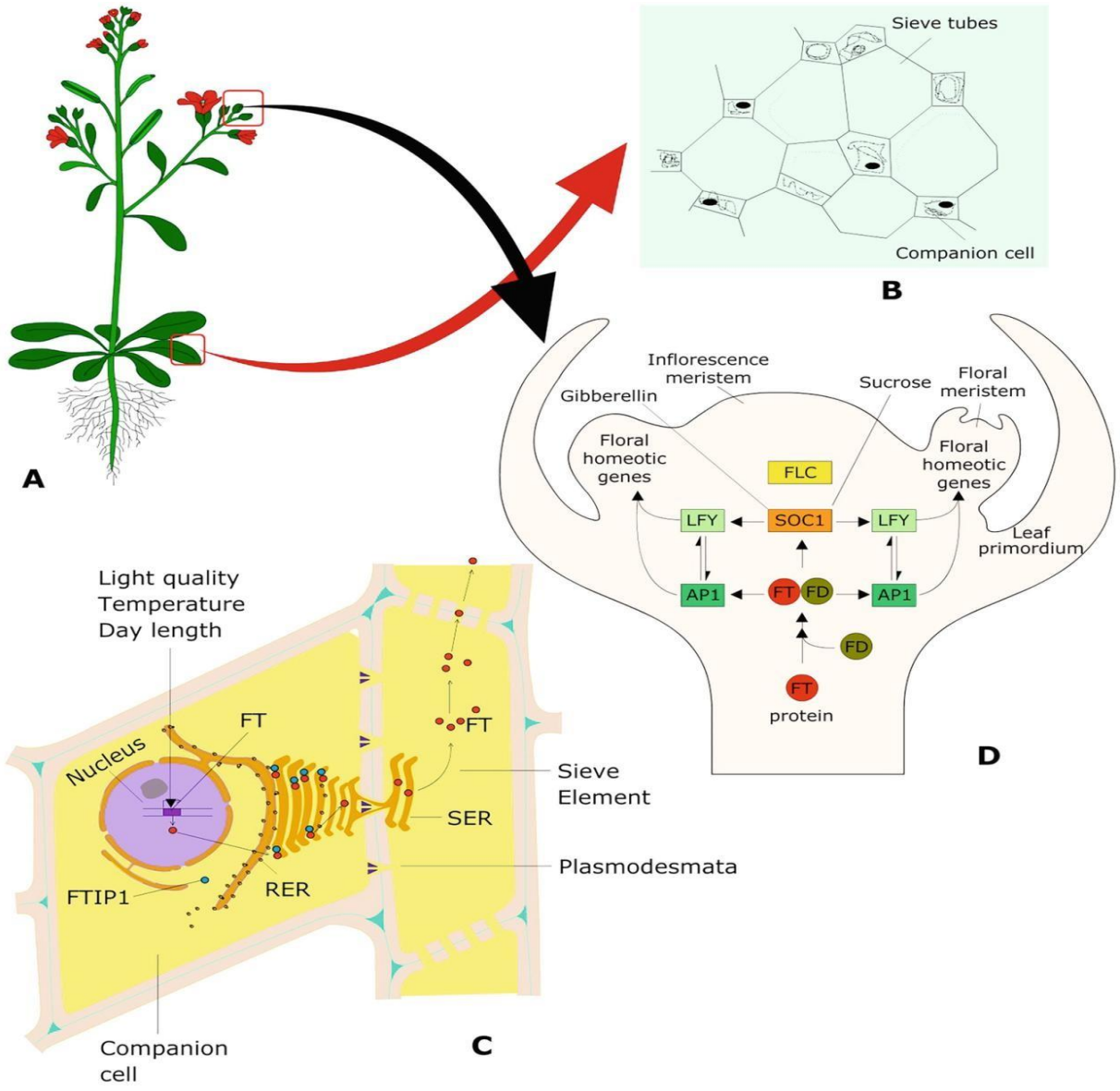


Fig: Flowering is regulated by multiple factors in *Arabidopsis* (**a**); (**b**) FT mRNA is expressed in companion cells of leaf vein in response to multiple signals, including day length, light quality, and temperature; and (**c**) FTIP1 mediates through a continuous ER network between the companion cells and the sieve tube elements. FT moves in the phloem from the leaves to the apical meristem. (**d**) FT is unloaded from the phloem in the meristem and interacts with FD. Then FT-FD complex activates SOC1 in the inflorescence meristem and AP1 in the floral meristem, which triggers LFY gene expression. LFY and AP1 trigger expression of the floral homeotic genes. The autonomous and vernalization pathways negatively regulate FLC, which acts as a negative regulator of SOC1 in the meristem and as a negative regulator of FT in the leaves. *FD* FLOWERING LOCUS D, *FT* FLOWERING LOCUS T, *FTIP1* FT-interacting protein 1, *SOC1* suppressor of constans1, *AP1* apetella1, and *LFY* leafy.

Role of Gibberellins

Gibberellins play important role/s during transition of vegetative to reproductive meristem. This includes their role in competence, promotion of bolting, and flowering in *Arabidopsis* and many other long-day plants. Flowering in perennial species tends to be insensitive to gibberellins. In an interesting observation, when extract from photoinduced leaves of *Xanthium* is applied, it induces flowering in *Lemna* kept under noninductive conditions. However, the extracts need to be supplemented with gibberellin. The leaf extract alone or gibberellin alone has no effect. Extract prepared from spinach leaves grown under short days suggests that a critical step in GA biosynthesis is inhibited. Plants remain vegetative and rosetted under short days. This shows that gibberellin is partially responsible for flowering. There is a possibility that GA is a mobile signal that transmits the photoperiodic floral stimulus and its action is independent of FT, the phloem mobile protein that relays the floral induction signal from leaf to shoot apex. Expression of both SOC1 and LFY in *Arabidopsis* is promoted by GA via DELLA-mediated signaling mechanism. SOC1 is thus regulated in a multifactorial manner and integrates the autonomous, vernalization, and GA pathways. Chailakhyan stated that vernalin hormone may be a precursor of gibberellin. Under long-day conditions, it is converted to gibberellin. Another hormone called **anthesin** is present in long-day plants which, along with vernalin, causes flowering in long-day plants. In short-day conditions, vernalin is not converted to gibberellin. Hence, flowering does not occur. Gibberellin treatment to long-day non-vernalized plants kept under long day leads to flowering as these plants possibly contain anthesin. Gibberellin is ineffective in flower induction in short-day plants as they lack anthesin. Auxin application induces flowering in pineapple and litchi. In pineapple, the effect of auxin may be due to stimulation of ethylene production.

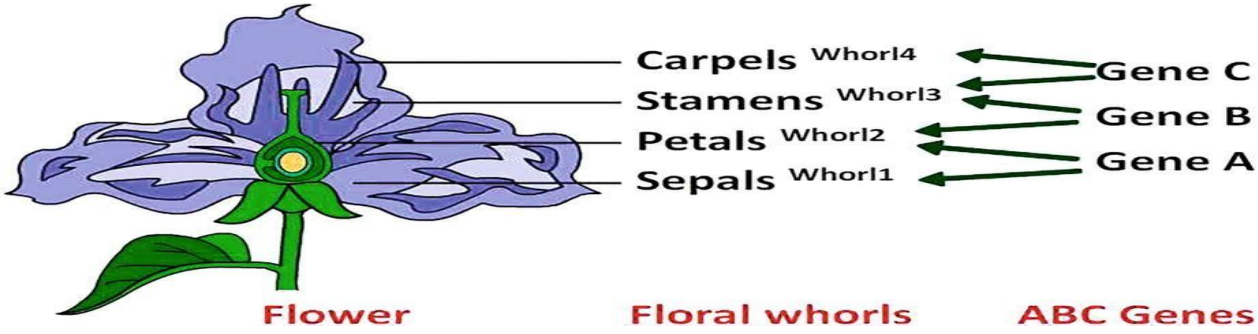


Fig: ABC model whereby floral organ identity is controlled by three homeotic genes, namely, A, B, and C.

Flower Development

Two categories of genes are responsible for flower development ,viz., floral meristem identity genes and floral organ identity genes. The floral meristem identity genes are responsible for the transition of vegetative meristem to floral meristem. In *Arabidopsis*, these genes include LEAFY (LFY), FLOWERING LOCUS D (FD), SOC1, and APETALA1 (AP1). LFY, FD, and SOC1 play a critical role in integrating the signals—both environmental and internal. These genes act as master regulators for the initiation of floral development. Floral meristems can be distinguished from vegetative meristem by its larger size. The transition from vegetative to reproductive phase is marked by an increase in the frequency of cell division within the central zone of shoot apical meristem. Four different types of floral organs are initiated in separate whorls, namely, sepals, petals, stamens, and carpels. They develop in concentric rings called **whorls**, numbered 1, 2, 3, and 4, respectively. Molecular basis of floral morphogenesis has been studied extensively in *Arabidopsis*. Floral organ identity genes were discovered in **homeotic gene** mutants. Homeotic genes encode transcription factors that determine the location where specific structures develop. Five key genes have been identified in *Arabidopsis* which specify floral organ identity, namely, APETALA1 (AP1), APETALA2 (AP2), APETALA3 (AP3), PISTILLATA (P1), and AGAMOUS (AG). Influence of organ identity genes on floral development in *Arabidopsis* can be understood by loss-of-function mutants of these genes. Mutations in these genes change the floral organ identity without affecting the initiation of flowers. The genes that determine the four basic whorls in flower have been grouped into three classes, A, B, and C. Each group does not necessarily represent a single gene. This view is expressed as **ABC model**. This model postulates that organ identity in each whorl is determined by a unique combination of the activities of three organ identity genes. Type A gene alone specifies sepals, while A and B together are required for petal formation. Genes of B and C category are required for stamen differentiation, while type C genes are responsible for carpel formation. According to ABC model, Class A and C genes are mutually repressive to each other. Loss of type A activity (encoded by AP1 and AP2) results in the formation of carpels instead of sepals in the first whorl and stamens instead of petals in the second whorl. Loss of type B activity (encoded by AP3 and PI) results in the formation of sepals instead of petals in the second whorl and carpels instead of stamens in the third whorl since the genes belonging to this category control organ determination in the second and third

whorl. Type C gene (AG) controls events in the third and fourth whorl. Loss of type C gene activity results in the formation of petals instead of stamens in the third whorl and replacement of fourth whorl by a new flower such that this whorl is occupied by sepals.

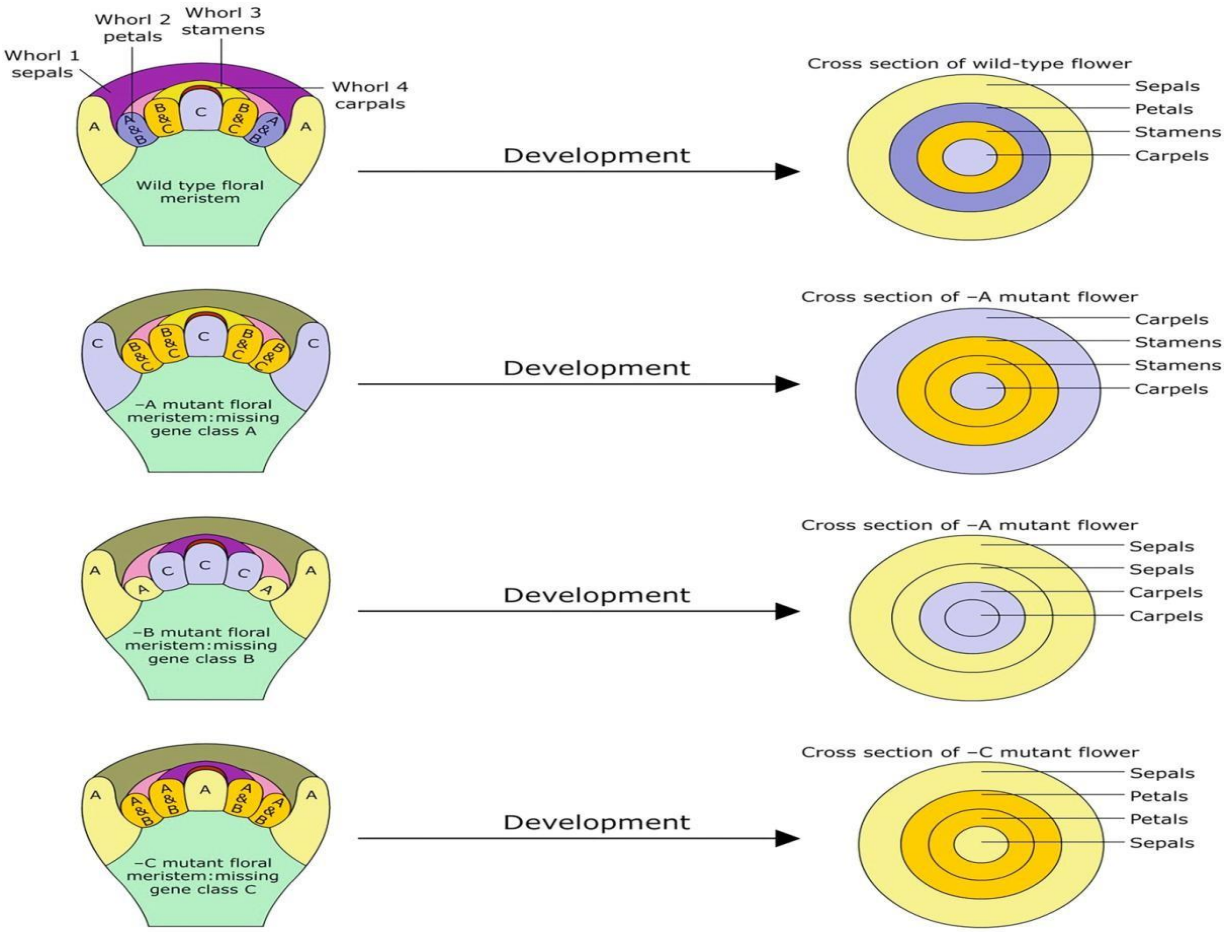


Fig: Letters within the whorls indicate active genes. In case of loss of function of A, the role of C expands to the first and second whorls; in case of loss of B gene activity, the outer two whorls will have function of A; loss of function of C, A expands into the inner two whorls.

To sum up, photoperiodism and vernalization facilitate plants to synchronize their life cycle with the time of the year. It is clear that the process of flower formation is an interplay of various transcriptional networks that regulate organ-specific gene expression. Such altered expression of floral homeotic genes also explains the floral diversity that we observe in our daily life. Flowering plants constitute an enormous range which needs to be explored with

reference to gene networks that regulate floral development. Future challenge is to explore the variability found in nature which is due to gene network regulating the floral development.

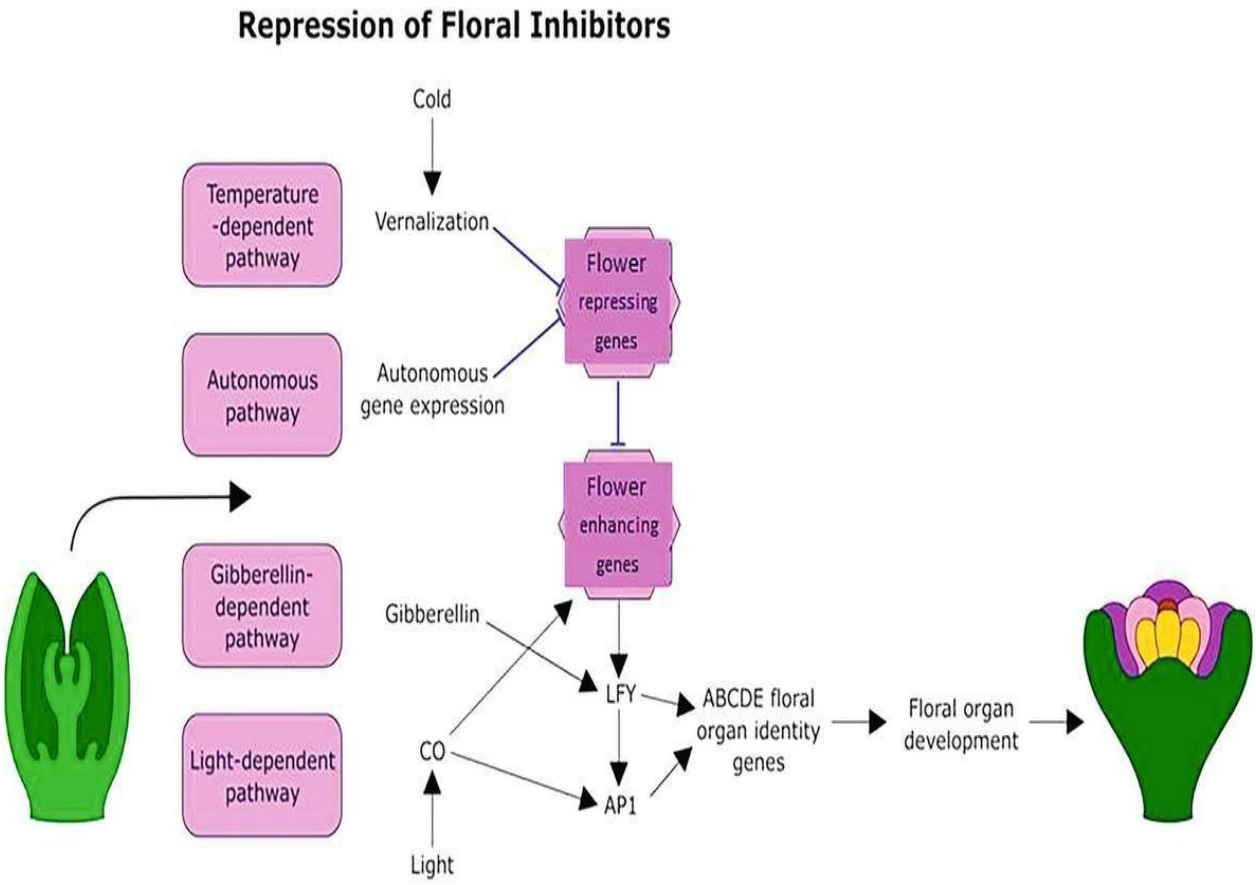


Fig: Temperature, light, and gibberellin-dependent pathways work through repression of floral inhibitors for flower formation as well as by activating floral meristem identity genes