Chloroplast retrograde signal regulates flowering

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Light is a major environmental factor regulating flowering time, thus ensuring reproductive success of higher plants. In contrast to our detailed understanding of light quality and photoperiod mechanisms involved, the molecular basis underlying high light-promoted flowering remains elusive. Here we show that, in *Arabidopsis*, a chloroplast-derived signal is critical for high light-regulated flowering mediated by the *FLOWERING LOCUS C* (*FLC*). We also demonstrate that PTM, a PHD transcription factor involved in chloroplast retrograde signaling, perceives such a signal and mediates transcriptional repression of *FLC* through recruitment of FVE, a component of the histone deacetylase complex. Thus, our data suggest that chloroplasts function as essential sensors of high light to regulate flowering and adaptive responses by triggering nuclear transcriptional changes at the chromatin level.

chloroplast retrograde signals | flowering regulation | *FLC* | chromatin remodeling | high light

The transition from the vegetative phase to the reproductive phase, also called flowering, is a crucial developmental switch in higher plants and is profoundly affected by various environmental and endogenous factors, including light, temperature, hormone status, and age (1, 2). In the model dicotyledonous plant *Arabidopsis thaliana*, genetic networks defining the intricate mechanisms by which plants initiate the floral induction have been studied extensively over the past several decades (3, 4). The photoperiod and vernalization pathways monitor the seasonal changes in day length and prolonged exposure to winter cold, respectively, to control flowering time, whereas ambient growth temperature regulates flowering independently. In addition, flowering time also responds to intrinsic signals, including the growth regulator gibberellin, endogenous carbohydrate levels, age-dependent changes in the expression of specific microRNAs, and the autonomous pathway. These different genetic pathways ultimately converge to regulate a set of floral integrator genes, *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF CONSTANS 1* (*SOC1*), which in turn activate the expression of floral meristem identity genes to trigger the formation of flowers (5–10).

Among the central players in flowering regulation, *FLOWERING LOCUS C* (*FLC*) is the potent floral repressor gene encoding a MADS-box transcription factor (11, 12). In winter-annual *Arabidopsis*, *FLC* expression is stably silenced by prolonged cold exposure during winter and then maintained until embryogenesis in an epigenetic-dependent manner. This process involves the polycomb-mediated multiple chromatin regulation and different long noncoding RNA (lncRNA) transcription to quantitatively repress the *FLC* gene expression, thereby enabling other floral promotion signals to induce flowering in the spring (13, 14).

Light is one of the most prominent environmental factors in the regulation of flowering at multiple levels, including light quality, intensity, and duration. Intensive molecular and genetic studies have provided considerable insight into the relevant mechanisms, particularly with regard to light quality and photoperiod (6, 15). For photoperiodic flowering, light is perceived in leaves by the sensory photoreceptors, phytochromes and cryptochromes, to coincide with the rhythmic expression of *CONSTANS* (*CO*), mediated by the cooperation of two circadian clock components, *GIGANTEA* (*GI*) and *FLAVIN KELCH F BOX1* (*FKF1*) (16–18). The CO protein in turn is stabilized by light and accumulates only under long days to activate transcription of the *FT* gene, whose product then acts as a mobile signal and travels to the shoot apical meristem to induce floral transition through interaction with the bZIP transcription factor FD (19–21).

The flowering transition is also regulated by light quality, mainly shade light conditions with an altered ratio of red to far-red light (R:FR). Under shade conditions, the red light photoreceptor phytochrome B (phyB) acts through *PHYTOCHROME AND FLOWERING TIME 1* (*PFT1*) to increase *FT* expression and promote flowering, in part by enhancing the CO-dependent photoperiodic response (22, 23).

As a key parameter of light, light intensity also plays independently essential roles in flowering time regulation (24). *Arabidopsis*, like many higher plants, responds to high light by increasing the vegetative growth rate and accelerating its reproductive transition (25). The molecular mechanisms involved in light intensity control of flowering remain largely unknown, however.

In this study, we used a combination of biochemical and genetic approaches to reveal an unexpected role of chloroplasts in high light-mediated flowering, and have established a molecular framework that links the functional state of the endosymbiotic

Significance

Proper timing of flowering transition is vital for the reproductive success of plants and orchestrated by endogenous and external factors; however, the mechanisms of how plants regulate flowering under high light are not well understood. In this study, we show that promotion of flowering by high light involves the coupling of chloroplast retrograde signals and transcriptional silencing of the floral repressor *FLOWERING LOCUS C* (*FLC*). In response to high light, a chloroplast envelope-localized transcription factor, PTM, releases its N-terminal fragment through processing to associate with the chromatin remodeler FVE and suppresses *FLC* transcription. This report describes the molecular basis for a unique intracellular signaling pathway derived from chloroplasts in which plants regulate the developmental timing of the flowering transition.

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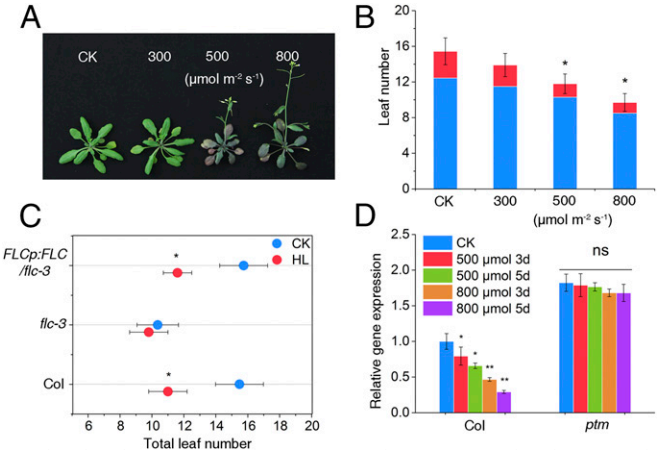

Fig. 1. *FLC* is required for high light-induced flowering in *Arabidopsis*. (*A*) Images of WT plants showing their flowering phenotype under different light irradiances (300, 500, and 800 μmol m^{-2} s^{-1}). CK represents normal light (100 μmol m^{-2} s^{-1}). (*B*) Flowering times of WT plants under different light irradiances assessed by leaf number. Red bars represent cauline leaves, and blue bars represent rosette leaves. Total leaf numbers were counted using at least 16 plants. (*C*) Flowering times of Col-0, *flc-3*, and the complemented plants under normal light (100 μmol m^{-2} s^{-1}) and high light (800 μmol m^{-2} s^{-1}). CK, normal light; HL, high light. (*D*) Effects of high light treatment on *FLC* expression. Plants treated with different light intensities (*Left*) under LD (16-h light/8-h dark) conditions were used for extraction of total RNA, and mRNA levels of *FLC* were determined using qRT-PCR. Values shown are mean ± SD; $n = 3$. The results were statistically treated using Student's *t* test. $^*P < 0.05$; $^{**}P < 0.01$; ns, not significant.

organelles to the nuclear transcriptional regulation of *FLC* through a retrograde signaling pathway. Our study also provides a unique perspective on how plastid information is perceived and translated into the histone code through intracellular coordination to control plant developmental reprogramming and growth.

Results

High Light-Induced Flowering Requires *FLC* Activity. To explore the molecular mode of high light action on flowering, we examined the flowering time of 57 wild accessions of *A. thaliana* globally distributed in specific geographic locations at different light intensities (normal light, 100 μmol m^{-2} s^{-1}; high light, 800 μmol m^{-2} s^{-1}) under long-day (LD) conditions. Seedlings were grown for 3 wk under a 16-h light/8-h dark cycle under normal light and subsequently subjected to normal light or high light for 5 d. In most cases, flowering occurred during this 5-d period. Our results show that most accessions flowered earlier on average under 800 μmol m^{-2} s^{-1} photons than under 100 μmol m^{-2} s^{-1} photons, as measured by total leaf number at bolting (Fig. S1), of which Columbia-0 (Col-0) is a typical genotype with a robust response (Fig. 1 *A* and *B*), consistent with the earlier finding of promotion of floral transition by high light (25). Notably, within these accessions, we observed that a subset of isolates, including Landsberg *erecta* (L*er*), Da(1)-12, and Shakdara, which contain nonfunctional alleles of *FLC*, did not flower earlier under high light (26, 27) (Fig. S2*A*).

This result raised the possibility that the high light-induced flowering response might depend on *FLC* activity. To address this point, we further analyzed the flowering behavior of *flc-3*, a loss-of-function mutant of *FLC* in a Columbia background (11). As expected, the *flc-3* mutant did not show any significant difference in flowering time with or without high light treatment, whereas the rescued transgenic lines with the *FLC* gene driven by its promoter (*FLCp:FLC*) restored the impaired high light response of *flc-3* (Fig. 1*C*). We obtained similar results using the *flc-20* mutant in C24 background (28) (Fig. S2*B*). Interestingly, we also observed that the *FRI*-Col allele, which leads to high expression of *FLC*, had a normal response to high light only after a vernalization treatment of 30 d, which is well known to repress *FLC* transcription (11) (Fig. S2 *C* and *D*). These results suggest that high light-induced flowering requires *FLC* activity, and led us to investigate whether high light regulates *FLC* expression to control flowering.

We next examined the level of *FLC* gene expression on high light treatment and found that compared with plants grown under normal light irradiance, *FLC* transcript levels were 2.5-fold lower in plants treated with 500 μmol m^{-2} s^{-1} photons and 4-fold lower in plants treated with 800 μmol m^{-2} s^{-1} photons (Fig. 1*D*). Consistently, examination of *FLC* expression patterns in vivo using a transgenic reporter of *FLCp:LUC* showed that LUC activity was substantially decreased by high light treatment (Fig. S3). These data suggest that high light promotes flowering through transcriptional repression of *FLC*.

High Light Regulates Flowering Through Chloroplast Retrograde Signals. High light is known to perturb the photosynthetic electron transport chain activity, which in turn is known to trigger chloroplast retrograde signaling (29, 30). In addition, our previous study revealed that a chloroplast envelope-bound PHD-type transcription factor with transmembrane domains, PTM, mediates high light-triggered plastid signals to regulate photosynthesis and stress responsive gene expression (31). To test whether PTM-mediated plastid signaling is involved in high light-induced flowering, we examined the flowering behavior of the *ptm* mutant and observed that high light treatment promotes significantly earlier flowering in wild type (WT) plants but has a minor effect on the *ptm* mutant (Fig. 2*A*), suggesting that *PTM* is critical for high light induction of flowering. In addition, quantitative PCR (qPCR) assays and luciferase imaging data showed that changes in *FLC* expression under high light treatment are significantly reduced in the *ptm* mutants compared with WT plants (Fig. 1*D* and Fig. S3). To test whether *PTM* and *FLC* genetically interact to regulate flowering, we introduced the *ptm* mutant into the *flc-3* background and determined the flowering time of the *ptmflc-3* double mutant. The *ptmflc-3* double mutant flowered as early as the *flc-3* single mutant, and exhibited an impaired response to high light-induced flowering (Fig. 2*B*), suggesting that *FLC* acts downstream of *PTM* to regulate flowering. Taken together, these results indicate that plastid signals mediated by *PTM* participate in high light-accelerated flowering through the repression of *FLC*.

As one of the main photosynthetic products, sugars accumulate under high light conditions and play a key role in the regulation of flowering (32). However, we found no significant

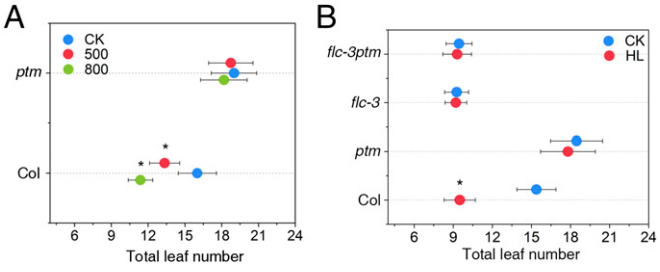

Fig. 2. *ptm* is impaired in high light-regulated flowering. (*A*) Effects of high light treatment on the flowering time of WT and *ptm* mutant plants. (*B*) Flowering times of the indicated genotypes assessed by total leaf numbers under LD conditions. All plants were grown under LD conditions at 100 μmol m^{-2} s^{-1} for 3 wk. Total leaf numbers of Col-0, *ptm*, *flc-3*, and *ptmflc-3* plants under normal light (100 μmol m^{-2} s^{-1}) and high light (800 μmol m^{-2} s^{-1}) conditions were determined using at least 16 plants of each line.

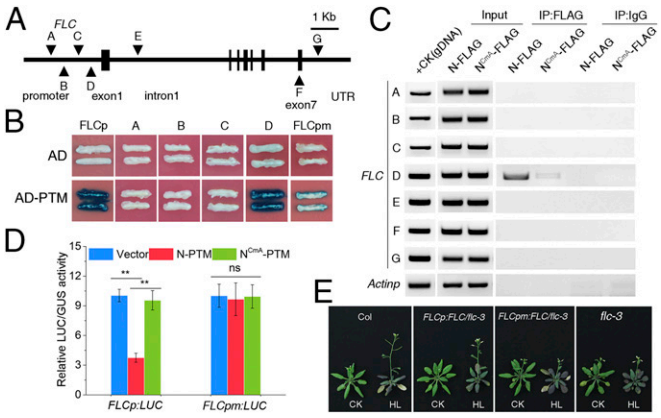

Fig. 3. N-PTM binds directly to the *FLC* promoter and represses its transcription. (*A*) Schematic representation of the *FLC* genomic region and positioning of qPCR amplicons for ChIP analysis. Triangles indicate primers for ChIP-qPCR. (*B*) Yeast one-hybrid assay of N-PTM binding to the proximal region of the *FLC* promoter. *FLCp* represents the 1-kb *FLC* promoter upstream of the transcriptional start site, and *FLCpm* represents *FLC* promoter lacking the 39-bp critical region. (*C*) ChIP assay of N-PTM binding to the *FLC* gene locus in vivo. Chromatin fragments (~500 bp) were prepared from 2-wk-old seedlings of *35S:PTM-N-FLAG* and *35S:PTM-N^{CmA}-FLAG* transgenic plants and immunoprecipitated with anti-FLAG antibody. Precipitated DNA was amplified using specific primers as indicated in *A*. The *ACTIN12* promoter served as a negative control. (*D*) N-PTM represses *FLC* gene transcription in transient luciferase reporter assays. The relative luciferase activity was normalized to the GUS activity and shown in LUC/GUS. Error bars represent SD. (*E*) Effect of the 39-bp critical region on the flowering phenotype under high light conditions. Plants transformed with *FLCp:FLC* and *FLCpm:FLC* in the *flc-3* background were grown at 100 μmol m^{-2} s^{-1} under LD conditions for 2 wk and then exposed to 800 μmol m^{-2} s^{-1} high light.

difference in sucrose accumulation with high light treatment or in the flowering response to sucrose application between WT and the *flc-3* mutant (Fig. S4 *A* and *B*). These results suggest that *FLC*-dependent regulation of flowering by high light cannot be attributed to increased sugar production. To further examine whether thermo induction or light quality alteration is involved, we analyzed the flowering time of mutants deficient in these two flowering pathways (*pif4*, *arp6*, and *phyB*, *pft1*) in response to high light (22, 33, 34). Unlike *flc-3*, these mutants all flowered earlier under high light conditions (Fig. S5 *A* and *B*), suggesting that high light-regulated flowering is distinct from these two flowering pathways.

PTM Is a Negative Regulator of *FLC*. Given that *PTM* encodes a PHD-type transcription factor that requires a proteolytic cleavage at the N terminus to produce a protein targeted to the nucleus with DNA binding activities (31), we next tested whether PTM is a potential regulator of *FLC* through direct binding to its promoter. Yeast one-hybrid assays revealed that N-PTM (the N terminus of PTM protein) specifically binds to the proximal region (designated as the D region) of the *FLC* promoter (Fig. 3 *A* and *B*); however, no obvious binding with promoters of other *FLC* clade members, including *MADS AFFECTING FLOWERING* (*MAF*) genes, was observed (Fig. S6*A*), supporting the binding specificity. We subsequently confirmed this binding in vivo by chromatin immunoprecipitation (ChIP) assays using transgenic plants constitutively expressing FLAG-tagged N-PTM, whereas the mutated form of N-PTM with the change in two key cysteines displayed lower binding activity (Fig. 3*C*) (31). We further determined the binding site of N-PTM through a deletion analysis with the yeast one-hybrid assay, and found that a sequence of approximately 39 bp near the transcriptional start site is critical for PTM binding (Fig. 3*B* and Fig. S6 *B* and *C*). In agreement with this observation, electrophoretic mobility shift assays (EMSAs) demonstrated that N-PTM binds to the 39-bp probe from the D region of the *FLC* promoter (Fig. S7).

We next carried out a transient transcription experiment in *Arabidopsis* plants using a dual luciferase assay to directly test the role of N-PTM in *FLC* expression (35). The expression level of luciferase driven by the *FLC* promoter was reduced by approximately threefold relative to that of the control when N-PTM was overexpressed, but not with the mutated form of N-PTM; however, this decrease was significantly abolished when we used the *FLC* promoter lacking the 39-bp critical region to drive luciferase expression (Fig. 3*D*). These data indicate that PTM acts as a repressor of *FLC* expression through binding to the critical region.

To evaluate the biological function of this critical region in vivo, we generated transgenic lines with an *flc-3* background carrying an *FLC* genomic sequence driven by the full-length and mutated forms of the *FLC* promoter: *FLCp:FLC* and *FLCpm:FLC*, respectively. Similar levels of *FLC* transcript were detected in both transgenic plants compared with WT plants (Fig. S8*A*). Analysis of flowering time in response to high light showed that expression of *FLC* under control of the full-length promoter complemented the insensitive response of the *flc-3* mutant. In contrast, this rescue was strikingly reduced when the promoter was replaced with its mutated counterpart (Fig. 3*E* and Fig. S8*B*), indicating that the critical region by which N-PTM binds to *FLC* is crucial for high light-induced flowering. Taken together, these data lead us to conclude that PTM directly binds the *FLC* promoter in a critical region and represses *FLC* expression.

The PHD-type transcription factors, like PTM, are extensively involved in gene regulation in association with histone modifications (31, 36), whereas *FLC* expression is subject to multiple epigenetic regulations in response to various endogenous and environmental cues (37). Therefore, we tested whether

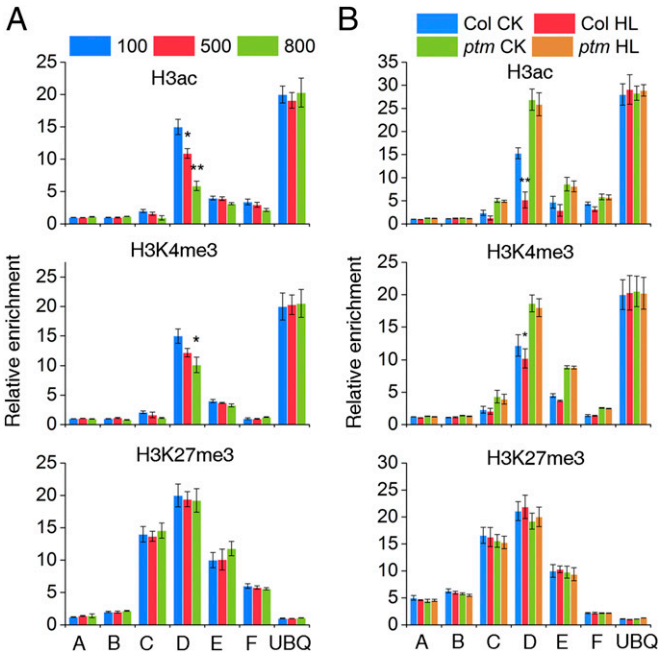

Fig. 4. Regulation of *FLC* by high light at the chromatin level. Shown are relative levels of histone modifications at the *FLC* locus under different light treatments. WT and *ptm* plants grown in soil for 2 wk and treated with high light (500 and 800 μmol m^{-2} s^{-1}) were used for chromatin preparation. ChIP assays were performed using anti-H3ac, anti-H3K4, and anti-H3K27 trimethylation antibodies. *$P < 0.05$; **$P < 0.01$. The three graphs in *A* show the effect of high light on the histone modification markers in WT, whereas *B* represents the changes compromised in the *ptm* mutant.

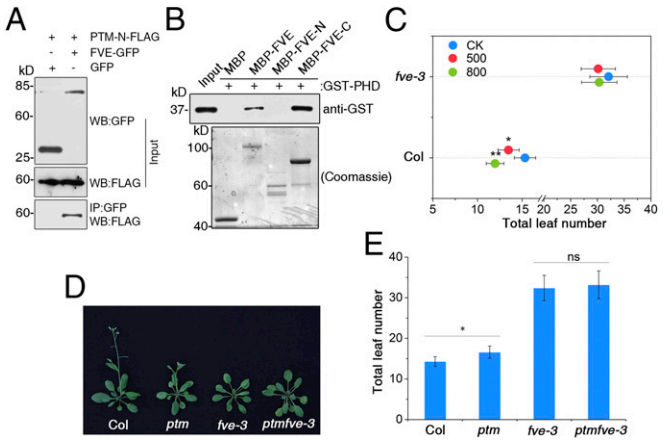

Fig. 5. N-PTM interacts with FVE in the nucleus. (*A*) Co-IP assay in tobacco showing the interaction between N-PTM and FVE. (*B*) FVE interacts directly with N-PTM in a pull-down assay in vitro. (*C*) Flowering time of Col-0 and *fve-3* mutant under different light irradiances (100, 500, and 800 μmol m^{-2} s^{-1}). (*D*) Images of Col-0, *ptm*, *fve-3*, and *fve-3ptm* mutants showing their flowering phenotypes under LD conditions. (*E*) Flowering times of indicated genotypes assessed by total leaf number. The total leaf number of each genotype was recorded using at least 16 plants and statistically treated using Student's *t* test. *$P < 0.05$; ns, not significant.

PTM represses *FLC* transcription through an epigenetic-related mechanism. ChIP assays using antibodies against specifically modified histone3 forms showed that the levels of two active histone marks on *FLC* chromatin, H3ac and H3K4me3, were decreased by high light treatment. In contrast, the level of the repressive mark H3K27me3 was almost unchanged (Fig. 4*A*) (38). Moreover, the H3ac and H3K4me3 levels in response to high light treatment were barely decreased in the *ptm* mutant, suggesting the involvement of PTM in this process (Fig. 4*B*). No obvious global changes in the levels of H3ac, H3K4me3, and H3K27me3 were detected in WT and *ptm* plants under high light treatment (Fig. S9), suggesting that PTM is targeted solely to a subset of unique genes in response to high light. Taken together, these results indicate that high light contributes to *FLC* silencing through modulation of H3ac and H3K4me3 levels on *FLC* chromatin in a PTM-dependent manner.

PTM-Mediated Repression of *FLC* Requires the Chromatin Remodeler FVE. It is generally accepted that functioning of the PHD-type transcription factors in histone modifications requires specific binding of chromatin remodelers (36, 39). To explore the molecular mechanisms underlying PTM functions in *FLC* repression, we screened for PTM-interacting partners with yeast two-hybrid assays using the N-terminal fragment of PTM as bait. This screen identified FVE as a PTM-interacting factor. FVE, a putative retinoblastoma-associated protein, has been shown to contribute to flowering promotion via repression of *FLC* (40, 41). Coimmunoprecipitation (co-IP) and bimolecular fluorescence complementation (BiFC) assays confirmed the physical interaction between FVE and N-PTM in the nucleus (Fig. 5*A* and Fig. S10*A*). Sequence analysis showed that the FVE protein harbors a series of WD40 domains at the C terminus that are known to mediate protein–protein interactions (40). Domain deletion analysis showed that the C terminus of FVE is required for its interaction with N-PTM (Fig. 5*B* and Fig. S10).

This physical interaction led us to further examine whether *FVE* is involved in the high light-triggered early flowering response. Indeed, similar to the *ptm* mutant, the *fve-3* null allele did not flower earlier when treated with high light, whereas other autonomous pathway mutants, including *fld-4*, *fca-9*, *fpa, ld*, *fy*, *flk*, and *skb1-1*, showed earlier flowering similar to WT plants (Fig. 5*C* and Fig. S11*A*) (42, 43), suggesting that *FVE* is specifically involved in this process. However, the *ptmfve-3* double mutant flowered later than the *ptm* mutant, but did not show any additive effects compared with the *fve-3* mutant (Fig. 5 *D* and *E*). Furthermore, constitutive expression of N-PTM induced early flowering in WT plants, but not in the *fve-3* mutants (Fig. S11 *B* and *C*), supporting the view that *PTM* might regulate flowering with a dependence on *FVE*.

High Light Increases the Level of PTM-FVE Complex to Modulate *FLC* Expression. PTM undergoes proteolytic cleavage on chloroplast retrograde signaling, and the processed ~58-kDa N-terminal fragment accumulates in the nucleus after high light treatment (Fig. 6*A*) (31). This cleavage also occurs when plants are treated with increased light intensities. In contrast to the increased nuclear accumulation of N-PTM, FVE protein levels were not significantly altered on high light treatment (Fig. S12). The interaction between PTM and FVE, as well as the repressive effect on *FLC*, prompted us to investigate whether high light represses *FLC* expression by regulating formation of the N-PTM–FVE complex. Therefore, we examined the level of this repressor complex in response to high light by co-IP assays using transgenic plants expressing *FVEp:FVE-GFP*. Indeed, FVE-GFP immunoprecipitated a greater amount of N-PTM protein in a time course experiment with high light treatment (Fig. 6*B*). Furthermore, we confirmed the induced level of this interacting complex during the treatment with increased light irradiance (Fig. 6*C*).

To investigate whether PTM-mediated repression of *FLC* is due to the induced binding of the N-PTM–FVE repressor complex to its chromatin, we performed a ChIP assay on high light treatment using an antibody against FVE. Increased light irradiance specifically enhanced the binding of FVE to the proximal region of the *FLC* promoter by approximately twofold

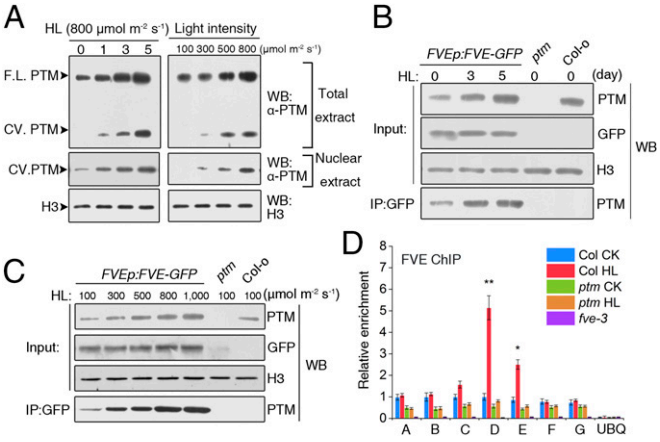

Fig. 6. High light regulates the accumulation of N-PTM in the nucleus and its interaction with FVE. (*A*) High light treatment accelerates the processing of full-length PTM protein and promotes PTM accumulation in the nucleus. Total protein and nuclear proteins were extracted separately from WT plants treated with high light (800 μmol m^{-2} s^{-1}) (*Left*) and different light irradiances (*Right*) and used for immunobloting with a specific PTM antibody. Histone3 protein was loaded as a control. FL, full-length; CV, cleaved; WB, immunoblot. (*B*) Effect of high light treatment on the level of the N-PTM–FVE complex. Nuclear proteins were extracted from *FVEp:FVE-GFP* transgenic plants treated with 800 μmol m^{-2} s^{-1} high light during a time course and immunoprecipitated with anti-GFP antibody. IP, immunoprecipitation; WB, immunoblot. (*C*) Effect of different light irradiances on the level of the N-PTMFVE complex. (*D*) ChIP analysis of FVE binding to the *FLC* locus using an FVE-specific antibody under high light treatment. Here 3-wk-old Col-0, *ptm*, and *fve-3* plants were used for chromatin extraction, and the immunoprecipitated DNA fragments were amplified using specific primers. *UBIQUITIN10* promoter primers served as a negative control.

to threefold, but no binding was detected to other regions or to the promoter of *POLYUBIQUITIN10* (*UBQ10*) (Fig. 6*D*); however, the increased abundance of FVE on the *FLC* proximal promoter was significantly compromised in the *ptm* mutant compared with WT (Fig. 6*D*), indicating that enrichment of FVE on *FLC* chromatin under high light relies on PTM. Taken together, our results suggest that high light silences *FLC* expression by regulating binding of the N-PTM–FVE complex to its chromatin.

Discussion

A prime challenge in plant biology is to understand the molecular mechanisms of how organelles perceive environmental cues to coordinate plant growth, development, and local adaptation once they have become fully integrated into the life cycle of the host eukaryotic cell after endosymbiosis (44). The onset of flowering, which determines the reproductive success of plants, is crucial for the viability, fecundity, and species persistence of angiosperms in the face of different natural environments. For this reason, this developmental transition is closely linked to crop yield and current agricultural productivity, and thus is of great importance both ecologically and economically (2, 4). Here we report that chloroplast retrograde signals triggered by high light regulate floral transition in *Arabidopsis* through *FLC* gene repression (Fig. S13).

As a potent repressor of the flowering pathway, *FLC* has been demonstrated to play a central role in integrating multiple endogenous and exogenous signals, and its expression is under complex control (37, 45). Numerous investigations have elucidated the molecular mechanisms governing the regulation of *FLC*, which involves multiple epigenetic modifications and noncoding RNA, thus providing a paradigm for chromatin-based control of other developmental genes (46). Vernalization, a prolonged period of cold exposure, represses *FLC* expression. This process involves recruitment of the Polycomb Repression Complex 2 (PRC2), which deposits H3K27me3 in the region around the first intron of *FLC* (13). Recent studies revealed that two different lncRNAs, *COOLAIR* and *COLDAIR*, are involved in this process (47, 48), making the mechanism of *FLC* regulation more complicated. *FLC* repression also can be achieved by the autonomous pathway members through chromatin remodeling and RNA processing, in which *FLD* and *FVE* act on H3K4me and H3ac modifications, respectively (40, 49).

Here we have provided evidence indicating that *FLC* is also involved in flowering regulation in response to high light irradiance and the resulting plastid signaling, which highlights the contribution of *FLC* in spatial and temporal adaptive responses. To ensure a timely reproductive transition in face of stresses that interfere with photosynthesis, higher plants are likely to direct the plastid information flow to the nuclear-encoded flowering network governed by *FLC* and initiate floral-related transcriptional reprogramming at the shoot apex. Interestingly, we observed that accessions carrying dominant *FRI* alleles also showed a compromised response similar to that of the *flc* null mutant; however, vernalization treatment for 30 d could restore the WT response to high light (Fig. S2*C* and *D*). Previous studies have identified *FRI* as a potent activator of *FLC*, and vernalization promotes flowering through repression of *FLC*, which involves the disassociation of FRI from *FLC* chromatin (50, 51). Temporal regulation of *FLC* gene expression likely is conferred by differential binding of various complexes at different stages of plant development.

We also have demonstrated that plastid signals are recruited by the chromatin remodeling machinery operating on the *FLC* gene and dedicated to this beneficial flowering. This is corroborated by our observation that *FLC* transcription is substantially repressed in response to high light treatment (Fig. 1*D*), with a concomitant decline in histone modifications that include H3K4me3 and H3ac on *FLC* chromatin (Fig. 4*A*). In addition, we found that a 39-bp region within the *FLC* promoter is critical for the induction of flowering by chloroplast retrograde signals (Fig. 3*E* and Fig. S8*B*), suggesting that the *FLC* promoter also contributes to its epigenetic regulation in addition to the first intron and noncoding RNA (14, 45). Thus, our study supports the idea that specific nucleotide sequence signatures in different parts of *FLC* may be recognized by distinct genetic mechanisms to facilitate a flexible evolutionary response to diverse environmental factors (52, 53).

PTM, a key component of the retrograde signaling pathway, is required for such flowering regulation by perceiving and mediating chloroplast retrograde signals through proteolysis, and by acting upstream of *FLC* to modulate its expression. Cleavage of PTM full-length protein in the chloroplast outer envelope membrane gives rise to N-PTM, which represses *FLC* through the erasure of two active histone marks. FVE, a putative retinoblastoma-associated protein, was further identified as a potential interacting partner of N-PTM that facilitates modification of the *FLC* chromatin status by PTM. Moreover, the interaction between N-PTM and FVE, which responds to light irradiance and plastid signals (Fig. 6 *B* and *C*) may build up a platform for *FLC* regulation at the chromatin level and provides a route for integration of chloroplast status into the histone code. An earlier study demonstrated that FVE plays dual roles in flowering regulation and cold acclimation to provide evolutionary fitness to plants in sensing intermittent cold stress (41). In addition, FVE is also implicated in the photoperiod flowering pathway and is postulated to bind chromatin as a large protein complex of ∼1.0 MDa (54, 55). Thus, it is likely that these versatile functions of FVE in environment-controlled flowering are achieved through specific interactions with distinct partners like HOS1 and CUL4-DDB1 (54, 56). Collectively, our findings suggest that PTM functions not only as a messenger between chloroplast and nucleus, but also as a multifaceted adaptor to recruit specific partners to trigger diverse transcriptional reprogramming events.

After the endosymbiotic engulfment, chloroplasts not only have significantly contributed to the genomic resources of the eukaryotic host cell, but also have evolved to function as environmental sensors to coordinate plant growth, development, and adaptive stress responses (44, 57). This coordination is orchestrated by the interactive exchange of information between the nucleus and organelles, in which retrograde signals are relayed from chloroplasts to inform the nucleus of their metabolic and functional state (58). When plants encounter environmental stresses that perturb photosynthetic functions like high light, adjustments in light-harvesting complex II (LHCII) phosphorylation and antenna size are needed to maintain the redox poise of the PQ pool and the excitation balance between PSII and PSI, further leading to the overall metabolic remodeling of chloroplasts. Chloroplast retrograde signals are subsequently triggered by these changes, ranging from plastid redox status to the levels of various metabolites to coordinate proper expression of nuclear genes involved in photosynthesis fine-tuning and stress response (29, 58). In this view, our results support a more dynamic function of chloroplasts as stress sensors that transmit interorgannellar retrograde signals to prime the floral machinery by eliciting the expression of flowering-related genes, thereby promoting higher plants to complete the reproductive transition under high light stress.

In conclusion, our study reveals a previously unreported cellular response to high light stress that modulates the timing of reproduction, and provides insight into how chloroplasts emerged as key players in plant growth and development after their integration into their host cells through endosymbiosis. Such signaling mechanisms may enable higher plants to more effectively adapt to the ever-changing environment and mitigate detrimental effects to fitness.

Materials and Methods

Plant Materials. *A. thaliana* plants were grown on soil or Murashige and Skoog (MS) plates with 2% sucrose (wt/vol) in a controlled culture room under LD conditions (16-h light/8-h dark) at 22 °C. The *flc-3*, *ptm*, and *fve-3* mutants were in a Columbia background. Transgenic plants were generated through *Agrobacterium tumefaciens*-mediated transformation by the floral dip method. Seeds

from transgenic plants were selected on MS medium supplemented with 20 mg/L hygromycin.

Analysis of Gene Transcript Levels. For real-time qPCR, total RNA was isolated using the Qiagen RNeasy Mini Kit according to the manufacturer's instructions. RNA was treated with DnaseI at 37 °C for 30 min and used for cDNA synthesis. qPCR was performed using 2× SYBR Green Supermix on a Roche LightCycler 480 system. Each sample was quantified in triplicate, and the relative transcript level of each gene was determined by normalization of the expression level vs. that of *PP2A* (*AT1G13320*). The primers used in gene expression analysis are listed in Table S1.

Isolation of Nuclear Protein Extracts. Nuclear protein extracts were isolated from 3-wk-old *Arabidopsis* leaves using the CelLytic PN Isolation/Extraction Kit (Sigma-Aldrich) and then used in the immunoblot and co-IP assays.

Yeast One-Hybrid Assay. To detect the binding activity of N-PTM, plasmid with GAL4 DNA-binding domain fusions were cotransformed with *LacZ* reporter genes driven by various *FLC* promoter fragments into the yeast strain *EGY48* using standard transformation techniques. Transformants were grown on proper dropout plates containing X-gal for blue color development.

More detailed information regarding the experimental procedures used in this study is provided in *SI Materials and Methods*.

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1. Amasino R (2010) Seasonal and developmental timing of flowering. *Plant J* 61(6): 1001–1013.
2. Simpson GG, Dean C (2002) *Arabidopsis*, the Rosetta Stone of flowering time? *Science* 296(5566):285–289.
3. Bäurle I, Dean C (2006) The timing of developmental transitions in plants. *Cell* 125(4): 655–664.
4. Srikanth A, Schmid M (2011) Regulation of flowering time: All roads lead to Rome. *Cell Mol Life Sci* 68(12):2013–2037.
5. Lee JH, et al. (2013) Regulation of temperature-responsive flowering by MADS-box transcription factor repressors. *Science* 342(6158):628–632.
6. Andrés F, Coupland G (2012) The genetic basis of flowering responses to seasonal cues. *Nat Rev Genet* 13(9):627–639.
7. Wang JW, Czech B, Weigel D (2009) miR156-regulated SPL transcription factors define an endogenous flowering pathway in *Arabidopsis thaliana*. *Cell* 138(4):738–749.
8. Mutasa-Göttgens E, Hedden P (2009) Gibberellin as a factor in floral regulatory networks. *J Exp Bot* 60(7):1979–1989.
9. Kim DH, Doyle MR, Sung S, Amasino RM (2009) Vernalization: Winter and the timing of flowering in plants. *Annu Rev Cell Dev Biol* 25:277–299.
10. Lee H, et al. (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. *Genes Dev* 14(18):2366–2376.
11. Michaels SD, Amasino RM (1999) *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell* 11(5):949–956.
12. Sheldon CC, et al. (1999) The *FLF* MADS box gene: A repressor of flowering in *Arabidopsis* regulated by vernalization and methylation. *Plant Cell* 11(3):445–458.
13. Kim DH, Sung S (2014) Genetic and epigenetic mechanisms underlying vernalization. *Arabidopsis Book* 12:e0171.
14. Berry S, Dean C (2015) Environmental perception and epigenetic memory: Mechanistic insight through *FLC*. *Plant J* 83(1):133–148.
15. Jiao Y, Lau OS, Deng XW (2007) Light-regulated transcriptional networks in higher plants. *Nat Rev Genet* 8(3):217–230.
16. Suárez-López P, et al. (2001) *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410(6832):1116–1120.
17. Sawa M, Nusinow DA, Kay SA, Imaizumi T (2007) FKF1 and GIGANTEA complex formation is required for day-length measurement in *Arabidopsis*. *Science* 318(5848):261–265.
18. Song YH, Smith RW, To BJ, Millar AJ, Imaizumi T (2012) FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. *Science* 336(6084): 1045–1049.
19. Corbesier L, et al. (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. *Science* 316(5827):1030–1033.
20. Abe M, et al. (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* 309(5737):1052–1056.
21. Wigge PA, et al. (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* 309(5737):1056–1059.
22. Cerdán PD, Chory J (2003) Regulation of flowering time by light quality. *Nature* 423(6942):881–885.
23. Halliday KJ, Salter MG, Thingnaes E, Whitelam GC (2003) Phytochrome control of flowering is temperature-sensitive and correlates with expression of the floral integrator *FT*. *Plant J* 33(5):875–885.
24. Levy YY, Dean C (1998) The transition to flowering. *Plant Cell* 10(12):1973–1990.
25. Castro Marín I, et al. (2011) Nitrate regulates floral induction in *Arabidopsis*, acting independently of light, gibberellin, and autonomous pathways. *Planta* 233(3):539–552.
26. Michaels SD, He Y, Scortecci KC, Amasino RM (2003) Attenuation of *FLOWERING LOCUS C* activity as a mechanism for the evolution of summer-annual flowering behavior in *Arabidopsis*. *Proc Natl Acad Sci USA* 100(17):10102–10107.
27. Gazzani S, Gendall AR, Lister C, Dean C (2003) Analysis of the molecular basis of flowering time variation in *Arabidopsis* accessions. *Plant Physiol* 132(2):1107–1114.
28. Finnegan EJ, Sheldon CC, Jardinaud F, Peacock WJ, Dennis ES (2004) A cluster of *Arabidopsis* genes with a coordinate response to an environmental stimulus. *Curr Biol* 14(10):911–916.
29. Li Z, Wakao S, Fischer BB, Niyogi KK (2009) Sensing and responding to excess light. *Annu Rev Plant Biol* 60(1):239–260.
30. Nott A, Jung HS, Koussevitzky S, Chory J (2006) Plastid-to-nucleus retrograde signaling. *Annu Rev Plant Biol* 57:739–759.
31. Sun X, et al. (2011) A chloroplast envelope-bound PHD transcription factor mediates chloroplast signals to the nucleus. *Nat Commun* 2:477.
32. King RW, Hisamatsu T, Goldschmidt EE, Blundell C (2008) The nature of floral signals in *Arabidopsis*, I: Photosynthesis and a far-red photoresponse independently regulate flowering by increasing expression of *FLOWERING LOCUS T* (*FT*). *J Exp Bot* 59(14): 3811–3820.
33. Kumar SV, et al. (2012) Transcription factor PIF4 controls the thermosensory activation of flowering. *Nature* 484(7393):242–245.
34. Kumar SV, Wigge PA (2010) H2A.Z-containing nucleosomes mediate the thermosensory response in *Arabidopsis*. *Cell* 140(1):136–147.
35. Yoo SD, Cho YH, Sheen J (2007) *Arabidopsis* mesophyll protoplasts: A versatile cell system for transient gene expression analysis. *Nat Protoc* 2(7):1565–1572.
36. Mellor J (2006) It takes a PHD to read the histone code. *Cell* 126(1):22–24.
37. Crevillén P, Dean C (2011) Regulation of the floral repressor gene *FLC*: The complexity of transcription in a chromatin context. *Curr Opin Plant Biol* 14(1):38–44.
38. Jarillo JA, Piñeiro M, Cubas P, Martínez-Zapater JM (2009) Chromatin remodeling in plant development. *Int J Dev Biol* 53(8-10):1581–1596.
39. Bienz M (2006) The PHD finger, a nuclear protein-interaction domain. *Trends Biochem Sci* 31(1):35–40.
40. Ausín I, Alonso-Blanco C, Jarillo JA, Ruiz-García L, Martínez-Zapater JM (2004) Regulation of flowering time by FVE, a retinoblastoma-associated protein. *Nat Genet* 36(2):162–166.
41. Kim HJ, et al. (2004) A genetic link between cold responses and flowering time through FVE in *Arabidopsis thaliana*. *Nat Genet* 36(2):167–171.
42. Koornneef M, Hanhart CJ, van der Veen JH (1991) A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol Gen Genet* 229(1):57–66.
43. Wang X, et al. (2007) SKB1-mediated symmetric dimethylation of histone H4R3 controls flowering time in *Arabidopsis*. *EMBO J* 26(7):1934–1941.
44. Lopez-Juez E, Pyke KA (2005) Plastids unleashed: Their development and their integration in plant development. *Int J Dev Biol* 49(5-6):557–577.
45. Ietswaart R, Wu Z, Dean C (2012) Flowering time control: Another window to the connection between antisense RNA and chromatin. *Trends Genet* 28(9):445–453.
46. He Y (2012) Chromatin regulation of flowering. *Trends Plant Sci* 17(9):556–562.
47. Heo JB, Sung S (2011) Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. *Science* 331(6013):76–79.
48. Swiezewski S, Liu F, Magusin A, Dean C (2009) Cold-induced silencing by long antisense transcripts of an *Arabidopsis* Polycomb target. *Nature* 462(7274):799–802.
49. He Y, Michaels SD, Amasino RM (2003) Regulation of flowering time by histone acetylation in *Arabidopsis*. *Science* 302(5651):1751–1754.
50. Lee I, Amasino RM (1995) Effect of vernalization, photoperiod, and light quality on the flowering phenotype of *Arabidopsis* plants containing the *FRIGIDA* gene. *Plant Physiol* 108(1):157–162.
51. Johanson U, et al. (2000) Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. *Science* 290(5490):344–347.
52. Coustham V, et al. (2012) Quantitative modulation of polycomb silencing underlies natural variation in vernalization. *Science* 337(6094):584–587.
53. Fournier-Level A, et al. (2011) A map of local adaptation in *Arabidopsis thaliana*. *Science* 334(6052):86–89.
54. Pazhouhandeh M, Molinier J, Berr A, Genschik P (2011) MSI4/FVE interacts with CUL4-DDB1 and a PRC2-like complex to control epigenetic regulation of flowering time in *Arabidopsis*. *Proc Natl Acad Sci USA* 108(8):3430–3435.
55. Jeon J, Kim J (2011) FVE, an *Arabidopsis* homologue of the retinoblastoma-associated protein that regulates flowering time and cold response, binds to chromatin as a large multiprotein complex. *Mol Cells* 32(3):227–234.
56. Jung JH, et al. (2013) The cold signaling attenuator HIGH EXPRESSION OF OSMOTICALLY RESPONSIVE GENE1 activates *FLOWERING LOCUS C* transcription via chromatin remodeling under short-term cold stress in *Arabidopsis*. *Plant Cell* 25(11):4378–4390.
57. Woodson JD, Chory J (2008) Coordination of gene expression between organellar and nuclear genomes. *Nat Rev Genet* 9(5):383–395.
58. Chi W, Sun X, Zhang L (2013) Intracellular signaling from plastid to nucleus. *Annu Rev Plant Biol* 64(1):559–582.
59. Sparkes IA, Runions J, Kearns A, Hawes C (2006) Rapid, transient expression of fluorescent fusion proteins in tobacco plants and generation of stably transformed plants. *Nat Protoc* 1(4):2019–2025.
60. Yamaguchi N, et al. (2014) PROTOCOLS: Chromatin immunoprecipitation from *Arabidopsis* tissues. *Arabidopsis Book* 12:e0170.