学位論文 (要約)

# FE regulates florigen-mediated flowering through multi-layered functions in *Arabidopsis thaliana*

（シロイヌナズナのフロリゲンを介した花成制御における FE の多層的な役割）

平成 29 年 12 月　博士（理学）申請

東京大学大学院理学系研究科

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Acknowledgements

I would like to express my sincerest appreciation to Prof. Dr. Mitsutomo Abe for giving me the opportunity to study in his group and for supervising and encouraging me throughout this study. I would also like to show my deepest gratitude to Prof. Dr. Hiroyuki Hirano, Prof. Dr. Yuichiro Watanabe, Prof. Dr. Munetaka Sugiyama and Prof. Dr. Kyoko Ohashi-Ito for their critical comments on this thesis. I am deeply indebted to all the members of the laboratory of genetics, the University of Tokyo, especially Prof. Dr. Tetsuji Kakutani, Dr. Taiko To, Dr. Taku Sasaki and Ms. Ayako Watanabe-Taneda, for the cooperation, discussion and advice. I thank Prof. Dr. J. M. Ward (University of Minnesota, USA) for the generous gift of seed materials and Dr. Yoshibumi Komeda, Dr. Hidetaka Kaya and Dr. Souichi Inagaki for technical support and useful advice. I wish to express my thanks to all of my friends and all the people that led me to be a scientist. Finally, I thank all of my family and my fiancé for their constant support all the time.

List of Abbreviations

| | |
|---|---|
| ChIP | Chromatin immunoprecipitation |
| CO | CONSTANS |
| CORE | CO responsive elements |
| CLF | CURLY LEAF |
| Cyc | Cycloheximide |
| DAG | Day after germination |
| Dex | Dexamethasone |
| EFM | EARLY FLOWERING MYB PROTEIN |
| EIF4 | EUKARYOTIC TRANSLATION INITIATION FACTOR 4A-1 |
| FE/APL | FE/ALTERED PHLOEM DEVELOPMENT |
| FLC | FLOWERING LOCUS C |
| FT | FLOWERING LOCUS T |
| FTIP1 | FT-INTERACTING PROTEIN 1 |
| GR | Glucocorticoid receptor |
| GUS | $\beta$-glucuronidase |
| H3K27me3 | Trimethylated Lys residues at position 27 of histone 3 |
| H3K36me2 | Dimethylated Lys residues at position 36 of histone 3 |
| HSP | Heat shock protein |
| JMJ | JUMONJI |
| JmjC | JUMONJI-C |
| JMJ30 | JUMONJI C-DOMAIN-CONTAINING PROTEIN 30 |
| JMJ32 | JUMONJI C-DOMAIN-CONTAINING PROTEIN 32 |
| LHP1 | LIKE HETEROCHROMATIN PROTEIN 1 |
| MS | Murashige and Skoog |
| NaKR1 | SODIUM POTASSIUM ROOT DEFECTIVE 1 |
| NF-Y | NUCLEAR FACTOR-Y |
| PIF4 | PHYTOCHROME INTERACTING FACTOR 4 |
| PRC1 | Polycomb repressive complex 1 |
| PRC2 | Polycomb repressive complex 2 |
| RAM | Root apical meristem |
| REF6 | RELATIVE OF EARLY FLOWERING 6 |

| | |
|---|---|
| RT-qPCR | Quantitative reverse transcription-PCR |
| SAM | Shoot apical meristem |
| SDs | Standard deviations |
| SD-leu/-trip | Synthetic defined medium lacking leucine and tryptophan |
| SD-leu/-trip/-his | Synthetic defined medium lacking leucine, tryptophan, and histidine |
| SWN | SWINGER |
| WT | Wild type |
| Y2H | Yeast two hybrid |
| ZT | Zeitgeber time |

Abstract

The proper timing of flowering is essential for reproductive success. In a facultative long-day plant, *Arabidopsis thaliana*, *FLOWERING LOCUS T* (*FT*) encodes the mobile hormone florigen, and plays a pivotal role in modulating the optimal timing of flowering. FT is synthesized in leaf phloem companion cells and transported from leaves to the shoot apical meristem (SAM) via phloem tissue to initiate flowering.

Since *FT* is the most important gene for flowering, multi-layered regulation mechanisms are needed to proper regulation of florigen activity. Under inductive long-day conditions, the abundance of *FT* mRNA shows unique diurnal expression pattern. CONSTANS (CO) plays an important role in the temporal regulation of *FT*. CO interacts with NUCLEAR FACTOR-Y (NF-Y), and CO/NF-Y complex binds to the *FT* promoter region to activate *FT* expression. Over the *FT* locus, a transcriptionally repressive histone modification, trimethylated Lys residues at position 27 of histone 3 (H3K27me3) is widely enriched. This transcriptionally repressive chromatin status is regulated by polycomb group proteins. After translation, FT is transported to the SAM through interaction with florigen transporters, such as SODIUM POTASSIUM ROOT DEFECTIVE 1 (NaKR1) and FT-INTERACTING PROTEIN 1 (FTIP1).

The mechanisms of temporal transcriptional regulation and setting the repressive chromatin status have been revealed to a large extent. However, mechanisms of phloem-specific spatial transcriptional regulation and setting active chromatin status remain unclear. In addition, there is room to elucidate the molecular mechanism that coordinates FT protein synthesis and transport for opportune flowering.

In this thesis, I focused on the phloem-specific Myb-related transcription factor, FE. In order to clarify molecular functions of FE in the florigen-mediated flowering, I performed experiment from three different points of view. Firstly, in transcriptional regulation, FE directly binds to the *FT* promoter together with CO/NF-Y to activate *FT*. Simultaneous induction of FE and CO strongly activates *FT* transcription not only in phloem tissue but also in some other tissues. Secondly, in chromatin remodeling, FE mediates removal of H3K27me3 with RELATIVE OF EARLY FLOWERING 6 (REF6) to set active chromatin state at the *FT* locus. And finally, in protein transport regulation, FE activates *NaKR1* and *FTIP1* for effective transport in CO-independent manners.

Given that the results of transcriptional regulation and chromatin remodeling, I suggest that FE and REF6 provide capability of accepting transcription factors for the *FT* locus through chromatin remodeling, and then FE recruits CO to induce *FT* expression in phloem tissue. From protein transport analysis, I suggest that FT protein synthesis and transport are governed by FE in CO-dependent/independent manners. Taken together, my results suggest that FE regulates the florigen mediated-flowering through multi-layered functions.

# Chapter I
# General introduction

The proper timing of flowering, which is the developmental transition from vegetative phase to reproductive phase, is crucial for reproductive success of flowering plants. This transition is precisely controlled by developmental and environmental cues (Andrés and Coupland, 2012). Perception of these signals induces the production of a mobile flowering hormone, florigen, in leaf phloem companion cells. This flowering hormone is transported from leave to the shoot apical meristem (SAM) via phloem tissue to initiate flowering. In a facultative long-day plant, *Arabidopsis thaliana*, FLOWERING LOCUS T (FT) protein is a major component of florigen (Kardailsky et al., 1999; Kobayashi et al., 1999; Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007; Jaeger and Wigge, 2007; Lin et al., 2007; Mathieu et al., 2007, Notaguchi et al., 2008). In order to induce flowering at the optimum timing, *FT* transcription and FT transportation are regulated by multi-layered mechanisms such as chromatin remodeling, transcriptional regulation and protein transport regulation.

Under long-day conditions, *FT* transcription is induced and shows diurnal oscillation with a significant peak at dusk; this unique expression pattern of *FT* mRNA is mainly controlled by CONSTANS (CO), a transcription factor with B-box type zinc fingers (Samach et al., 2000; Robson et al., 2001; Suárez-López et al., 2001). *CO* mRNA abundance is regulated by light signaling and circadian rhythms, and CO protein is stabilized by light signaling specifically in long-day afternoon (Putterill et al., 1995; Samach et al., 2000; Suárez-López et al., 2001; Valverde et al., 2004; Imaizumi, 2010; Song et al., 2013). This light-dependent control of CO activity leads to the long-day-specific induction of *FT* mRNA.

*FT* expression is spatially limited to phloem companion cells in distal leaf blade during vegetative phase (Takada and Goto, 2003; Yamaguchi et al., 2005; Adrian et al., 2010). A modification of transcriptionally repressive chromatin, trimethylated Lys residues at position 27 of

histone 3 (H3K27me3), is spread over the *FT* locus at high level and is involved with spatial regulation (Turck et al., 2007; Zhang et al., 2007). From phenotypic and genetic analyses, this H3K27me3 distribution is regulated by polycomb repressive complex 2 (PRC2). PRC2 has methyltransferase activity and trimethylates H3K27. Then PRC2 recruits polycomb repressive complex 1 (PRC1) to repress target genes (Hennig and Derkacheva, 2009; Simon and Kingston, 2009). Either CURLY LEAF (CLF) or SWINGER (SWN) comprises PRC2, which has histone methyltransferase activity responsible for H3K27me3 deposition. In the *FT* repression, CLF and SWN play partially overlapping roles (Jiang et al., 2008; Liu et al., 2010). A PRC1-like protein, LIKE HETEROCHROMATIN PROTEIN 1 (LHP1) contains both a chromo domain and a chromo shadow domain, and is involved with the maintenance of H3K27me3 distribution over various loci (Gaudin et al., 2001; Veluchamy et al., 2016). *LHP1* is strongly expressed in roots, hypocotyls, juvenile leaves, and proximal region of adult leaves (Takada and Goto, 2003). As *FT* is expressed in phloem tissue at distal region of leaf blade, spatial expression pattern of *FT* and *LHP1* are exclusive with each other (Takada and Goto, 2003). In *lhp1* mutants, the *FT*-transcribed region is expanded to phloem tissue at proximal region of leaf blade (Adrian et al., 2010). These results suggest that the unique spatial expression pattern of *FT* reflects the integration of transcriptional activation in leaf phloem tissue and chromatin mediated repression in proximal region of leaf blade (Fig. I-1).

As I referred above, the mechanisms of temporal transcriptional activation and repression of chromatin in *FT* regulation have been well understood. However, the mechanisms of phloem-specific spatial regulation of *FT* and the modification of active chromatin at the *FT* locus remain unclear. Although both CO and LHP1 act as regulators of *FT*, phloem-specific expression of *FT* is strongly maintained in both *CO* ectopic-expressing transgenic plants and *lhp1* mutants (Adrian et al.,

2010). Thus, there must be an unknown factor (described as 'X' in Fig. I-1) which make the *FT* locus capable of accepting transcriptional factors to regulate *FT*. (Fig. I-1).

After FT protein translation in companion cells, FT is transported to the SAM through a complex process. For example, FT is loaded from companion cells to sieve elements via plasmodesmata through the interaction with FT-INTERACTING PROTEIN 1 (FTIP1) (Liu et al., 2012; Song et al., 2017). In sieve elements, FT binds to SODIUM POTASSIUM ROOT DEFECTIVE 1 (NaKR1) and NaKR1 mediates long-distance transport of FT toward the SAM (Zhu et al., 2016). The mechanism of FT phloem transport has been gradually elucidated, however, molecular mechanisms that coordinate FT protein synthesis and transport are needed to be elucidated.

Our group recently reported that the phloem-specific Myb-related transcription factor, FE/ALTERED PHLOEM DEVELOPMENT (FE/APL), is a pivotal flowering regulator of *Arabidopsis* photoperiodic flowering (Abe et al., 2015). FE/APL is known as a key regulator of phloem development, namely, FE has important functions in the determination of identity of phloem tissue (Bonke et al., 2003). Since phloem tissue is essential, the null mutant of *FE* (*apl*) exhibited seedling lethal phenotypes. On the other hand, the one amino acid substitution mutant, *fe-1*, can grow normally except for timing of flowering (Abe et al., 2015). Therefore, I focused on the late flowering phenotype of *fe-1* seedlings, and I hypothesize that FE governs florigen function through a multi-layered approach. According to our expression analysis, FE protein is involved in the activation of both *FT* and FT protein transporter coding genes. Decreased expression of *FT* and attenuation of FT protein transport in *fe-1* are partly responsible for the long-day-specific late flowering phenotypes (Abe et al., 2015). However, further study is required to clarify detailed molecular functions of FE in activation of target genes.

In this thesis, in order to clarify the molecular functions of FE in florigen-mediated flowering, I performed experiments from three different points of view. To find out the factor 'X', I further investigated the molecular functions of FE in both transcriptional regulation and chromatin modification of *FT* in Chapter III and IV. I showed the results of genetic analysis of transcriptional regulation in Chapter III, and the results of epigenetic analysis of chromatin modification in Chapter IV, respectively. In Chapter III, I show that FE directly activates the transcription of *FT* via binding to its promoter region. I find that CO/NUCLEAR FACTOR-Y (CO/NF-Y) complex is necessary for FE-mediated *FT* activation under long-day conditions, and FE and CO regulate the phloem-specific expression of *FT*. Moreover, in Chapter IV, I suggest through epigenetic analyses that FE might be also involved with the chromatin remodeling at the *FT* locus through interaction with RELATIVE OF EARLY FLOWERING 6 (REF6), a H3K27me3 demethylase. In addition, in Chapter V, I show FE activates the transcription of florigen transporter genes, *NaKR1* and *FTIP1* in CO-independent manners. Based on my research, I propose that FE controls florigen functions through multi-layered regulation systems: chromatin remodeling at the *FT* locus, transcriptional regulation of *FT* gene, and regulation of phloem transport of FT protein through transcriptional regulation of downstream genes.

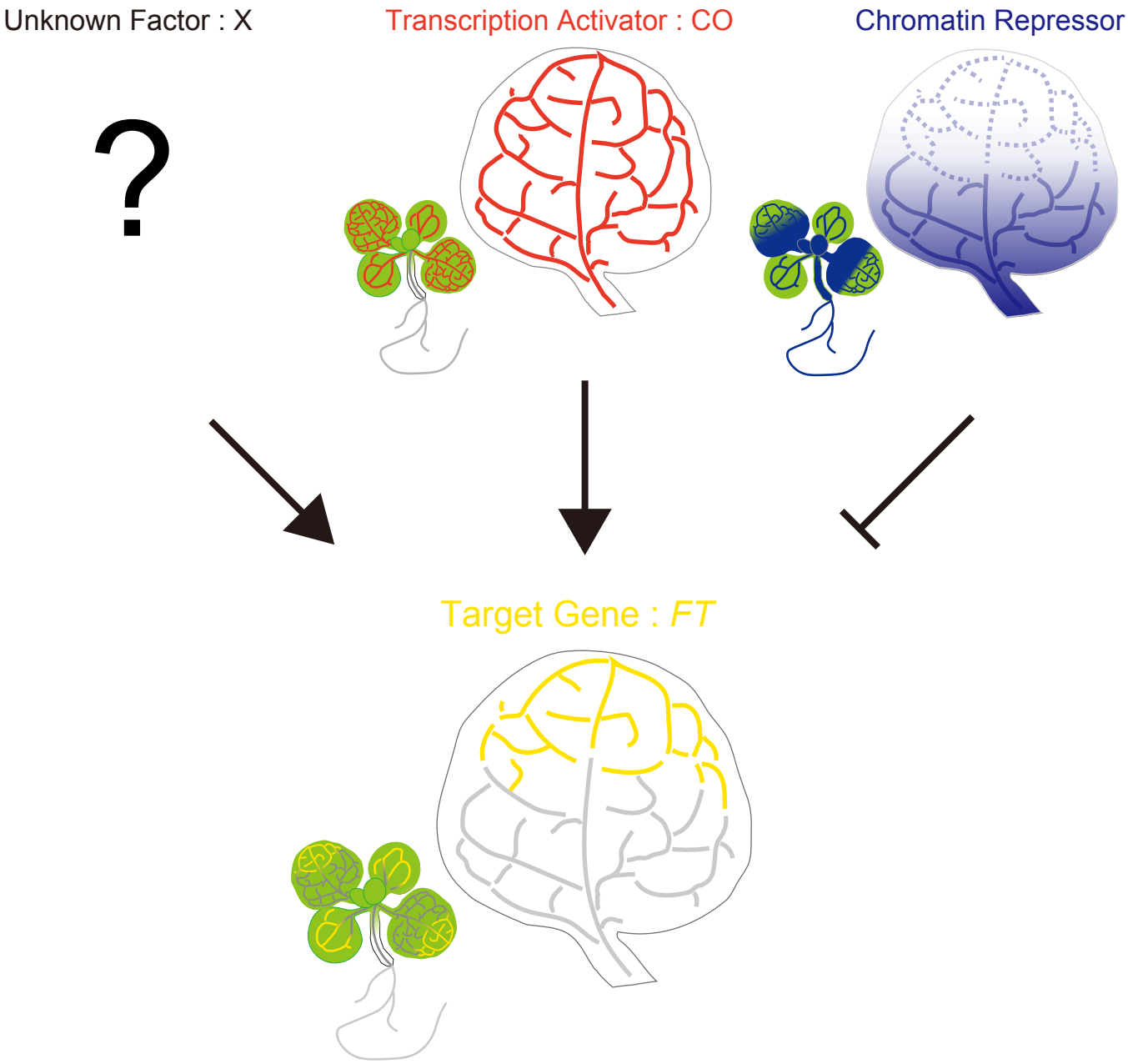


Fig. I-1. A prospected model describing the spatial expression pattern of *FT*. The spatial pattern may reflect the integration of transcriptional activation in the leaf phloem tissue and chromatin mediated repression in the proximal region of the leaf blade. However, there must be an unknown factor (described as 'X' ) for proper spatial expression of *FT*.

References

Abe, M., Kaya, H., Watanabe-Taneda, A., Shibuta, M., Yamaguchi, A., Sakamoto, T. et al. (2015) FE, a phloem-specific Myb-related protein, promotes flowering through transcriptional activation of *FLOWERING LOCUS T* and *FLOWERING LOCUS T INTERACTING PROTEIN 1*. Plant J. 83: 1059-1068.

Abe, M., Kobayashi, Y., Yamamoto, S., Daimon, Y., Yamaguchi, A., Ikeda, Y. et al. (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. Science. 309: 1052-1056.

Adrian, J., Farrona, S., Reimer, J.J., Albani, M.C., Coupland, G. & Turck, F. (2010) *cis*-Regulatory elements and chromatin state coordinately control temporal and spatial expression of *FLOWERING LOCUS T* in *Arabidopsis*. Plant Cell. 22: 1425-1440.

Andrés, F. & Coupland, G. (2012) The genetic basis of flowering responses to seasonal cues. Nat Rev Genet. 13: 627-639.

Bonke, M., Thitamadee, S., Mahonen, A.P., Hauser, M.T. & Helariutta, Y. (2003) APL regulates vascular tissue identity in *Arabidopsis*. Nature. 426: 181-186.

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I. et al. (2007) FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis*. Science. 316: 1030-1033.

Gaudin, V., Libault, M., Pouteau, S., Juul, T., Zhao, G., Lefebvre, D. et al. (2001) Mutations in *LIKE HETEROCHROMATIN PROTEIN 1* affect flowering time and plant architecture in *Arabidopsis*. Development. 128: 4847-4858.

Hennig, L. & Derkacheva, M. (2009) Diversity of Polycomb group complexes in plants: same rules, different players? Trends Genet. 25: 414-423.

Imaizumi, T. (2010) *Arabidopsis* circadian clock and photoperiodism: time to think about location. Curr Opin Plant Biol. 13: 83-89.

Jaeger, K.E. & Wigge, P.A. (2007) FT protein acts as a long-range signal in *Arabidopsis*. Curr Biol. 17: 1050-1054.

Jiang, D., Wang, Y., Wang, Y. & He, Y. (2008) Repression of *FLOWERING LOCUS C* and *FLOWERING LOCUS T* by the *Arabidopsis* Polycomb repressive complex 2 components. PLoS One. 3: e3404.

Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T. et al. (1999) Activation tagging of the floral inducer FT. Science. 286: 1962-1965.

Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M. & Araki, T. (1999) A pair of related genes with antagonistic roles in mediating flowering signals. Science. 286: 1960-1962.

Lin, M.K., Belanger, H., Lee, Y.J., Varkonyi-Gasic, E., Taoka, K., Miura, E. et al. (2007) FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits. Plant Cell. 19: 1488-1506.

Liu, C., Lu, F., Cui, X. & Cao, X. (2010) Histone methylation in higher plants. Annu Rev Plant Biol. 61: 395-420.

Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z. et al. (2012) FTIP1 is an essential regulator required for florigen transport. PLoS Biol. 10: e1001313.

Mathieu, J., Warthmann, N., Kuttner, F. & Schmid, M. (2007) Export of FT protein from phloem companion cells is sufficient for floral induction in *Arabidopsis*. Curr Biol. 17: 1055-1060.

Notaguchi, M., Abe, M., Kimura, T., Daimon, Y., Kobayashi, T., Yamaguchi, A. et al. (2008) Long-distance, graft-transmissible action of *Arabidopsis* FLOWERING LOCUS T protein to promote flowering. Plant Cell Physiol. 49: 1645-1658.

Putterill, J., Robson, F., Lee, K., Simon, R. & Coupland, G. (1995) The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell. 80: 847-857.

Robson, F., Costa, M.M., Hepworth, S.R., Vizir, I., Piñeiro, M., Reeves, P.H. et al. (2001) Functional importance of conserved domains in the flowering-time gene *CONSTANS* demonstrated by analysis of mutant alleles and transgenic plants. Plant J. 28: 619-631.

Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F. et al. (2000) Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. Science. 288: 1613-1616.

Simon, J.A. & Kingston, R.E. (2009) Mechanisms of polycomb gene silencing: knowns and unknowns. Nat Rev Mol Cell Biol. 10: 697-708.

Song, S., Chen, Y., Liu, L., Wang, Y., Bao, S., Zhou, X. et al. (2017) OsFTIP1-mediated regulation of florigen transport in rice is negatively regulated by the ubiquitin-like domain kinase OsUbDKγ4. Plant Cell. 29: 491-507.

Song, Y.H., Ito, S. & Imaizumi, T. (2013) Flowering time regulation: photoperiod- and temperature-sensing in leaves. Trends Plant Sci. 18: 575-583.

Suárez-López, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F. & Coupland, G. (2001) CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature. 410: 1116-1120.

Takada, S. & Goto, K. (2003) TERMINAL FLOWER2, an Arabidopsis homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of *FLOWERING LOCUS T* by CONSTANS in the vascular tissues of leaves to regulate flowering time. Plant Cell. 15: 2856-2865.

Turck, F., Roudier, F., Farrona, S., Martin-Magniette, M.L., Guillaume, E., Buisine, N. et al. (2007) Arabidopsis TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. PLoS Genet. 3: e86.

Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A. & Coupland, G. (2004) Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science. 303: 1003-1006.

Veluchamy, A., Jégu, T., Ariel, F., Latrasse, D., Mariappan, K.G., Kim, S.K. et al. (2016) LHP1 Regulates H3K27me3 Spreading and Shapes the Three-Dimensional Conformation of the Arabidopsis Genome. *PLoS One*. 11: e0158936.

Wigge, P.A., Kim, M.C., Jaeger, K.E., Busch, W., Schmid, M., Lohmann, J.U. et al. (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. Science. 309: 1056-1059.

Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M. & Araki, T. (2005) TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT. *Plant Cell Physiol*. 46: 1175-1189.

Zhang, X., Germann, S., Blus, B.J., Khorasanizadeh, S., Gaudin, V. & Jacobsen, S.E. (2007) The *Arabidopsis* LHP1 protein colocalizes with histone H3 Lys27 trimethylation. Nat Struct Mol Biol. 14: 869-871.

Zhu, Y., Liu, L., Shen, L. & Yu, H. (2016) NaKR1 regulates long-distance movement of FLOWERING LOCUS T in *Arabidopsis*. Nat Plants. 2: 16075.

# Chapter II
# Materials and Methods

Plant materials and growth conditions

The accession Columbia (Col) was used as wild type (WT). The *Arabidopsis* homozygous mutants *fe-1* (point mutation), *nakr1-1* (frameshift mutation), *co-10* (T-DNA insertion) and *ref6-3* (T-DNA insertion) were described previously (Noh et al., 2004; Laubinger et al., 2006; Tian et al., 2010; Abe et al., 2015). All transgenic lines used in this study were in Col background. Each transgenic line for *gFT:GUS*, *p35S:FE-GR* and *pNaKR1:GUS* was described previously (Notaguchi et al., 2008; Tian et al., 2010; Abe et al., 2015). Transgenic lines for *pHSP:FE*, *pHSP:CO*, *p35S:CO-GR*, and *gFE-3xFLAG* were obtained in this study (Fig. II-1A-D). *Arabidopsis* was grown on soil or Murashige and Skoog (MS) medium that contained 0.8% agar and was supplemented with 1% sucrose at 22℃ under long-day (16 h light / 8 h dark) or short-day (8 h light / 16 h dark) conditions. To induce callus, seeds were germinated on MS medium containing 0.8% agar and supplemented with 2% glucose, 4.5 μM 2,4-dichlorophenoxyacetic acid and 0.45 μM kinetin for 3 weeks.

Plasmid construction

Plasmids based on pTT101, pRI201-AN, pBI101 and pBI121 were introduced into *Agrobacterium tumefaciens* strain GV3101. Genes on these plasmids were transformed into Col by the floral dip method (Clough and Bent, 1998). In the T1 generation, seedlings carrying each transgene were identified on the basis of Kanamycin resistance.

Heat treatment

Seedlings grown on MS medium plates were incubated at 37℃ in the incubators (EYELA PERSONAL INCUBATOR LTI-2000) for 2 hours, then were incubated at 22℃ under long-day or short-day conditions, and then were harvested at the optional time points.

Dexamethasone (Dex) treatment

Under long-day or short-day conditions, seedlings were submerged for 30 min in water containing 1 μM Dex (Wako) and/or 10 μM Cyc (Wako), then excess liquid was removed and rinsed off, and then were harvested at the optional time points.

Quantitative reverse transcription-PCR (RT-qPCR)

Total RNA was extracted with TRIzol regent (Life technologies) from *Arabidopsis* seedlings. After Recombinant DNase (RNase-free) (TaKaRa) treatment, 100 ng of total RNA served as templates for cDNA synthesis using Super Script III (Life technologies). RT-qPCR reactions were performed with the Lightcycler 480II system (Roche) using THUNDERBIRD® Probe qPCR Mix (TOYOBO) or KAPA SYBR® FAST qPCR kit (NIPPON Genetics). The primers and probes used are listed in Table II-1. Levels of given mRNA were normalized by levels of *EUKARYOTIC TRANSLATION INITIATION FACTOR 4A-1* (*EIF4*) in each sample. Standard deviations (SDs) were calculated using at least three biological replicates.

GUS staining

Seedlings grown on MS medium were fixed in 90% acetone for 15 minutes on ice. After rinsing samples with 50 mM Phosphate Buffer (pH7.0) twice, samples were transferred to the staining buffer [50 mM Phosphate Buffer (pH7.0), 3 mM potassium ferrocyanide, 3 mM potassium ferricyanide, 0.1% Triton X-100, 0.5 mg $ml^{-1}$ X-Gluc]. After brief vacuum infiltration, the mix was incubated at 37°C overnight. GUS staining was observed under a microscope after destaining with 70% ethanol.

Yeast two-hybrid (Y2H) assay

The coding regions of *FE, CO*, *NF-YB2*, *REF6-N* and *REF6-C* were amplified and cloned into either pGADT7 or pGBKT7 (Clontech). Primers used in the Y2H assay are listed in Table I-1. Yeast Y2HGold strain was co-transformed with specific bait and prey constructs. Transformed yeast cells were grown on synthetic defined medium lacking leucine and tryptophan (SD/−Leu/−Trp ) or synthetic defined medium lacking leucine, tryptophan, and histidine (SD/−Leu/−Trp/−His ) for selections or assays.

Chromatin immunoprecipitation (ChIP) assay

Twelve-day-old seedlings were collected and powdered in liquid nitrogen, then fixed in room temperature for 10 min in 1% formaldehyde. Chromatin was isolated and sonicated to produce 200 to 500 DNA fragments. FE-3xFLAG, H3K27me3 and H3 was immunoprecipitated with anti-FLAG antibody (F1804), anti-H3K27me3 antibody (07-449; Millipore) and anti-H3 antibody (ab1791; Abcam), respectively. The relative enrichment was calculated from the ratio between levels of immunoprecipitated DNA with anti-FLAG antibody and those of input DNA, and from the ratio between levels of immunoprecipitated DNA with anti-H3K27me3 antibody and those with anti-H3 antibody by qPCR. Statistical analysis was performed using Student's *t*-test.

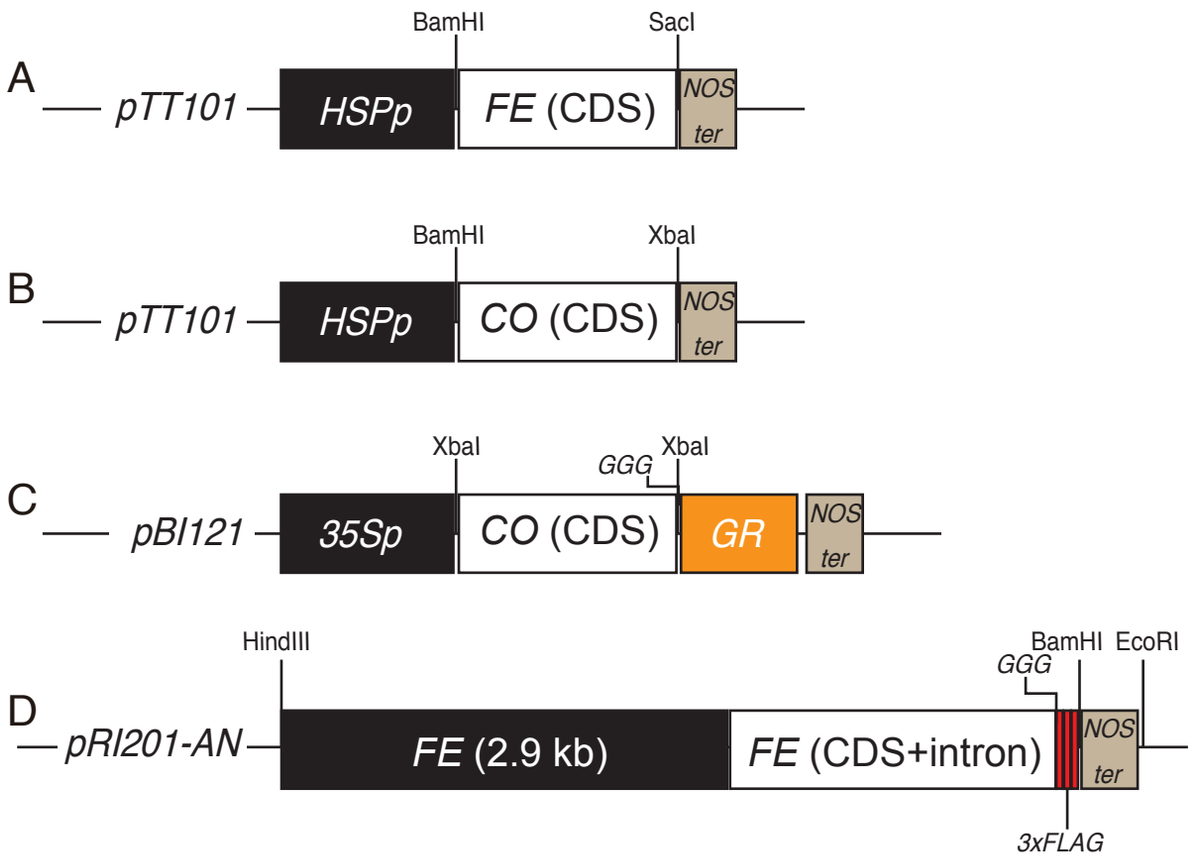


Fig. II-1. Plasmid maps. (A) *pHSP:FE*, (B) *pHSP:CO*, (C) *p35S:CO-GR*, and (D) *gFE-3xFLAG*. (Plant Cell Physiol. Nov 1; 58(11) 2017-2025, 2017 “FE Controls the Transcription of Downstream Flowering Regulators Through Two Distinct Mechanisms in Leaf Phloem Companion Cells” OXFORD UNIVERSITY PRESS)

Primer Sequences

| Target | Application | Forward primer | Reverse primer | Probe |
|---|---|---|---|---|
| ***NaKR1*** | RT-qPCR | CGGTTAGTTGTTTTAGCAGTGACACACCTC | CGGCTGGTCTCATTTCTGAATAATCTCAGG | |
| ***FT*** | RT-qPCR | TGGTGGAGAAGACCTCAGGAAC | GTGAGGGTTGCTAGGACTTGGA | #138 |
| ***FTIP1*** | RT-qPCR | GCGCAAGATGTTGAGCCTA | TTGTACTTTAACGAAAGCTTGAGG | #92 |
| ***eIF4*** | RT-qPCR | TCAAACTGTGTGCCTTCTGG | CGAGTTTGGGAGATTCAAGC | #67 |
| ***FE*** | RT-PCR | ATGTTCCATGCTAAGAAACCTTCAAG | TCACCCAAATGGCGAGTTTCTTCC | |
| ***TUB*** | RT-PCR | CTCAAGAGGTTCTCAGCAGTA | TCACCTTCTTCATCCGCAGTT | |
| ***Fp-1*** | ChIP-qPCR | GGCCAACATTAGAAGAAGATTCC | TCTTGACATGGAGCGAAAGA | |
| ***Fp-2*** | ChIP-qPCR | TTCTATGATTTCTCATGAACCG | GAACACTAAAATGTAGAAGAACC | |
| ***Fp-3*** | ChIP-qPCR | GTGGCTACCAAGTGGGAGAT | TAACTCGGGTCGGTGAAATC | |
| ***Np-1*** | ChIP-qPCR | TTTCTGGCTCCAAACCCTAA | GAAATAACTATTGGGCTATTACACGG | |
| ***Np-2*** | ChIP-qPCR | AAAGGTGTGAATAGTGAAAGCTAATACGA | TAGAAAGGCCAATTCTTTTTGCC | |
| ***Np-3*** | ChIP-qPCR | CAATCTCTCACACATTGGTTTGATAAG | GTCTTTAGTGTTTGCAATGAAAATGTG | |
| ***p-1*** | ChIP-qPCR | CTGCGACTGCGACCTATTTT | GCCACTGTTCTACACGTCCA | |
| ***gb-1*** | ChIP-qPCR | CCAAGAGTTGAGATTGGTGGA | GGGCATTTTTAACCAAGGTCT | |
| ***gb-2*** | ChIP-qPCR | GCTCAAACATGTTGCTCGAA | TGCGATCAGTAAAATACACAGACA | |
| ***gb-3*** | ChIP-qPCR | GATCTACAATCTCGGCCTTCC | ATCATCACCGTTCGTTACTCG | |
| ***u-1*** | ChIP-qPCR | TTCTGTGCATTCAACCGATA | CAGTTTTTGGGACGCAAAGT | |
| ***AD-CO*** | Y2H cloning | CCAGATTACGCTCATATGTTGAAACAAGAG | CCCACCCGGGTGGAATTCTCAGAATGAAGGAAC | |
| ***AD-REF6-N*** | Y2H cloning | ATCGAATTAGGATCCACAGCAACTTCGTC | ACTAATACTCTCGAGTCACCTTTTGTTGGTC | |

| | | | | |
|---|---|---|---|---|
| ***AD-REF6-C*** | Y2H cloning | ATCGAATTAGGATCCATGGCG GTTTCAGAG | ACTAATACTCTCGAGTCCACT TCCGCAGC | |
| ***AD-NF-YB2*** | Y2H cloning | CCAGATTACGCTCATATGGAG CAGTCAGAAG | CCCACCCGGGTGGAATTCTT AAGACTCATCAGGG | |
| ***BD-FE*** | Y2H cloning | GAGGAGGACCTGCATATGTTC CATGCTAAG | CGGCCGCTGCAGGTCGACTC ACCCAAATGGCGA | |

References

Abe, M., Kaya, H., Watanabe-Taneda, A., Shibuta, M., Yamaguchi, A., Sakamoto, T. et al. (2015) FE, a phloem-specific Myb-related protein, promotes flowering through transcriptional activation of *FLOWERING LOCUS T* and *FLOWERING LOCUS T INTERACTING PROTEIN 1*. Plant J. 83: 1059-1068.

Clough, S.J. & Bent, A.F. (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. Plant J. 16: 735-743.

Laubinger, S., Marchal, V., Le Gourrierec, J., Wenkel, S., Adrian, J., Jang, S. et al. (2006) *Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability. Development. 133: 3213-3222.

Noh, B., Lee, S.H., Kim, H.J., Yi, G., Shin, E.A., Lee, M. et al. (2004) Divergent roles of a pair of homologous jumonji/zinc-finger-class transcription factor proteins in the regulation of Arabidopsis flowering time. Plant Cell. 16: 2601-2613.

Notaguchi, M., Abe, M., Kimura, T., Daimon, Y., Kobayashi, T., Yamaguchi, A. et al. (2008) Long-distance, graft-transmissible action of *Arabidopsis* FLOWERING LOCUS T protein to promote flowering. Plant Cell Physiol. 49: 1645-1658.

Tian, H., Baxter, I.R., Lahner, B., Reinders, A., Salt, D.E. & Ward, J.M. (2010) *Arabidopsis* NPCC6/NaKR1 is a phloem mobile metal binding protein necessary for phloem function and root meristem maintenance. Plant Cell. 22: 3963-3979.

# Chapter III

# FE controls spatiotemporal expression of *FLOWERING LOCUS T* via interaction with CONSTANS.

Introduction

Under inductive photoperiodic conditions, CONSTANS (CO) plays an important role in temporal regulation of *FLOWERING LOCUS T* (*FT*) expression (Putterill et al., 1995; Samach et al., 2000; Suárez-López et al., 2001; Valverde et al., 2004; Imaizumi, 2010; Song et al., 2013). A 5.7-kb sequence upstream of the *FT* initiation codon contains all regulatory elements necessary for the spatiotemporal expression of *FT* (Adrian et al., 2010). Chromatin immunoprecipitation (ChIP) assays and electrophoretic mobility shift assays demonstrated that CO protein directly binds to CO responsive elements (CORE) located approximately 250 bp upstream of the FT translation start site (Tiwari et al., 2010; Song et al., 2012). CO protein requires physical association with components of NUCLEAR FACTOR-Y (NF-Y) complex, which bind to a CCAAT motif, to activate *FT* expression (Ben-Naim et al., 2006; Wenkel et al., 2006; Kumimoto et al., 2010; Cao et al., 2014; Zhao et al., 2017). The putative NF-Y complex binding site is not found in the proximal region including CORE site, although multiple CCAAT *cis* elements are located in the distal region of the *FT* promoter. Therefore, CO-mediated activation of *FT* transcription is proposed to involve formation of long-distance chromatin looping, which brings the distal CCAAT sites into proximity to CORE sites. However, more work is needed to understand the proper mechanism of the *FT* transcription because previous studies suggest existence of additional factors which assist effective formation of looping structure (Kumimoto et al., 2010; Tiwari et al., 2010; Cao et al., 2014; Siriwardana et al., 2016).

In spite of the substantial contribution of CO in the temporal regulation of *FT* mRNA abundance, the spatial expression of *FT* cannot be perfectly explained by CO activity. Unlike *CO*, which is expressed in phloem tissues at the distal and proximal region of leaf blade, the *FT* expression in phloem tissue at the proximal region of leaf blade is relatively repressed. Even though *CO* is induced ectopically in all tissues, *FT* is activated only in phloem tissue (Yamaguchi et al.,

2005; Adrian et al., 2010). Furthermore, *FT* expression is not observed in hormone-induced callus; this suggests that a key of spatial regulation of *FT* is the proper development and differentiation of phloem tissue (Farrona et al., 2011). However, in order to regulate the phloem-specific expression pattern of *FT*, there must be an additional positive regulator of *FT* besides CO (described as factor 'X' in Fig. I-1).

I consider that FE has a potential for factor 'X', because FE plays roles in both flowering and phloem development. Therefore, in this chapter, to discover the factor 'X', I investigated molecular functions of FE in transcriptional regulation of *FT* gene. I showed that FE directly activates the transcription of *FT* by binding to its promoter regions to form the chromatin looping. Furthermore, my experimental data strongly suggest that FE and CO activate *FT* expression interdependently under long-day conditions and play a pivotal role in the regulation of phloem-specific expression of *FT*.

Results

**FE and CO regulate *FT* expression in a mutually depending manner**

Our group previously showed that *FT* is an immediate target of FE protein (Abe et al., 2015). To further understand roles of FE in *FT* activation, I initially analyzed effects of the *fe-1* mutation on the expression rhythms of the *FT* transcription by quantitative reverse transcription–PCR (RT–qPCR). The diurnal expression pattern of *FT* mRNA, which shows a significant peak at dusk in WT seedlings, was eliminated in *fe-1* mutants even under long-day conditions (Fig. III-1). This strongly suggests that FE is necessary for the activation of the *FT* transcription at dusk under long-day conditions. Next, I constructed a transgenic plant overexpressing glucocorticoid receptor (GR)-fused FE (*p35S:FE-GR*), which can induce FE activity by dexamethasone (Dex) treatment. In *p35S:FE-GR* seedlings, *FT* expression was immediately induced after application of Dex at dusk (Fig. III-2). These observations suggest that FE is involved in induction of *FT*.

To understand the molecular mechanism of FE-mediated *FT* induction, I constructed another FE-inducible system, in which *FE* expression was driven under the control of the *heat shock protein 18.2* promoter (*pHSP:FE*) (Takahashi and Komeda, 1989). In *pHSP:FE* seedlings, a large amount of *FE* mRNA was rapidly induced by heat treatment, followed by accumulation of FE protein (Fig. III-3). Use of this heat-inducible *FE* expression system demonstrated that heat treatment at 4 hours after the beginning of day light [zeitgeber time (ZT) 4] could not immediately increase amount of *FT* mRNA in *pHSP:FE* seedlings (Fig. III-4). However, higher mRNA levels of *FT* in heat-treated *pHSP:FE* seedlings than those in heat-treated WT seedlings were observed at dusk (ZT16) (Fig. III-4), when CO protein endogenously activates *FT* expression under long-day

conditions (Shim et al., 2017). Furthermore, under short-day conditions, heat treatment at ZT4 did not lead to the accumulation of *FT* mRNA at ZT16 (Fig. III-5). The suppression of CO-mediated diurnal expression of *FT* in *fe-1* seedlings and the day-length-dependency of FE-mediated *FT* induction in *p35S:FE-GR* seedlings suggest that *CO* is involved in FE-mediated *FT* induction.

To address whether *CO* is involved in FE-mediated *FT* induction, expression analysis was performed using the *p35S:FE-GR* system in WT and a T-DNA insertion allele in *CO* (*co-10*) background. In *p35S:FE-GR co-10* seedlings, the amount of *FT* mRNA induced by application of Dex was dramatically lower compared to that in WT background (Fig. III-6). In addition, to investigate whether *FE* is required for the CO-mediated *FT* activation, I also constructed a *CO*-inducible transgenic plant, *pHSP:CO*. In *pHSP:CO* seedlings, a large amount of *FT* mRNA was observed after heat treatment, however, in *pHSP:CO fe-1* seedlings, the amount of induced *FT* mRNA by heat treatment was lower compared to that in *pHSP:FE* seedlings (Fig. III-7). Therefore, we concluded that FE could not effectively induce *FT* mRNA in absence of CO function, and similarly CO could not effectively induce *FT* mRNA in absence of FE function. These results suggest that FE and CO act closely together in the transcriptional activation of *FT* under inductive photoperiods. This was further supported by the observation that *fe-1* did not markedly enhance flowering phenotypes of *co-10* (Fig. III-8A and B).

**FE protein physically associates with the distal region of the *FT* promoter**

Previous studies suggested that putative components of CO/NF-Y complex bind to several regions of the *FT* promoter to form long-distance chromatin loops to stabilize the *FT* transcription (Tiwari et al., 2010; Song et al., 2012; Cao et al., 2014). Since the amount of *CO* mRNA was not changed in *fe-1* seedlings compared with WT seedlings (Abe et al., 2015), I considered that *CO* was

not a downstream target of FE in regulation of *FT*. Then I hypothesized that FE acts together with CO/NF-Y complex in activation of *FT*. Thus, I next investigated whether FE directly binds to the *FT* promoter by chromatin immunoprecipitation (ChIP) analysis using the *gFE:3xFLAG* transgenic line, which can rescue the defect of a null allele of *FE* (*apl*) (Fig. III-9A and B). Three DNA fragments in the *FT* promoter region, designated *Fp-1* to *Fp-3*, were used for the ChIP assays (Fig. III-10A). The ChIP-qPCR experiment showed that the FE-3xFLAG protein was strongly associated with the *Fp-2* region which is 4.5 kb upstream from the translation initiation site (Fig. III-10A and B). FE-3xFLAG protein also physically bound to the *Fp-1* region which contains several NF-Y complex binding sites (*CCAAT* sites) located 5.3 kb upstream from the ATG site (Fig. III-10A and B). According to previous reports, *Fp-1* and *Fp-3* are important regions for the formation of the chromatin looping structure by CO/NF-Y complex on the *FT* promoter (Cao et al., 2014, Gnesutta et al., 2017); however, a significant enrichment of the *Fp-3* region containing CORE sites was not observed in the ChIP-qPCR analysis (Fig. III-10A and B).

Finally, I performed yeast two hybrid (Y2H) assays to test whether FE physically interacts with components of CO/NF-Y complex. *NF-YB2* is strongly expressed in phloem tissue (Siefers et al., 2009), and NF-YB2 interacts with CO, and then binds to 5.3 kb upstream from the ATG site to form the chromatin looping at the *FT* promoter (Cao et al., 2014). In this assay, FE protein reproducibly interacted with CO or NF-YB2 in yeast cells (Fig. III-11). Therefore, it is likely that FE forms a chromatin loop together with components of CO/NF-Y complex and directly binds to several regions of the *FT* locus to promote its expression under long-day conditions.

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Discussion

Flowering plants sense seasonal cues for reproductive success. In *Arabidopsis,* CO is a key activator of *FT* transcription in long-day-specific flowering. FE is also a positive regulator of *FT* transcription, and here, I show the function of FE through the interaction with CO in *FT* activation. Previous studies suggested that NF-Y subunit play a 'pioneering' role by forming chromatin looping structures together with CO, which bind to CORE sites in the proximal region of the *FT* promoter (Ben-Naim et al., 2006; Wenkel et al., 2006; Adrian et al., 2010; Cao et al., 2014; Siriwardana et al., 2016). As for CO/NF-Y complex, hypothetical models for the activation of *FT* were proposed by several groups. However, further identification of transcription factors and *cis* elements that stabilize the looping structure on the *FT* promoter is required for the following reasons: (i) High levels of *CO* overexpression can partially overcome late-flowering phenotypes in various *nf-y* mutants, (ii) the chromatin-looping structure does not completely disappear in *co* or *nf-y* mutants, and (iii) mutations at −5.3 kb distal *CCAAT* sites cause earlier flowering than in *nf-y* mutants (Kumimoto et al., 2010; Tiwari et al., 2010; Cao et al., 2014; Siriwardana et al., 2016). I demonstrated that FE can potentially interact with both CO and NF-YB2 (Fig. III-11), an important component of the flowering-related NF-Y (Cao et al., 2014), and that FE associates with a −5.3 kb distal region where NF-YB2 binds to assist chromatin-loop formation (Fig. III-10A and B). In addition, FE activates *FT* in a CO-dependent manner (Fig. III-6) and CO also activates *FT* in a FE-dependent manner (Fig. III-7). In addition, *fe-1* causes reduced expression of *FT* despite unaltered *CO* mRNA levels (Abe et al., 2015). These results led me to hypothesize that FE is involved in the chromatin-loop formation in cooperation with CO/NF-YB2 complexes. In my scenario, FE may act as an additional scaffold at the *Fp-2* region to generate a robust looping structure for stabilizing CO binding to the proximal CORE sites.

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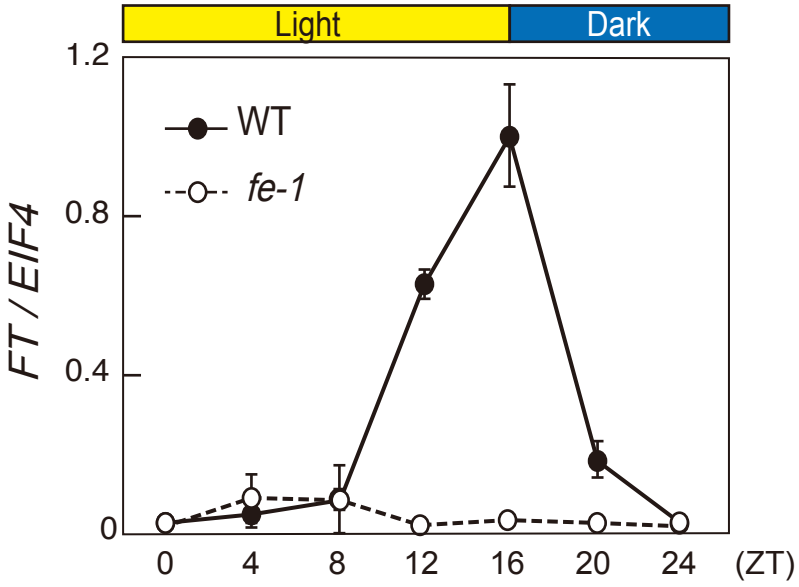


Fig. III-1. Diurnal mRNA levels of *FT* in WT and *fe-1* seedlings collected at 10 DAG under long-day conditions every 4 h over a 24-h period after dawn (ZT0). Error bars show the SDs (*n* =3). An yellow box indicates a ight period, and a blue box indicates a dark period. (Plant Cell Physiol. Nov 1; 58(11) 2017-2025, 2017 “FE Controls the Transcription of Downstream Flowering Regulators Through Two Distinct Mechanisms in Leaf Phloem Companion Cells” OXFORD UNIVERSITY PRESS)

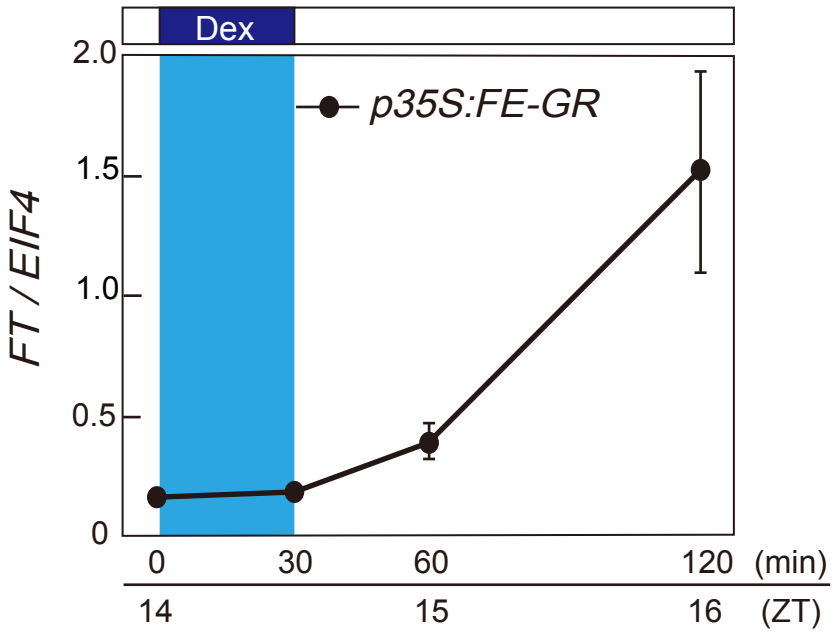


Fig. III-2. *FT* mRNA levels in *p35S:FE-GR* seedlings 30, 60 and 120 min after Dex treatment under long-day conditions. Transgenic plants were treated with Dex in ZT14-14.5. Error bars show the SDs (*n* = 3). A blue box indicates the period of Dex treatment.

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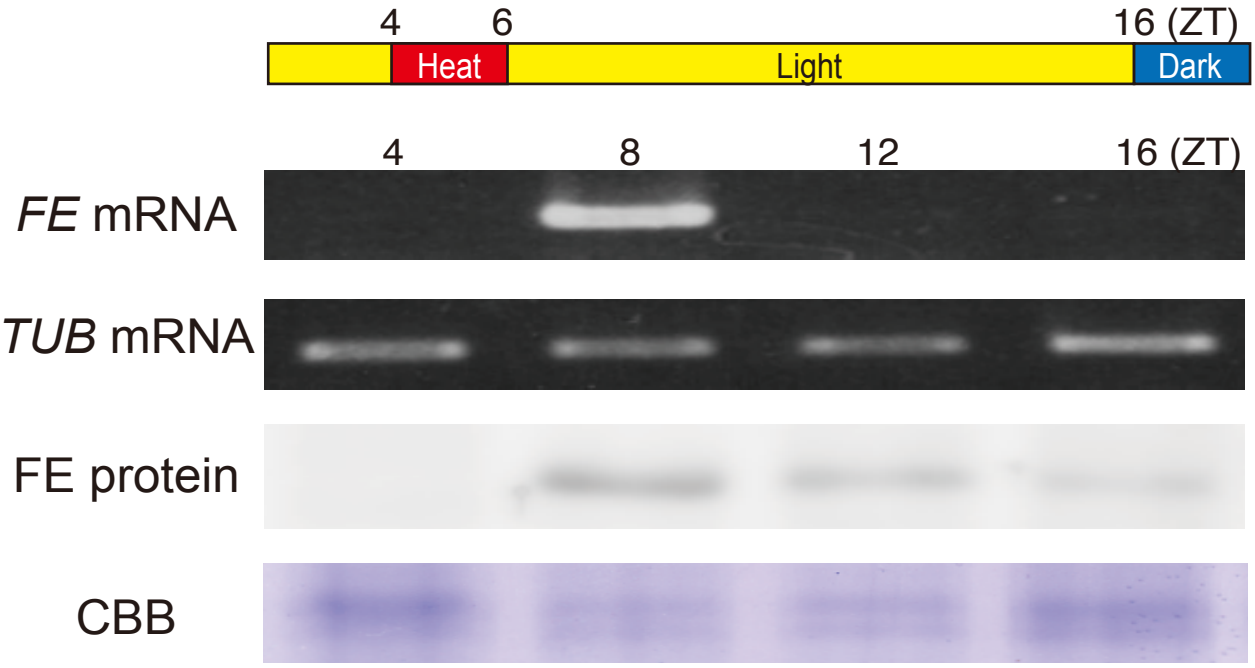


Fig. III-3. *FE* mRNA and FE protein abundance in *pHSP:FE* seedlings every 4h after heat treatment under long-day conditions. Transgenic plants were heat-treated in ZT4-6 at 37°C. A red box indicates the period of heat treatment, yellow boxes indicate light periods, and a blue box indicates a dark period.
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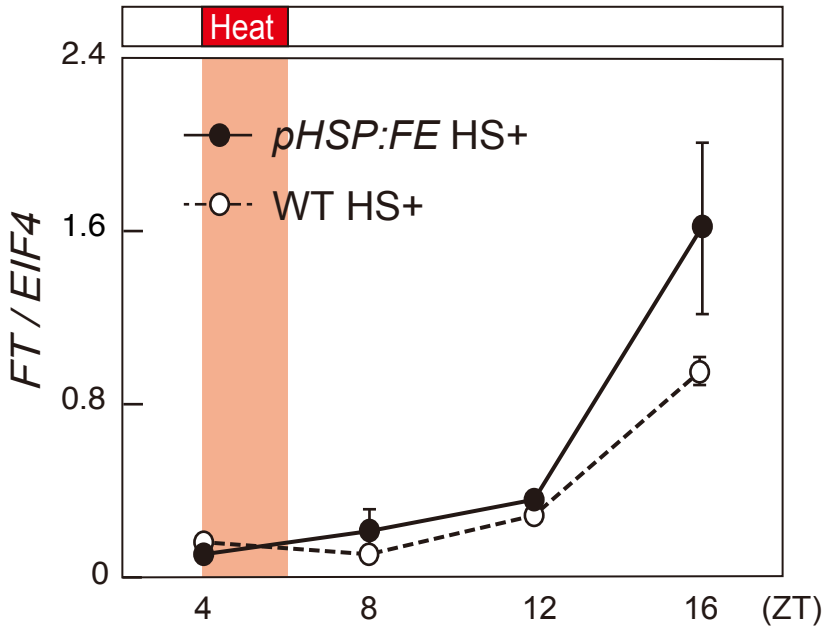


Fig. III-4. *FT* mRNA levels in WT and *pHSP:FE* seedlings measured every 4 h after heat treatment under long-day conditions. Seedlings were heat-treated at ZT4–6 at 37 °C. Error bars show the SDs (*n* =3). A red box indicates the period of heat treatment.
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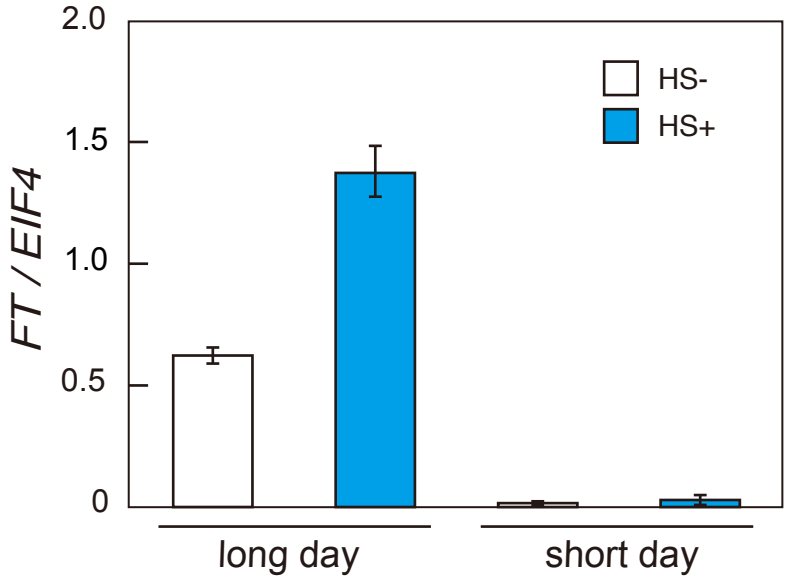


Fig. III-5. *FT* mRNA levels in *pHSP:FE* seedlings 12h after heat shock treatment under long-day or short-day conditions. Transgenic plants were heat-treated in ZT4-6 at 37°C. Error bars show the SDs (*n* = 3).

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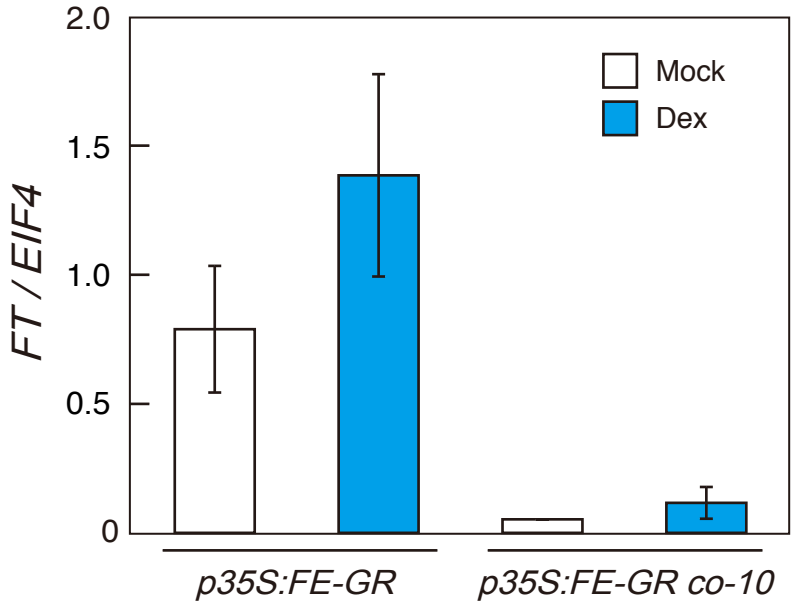


Fig. III-6. *FT* mRNA levels in *p35S:FE-GR* seedlings under long-day conditions. Transgenic plants were treated with Mock or Dex at ZT12–12.5 and harvested at ZT16. Error bars show the SDs (*n* =3).

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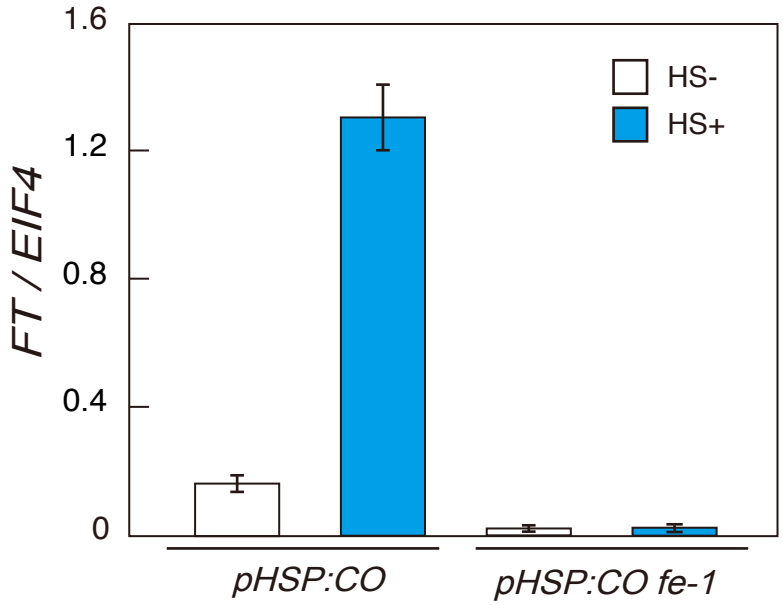


Fig. III-7. *FT* mRNA levels in *pHSP:CO* seedlings 4h after heat treatment under long-day conditions. Transgenic plants were heat-treated in ZT12-14 at 37°C. Error bars show the SDs (*n* = 3).

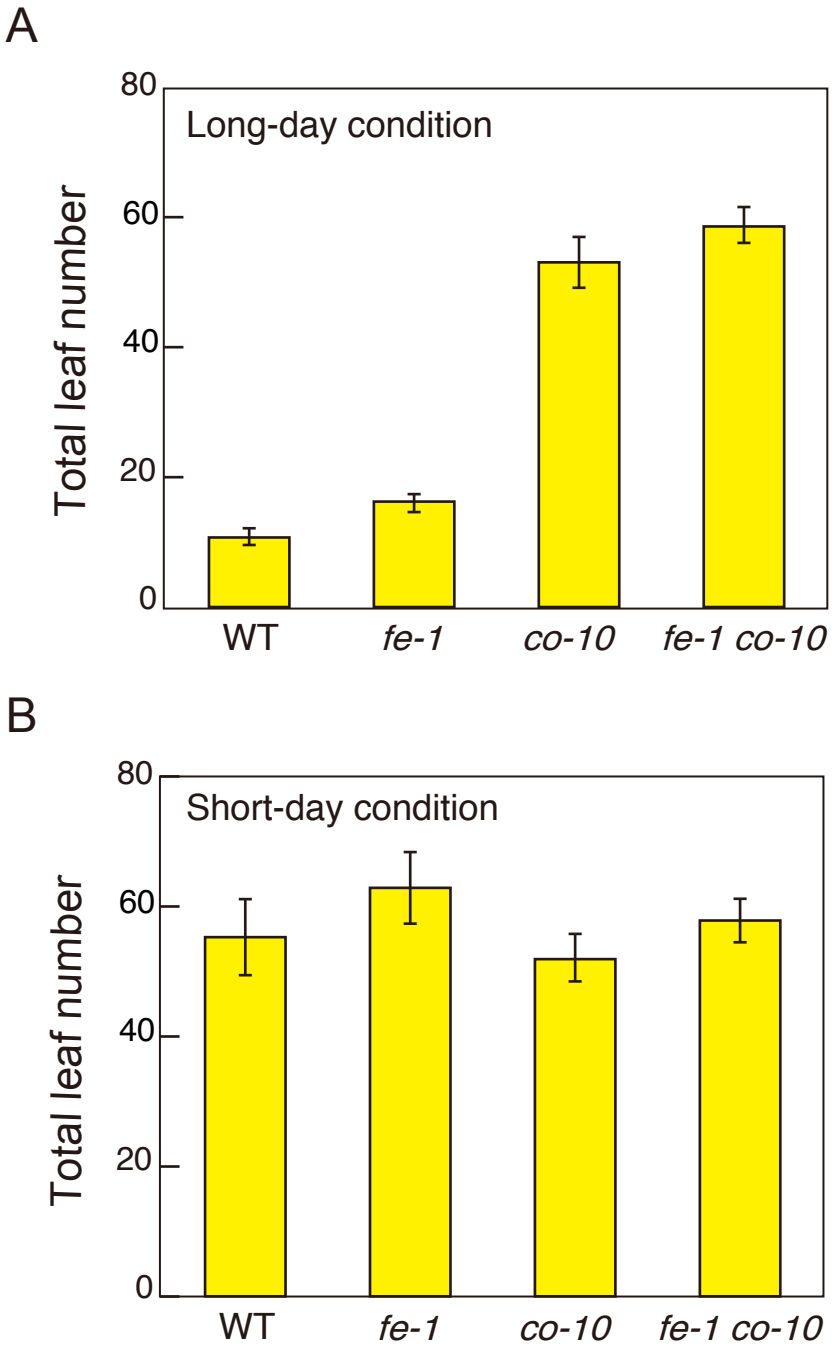


Fig. III-8. Flowering phenotypes of WT, *fe-1*, *co-10*, and *fe-1 co-10* seedlings under long-day (A) and short-day (B) conditions. Total leaves at the time of flowering were counted. Error bars show the SDs (*n* =12).

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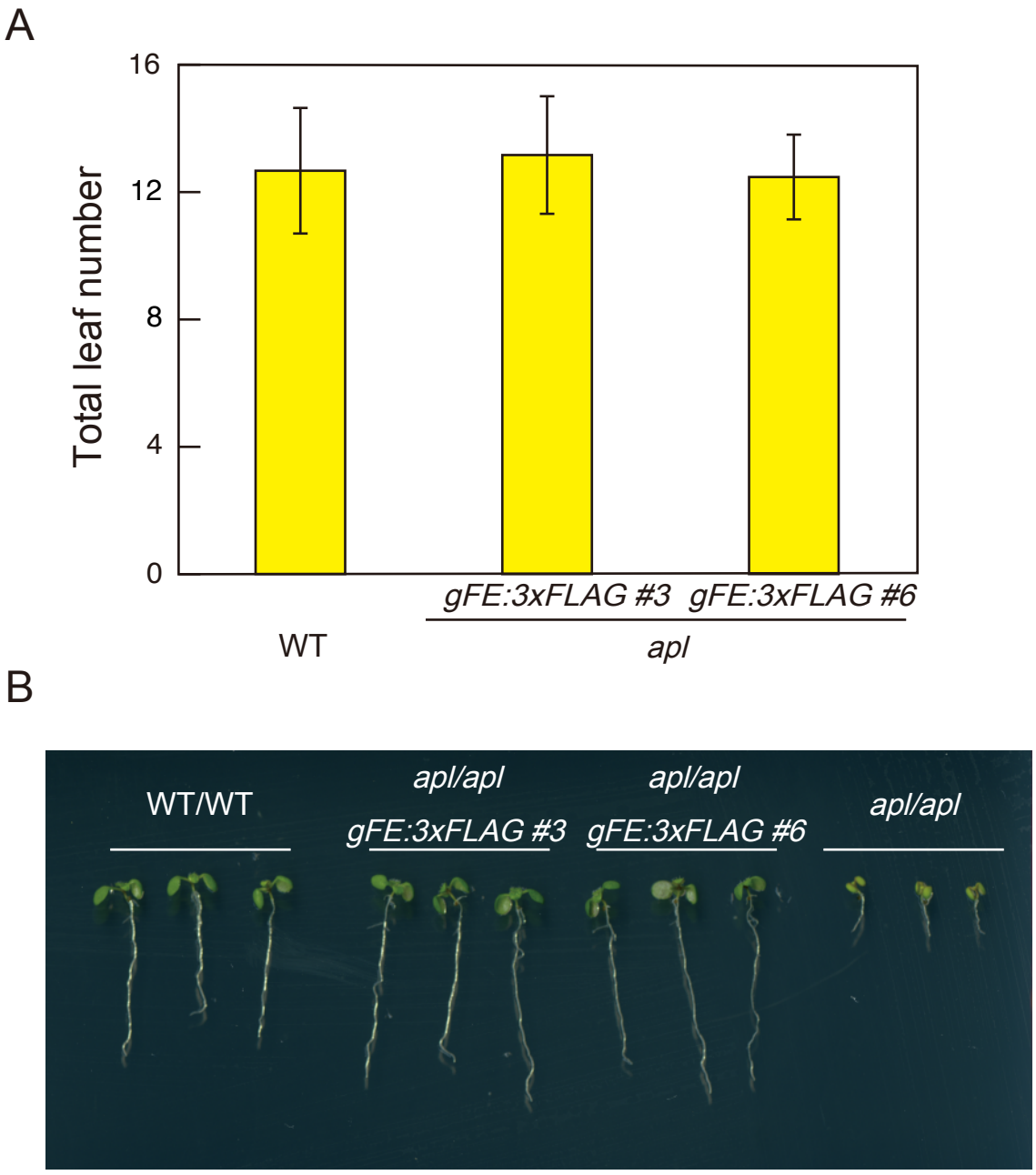


Fig. III-9. Comparision of the *gFE:3xFLAG apl/apl* complementation lines and WT seedlings. (A) Flowering phenotypes of WT, *apl gFE:3xFLAG #3, apl gFE:3xFLAG #6* seedlings under long-day conditions. The number of total leaves at the time of flowering was measured. Error bars show the SDs (*n* = 12). (B) Seedlings in 10DAG.
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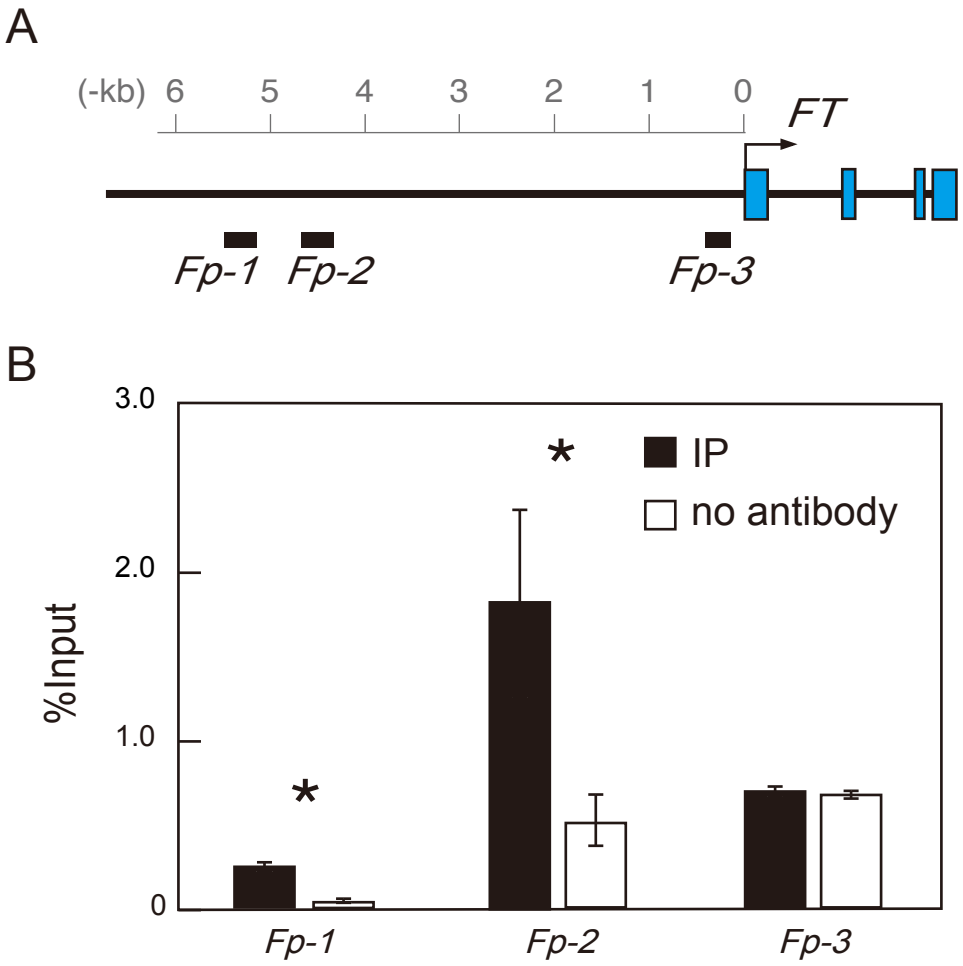


Fig. III-10. ChIP analysis of binding of FE to the *FT* promoter. (A) Schematic diagram showing *FT* gene structure. (B) FE-3×FLAG accumulation at the *FT* locus in *gFE:3xFLAG* seedlings. Target DNA from *gFE:3xFLAG* immunoprecipitated against input DNA was quantified by qPCR. Asterisks indicate statistically significant differences (Student' s *t*-test, *: $p<0.05$). Error bars show the SDs ($n$ =3).

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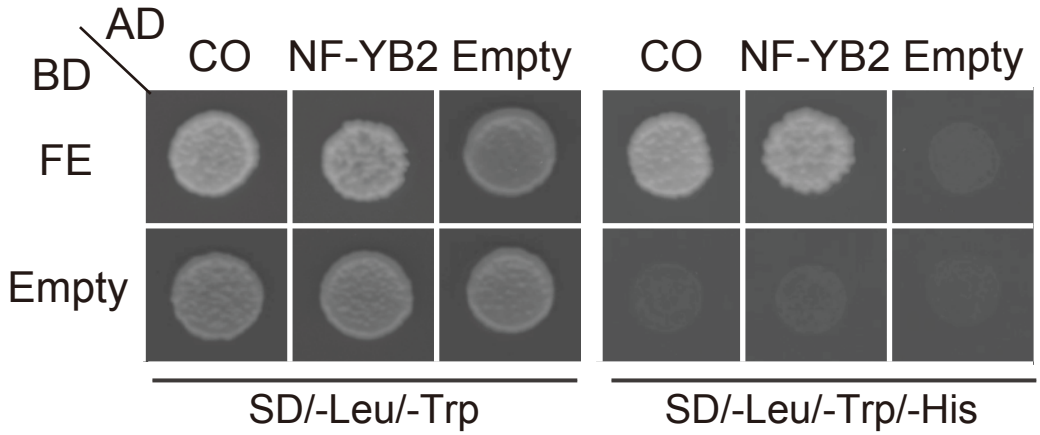


Fig. III-11. Interaction between FE and CO or NF-YB2 in the Y2H analysis. Yeast cells were co-transformed with the indicated bait and prey combination and grown on SD/−Leu/−Trp (left) or SD/−Leu/−Trp/−His (right).
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References

Abe, M., Kaya, H., Watanabe-Taneda, A., Shibuta, M., Yamaguchi, A., Sakamoto, T. et al. (2015) FE, a phloem-specific Myb-related protein, promotes flowering through transcriptional activation of *FLOWERING LOCUS T* and *FLOWERING LOCUS T INTERACTING PROTEIN 1*. Plant J. 83: 1059-1068.

Adrian, J., Farrona, S., Reimer, J.J., Albani, M.C., Coupland, G. & Turck, F. (2010) *cis*-Regulatory elements and chromatin state coordinately control temporal and spatial expression of *FLOWERING LOCUS T* in *Arabidopsis*. Plant Cell. 22: 1425-1440.

An, H., Roussot, C., Suárez-López, P., Corbesier, L., Vincent, C., Piñeiro, M. et al. (2004) CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of *Arabidopsis*. Development. 131: 3615-3626.

Ben-Naim, O., Eshed, R., Parnis, A., Teper-Bamnolker, P., Shalit, A., Coupland, G. et al. (2006) The CCAAT binding factor can mediate interactions between CONSTANS-like proteins and DNA. Plant J. 46: 462-476.

Cao, S., Kumimoto, R.W., Gnesutta, N., Calogero, A.M., Mantovani, R. & Holt, B.F.,3rd. (2014) A distal *CCAAT*/NUCLEAR FACTOR Y complex promotes chromatin looping at the *FLOWERING LOCUS T* promoter and regulates the timing of flowering in *Arabidopsis*. Plant Cell. 26: 1009-1017.

Farrona, S., Thorpe, F.L., Engelhorn, J., Adrian, J., Dong, X., Sarid-Krebs, L. et al. (2011) Tissue-specific expression of *FLOWERING LOCUS T* in *Arabidopsis* is maintained independently of polycomb group protein repression. Plant Cell. 23: 3204-3214.

Gnesutta, N., Kumimoto, R.W., Swain, S., Chiara, M., Siriwardana, C., Horner, D.S. et al. (2017) CONSTANS imparts DNA sequence specificity to the histone fold NF-YB/NF-YC dimer. Plant Cell. 29: 1516-1532.

Imaizumi, T. (2010) *Arabidopsis* circadian clock and photoperiodism: time to think about location. Curr Opin Plant Biol. 13: 83-89.

Kumimoto, R.W., Zhang, Y., Siefers, N. & Holt, B.F.,3rd. (2010) NF-YC3, NF-YC4 and NF-YC9 are required for CONSTANS-mediated, photoperiod-dependent flowering in *Arabidopsis thaliana*. Plant J. 63: 379-391.

Putterill, J., Robson, F., Lee, K., Simon, R. & Coupland, G. (1995) The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell. 80: 847-857.

Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F. et al. (2000) Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. Science. 288: 1613-1616.

Shim, J.S., Kubota, A. & Imaizumi, T. (2017) Circadian clock and photoperiodic flowering in Arabidopsis: CONSTANS is a hub for signal integration. Plant Physiol. 173: 5-15.

Siefers, N., Dang, K.K., Kumimoto, R.W., Bynum, W.E.,4th, Tayrose, G. & Holt, B.F.,3rd. (2009) Tissue-specific expression patterns of Arabidopsis NF-Y transcription factors suggest potential for extensive combinatorial complexity. Plant Physiol. 149: 625-641.

Siriwardana, C.L., Gnesutta, N., Kumimoto, R.W., Jones, D.S., Myers, Z.A., Mantovani, R. et al. (2016) NUCLEAR FACTOR Y, Subunit A (NF-YA) proteins positively regulate flowering and act through *FLOWERING LOCUS T*. PLoS Genet. 12: e1006496.

Song, Y.H., Ito, S. & Imaizumi, T. (2013) Flowering time regulation: photoperiod- and temperature-sensing in leaves. Trends Plant Sci. 18: 575-583.

Song, Y.H., Smith, R.W., To, B.J., Millar, A.J. & Imaizumi, T. (2012) FKF1 conveys timing information for CONSTANS stabilization in photoperiodic flowering. Science. 336: 1045-1049.

Suárez-López, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F. & Coupland, G. (2001) *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature. 410: 1116-1120.

Sugimoto, K., Jiao, Y. & Meyerowitz, E.M. (2010) *Arabidopsis* regeneration from multiple tissues occurs via a root development pathway. Dev Cell. 18: 463-471.

Takahashi, T. & Komeda, Y. (1989) Characterization of two genes encoding small heat-shock proteins in *Arabidopsis thaliana*. Mol Gen Genet. 219: 365-372.

Tiwari, S.B., Shen, Y., Chang, H.C., Hou, Y., Harris, A., Ma, S.F. et al. (2010) The flowering time regulator CONSTANS is recruited to the *FLOWERING LOCUS T* promoter via a unique *cis*-element. New Phytol. 187: 57-66.

Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A. & Coupland, G. (2004) Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. Science. 303: 1003-1006.

Wenkel, S., Turck, F., Singer, K., Gissot, L., Le Gourrierec, J., Samach, A. et al. (2006) CONSTANS and the CCAAT box binding complex share a functionally important domain and interact to regulate flowering of *Arabidopsis*. Plant Cell. 18: 2971-2984.

Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M. & Araki, T. (2005) TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT. Plant Cell Physiol. 46: 1175-1189.

Zhao, H., Wu, D., Kong, F., Lin, K., Zhang, H. & Li, G. (2017) The *Arabidopsis thaliana* nuclear factor Y transcription factors. Front Plant Sci. 7: 2045.

# Chapter IV

# FE plays a role in chromatin modification at the *FLOWERING LOCUS T* locus before transcriptional activation with CONSTANS.

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# Chapter V

# FE is involved with florigen phloem transport regulation through the regulation of florigen transporter genes, *SODIUM POTASSIUM ROOT DEFECTIVE 1* and *FT-INTERACTING PROTEIN 1* expressions independent of CONSTANS function.

Introduction

Under inductive conditions, our phenotypic analysis revealed that the *fe-1* mutation impaired the long-distance transport of FLOWERING LOCUS T (FT) protein, in addition to the activation of *FT* transcription (Abe et al., 2015). Recently, the phloem-localized FT protein interactors, FT-INTERACTING PROTEIN 1 (FTIP1) and SODIUM POTASSIUM ROOT DEFECTIVE 1 (NaKR1) were reported as florigen transporters required for the long-distance transport of FT (Liu et al., 2012; Zhu et al., 2016; Song et al., 2017). *FTIP1* encodes a plasmodesmata-localized protein containing three C2 domains and one phosphoribosyltransferase C-terminal domain, and FTIP1 associates with FT at plasmodesmata, mediating loading of FT from companion cells to sieve elements (Liu et al., 2012; Song et al., 2017). *ftip1* mutants show late flowering phenotypes under long-day conditions, which are caused by inefficient FT transportation through plasmodesmata. Attenuation of the long-distance transport of FT in the *fe-1* mutant is at least partly caused by the lower expression of *FTIP1*, an immediate target gene of FE (Abe et al., 2015). Another FT protein transporter, NaKR1, is a member of the family of metallochaperone-like proteins with a heavy-metal-associated domain (Tian et al., 2010). NaKR1 localizes in both companion cells and sieve elements and interacts with FT in its C-terminal heavy-metal-associated domain, promoting FT phloem transport (Zhu et al., 2016). Zhu et al. reported that the transcription of *NaKR1* exhibits an obvious circadian rhythm under long-day conditions and is regulated by CONSTANS (CO) protein. These observations suggest that the FT transport to the SAM is regulated in a day-length dependent manner. Recent studies, including ours, revealed that long-distance transport of FT protein involves multiple steps and various regulators; however, the transcriptional regulation of FT protein transporter genes needs to be unraveled in a molecular level.

Our group reported that FE plays a key role in coordinating the synthesis and transport of FT via transcriptional regulation (Abe et al., 2015). In this chapter, I further investigated molecular functions of FE in transcriptional regulation of downstream targets. I show that FE directly activated the transcription of *NaKR1* via binding to its promoter region. In Chapter III, I showed that CO is necessary for FE-mediated *FT* activation under long-day conditions and abundance of *FT* mRNA exhibited an obvious diurnal rhythm. On the other hand, the transcription of florigen transporters, *NaKR1* and *FTIP1* mRNA, showed non-diurnal rhythm in WT seedlings. Furthermore, genetic analysis revealed that CO function is not required for the transcription of *NaKR1* and *FTIP1* via FE protein. Based on these results, I propose that FE regulates the transcription of *FT* and florigen transporter genes in different manners in leaf phloem companion cells.

Results

### *fe-1* affects the expression of *NaKR1*

FT protein phloem transport from leaf to the SAM is delayed in the *fe-1* mutant, since *fe-1* results in reduced transcription of *FTIP1* (Fig. V-1A). Recently, NaKR1, a heavy-metal-associated protein, was identified as another florigen transporter (Tian et al., 2010; Zhu et al., 2016). Therefore, I investigated whether *fe-1* affects the expression of *NaKR1* as well as that of *FTIP1*.

I first compared amount of *NaKR1* mRNA in WT and *fe-1* seedlings by quantitative reverse transcription PCR (RT–qPCR). The transcription of *NaKR1* is reported to be regulated by CO and shows a long-day-specific diurnal oscillation with a significant peak at ZT10 in WT seedlings (Zhu et al., 2016); however, the amount of *NaKR1* mRNA in WT seedlings did not exhibit an obvious circadian rhythm under long-day conditions in my experiments (Fig. V-1B). Consistently, public microarray data re-analyzed with a web-based tool provided by the Mockler Lab, Donald Danforth Plant Science Center (http://diurnal.mocklerlab.org) (Mockler et al., 2007), did not show the distinct peak of *NaKR1* around ZT10 under long-day conditions (Fig. V-2A-D). In addition, the amount of *FTIP1* mRNA did not exhibit an obvious circadian oscillation in long days (Fig. V-1A). The rhythm of both *NaKR1* and *FTIP1* mRNA levels were not markedly affected by the *fe-1* mutation, although the abundance of each mRNA in *fe-1* was lower than that in WT throughout a day (Fig. V-1A and B).

To further examine the effects of *fe-1* on *NaKR1* expression, *β-glucuronidase* (*GUS*) expression was analyzed in transgenic *Arabidopsis* expressing the *GUS* reporter gene under the

control of the *NaKR1* promoter (*pNaKR1:GUS*) (Tian et al., 2010). GUS expression in WT seedlings was detected in the phloem tissue of various organs, including the root (Fig. V-3A-C). In *fe-1* background, however, GUS activity was greatly reduced compared with that in WT background in the phloem tissue (Fig. V-3D-F). This was consistent with the results obtained by RT–qPCR analysis of *NaKR1* mRNA levels (Fig. V-1B). According to these expression analyses, *fe-1* reduced the mRNA levels of *NaKR1*, as it did also with *FTIP1*. These data suggest that *NaKR1* is a possible downstream target of FE.

**FE directly induces *NaKR1* mRNA**

To test whether FE protein can activate the transcription of *NaKR1*, RT–qPCR analysis was performed using a heat-inducible *FE* expression system (*pHSP:FE*). After induction of FE by heat treatment in the morning (ZT4), *NaKR1* mRNA levels increased rapidly within 4 h and were maintained at a high level until the evening (ZT16) (Fig. V-4A). This *NaKR1* up-regulation was not observed in the heat-treated WT (Fig. V-4A).

In *p35S:FE-GR* seedlings, the amount of *NaKR1* mRNA obviously increased after application of dexamethasone (Dex) even in the presence of cycloheximide (Cyc), a protein translation inhibitor (Fig. V-4B). These analyses indicated that FE is involved in the immediate activation of *NaKR1*. The late-flowering phenotype of *nakr1-1* was enhanced by *fe-1* (Fig. V-5). The defect of FTIP1 function in the *fe-1* mutant might be responsible for this enhancement, because *nakr1-1 ftip1-1* double mutants flowered later than each single mutant under long-day conditions (Zhu et al., 2016).

To determine whether FE binds directly to the *NaKR1* promoter, I performed chromatin immunoprecipitation (ChIP) analysis using *gFE-3×FLAG* seedlings. I used three DNA fragments in the *NaKR1* promoter region, designated *Np-1* to *Np-3*, for the ChIP assays (Fig. V-6A). As shown in Fig. V-6B, ChIP–qPCR experiments revealed that *Np-1* and *Np-2* fragments were significantly enriched using an anti-FLAG antibody, indicating that FE-3×FLAG protein was physically associated with sites 600 (*Np-1*) and 400 bp upstream (*Np-2*) from the translation initiation site of *NaKR1*, but not with the proximal region of the ATG site (*Np-3*) (Fig. V-6A and B).

The diurnal oscillation and spatial expression patterns of *NaKR1* were similar to those of *FE* (Fig. IV-4A). In addition, I revealed that FE protein can activate *NaKR1* expression, and FE has the ability to bind to the *NaKR1* promoter region. Taken together, these results indicate that FE protein directly promotes *NaKR1* transcription in companion cells .

**CO function is not required for FE-mediated *NaKR1* mRNA induction**

To determine whether CO function is required for FE-mediated *NaKR1* activation, I compared *NaKR1* mRNA levels between WT and *co-10* seedlings by RT–qPCR. An earlier study reported that the transcription of *NaKR1* was downregulated during daytime in the *co* mutant or under short-day conditions (Zhu et al., 2016); however, no significant difference in *NaKR1* mRNA levels was observed between WT and *co-10* seedlings under long-day conditions in the present study (Fig. V-7A). Even in the short-day-grown WT seedlings, *NaKR1* was expressed at the same level as in the long-day-grown WT seedlings (Fig. V-7B). These results suggest that CO function and long-day conditions rarely affect the transcription of *NaKR1* in companion cells.

Next, whether CO function is required for FE-mediated *NaKR1* induction was investigated using the *p35S:FE-GR* system. In *p35S:FE-GR* seedlings, the amount of *NaKR1* mRNA immediately increased after a single application of Dex (Fig. V-8A). Even in *co-10 p35S:FE-GR* seedlings, *NaKR1* mRNA increased after Dex treatment at the same level as in *p35S:FE-GR* seedlings (Fig. V-8A). In addition, even in the short-day-grown seedlings, Dex application on *p35S:FE-GR* seedlings activated the *NaKR1* expression at the same level of that of long-day-grown seedlings (Fig. V-8B). The same experiments were performed with *FTIP1* gene, and showed that activation of *FTIP1* was also independent of both CO function and short-day conditions (Fig. V-8C and D).

My expression analysis showed that induction of *NaKR1* and *FTIP1* mRNA in the *FE*-inducible systems was affected by neither the *co* mutation nor short-day conditions. Therefore, CO function is not necessary for FE-mediated activation of *NaKR1* and *FTIP1*, indicating that FE can effectively promote *NaKR1* and *FTIP1* expression independently of *CO* in companion cells.

Discussion

FE activates the transcription of *NaKR1* and *FTIP1*, florigen transporter genes, in phloem tissue (Fig. V-9). My expression analyses revealed that neither *NaKR1* nor *FTIP1* shows a diurnal expression pattern at the mRNA level under long-day conditions (Fig. V-1A and B and Fig. V-2 A and B). Although Zhu et al. reported that CO directly regulates *NaKR1* expression (Zhu et al., 2016), my results showed that CO function was not required for the upregulation of *NaKR1* (Fig. V-8A).

FE contains a Myb domain, a well-known DNA binding motif, at the N-terminus, although a definite transcription-activation domain is not found in its sequence. Hence, a functional partner containing a transcription-activation domain other than CO would be required for the transcriptional activation of *NaKR1* or *FTIP1* (described as 'A' in Fig. V-9).

Whether the activity level of florigen transporters is constant throughout a day remains unclear; however, a previous study suggested that there is a strict time point at which FT protein sufficiently initiates flowering (Krzymuski et al., 2015). Additionally, NaKR1 is involved in the transport of sucrose from source to sink tissues, which is deeply associated with photosynthetic product translocation during night (Tian et al., 2010; Zhu et al., 2016). Hence, it is natural to consider that most phloem mobile substrates, such as florigen or photosynthetic products, are transported to the SAM or sink tissues under photoperiodic regulation, which reflects the diurnal pattern of *FT* expression or activity in photosynthesis. My data indicate that *NaKR1* and *FTIP1* are not controlled by photoperiodic regulation, at least at mRNA level. Therefore, unknown post-transcriptional and/or post-translational modifications of these transporter genes must be required for the effective regulation of time-point-dependent transportation.

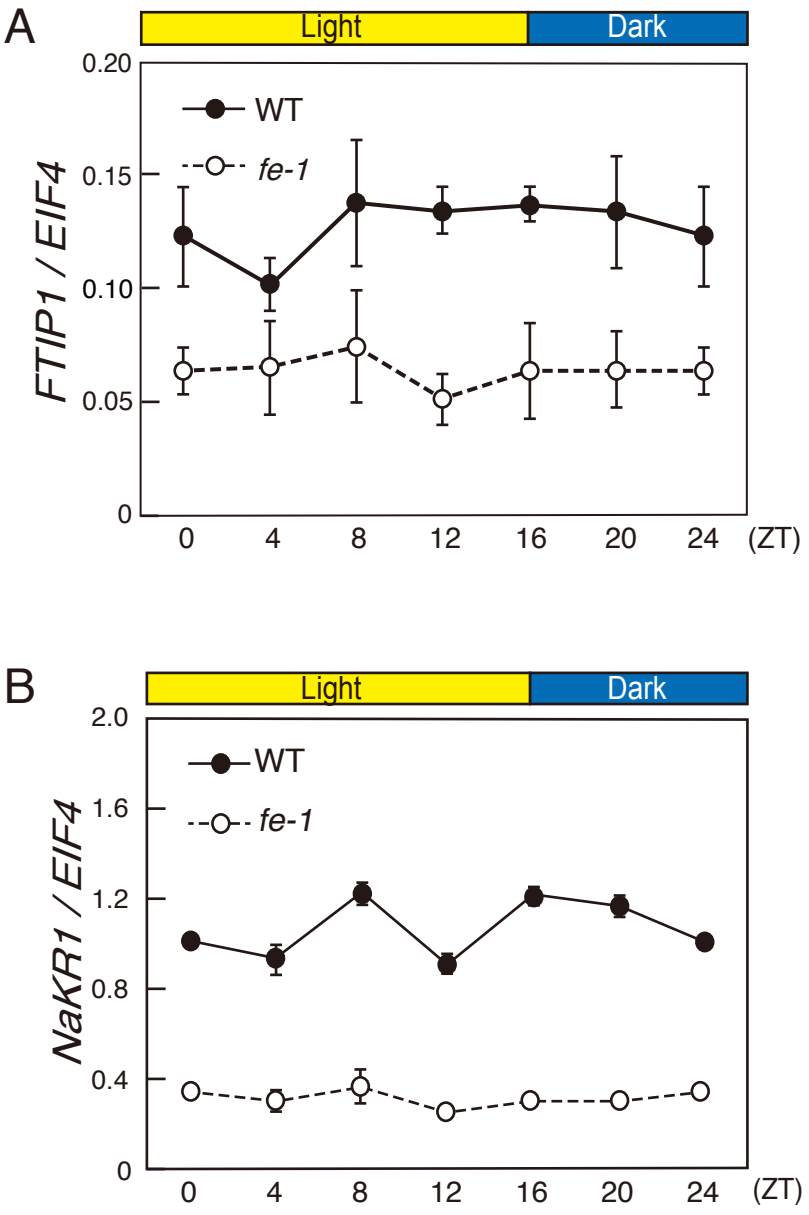


Fig. V-1. Diurnal mRNA levels of *FTIP1* (A) and *NaKR1* (B) in WT or *fe-1* seedlings collected at 10DAG under long-day conditions every 4h over a 24-h period after dawn (ZT=0). Error bars show the SDs (*n*= 3). Yellow boxes indicate light period, and blue boxes indicate dark periods.

(Plant Cell Physiol. Nov 1; 58(11) 2017-2025, 2017 “FE Controls the Transcription of Downstream Flowering Regulators Through Two Distinct Mechanisms in Leaf Phloem Companion Cells” OXFORD UNIVERSITY PRESS)

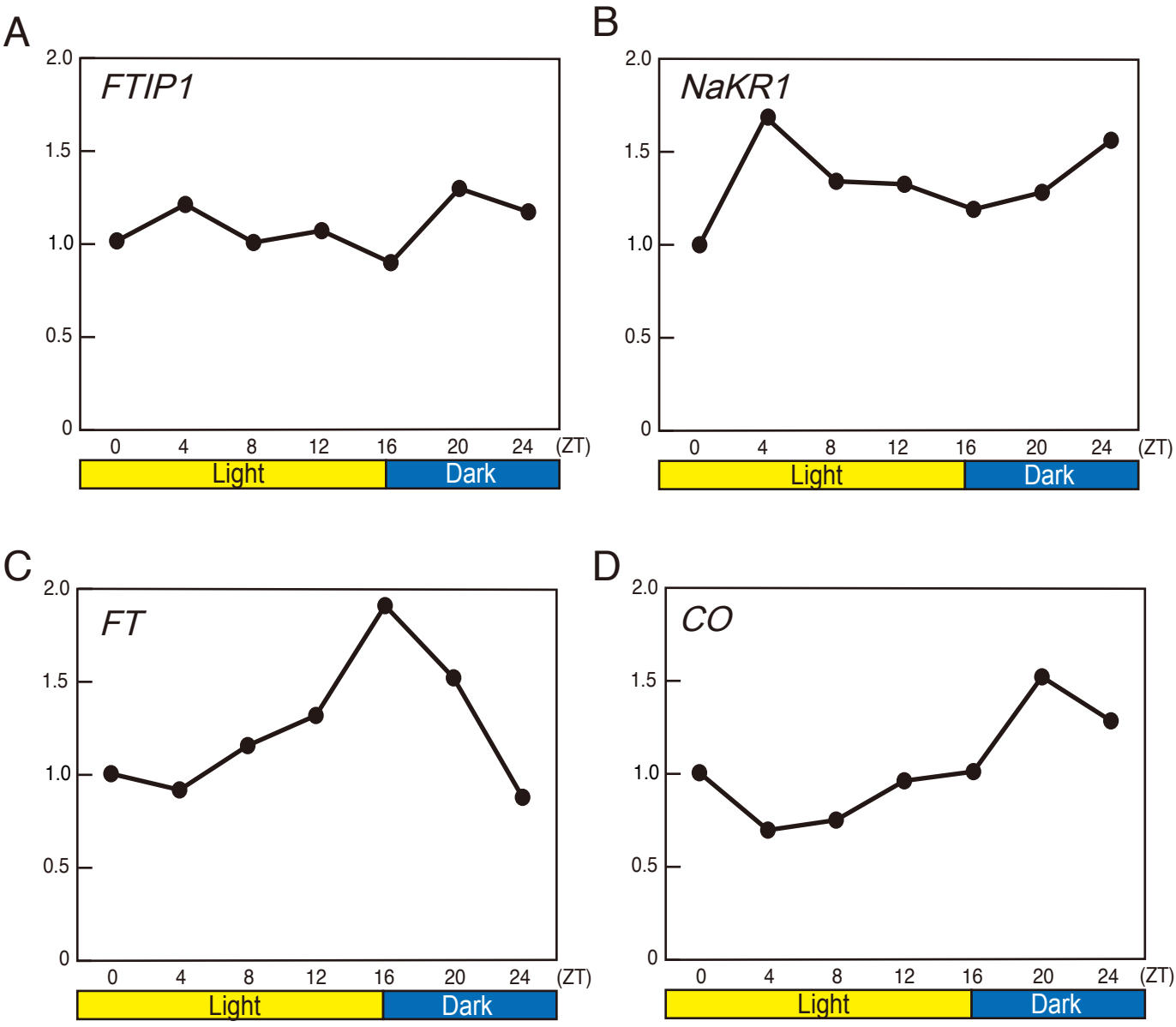


Fig. V-2. Diurnal expression of *FTIP1* (A), *NaKR1* (B), *FT* (C) and *CO* (D) under long-day conditions according to the diurnal database on the website of Mockler lab, Donald Danforth Plant Science Center (http://diurnal.mocklerlab.org). Data were further normalized to the that of ZT0. Yellow boxes indicate light period, and blue boxes indicate dark periods. (Plant Cell Physiol. Nov 1; 58(11) 2017-2025, 2017 “FE Controls the Transcription of Downstream Flowering Regulators Through Two Distinct Mechanisms in Leaf Phloem Companion Cells” OXFORD UNIVERSITY PRESS)

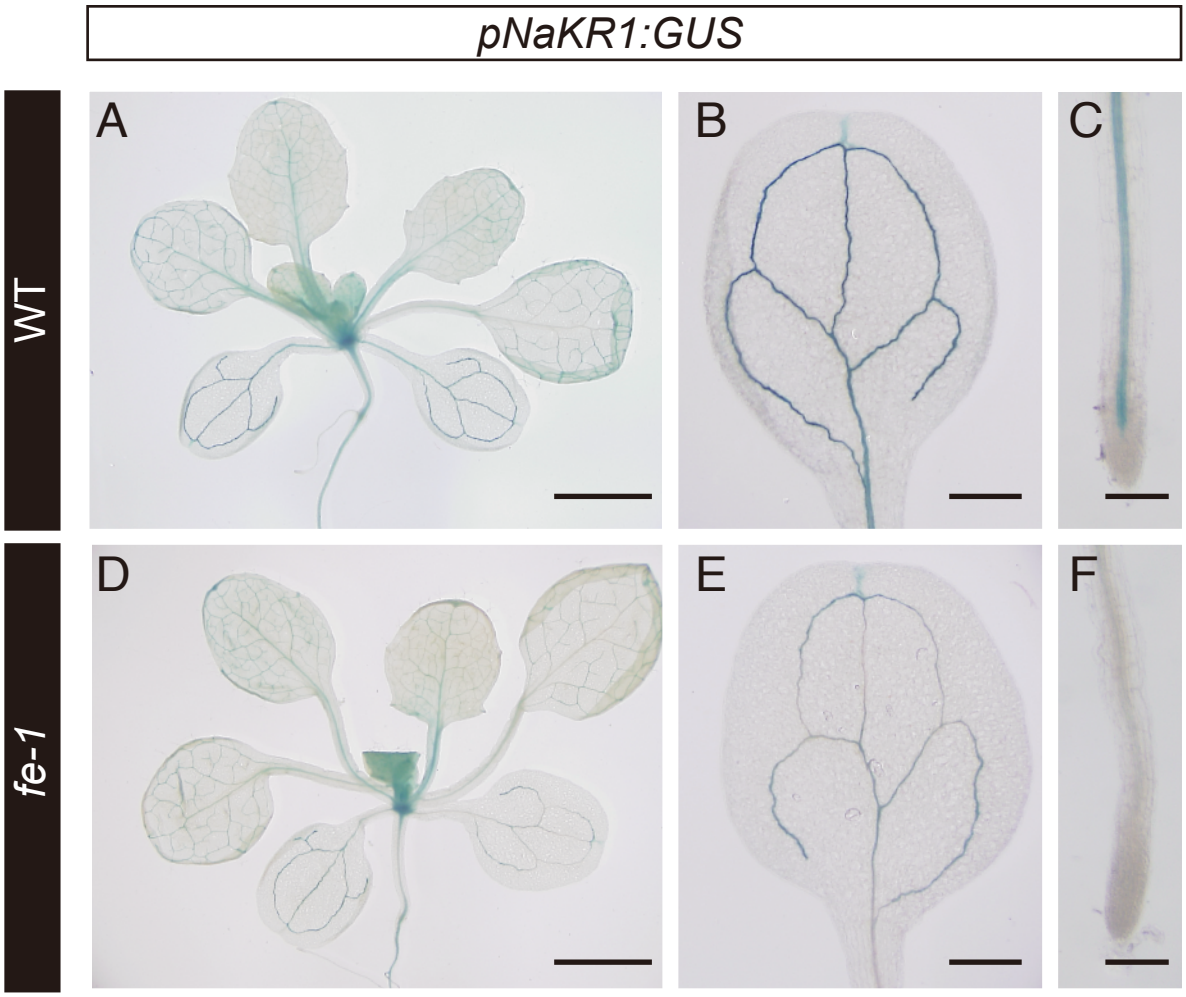


Fig. V-3. GUS activity in *pNaKR1:GUS* (A-C) and *fe-1 pNaKR1:GUS* (D-F) seedlings. Scale bars = 2.0 mm (A, D), 500 μm (B, E), 150 μm (C, F).
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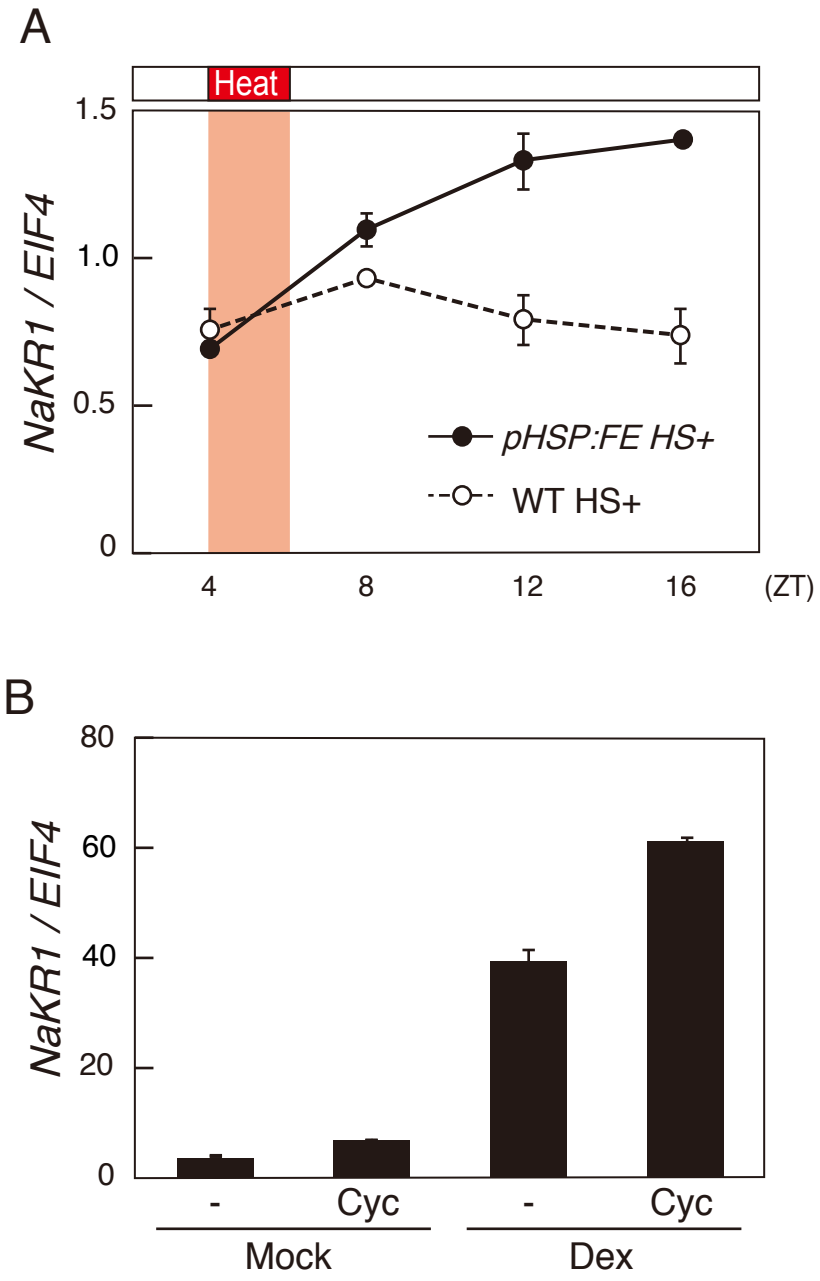


Fig. V-4. (A) *NaKR1* mRNA levels in *pHSP:FE* seedlings measured every 4 h after heat treatment under long-day conditions. Transgenic plants were heat-treated at ZT4–6 at 37°C. Error bars show the SDs (*n* =3). A red box indicates the period of heat treatment. (B) *NaKR1* mRNA levels in *p35S:FE-GR* seedlings at ZT16 under long-day conditions. Transgenic plants were treated with Mock or Dex in the absence (–) or presence (Cyc) of cycloheximide in ZT12-12.5. Error bars show the SDs (*n* =3).

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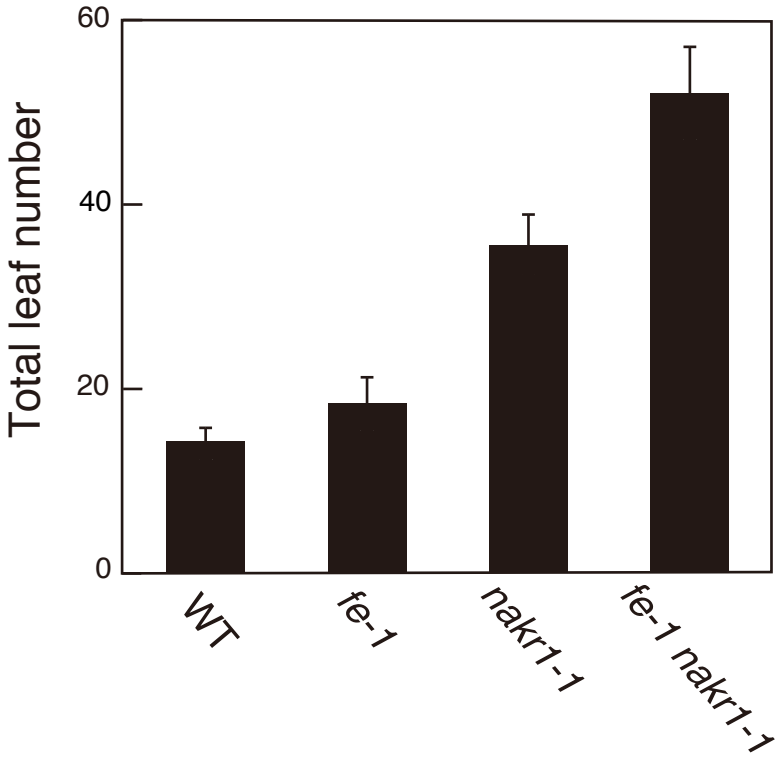


Fig. V-5. Flowering phenotypes of WT, *fe-1*, *nakr1-1* and *fe-1 nakr1-1* seedlings under long-day conditions. The number of total leaves at the time of flowering was measured. Error bars show SDs (*n* =12).

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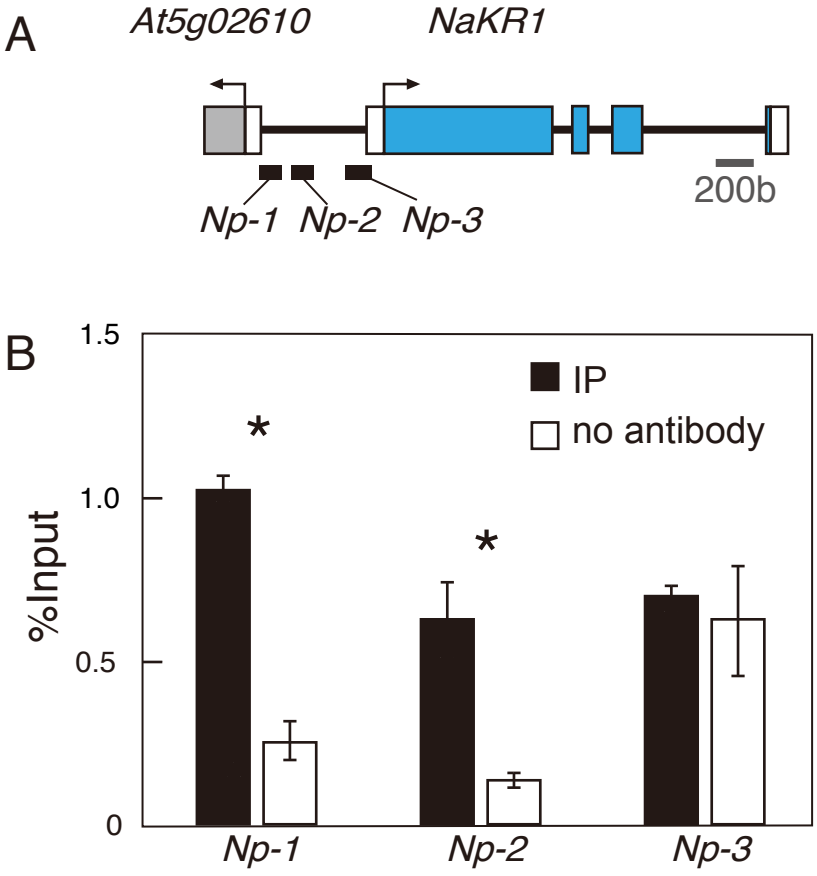


Fig. V-6. ChIP analysis of binding of FE to the *NaKR1* promoter. (A) A schematic diagram showing the *NaKR1* gene structure. (B) FE-3×FLAG accumulation at the *NaKR1* locus in *gFE:3xFLAG* seedlings. Target DNA from gFE:3xFLAG immunoprecipitated against the input DNA was quantified by qPCR. Asterisks indicate statistically significant differences (Student' s *t*-test, *: $p< 0.05$). Error bars show the SDs ($n$ =3).
(Plant Cell Physiol. Nov 1; 58(11) 2017-2025, 2017 "FE Controls the Transcription of Downstream Flowering Regulators Through Two Distinct Mechanisms in Leaf Phloem Companion Cells" OXFORD UNIVERSITY PRESS)

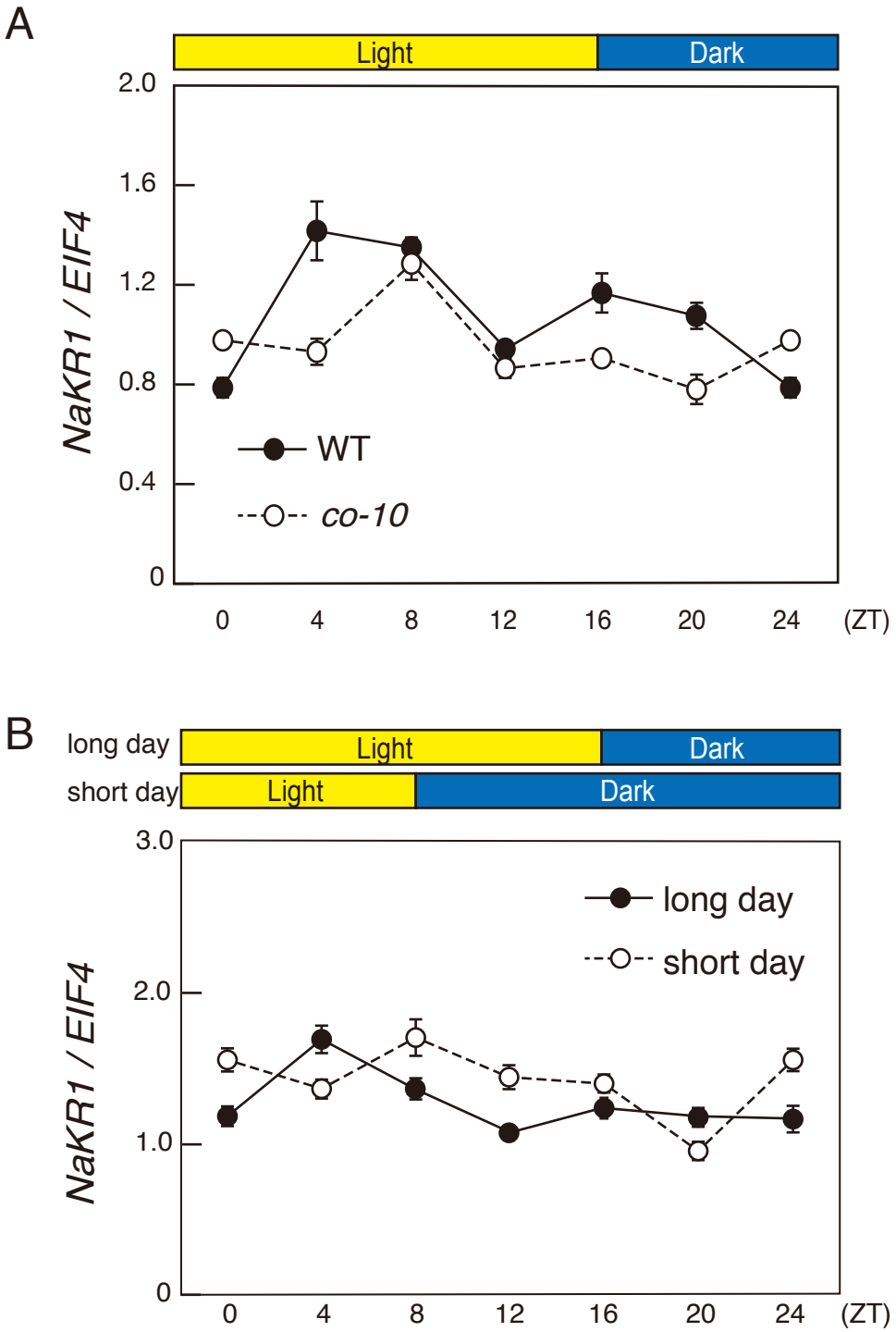


Fig. V-7. Diurnal mRNA levels of *NaKR1* in WT and *fe-1* seedlings under long-day conditions (A) and WT seedlings under long-day or short day conditions (B) collected at 10DAG every 4h over a 24-h period after dawn (ZT=0). Error bars show the SDs ($n$ = 3). Yellow boxes indicate light period, and blue boxes indicate dark periods.
[(B) is cited from Plant Cell Physiol. Nov 1; 58(11) 2017-2025, 2017 “FE Controls the Transcription of Downstream Flowering Regulators Through Two Distinct Mechanisms in

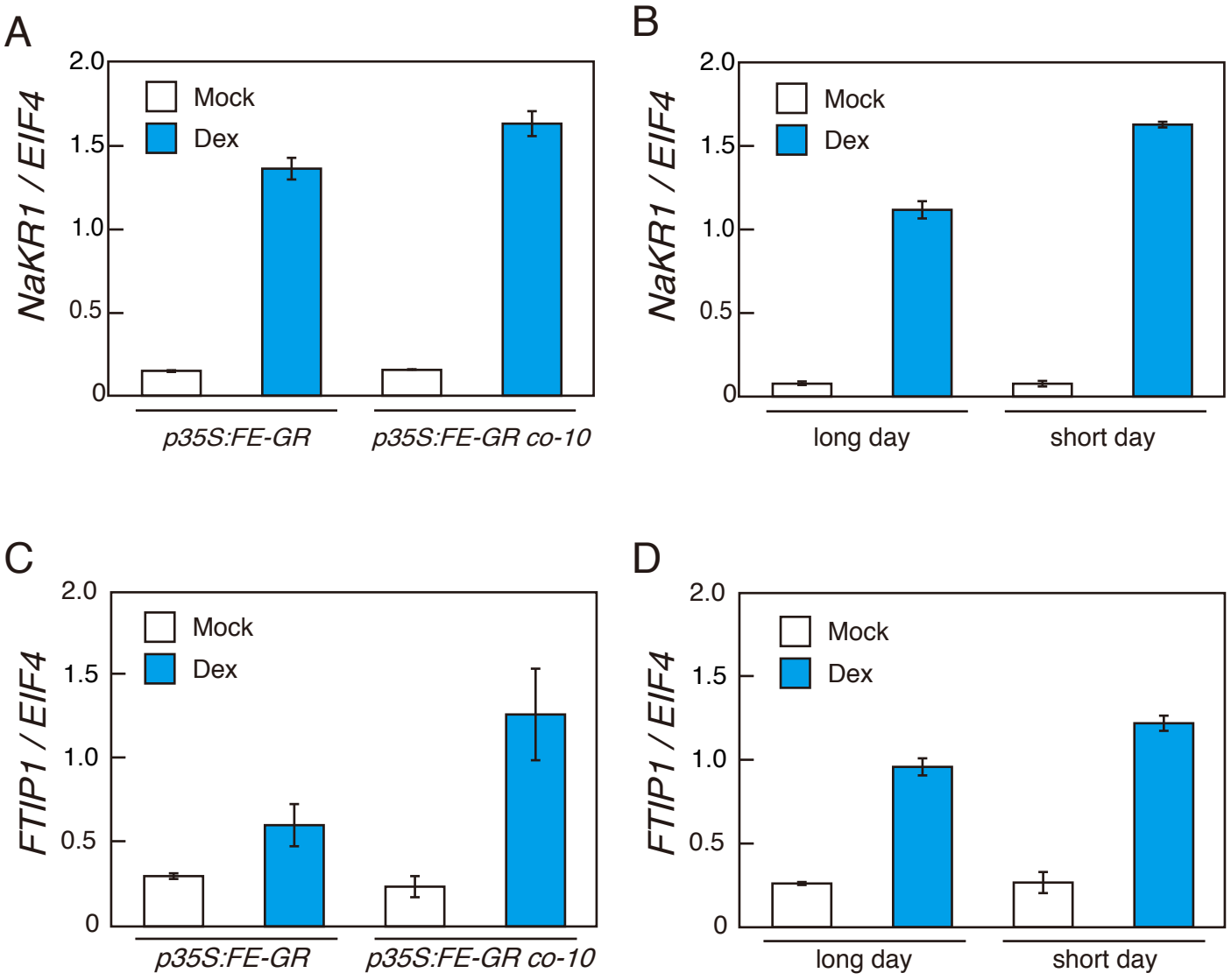


Fig. V-8. *NaKR1* (A and B) and *FTIP1* (C and D) mRNA levels in *p35S:FE-GR* and *p35S:FE-GR co-10* seedlings under long-day conditions (A and C) and in *p35S:FE-GR* seedlings under long-day or short-day conditions (B and D) at ZT16. Transgenic plants were treated with Mock or Dex at ZT12–12.5. Error bars show the SDs (*n* =3).
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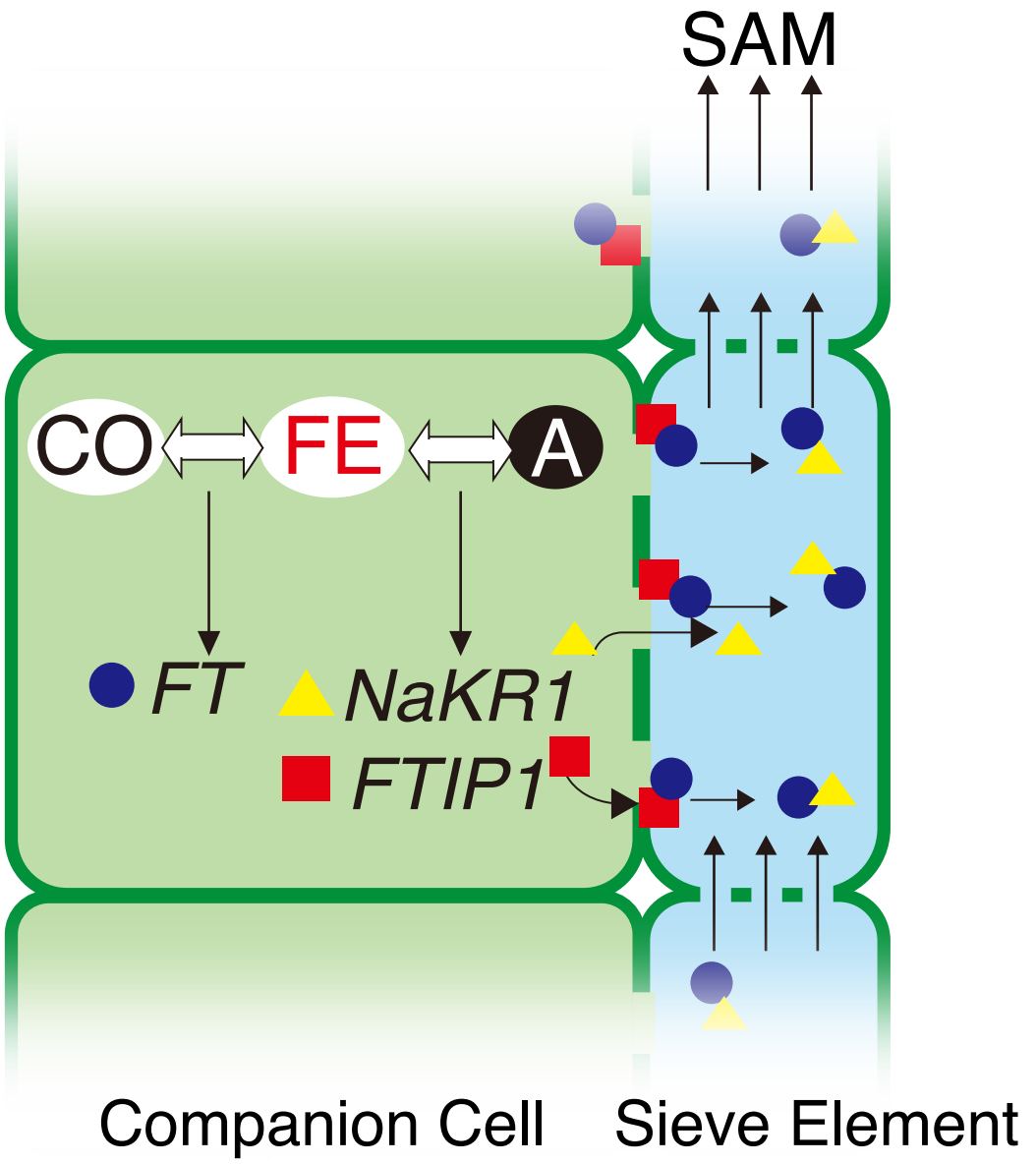


Fig. V-9. FE regulates *FT* and florigen transporter genes in distinct manners. FE activates the florigen transporter genes, *NaKR1* and *FTIP1,* in a CO-independent manner to transport the FT protein smoothly from the leaf to the SAM via the phloem tissues. FT protein is loaded from the companion cells to the sieve elements by FTIP1, then travels through the sieve elements with the help of NaKR1. In *NaKR1* and *FTIP1* regulation, FE needs a functional partner containing a transcription-activation domain (discrived as 'A' ). (Plant Cell Physiol. Nov 1; 58(11) 2017-2025, 2017 "FE Controls the Transcription of Downstream Flowering Regulators Through Two Distinct Mechanisms in Leaf Phloem Companion Cells" OXFORD UNIVERSITY PRESS)

References

Abe, M., Kaya, H., Watanabe-Taneda, A., Shibuta, M., Yamaguchi, A., Sakamoto, T. et al. (2015) FE, a phloem-specific Myb-related protein, promotes flowering through transcriptional activation of *FLOWERING LOCUS T* and *FLOWERING LOCUS T INTERACTING PROTEIN 1*. Plant J. 83: 1059-1068.

Krzymuski, M., Andrés, F., Cagnola, J.I., Jang, S., Yanovsky, M.J., Coupland, G. et al. (2015) The dynamics of *FLOWERING LOCUS T* expression encodes long-day information. Plant J.

Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z. et al. (2012) FTIP1 is an essential regulator required for florigen transport. PLoS Biol. 10: e1001313.

Mockler, T.C., Michael, T.P., Priest, H.D., Shen, R., Sullivan, C.M., Givan, S.A. et al. (2007) The DIURNAL project: DIURNAL and circadian expression profiling, model-based pattern matching, and promoter analysis. Cold Spring Harb Symp Quant Biol. 72: 353-363.

Song, S., Chen, Y., Liu, L., Wang, Y., Bao, S., Zhou, X. et al. (2017) OsFTIP1-mediated regulation of florigen transport in rice is negatively regulated by the ubiquitin-like domain kinase OsUbDKγ4. Plant Cell. 29: 491-507.

Tian, H., Baxter, I.R., Lahner, B., Reinders, A., Salt, D.E. & Ward, J.M. (2010) *Arabidopsis* NPCC6/NaKR1 is a phloem mobile metal binding protein necessary for phloem function and root meristem maintenance. Plant Cell. 22: 3963-3979.

Zhu, Y., Liu, L., Shen, L. & Yu, H. (2016) NaKR1 regulates long-distance movement of FLOWERING LOCUS T in *Arabidopsis*. Nat Plants. 2: 16075.

# Chapter VI
# Conclusion

本章については、5 年以内に雑誌等で刊行予定のため、非公開。

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