Enabling photoperiodic control of flowering by timely chromatin silencing of the florigen gene

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Many plants synchronize their flowering times with changing seasons to maximize reproductive success. A key seasonal cue is the change in day length (photoperiod), that induces the production of a systemic flowering signaling molecule called florigen. A major florigen component is FLOWERING LOCUS T (FT) or its orthologs. In the long-day plant Arabidopsis thaliana, FT expression is well known to be activated by the photoperiod pathway output specifically near dusk in long days; however, underappreciated is the importance of FT silencing at other times of the day, in enabling Arabidopsis to respond only to long days in flowering. We have recently reported that a plant-specific chromatin-silencing complex called EMF1c represses FT expression at times other than around dusk in long days to prevent its temporal ectopic expression from “spoiling” the long-day floral induction in Arabidopsis. Here I further discuss in other day-length sensitive plants the potential involvement of a chromatin mechanism similar to the Arabidopsis EMF1c-mediated silencing, in repressing the expression of FT orthologs to enable diverse photoperiodic control of flowering.

Many plants synchronize their timing of developmental transition from vegetative to reproductive growth (i.e., flowering) with changing seasons to maximize reproductive success. A key seasonal cue is the change in day length (photoperiod) at different times of the year, quite noticeable at higher latitudes.1,2 Through a photoperiod pathway of floral induction, plants sense such cue to flower at a right season.2 According to their flowering responses to photoperiodic changes, plants are classified into long-day, short-day and day-neutral plants. Long-day plants flower when days getting longer than a threshold length or flower rapidly in long days, whereas short-day plants flower when the day becomes shorter; plants that flower regardless of day length changes are day-neutral.1

The day-length changes are perceived in leaves by photoreceptors such as phytochrome and cryptochrome.3,4 Upon perception of inductive photoperiods, plants produce a systemic flowering signaling molecule called florigen in leaf phloem tissues, which is subsequently transported through the phloem to shoot apex to induce flowering.5-7 A key component of the mobile florigen is FT, first discovered in the model flowering plant Arabidopsis thaliana and conserved among flowering plants so far examined.8,9 In the long-day plant Arabidopsis, FT expression is activated by the photoperiod pathway output CONSTANS (CO).10 In long days, CO mRNA expression is set at a high level during late afternoon by the interplay of several circadian clock-controlled activities of gene regulation.10 The coincidence of high-level CO mRNAs with light exposure that stabilizes the CO protein, results in CO accumulation in leaf veins near dusk.12,13 This CO-triggered FT expression near dusk is well known to be essential for floral induction in Arabidopsis by the long-day photoperiods; however, underappreciated is the importance of FT silencing at other times of the day (i.e., prior to late afternoon...
and after dusk), in enabling *Arabidopsis* to respond to long days in floral induction.

The silencing of gene expression through covalent chromatin modifications plays a crucial role in eukaryotic gene regulation. Various repressive modifications of the chromatin constituents including DNA and histones such as DNA methylation and Histone 3 lysine-27 trimethylation (H3K27me3) can lead to gene silencing. The repressive H3K27me3 can lead to gene silencing and Histone 3 lysine-27 trimethylation. Various repressive modifications plays a crucial role in eukaryotic gene regulation through covalent chromatin modifications.

In addition, a JmjC domain-containing H3 lysine-4 demethylase known as JMJD14/PKDM7B localizes at the *FT* locus to mediate H3K4 demethylation and repress *FT* expression. Recently, we have uncovered that LHP1 and JMJD14 are part of a PcG complex that silences *FT* expression.

In plants and animals PRC2 subunits are well conserved, but there are no apparent homologs of most animal PRC1 components in plants. PRC2, conserved from plants to animals, catalyzes H3K27me3, whereas PRC1 typically acts to maintain this mark, catalyze another repressive modification-Histone 2A monoubiquitination (H2Aub), and/or compact chromatin to repress gene expression. PRC2, composed of five Polypeptide of complex-mediated chromatin silencing plays a critical role in developmental gene regulation in plants and animals.

The expression control of florigen gene *FT* involves not only CO-triggered activation, but also chromatin silencing mechanisms. In *Arabidopsis*, a PRC2 complex catalyzes H3K27me3 at the *FT* locus, and is required for *FT* repression; moreover, LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) recognizes and binds to H3K27me3 and mediates *FT* silencing. In addition, a JmjC domain-containing H3 lysine-4 demethylase known as JMJD14/PKDM7B localizes at the *FT* locus to mediate H3K4 demethylation and repress *FT* expression. Recently, we have uncovered that LHP1 and JMJD14 are part of a PcG complex that silences *FT* expression.

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To explore the biological function of EMF1c-PcG in photoperiodic flowering control, we undertook a genetic approach by knocking down the core complex subunit EMF1 in leaf phloem tissues, where *FT* expression is activated by CO specifically near dusk in long days. This phloem-specific knockdown resulted in largely constitutive *FT* expression across day and night (from dawn to dusk to dawn again). Apparently, EMF1c is essential for keeping *FT* silenced. The EMF1c knockdown plants flowered rapidly and around the same time under long-day and short-day conditions (note that *Arabidopsis* typically flowers rapidly in long days, whereas the short-day condition inhibits its flowering); in other words, these plants became day-neutral, and flowered rapidly regardless of day length conditions. Loss of EMF1c function in the leaf phloem tissues converted *Arabidopsis* from a long-day plant to day-neutral plant. Therefore, in *Arabidopsis* EMF1c-mediated *FT* silencing enables the photoperiodic control of flowering (i.e., long-day induction of the floral transition).

We have further determined at what times of the day (in long days) EMF1c acts to silence *FT* expression by examining the temporal localization pattern of EMF1 at the *FT* locus. In the morning, EMF1 binds to *FT* chromatin to silence *FT* expression. When the day advances, CO starts to accumulate in late afternoon with a peak at dusk; CO, together with a dimer of NF-YB and NF-YC, forms a trimer (CO-NF-Y) that binds directly to the *FT* promoter to disrupt EMF1 binding to *FT* chromatin, as suggested by the ectopic accumulation of CO in the morning resulting in an elimination of EMF1 binding to *FT* chromatin. This CO-triggered reduction of EMF1 abundance at the *FT* locus gives rise to *FT* expression activation near dusk to induce flowering, conferring a long-day flowering induction in *Arabidopsis*. When it reaches night, the CO protein is degraded rapidly by proteasomes, EMF1/EMF1c comes back to *FT* chromatin again to silence *FT* expression. In short, *FT* repression at times other than around dusk in long days is not by default, but achieved by ‘active’ EMF1c-mediated chromatin silencing. This silencing is relieved only when the CO level reaches a threshold near dusk; thus, it creates a situation where only long days can induce the florigen expression.

![Figure 1](image-url)
because of the silencing breaker – CO reaching a threshold level only near the end of day time (Fig. 1). FT silencing at other times of the day prevents its temporal ectopic expression from ‘spooling’ the long-day floral induction in Arabidopsis; in other words, EMF1c-mediated FT silencing enables such photoperiodic response of flowering.

The EMF1c components including EMF1, LHP1, JMJ14 and BMI1s are widely conserved in angiosperms.21-23,25-27 This raises a possibility that EMF1c-mediated silencing of FT (or FT orthologs) might be conserved in other day-length sensitive plants as well. In the model short-day plant Oryza sativa (rice), the expression of an FT ortholog called Hd3a, which plays a key role in rice floral induction,28 is activated around dawn in short days (8-hour light/16-hour night) by concerted action of a rice ortholog of the Arabidopsis CO and a B-type response regulator known as Eh1 (see illustration in Fig. 2).1,29,30 It would be interesting to determine whether in rice there is an EMF1c-like PcG complex with subunit composition and function similar to the Arabidopsis EMF1c, which silences Hd3a expression at times other than around dawn to enable short-day induction of rice flowering (Fig. 2). Future study of the role of EMF1c-like complexes in silencing of FT orthologs in various long-day and short-day plants will further our understanding of whether EMF1c-mediated silencing of FT or FT orthologs in leaf veins is a general feature in enabling photoperiodic control of flowering in angiosperms.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

References
1. Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T. Photoperiodic flowering: time measurement mechanisms in leaves. J Exp Bot 2014; 66:441-64. PMID:25534513; http://dx.doi.org/10.1093/oxforduniversity-press/9780199682202.0011.0032
2. Turck F, Fornara F, Coupland G. Photoresponse regulation of CONSTANS protein in photoperiodic flowering. Science 2004; 303:1003-6; PMID:14963328; http://dx.doi.org/10.1126/science.1091761
3. Li X, Wang Q, Yu X, Liu H, Yang H, Zhao C, Liu X, Tan C, Kiyono J, Zhang D, et al. Arabidopsis cryptochrome 2 (CRY2) functions by the photoactivation mechanism distinct from the cryptophan (trp) triad-related photoreceptor. Proc Natl Acad Sci U S A 2011; 108:20844-9; PMID:22129370; http://dx.doi.org/10.1073/pnas.1114579118
4. Corbesier L, Vincent C, Jagd, Sornara F, Fan Q, Searle I, Giakoumis A, Sornara S, Gisot L, Turnbull C, et al. FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science 2007; 316:1030-3; PMID:17446353; http://dx.doi.org/10.1126/science.1141751
5. Jaeger KE, Wigge PA. FT protein acts as a long-range signal in Arabidopsis.Curr Biol 2007; 17:1050-4; PMID:17540569; http://dx.doi.org/10.1016/j.cub.2007.05.008
6. Mathieu J, Werthmann N, Kurrer F, Schmid M. Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Curr Biol 2007; 17:1055-6; PMID:17540570; http://dx.doi.org/10.1016/j.cub.2007.05.009
7. Kardailsky I, Shulka VK, Akin JH, Daganais N, Christensen SK, Nguyen JY, Clory J, Harrison MJ, Weigel D. Activation tagging of the floral inducer FT. Science 1999; 286:1962-5; PMID:10583956; http://dx.doi.org/10.1126/science.286.5446.1962
8. Kobayashi Y, Kaya H, Gooto K, Iwabuchi M, Araki T. A pair of related genes with antagonistic roles in mediating flowering signals. Science 1999; 286:1960-2; PMID:10583960; http://dx.doi.org/10.1126/science.286.5446.1960
9. Yanovsky MJ, Kay SA. Molecular basis of seasonal time measurement in Arabidopsis. Nature 2002; 419:308-12; PMID:12239570; http://dx.doi.org/10.1038/nature0096
10. Song YH, Estrada DA, Johnson RS, Kim SK, Lee SY, MacCoss MJ, Imaizumi T. Distinct roles of FKF1, GIGANTEA, and ZEITLUPE proteins in the regulation of CONSTANS stability in Arabidopsis photoperiodic flowering. Proc Natl Acad Sci U S A 2014; 111:17672-7; PMID:25524219; http://dx.doi.org/10.1073/pnas.14153711
11. Simon JA, Kington RE. Mechanisms of Polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol 2009; 10:697-708; PMID:19738629; http://dx.doi.org/10.1038/nrm2731
12. Zhang H, Zhu JK. RNA-directed DNA methylation. Curr Opin Plant Biol 2011; 14:42-7; PMID:21420334; http://dx.doi.org/10.1016/j.cub.2011.02.003
13. Bentokey Y, Ohad N. Polycomb-group mediated epigenetic mechanisms through plant evolution. Biochim Biophys Acta 2011; 1809:395-406; PMID:21664995; http://dx.doi.org/10.1016/j.bbalip.2011.06.004
14. Jiang D, Wang Y, Wang Y, He Y. Repression of FLOWERING LOCUS C and FLOWERING LOCUS T by the Arabidopsis Polycomb repressive complex 2 components. PLoS ONE 2008; 3:e3404; PMID:18852898; http://dx.doi.org/10.1371/journal.pone.0003404
15. Turck F, Roudiere F, Sarrona S, Martin-Magniette ML, Guillaume E, Buisne N, Gagnot S, Maritesen RA, Coupland G, Color V. Arabidopsis TRU/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 4. FASEB J 2007; 21:2084-5; PMID:17542647; http://dx.doi.org/10.1096/fasebj.050086
16. Jiang JH, Song HR, Ka JH, Jeong YM, Kwon YE, Seol JH, Amasino RM, Noh B, Noh Y. Repression of FLOWERING LOCUS C and FLOWERING LOCUS T by the Arabidopsis Polycomb repressive complex 2 components. PLoS ONE 2008; 3:e3404; PMID:18852898; http://dx.doi.org/10.1371/journal.pone.0003404
17. Jiang D, Wang Y, Wang Y, He Y. Plant-specific histone H3 lysine 4 demethylases repress the floral transition through a distinct polycomb repressive complex 2 component. Dev Cell 2014; 26:1009-17; PMID:24610724; http://dx.doi.org/10.1016/j.devcel.2014.01.029
18. Molitor A, Shen WH. The Polycomb complex PRC1: composition and function in plants. J Genet Genomics 2005; 32:231-4; PMID:15976214; http://dx.doi.org/10.1016/j.jgg.2005.05.001
19. Aubert D, Chen L, Moon YH, Martin D, Castle LA, Yang CH, Sung ZR. EMF1, a novel protein involved in the control of shoot architecture and flowering in Arabidopsis. Plant Cell 2001; 13:1865-75; PMID:11487698; http://dx.doi.org/10.1105/tpc.13.8.1865
24. Beh LY, Colwell LJ, Francis NJ. A core subunit of Polycomb repressive complex 1 is broadly conserved in function but not primary sequence. Proc Natl Acad Sci U S A 2012; 109:E1063-71; PMID:22517748; http://dx.doi.org/10.1073/pnas.1118678109

25. Gaudin V, Libault M, Pouteau S, Juul T, Zhao G, Lefebvre D, Grandjean O. Mutations in LIKE HETEROCHROMATIN PROTEIN 1 affect flowering time and plant architecture in Arabidopsis. Development 2001; 128:4847-58; PMID:11731464

26. Zhou X, Ma H. Evolutionary history of histone demethylase families: distinct evolutionary patterns suggest functional divergence. BMC Evol Biol 2008; 8:294; PMID:18950507; http://dx.doi.org/10.1186/1471-2148-8-294

27. Bratze F, Lopez-Torrejon G, Koch M, Del Puno JC, Calonje M. Keeping cell identity in Arabidopsis requires PRC1 RING-finger homologs that catalyze H2A monoubiquitination. Curr Biol 2010; 20:1853-9; PMID:20933424; http://dx.doi.org/10.1016/j.cub.2010.09.046

28. Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K. H43a protein is a mobile flowering signal in rice. Science 2007; 316:1033-6; PMID:17446351; http://dx.doi.org/10.1126/science.1141753

29. Kojima S, Takahashi Y, Kobayashi Y, Monna L, Sasaki T, Araki T, Yano M. H33a, a rice ortholog of the Arabidopsis FT gene, promotes transition to flowering downstream of Hid1 under short-day conditions. Plant Cell Physiol 2002; 43:1096-105; PMID:12407188; http://dx.doi.org/10.1093/pcp/pcf156

30. Doi K, Izawa T, Fuse T, Yamanouchi U, Kubo T, Shimatani Z, Yano M, Yoshimura A. EhD1, a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hid1. Genes Dev 2004; 18:926-36; PMID:15078816; http://dx.doi.org/10.1101/gad.1189604

**Wang Y, Gu X, Yuan W, Schmitz R, He Y. Photoperiodic control of the floral transition through a distinct Polycomb repressive complex. Dev Cell 2014; 28:727-36; PMID:24613395; http://dx.doi.org/10.1016/j.devcel.2014.01.029**