Balancing forces in the photoperiodic control of flowering†

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Received 10th August 2010, Accepted 4th November 2010
DOI: 10.1039/c0pp00252f

In many plant species, the duration of the daily exposure to light (photoperiod) provides a seasonal cue that helps to adjust flowering time to the most favourable time of the year. In *Arabidopsis thaliana*, the core mechanism of acceleration of flowering by long days involves the stabilisation of the CONSTANS (CO) protein by light reaching the leaves, the direct induction of the expression of *FLOWERING LOCUS T* (*FT*) by CO and the migration of FT to the apex to promote flowering. In rice (*Oryza sativa*), the promotion of flowering by short days depends on the interplay between light conditions, and the genes *Grain number, plant height and heading date locus 7* (*Ghd7*) and *Early heading date 1* (*Ehd1*). In both cases, other day length-induced changes reinforce the core photoperiodic pathway of promotion of flowering. However, there are regulators of flowering time, quantitatively less important than the core pathways but still significant, which impact in the opposite direction, *i.e.* favouring rice flowering under long days or *Arabidopsis* flowering under short days. We show, for instance, that short days enhance leaf expression of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3* (*SPL3*), which stimulates *Arabidopsis* flowering under these conditions. We propose that fine tuning of flowering time depends on the balance of a hierarchy of multiple points of action of photoperiod on the network controlling flowering.

Seasonal cues provided by the light environment

In plants, the reproductive phase is particularly sensitive to the stress imposed by extreme conditions. High temperatures, low (freezing) temperatures, or drought are among the stressful conditions that are more likely to occur in a given season. Therefore, by flowering at the time of the year when these stressful events are less likely, plants reduce the chances of putting at risk the completion of their cycle and the transmission of their genes to the next generation. In many species, the initiation of the life cycle, triggered by seed germination, is affected by the temperature patterns and therefore it is more likely to occur within selected times of the year (see, for instance, ref. 1). The control of seed germination by seasonal cues helps to avoid stressful conditions during seedling establishment. However, this control is not enough to optimize the initiation of the reproductive phase. If the period where the conditions are benign is extended, delayed flowering favours the development of vegetative structures (leaves, roots) able to capture more resources to support the subsequent reproductive effort. If the period of benign conditions is restricted, delayed flowering would put the fate of the reproductive phase at risk and therefore a shorter cycle, although paying a cost in terms of the ability to capture resources, would increase the chance of completing the cycle. Since in different locations the duration of the favourable period can be different, a specific control by seasonal cues of the transition to the reproductive stage is required. The most important seasonal cues in the control of flowering are day length or photoperiod and temperature patterns.[2]

Day length reaches minimum values at the end of autumn/beginning of winter and maximum values at the end of spring/beginning of summer. The magnitude of these fluctuations increases with latitude. The so-called long-day plants flower or flower earlier when the days are long (above a critical daylength), whereas short-day plants flower or flower earlier when the days are short (below a critical day length).[3] Some plants (day-neutral plants) are insensitive to photoperiod. The photoperiodic requirement can be absolute (if flowering only occurs under the inductive photoperiods) or quantitative (if flowering is accelerated by adequate photoperiods but it will still take place under unfavourable photoperiods). These different classes of photoperiodic response can be observed among different species and in some cases within a given species.[3,4] Quantitative variations in critical day length are easily observed within species, are fundamental for plant adjustment to different regions and have major implications in agriculture.[5,6]

There are other features of the light environment that control flowering time. In several species, low red to far-red ratios accelerate flowering.[7] Light reflected and transmitted by green leaves is proportionally enriched in far-red light due to the absorption of red light by photosynthetic pigments. Therefore, plants shaded by taller plants are exposed to a low red/far-red ratio that may accelerate flowering. A plant that is not shaded may receive far-red light reflected on nearby neighbours and this reduction in red to far-red ratio is enough to induce responses that prepare the plant for the impending competition.[8] This may include the acceleration of flowering, which could then take place before competition becomes severe and the resources to support the reproductive development are scant. To investigate the effects of combining photoperiodic and shade-light signals

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† Electronic supplementary information (ESI) available: Analysis of gene expression by qRT–PCR. See DOI: 10.1039/c0pp00252f

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we cultivated plants of *Arabidopsis thaliana* in a glasshouse either under natural radiation or under simulated shade-light (Fig. 1, inset), and plotted flowering time (on a biological scale, *i.e.* number of leaves at flowering) against photoperiod at sowing. Shade-light accelerated flowering but this effect was quantitatively more important for plants sown under the short days of winter than under the long days of late spring (Fig. 1). This indicates that photoperiodic and shade signals are to some extent redundant in terms of flowering promotion in *Arabidopsis thaliana*. The latter can be accounted for by the observation that both inductive photoperiods[9,10] and shade-light[11] enhance the expression of the flowering promoter gene *FLOWERING LOCUS T* (*FT*).

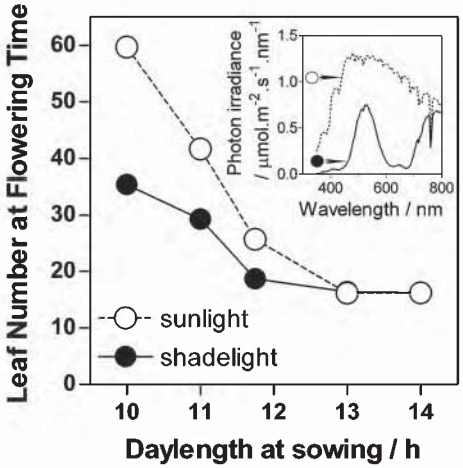

Fig. 1 Redundant promotion of flowering of *Arabidopsis thaliana* by long days and shade-light. Plants of *Arabidopsis thaliana* were grown in pots[14] in a heated glasshouse at different dates. When the plants were 3 weeks old, half of them were moved to simulated shade-light conditions provided by a green filter (Lee filters number 089) placed above the plants (lateral ventilation) within the glasshouse. The spectral photon irradiance measured at midday with a spectroradiometer (FieldSpec Pro FR; Analytical Spectral Devices [ASD], Boulder, CO, USA.) under sunlight or simulated shade-light conditions is shown in the inset. Flowering time measured as the number of rosette leaves at time when the flower bolt was 1 cm high is plotted against photoperiod at sowing. Data are means of 10–18 plants and ±SE bars are smaller than the symbols. Two-way ANOVA indicates significant interaction between photoperiod and shade-light treatments ($P < 0.001$).

At the extremes of the photoperiod the red/far-red ratio decreases due to atmospheric reasons. The extent of this decrease depends on the time of the year, suggesting that it could provide seasonal cues.[12] However, a role of these restricted changes in red/far-red ratio in the control of flowering time has not been demonstrated. Actually, at least for some physiological responses, if the decrease in red/far-red ratio is restricted to the end of the photoperiod, to be effective this reduction has to be much more severe (almost pure far-red) than that caused by atmospheric factors.[13]

Due to changes in cloudiness and in solar elevation, the level of irradiance to which the plants are exposed is lower in winter than in summer months, suggesting that overall irradiance could provide a seasonal cue. However, although low irradiance levels cause delayed growth and development, including flowering time, if flowering time is measured on a biological scale (number of leaves) to correct for general growth effects and focus on specific effects on flowering, the impact is not very strong, at least for *Arabidopsis thaliana*.[14] Therefore, day length, and not red/far-red ratio or irradiance, is the major seasonal clue provided by the light environment.

The lesson taught by Maryland Mammoth

In the discovery of photoperiod as a critical factor regulating flowering time, the characterisation of the floral transition in a *Nicotiana tabacum* cultivar called Maryland Mammoth played a decisive role. These plants were able to flower when grown during the autumn and winter in the greenhouse but not during the summer in the field. The search for the condition(s) that made the difference finally pointed to the requirement of short days.[15,16]

The response observed in Maryland Mammoth has an additional and often overlooked potential implication. The parental *Nicotiana tabacum* plants, from which the Maryland Mammoth cultivar emerged as a spontaneous mutant, normally flowers at the same time under long- and short-day conditions. The fact that a mutation within a day-neutral plant generated a plant that flowered only under short-day conditions indicates that tobacco plants normally contain regulatory pathways that simultaneously promote and inhibit flowering time under both long and short day conditions, and that the time it takes these plants to flower depends on the final balance between these pathways. Following this argument, the recessive Maryland Mammoth mutation could have turned a day-neutral plant into a short-day plant by affecting a component that acts to promote flowering specifically under long-day conditions.

Promotion of flowering by long days in *Arabidopsis thaliana*: coincidence between *CONSTANS* expression phase and the presence of light

Arabidopsis thaliana is a long-day plant, which perceives that the days are long when the presence of light coincides with the period of the day when the plants are sensitive to light (as far as the photoperiodic control of flowering is concerned). The diurnal variation in sensitivity to light is caused by the diurnal variation in the expression of the flowering promoter gene *CONSTANS* (*CO*), which is under the control of the circadian clock.[17,18] In seedlings entrained under day-night cycles and then transferred to free running conditions (constant light and temperature), *CO* expression increases during the evening and the first part of the subjective night. A similar pattern is observed in seedlings that remain under day-night cycles. When the days are short, the rising phase of *CO* expression occurs when the seedlings are already in darkness. However, under long days the rising phase of *CO* expression coincides (*i.e.* it shows significant overlap) with the presence of light and this induces flowering.[18]

The molecular mechanism of this coincidence model is based on the fact that in darkness, the active E3 ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) causes the degradation of CO protein in the proteasome.[19,20] Light inactivates COP1 in part by causing its migration from the nucleus to the cytoplasm,[21] but the occurrence of faster mechanisms of inactivation has been suggested at least for young seedlings. Therefore, light perceived

by cryptochrome 2 (cry2), cryptochrome 1 and phytochrome A stabilises CO,[22] promotes the expression of *FT*[17,18,23] and flowering.[24,25] The phase of high expression of *CO* and the presence of light to stabilise the newly synthesised CO protein coincide under long days but not under short days.

Interestingly, COP1 also contributes to regulate flowering time through its effects on the circadian clock. *cop1* mutants have a short circadian period phenotype and also flower early under short-day conditions.[26] That this early flowering is in part due to COP1 effects on clock function, and not only on its effect on CO stability, is revealed by the observation that the photoperiodic regulation of flowering time in *cop1* mutants is, to some extent, restored in *cop1* mutant plants grown in daily cycles with a total duration matching more closely the circadian period of the mutant (*i.e.* 18 instead of 24 h).[26] COP1 effects on circadian rhythms and flowering appear to be mediated by its interactions with EARLY FLOWERING 3 (ELF3) and GIGANTEA (GI). ELF3 has been proposed to act as a component of the central oscillator,[27] and it may act at the biochemical level as an adaptor/scaffold protein facilitating COP1 negative regulation of GI stability.[26] GI, in turn, regulates circadian rhythms and flowering time through different mechanisms (see below) and, therefore, regulation of its stability will contribute to the fine tuning of both processes simultaneously.

The Raf kinase inhibitor protein gene *FT*[9,10] and the MADS-box transcription factor gene *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*)[23,28] strongly promote flowering. These genes act as integrators of different endogenous and environmental cues that control flowering, including photoperiodic signals. The photoperiodic signal is perceived by the leaves.[3] The photoreceptors[29] and CO[30] present in the vascular bundles of the leaves promote *FT* expression under long days. Under long days, CO binds the regulatory region of the *FT* gene and promotes its expression in the leaf veins. The 5.7 kb sequence upstream of the translation start site of *FT* contains the *cis*-regulatory elements necessary to restrict *FT* expression to the leaf phloem and for CO-mediated enhancement of expression by long days.[31] Chromatin modifications at the *FT* locus enhance its expression and kinetic analysis has lead to the suggestion that these changes would be part of a positive feed-back loop rather than a requisite for the initial promotion of *FT* expression.[31] Then, the FT protein migrates *via* the phloem from the leaves to the vegetative apex, carrying the photoperiodic signal inducing flowering.[32–34] *TWIN SISTER OF FT* (*TSF*) shows strong sequence similarity to *FT* and would be functionally equivalent but quantitatively less important.[35]

In the apex, FT interacts physically with the bZIP transcription factor FD and directly promotes the transcription of the MADS-box transcription factor *APETALA1* (*AP1*),[36,37] which is involved in the change of identity of the apex (the transition from the vegetative to the reproductive phase of development).[38] In the apex, long days also promote the expression of *SOC1*, a response that might be mediated by the arrival of FT.[39] In turn, SOC1 forms heterodimers with AGAMOUS-LIKE 24 (AGL24) to promote the expression of *LEAFY* (*LFY*),[40] which is a floral-identity gene.[41]

FLOWERING LOCUS C (FLC) represses the expression of *FT* in the leaf and of *SOC1* and *FD* in the meristem.[42] FLC is a MADS-box transcription factor and its expression is reduced by vernalisation[43,44] both in the apex and in the leaves.[42] Therefore, in *Arabidopsis* accessions with strong FLC activity, vernalisation is required to allow a photoperiodic response.

Reinforcing the response: regulation of the waveform of *CO* expression by light in *Arabidopsis*

CO mRNA expression is complex as it shows low levels during daytime in either short or long days, a rise during the afternoon under long days, and a further increment at the beginning of the dark period. FLAVIN-BINDING, KELCH REPEAT, F BOX 1 (FKF1) contributes to increase *CO* mRNA levels, particularly during the late afternoon.[45] FKF1 is part of an E3 ubitiquitin ligase that targets the transcription factor CYCLING DOF FACTOR 1 (CDF1) for degradation through the proteasome.[46] In turn, CDF1 binds to the promoter of *CO* and represses its expression.[46] The expression of both *CDF1* and *FKF1* is regulated by the clock but maximum peak levels occur early in the morning and late in the afternoon, respectively. Late in the afternoon of long days, increased *FKF1* levels reduce CDF1 levels increasing *CO* expression. Interestingly, in plants that constitutively overexpress CDF1, constitutive FKF1 overexpression fails to reduce CDF1 levels in the early morning or during the night. This is so because FKF1 interacts with GI, another clock-regulated factor, to trigger CDF1 degradation.[47] The formation of the GI-FKF1 complex that triggers CDF1 degradation requires light perception by the LOV domain of FKF1. Thus, the coincidence of light with *FKF1* expression constitutes another layer of control ensuring seasonal regulation of *CO* expression and flowering time.[47]

Interestingly, there is evidence that GI also mediates the photoperiodic regulation of flowering in *Arabidopsis* through a CO-independent mechanism. Indeed, GI is required for the photoperiodic regulation of miR172 expression, whose abundance increases under long compared to short days.[48] This micro RNA promotes the floral transition by post-transcriptionally repressing *APETALA 2*-like genes, such as *TARGET OF EAT* (*TOE*) *1*, *2* and *3*, which negatively regulate *FT* expression. The photoperiodic regulation of miR172 abundance requires GI but not CO, and overexpression of miR172 accelerates flowering even in a *co* mutant background.[48] Thus, GI mediates the long-day induction of flowering through two apparently independent mechanisms, one based on *CO* expression and the other on miR172 activity.

Under long days *CO* expression is enhanced by the GI-FKF1 complex that triggers degradation of the negative regulator of CO, CDF1. Complementary, CO expression is repressed under short days by the membrane-bound E3 ligase DAY NEUTRAL FLOWERING (DNF).[49] Under long days, DNF has no obvious effects on *CO* or *FT* expression. However, under short days DNF is required to avoid the early increase of *CO* expression (4 h after dawn) and its high levels before the end of the short day (8 h), which result in high levels of *FT* expression and early flowering under short days.[49]

Reinforcing the response: light-dependent interaction between cry2 and transcription factors promotes *FT* expression in *Arabidopsis*

In addition to its function as positive regulator of CO, cry2 interacts with CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX (CIB1) protein and the related protein CIB5.[50] This interaction requires blue light and is therefore predicted to be more persistent under long than under short days.

Furthermore, the *cib1 cib5* double mutant flowers slightly late under long days. This phenotype would result from CIB1 interaction with the promoter of *FT*, which enhances *FT* expression levels.[50]

Counteracting the response: the SPL3 pathway promotes flowering under short days in *Arabidopsis*

The transcription factor *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 3* (*SPL3*) promotes the floral transition when expressed above certain threshold levels.[51] In the shoot apex, *SPL3* expression increases with age under short-day conditions[52] and when the plants are transferred from short to long day conditions.[53] The latter is part of the network that triggers the floral transition by promoting the expression of the meristem-identity gene *LEAFY*.[54] *SPL3* mRNA levels are regulated by micro RNA; in particular, miR156 reduces SPL3 levels through post-transcriptional regulatory mechanisms.[55–57] Despite the increased expression of *SPL3* transcript levels in the apex of plants transferred from short- to long-day conditions,[53] constitutive overexpression of miR156 delays flowering time particularly under short-day, rather than under long-day conditions.[56,58] We have observed a similar phenotype in a mutant recently isolated in our lab (Fig. 2). These plants have enhanced expression of the endogenous miR156c precursor, caused by the nearby insertion of tandem repeats of the 35S promoter (Fig. 2B) and, as expected, decreased levels of *SPL3* mRNA (Fig. 2B). We have conducted a careful evaluation of flowering time under different photoperiodic conditions in these plants, which clearly indicates that *miR156c* overexpression delays flowering time particularly under short-day conditions, and this effect decreases as the days become longer (Fig. 2A, C). Challenged by the observation that while *SPL3* expression increases in the apex upon transfer to long days, the phenotype of *miR156c* overexpression on flowering time is stronger under short days conditions, and taking into account the recent demonstration that *miR156c* regulates flowering time through its effect on *SPL3* not only in the shoot apical meristem but also in the leaves,[58] we investigated *SPL3* expression in the leaves. The examination of the photoperiodic regulation of expression of *SPL3* in the aerial part of three-week-old seedlings, which represents mRNAs expressed predominantly in leaves, revealed strongly depressed levels of mRNA in plants grown under long-day conditions and a significant increase during the night in plants grown under short-day conditions (Fig. 2D). In agreement with our observations, publicity available microarray data show that *SPL3* expression in young entire seedlings shows enhanced levels under short- compared to long-day conditions. Taken together, these observations indicate that photoperiodic regulation of *SPL3* in the leaves of *Arabidopsis* plants constitutes a short-day flowering promoting pathway in a long-day plant.

Photoperiodic regulation of the expression of other genes involved in the control of flowering in *Arabidopsis*

To investigate whether, in addition to its effects on *CO*, *FT* and *SPL3*, day length modifies the expression of other genes that regulate flowering time in *Arabidopsis*, we used a TAIR (The Arabidopsis Information Resource, www.arabidopsis.org) list of flowering-time-related genes and publicity available data of diurnal patterns of expression under short or long days.[59] The full list of genes is presented in Fig. S1 (ESI†), and selected cases are shown in Fig. 3. Long, compared to short days increased the expression of genes such as *FZO-LIKE* (*FZL*) and *SAL1/FIERY1* (*FRY1*) (Fig. 3A) that accelerate flowering as indicated by the delayed flowering of their loss-of-function mutants.[60,61] *FZL* is a plant-specific member of the dynamin superfamily, which is targeted to chloroplasts[22] and the connection to the control of the photoperiodic pathway has not been established. *SAL1/FRY1* encodes an enzyme with inositol polyphosphate 1-phosphatase and 3′(2′),5′-bisphosphate nucleotidase activity,[62] which promotes the expression of *FT* without affecting the expression of *CO*.[61]

Long, compared to short days, reduced the expression of genes that delay flowering such as *TERMINAL FLOWER 2* (*TFL2*)/*LIKE HETEROCHROMATIN PROTEIN 1*, *EARLY FLOWERING 6* (*ELF6*), *ELF3* (see above), *EMPFINDLICHER IM DUNKELROTEN LICHT 1* (*EID1*), *FRIGIDA-LIKE 2* (*FRL2*), *CONSTANS-LIKE 9* (*COL9*) and *TEMPRANILLO* (*TEM*) (Fig. 3B). The *tfl2* mutant flowers much earlier than the wild type under long or short days and shows very high expression of *FT*.[63] TFL2 co-localizes with genes (including *FT*) that possess nucleosomes with trimethylated lysine residues at position 27 of histone 3,[64] and is involved in the repression of *FT* expression in the middle vein, due to a negative effect on an enhancer element located between 1.0 and 4.0 kb upstream of the start codon of *FT*.[31] ELF6 is a jumonji/zinc-finger-class transcription factor that acts as repressor of the photoperiodic pathway,[65] as it is involved in the repression of *FT* transcription by removing methyl groups from histone H3 lysine 4 at the *FT* locus.[66] EID1 is an F-box protein that delays flowering, likely *via* its effects on phytochrome A signalling.[67,68] *FRL2* promotes the activation of *FLC* mediated by *FRIGIDA*,[69] probably by forming a complex with FRI.[70] COL9 is a nuclear protein that represses flowering by reducing the expression levels of *CO*, *FT* and *SOC1*.[71] TEM binds the 5 UTR of *FT* and represses *FT* expression.[72]

Long, compared to short days, enhanced the expression of *RELATED TO AP2.7* (*RAP2.7*)/*TOE1* and *ARABIDOPSIS THALIANA HOMEOBOX GENE 1* (*ATH1*) (Fig. 3C), which actually delay flowering. TOE1 is an APETALA 2 transcription factor negatively regulated by miRNA172.[73] *TOE1* negatively regulates *FT* expression independently of CO.[48] ATH1 is a transcription factor that represses flowering by activating *FLC* expression.[74]

Finally, long, compared to short days, reduced the expression of *GI*, *SET DOMAIN GROUP 26* (*SDG26*)/*ASH1-RELATED PROTEIN 1* (*ASHH1*) and *VERNALIZATION INSENSITIVE 3* (*VIN3*) (Fig. 3D), which are positive regulators of flowering. *SDG26* is a histone methyltransferase that down-regulates the expression of *FLC* and other MADS-box flowering repressors but without showing obvious effects on the levels of methylation in these genes.[75] *VIN3* is a homeodomain finger-containing protein required for *FLC* repression by vernalisation and the associated changes in *FLC* chromatin.[76]

In summary, in addition to favour the coincidence between *CO* expression and light leading to CO stability and CO-induced *FT* expression, long days reduce the expression of genes that delay flowering and promote the expression of genes that accelerate flowering, potentially reinforcing the photoperiodic response. Long days also increase the expression of genes that delay

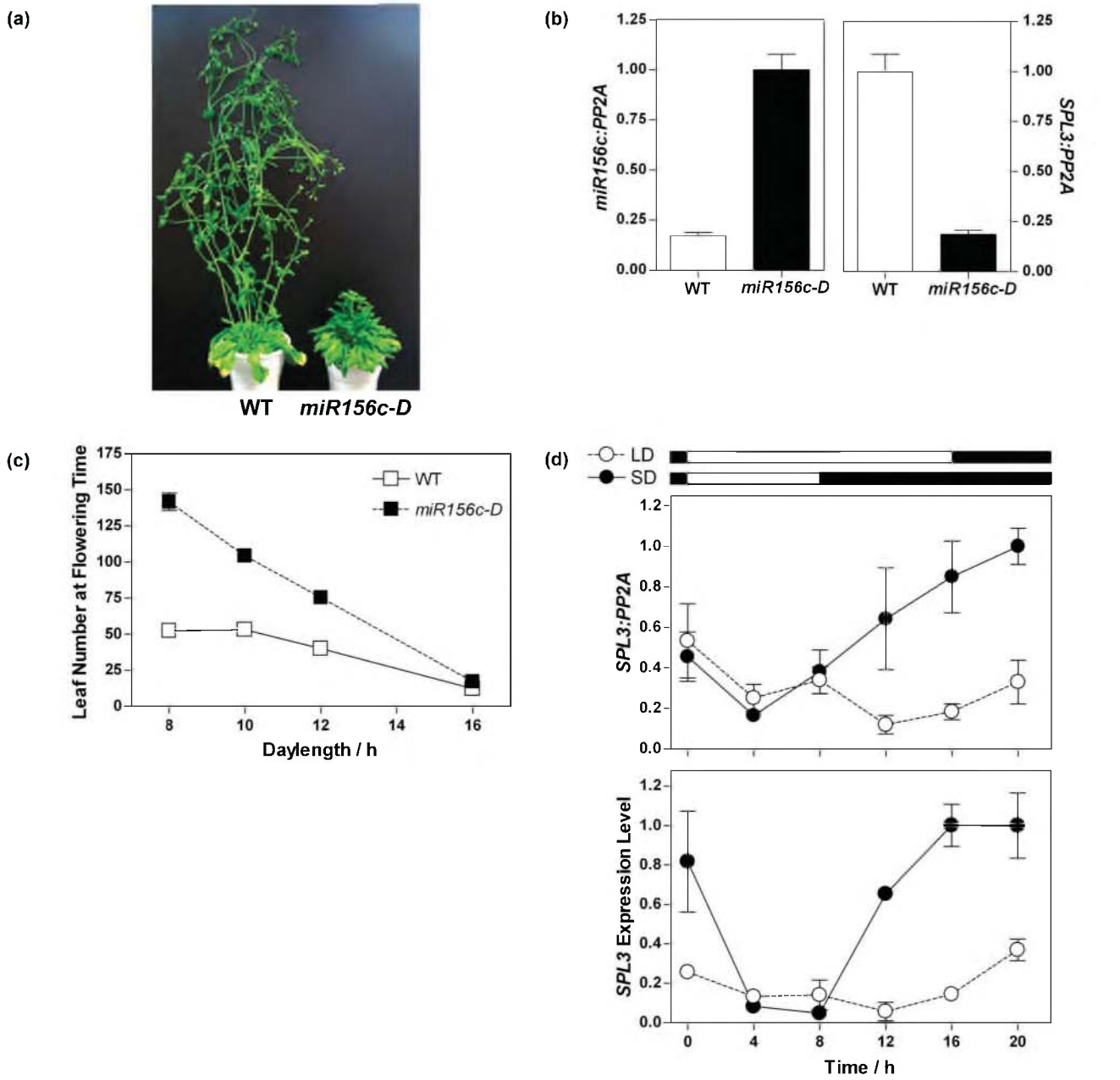

Fig. 2 The *miR156c-SPL3* pathway promotes flowering of the long-day plant *Arabidopsis thaliana* under short days. (a) Representative wild type (WT, Columbia) and *miR156c-D* mutant plants grown under a photoperiod of 10 h (white light from fluorescent tubes, 48 µmol m^{-2} s^{-1}) for 3 months. We isolated the *miR156c-D* mutant by screening a pool of activation tag T-DNA mutagenised plants (stock no. CS31100, Arabidopsis Biological Resource Center). The location of the T-DNA was determined by TAIL-PCR[96] and verified by DNA sequencing. (b) Expression of *miR156c* (left) and its target, the *SPL3* gene (right) measured by qPCR in seedlings grown under a photoperiod of 16 h. 21-day-old seedlings were harvested 20 h after the beginning of the photoperiod. (c) Flowering time of WT and *miR156c-D* seedlings grown under different photoperiods. Irradiance was adjusted to equalise the daily integral for all photoperiods (1,728 mol m^{-2} day^{-1}). Flowering time measured as the number of rosette leaves at time when the flower bolt was 1 cm high is plotted against photoperiod. (d) Photoperiodic control of *SPL3* expression. Expression levels measured by qPCR in WT Columbia seedlings (top) or in microarray experiments with WT plants of the accession Landsberg *erecta* (bottom, drawn after reference[59,97]) under short days (SD, 8 h) or long days (LD; 16 h). qPCR data are expressed relative to *PP2A* levels and normalized to the maximum level in each experiment (for methods see supplementary information). Each datum point is means ±SE (whenever larger than the symbols) of 2 (b, d bottom) or 3 (d top) independent biological samples or of at least 15 plants (c). Plants were grown at 22 °C in pots as described[14] (a–d).

flowering and reduce the expression of genes that accelerate flowering, potentially counteracting the photoperiodic response. Some of these photoperiodically-controlled genes act directly on *FT* itself, others would act upstream *FT*, and in other cases the connection to the established points of the control of flowering remains to be elucidated.

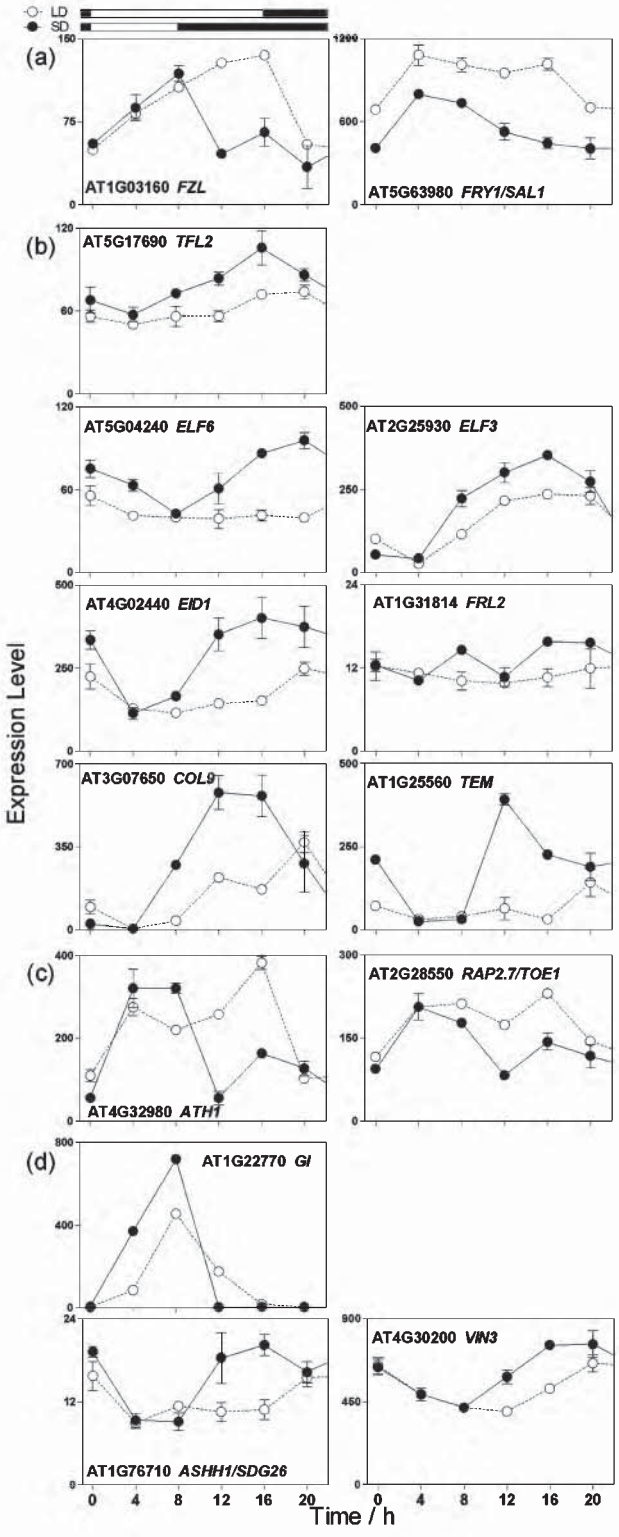

Fig. 3 Photoperiodic regulation of the expression of genes involved in the control of flowering in *Arabidopsis thaliana*. (a) Promotion by long days (LD, 16 h) compared to short days (SD, 8 h) of the expression of genes that accelerate flowering. (b) Inhibition by long days of the expression of genes that delay flowering. (c) Promotion by long days of the expression of genes that delay flowering. (d) Inhibition by long days of the expression of genes that promote flowering. Diurnal pattern of expression were drawn after publicity-available data.[59] Each datum point is mean ± SE of two biological replicates. An extended set of genes is shown in Fig. 1 (ESI).

Rice: promotion under short days, active repression under long days

The current hypothesis to explain the promotion of flowering time by short days in rice is based on the combined action of pathways converging to control the expression *HEADING DATE 3a* (*Hd3a*). *Hd3a* is a rice ortholog of *FT* detected as a quantitative trait locus in a cross between rice cultivars Nipponbare and Kasalath, which promotes flowering.[77] The analysis of 64 rice cultivars that include the genetic diversity from around the world has revealed a strong direct correlation between flowering time and *Hd3a* expression level.[78] Under long days, phytochrome perception of the light signal represses the expression of six *FT*-like genes, including *Hd3a*, *RICE FLOWERING LOCUS T 1* (*RFT1*) and *FT-LIKE* (*FTL*).[79] However, under long days, *RFT1* retains a peak of expression in the morning and *FTL* retains a peak of expression at the beginning of the night.[79] Morning *Hd3a* mRNA levels are high in plants grown under day lengths of ≤13 h and show a steep decrease to about one-tenth of the high expression, at a day length of 13.5 h and to undetectable values at day lengths of ≥14 h.[6] Under short days, *Hd3a* is expressed in the leaves to higher levels than in other organs and the Hd3a protein moves through the phloem from the leaf to the shoot apical meristem to induce flowering.[80] RFT1 is also produced in the leaves and migrates to the apex to induce flowering, but in contrast to Hd3a, it is more important to promote flowering under long days than under short days[81] (see below). In the apex, Hd3a[82] and RFT1[81] induce the expression of *OsMADS14* and *OsMADS15*, which are rice orthologs of *AP1*.

Early heading date 1 (*Ehd1*) encodes a B-type response regulator with no clear *Arabidopsis* orthologues that promotes *Hd3a* expression[83] and is central to the pathway that positively controls flowering under short days. *Ehd1* was originally identified as a flowering time quantitative trait locus in crosses between T65, which apparently bears a loss of function allele and either an accession of African rice (*Oryza glaberrima* Steud) or Nipponbare, which bear functional alleles.[83] A near isogenic line bearing a functional allele flowers early particularly under short days and shows enhanced expression of *Hd3a* and *RFT1* under short days.[83] *Ehd1* is expressed at low levels under long days and its expression is promoted by one week of exposure to short days.[83] Repression under long days requires phytochrome.[6] The expression of both *Ehd1* and *Hd3a* decreases sharply with increasing day length above 13 h, reaching undetectable values at 14 h.[6] Under short days, the expression of *Ehd1* (and hence the expression of *Hd3a* and the transition to flowering) is positively regulated by *OsMADS51*, which shows a maximum peak of expression at the end of short days.[84] In turn, *OsMADS51* expression is promoted by GI mainly under short days.[84] Under short days, GI promotes the expression of *OsMADS51*, which promotes the expression of *Ehd1*, which promotes the expression of *Hd3a*, which promotes flowering. Flowering, and the expression of *Ehd1* and *Hd3a* require *Oryza sativa INDETERMINATE1* (*OsId1*) both under short and long days.[85–87]

The *Grain number, plant height and heading date locus 7* (*Ghd7*) encodes a CCT domain protein with no clear *Arabidopsis* orthologues that represses *Ehd1* and hence *Hd3a* expression[6,88] and is central to the pathway that negatively controls flowering under long days. The expression of *Gdh7* is strongly promoted by

phytochromes under day length above 13 h (particularly during the light period),[6,88] but *Gdh7* expression retains a significant level under short days.[6] Under long days, active *Gdh7* alleles almost completely suppress the expression of *Hd3a via* the suppression of the morning peak of *Ehd1* expression.[6,88] The expression of *Gdh7* is positively regulated by GI.[6]

Ito *et al.*[6] have recently presented a model of the interplay between light conditions *Gdh7* and *Ehd1*. Under short days, dawn activation of blue-light photoreceptors rapidly induces a peak of *Ehd1* expression in a response that requires GI. This light induction is under circadian control and dusk light is not effective. The rhythm of sensitivity is similarly entrained by either short or long days. The promotion of *Gdh7* expression by light absorbed by phytochrome is also controlled by a rhythm of sensitivity but, in contrast to the case of *Ehd1*, this rhythm depends on the photoperiod. Under long days, maximum sensitivity occurs at dawn and therefore, there is a coincidence between the presence of light and the sensitivity to light. Under short days, maximum sensitivity occurs during the night and therefore, there is no coincidence between the presence of light and the sensitivity to light. However, a pulse of red light given during the night of a short day is effective to promote *Gdh7* and repress flowering. Morning induction of *Gdh7* under long days blocks the expression of *Ehd1* the following morning.

Heading date 1 (*Hd1*) is a homolog of *CO* identified as quantitative trait loci, allelic to *photoperiod sensitivity 1* (*se1*), which promotes flowering under short days and inhibits flowering under long days.[89] The *Hd1*-mediated promotion of flowering under short days involves enhanced expression of *Hd3a*[77,79] independently of *Ehd1*.[83] The Hd1-mediated inhibition of flowering under long days correlates with its reduction of *Hd3a*, *RTF1* and *FTL* expression.[79] The expression of *Hd1* is not strongly affected by day length but it could be fine-tuned by GI[90] as observed for *CO* in *Arabidopsis*. The transcriptional activity of Hd1 might be post-transcriptionally regulated depending on the coincidence between diurnal changes in expression and phytochrome activation by light.[79] A CO-like protein from a short day plant can complement the *co* mutant phenotype of a long day plant[91] and *vice versa*.[92] Therefore it must be assumed that the switch between long-day and short-day plant responses lies in some auxiliary factor(s) that regulate(s) CO activity such that it represses instead of promotes *FT* expression in short day plants. The idea of additional factors might also explain the contrasting action of Hd1 under short or long days in rice.

Rice: promotion of flowering under long days

In accordance with the patterns of *Hd3a* expression described above, RNAi lines with reduced *Hd3a* levels flower late under short days and only slightly late under long days. Under short days, *Hd3a* and *RTF1* show redundancy as the expression of *RTF1* increases in lines with reduced *Hd3a*.[82] RNAi lines with reduced levels of *RTF1* flower slightly late under short days. However, these lines flower very late under long days.[81] A similar pattern is shown by mutants in *OsMADS50*, a rice ortholog of *Arabidopsis SUPPRESOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*), which show reduced expression of *Ehd1*, *Hd3a* and *RTF1*. Therefore, under long days, the expression of *Ehd1* is inhibited by *Gdh7* and promoted by *OsMADS50*. Although *Hd3a* and *RTF1* are downstream *Ehd1*, the former is more important under short days and the latter is more important under long days. The molecular origin of this differential function is not clearly established.[81]

Other ways of controlling FT

The previous sections, which concentrate on the mechanisms of photoperiodic control of flowering in *Arabidopsis* and rice, demonstrate the key role of *FT* or *FT*-like genes as integrators of photoperiodic signals. Here, we present two additional cases where FT is central to the induction of flowering but it is controlled apparently *via* different mechanisms. Pharbitis (*Ipomoea nil*) is a short-day plant used in classical physiological studies (when it was known by its former name *Pharbitis nil*). Two orthologs of *FT* (*Pn FT1* and *Pn FT2*) are expressed during the long nights that accompany inductive short days.[93] When day length is varied, the expression of these genes correlates positively and precisely with the physiological response. Furthermore, if an inductive night is interrupted by a light pulse, both the expression of *Pn FT1* and *Pn FT2* and flowering are repressed. Overexpression of *Pn FT1* promotes flowering in Pharbitis. Thus, physiological and genetic evidence is consistent with a role of *Pn FT1* and *Pn FT2* in the induction of flowering in Pharbitis. Compared to *Arabidopsis* and rice, the difference appears to be in the mechanisms *Pn FT1* and *Pn FT2* control by day length. The expression of these genes shows a circadian rhythm that is manifested under free-running conditions of continuous darkness. When plants entrained under long days are transferred to continuous light for periods of different duration and then transferred to continuous darkness, *Pn FT1* and *Pn FT2* expression increase in darkness at a constant time after the beginning of darkness and independently of the timing of the previous light-on signal. This indicates that the rhythm is set by the transition from light to darkness, or dusk signal. If the night is short, the plant is exposed to light before the rising phase of *Pn FT1* and *Pn FT2* expression and light blocks the occurrence of this increased expression. Even a brief exposure to light (*e.g.* a 10 min night break) is enough to repress the expression of these genes *via* mechanisms that remain to be elucidated.

Cucurbita moschata is a short-day plant where flowering is promoted by FT-LIKE proteins transported in the phloem.[94] These *FT-LIKE* genes also promote flowering when expressed in *Arabidopsis*. In stem vascular tissues, the mRNA levels of the *FT-LIKE1* gene of *Cucurbita moschata* are slightly increased, rather than reduced by long compared to short days. However, the level of protein in excised vascular tissues as well as in the phloem sap is higher in plant grown under inductive short days. This indicates a post-transcriptional control of *FT-LIKE1*, which could involve changes in translocation.[94]

Conclusions

Day length is the most important seasonal cue from the light environment controlling flowering in sensitive plants. A feature that would be common in the species analysed so far is that elevated levels of FT or FT-like proteins are predicted to reach the apex when the leaves are exposed to inductive conditions (although not all the pieces of evidence are available for each case). In each case, there is a core mechanism controlling the

levels of FT. In *Arabidopsis*, the key is the direct transcription control of *FT* expression by CO. In rice, the induction of flowering by short days results from the interplay between light conditions *Gdh7* and *Ehd1*, which controls homologues of *Arabidopsis FT*, like *Hd3a*, *RFT1* and *FTL*. In Pharbitis, a circadian rhythm and light control *FT* homologues and in *Cucurbita*, the post-transcriptional control of FT appears to be important. In addition to the core mechanisms, the regulation of *CO* waveform by light in *Arabidopsis*, and the rice homologue of *CO* (*Hd1*) reinforce the photoperiodic response. Long, compared to short days, enhance the expression of other positive regulators of flowering and reduce the expression of negative regulators at least in *Arabidopsis*. The observations in *Cucurbita* suggest that the occurrence of post-transcriptional photoperiodic controls of *FT* in *Arabidopsis* and rice should be evaluated.

In addition to the above pathways defining the inductive (or potentially inductive) forces acting under the photoperiodic conditions that trigger flowering, there are pathways that act in the opposite direction. In *Arabidopsis SPL3* promotes flowering under short days. Furthermore, long, compared to short days, enhance the expression of other negative regulators of flowering and reduce the expression of positive regulators. In rice, *RTF1* promotes flowering under long days. The existence of multiple pathways with contrasting photoperiodic effects on flowering time within a single species, suggests that the photoperiodic behaviour of plants results, at least in part, from the net balance of positive and negative effects of photoperiodic conditions on multiple regulatory pathways. This concept does not contradict the existence of a clear hierarchy, where some of these forces are stronger than others. The occurrence of multiple positive and negative pathways would offer versatile tools[95] to adjust flowering time more precisely to the most favourable period of the year under which plant fitness is maximized at each particular geographic location.[6,81]

Acknowledgements

This work was supported by grants from the University of Buenos Aires (grant no. G044 to J.J.C.), International Centre for Genetic Engineering and Technology (grant no. CRP/ARG07–02 to J.J.C.), Agencia Nacional de Promoción Científica y Tecnológica (grant no. PICT 1913 to J.J.C. and PICT 1026 to M.J.Y.) and Howard Hughes Medical Institute International Scholar award to M.J.Y.

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