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Short Communication

Possible involvement of *FLC* in natural variation of activity to enhance the late flowering phenotype of the clock mutant *lhy cca1* under continuous light

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Abstract LHY and CCA1 play key roles in circadian clock functions and photoperiodic flowering in *Arabidopsis*. Double loss of function of *LHY* and *CCA1* genes (*lhy cca1*) accelerated flowering under long days or short days, but the *lhy cca1* delayed flowering time under constant-light (LL) conditions. *FCA* encodes an RNA binding protein that plays key roles in the autonomous pathway. Loss of function of *FCA* increases mRNA level of a major floral repressor gene, *FLC*. A mutation in *FLC* gene partially suppressed the late flowering phenotype of the *lhy cca1* in LL. Based on this result, we have proposed that FLC may be involved in this process. Increased level of *FLC* mRNA in *fca* is responsible for the delay of flowering and the late flowering phenotype of the *fca* is suppressed by vernalization. In this paper, we isolated an enhancer of the late-flowering phenotype of the *lhy cca1* in LL based on natural variation of two *Arabidopsis* accessions, Columbia and Landsberg *erecata*. The enhancer was named *ELLCL* and mapped near the *FLC*. This result suggested that the gene responsive to *ELLCL* might be *FLC* and was consistent with our previous results. The late-flowering phenotype of *lhy cca1* was insensitive to vernalization. By contrast, flowering time of the *fca* was accelerated by vernalization as reported. These results suggested that posttranslational, but not the transcriptional, regulation of FLC might be involved in this process.

Key words: CCA1, circadian rhythms, FLC, LHY, natural variation.

Fluctuations in the length of the day affect developmental processes and behaviors of many organisms. This phenomenon, called photoperiodism, allows the detection of seasonal changes and anticipation of environmental conditions (Boss et al. 2004; Mizoguchi et al. 2006; Mizoguchi et al. 2007). Photoperiodism was first described in detail by Garner and Allard (1920), who found that many plants flowered in response to changes in day length. The photoperiodic control of flowering time is closely linked to the circadian clock (Mizoguchi et al. 2002; Mizoguchi et al. 2005; Mizoguchi et al. 2006; Niinuma et al. 2007; Súarez-López et al. 2001; Yanovsky and Kay 2002). In *Arabidopsis*, *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) encode myb proteins essential for clock function (Schaffer et al. 1998; Wang and Tobin 1998). *FLOWERING LOCUS T* (*FT*) plays a pivotal role in the photoperiodic flowering affected by the circadian clock (Fujiwara et al. 2005a, 2005b, 2005c; Mizoguchi et al. 2005; Oda et al. 2007) and is an important component of the elusive "florigen" signal (Abe et al. 2005; Huang et al. 2005; Wigge et al. 2005). GIGANTEA (GI) and CONSTANS (CO) are mediators between the circadian clock and the FT in photoperiodic control of flowering in *Arabidopsis* under conditions of light/dark cycling (Calvino et al. 2005; Mizoguchi et al. 2005; Súarez-López et al. 2001; Yanovsky and Kay 2002).

Under conditions of short day length (SD), the *lhy cca1* plants flowered much earlier than did wild-type plants (Fujiwara et al. 2005a, 2005b, 2005c; Mizoguchi et al. 2002). The early flowering of *lhy cca1* was associated with and explained in part by phase shifting of *GI* and *CO* expression and upregulation of *FT* expression (Fujiwara et al. 2005a, 2005b, 2005c; Mizoguchi et al. 2005). Furthermore, we demonstrated that impairing the function of circadian clock components *LHY* and *CCA1* delayed flowering under conditions of continuous light (LL) and inverted the photoperiodic response from the LD type to a unique SD type in *Arabidopsis* (Fekih et al. 2009b; Fujiwara et al. 2008; Mizoguchi and Yoshida 2009; Yoshida et al. 2009). We identified three loss-of-function mutations, *short vegetative phase* (*svp*; Fekih et al. 2009a; Fujiwara et al. 2008; Yoshida et al. 2009), *flowering locus c* (*flc*; Fujiwara et al. 2008), and *early flowering 3* (*elf3*; Yoshida et al. 2009), which suppressed

Abbreviations: CCA1, CIRCADIAN CLOCK ASSOCIATED 1; FLC, FLOWERING LOCUS C; LHY, LATE ELONGATED HYPOCOTYL; LD, long-days, LL, continuous light, SD, short-days; SVP, SHORT VEGETATIVE PHASE.
This article can be found at http://www.jspcmb.jp/

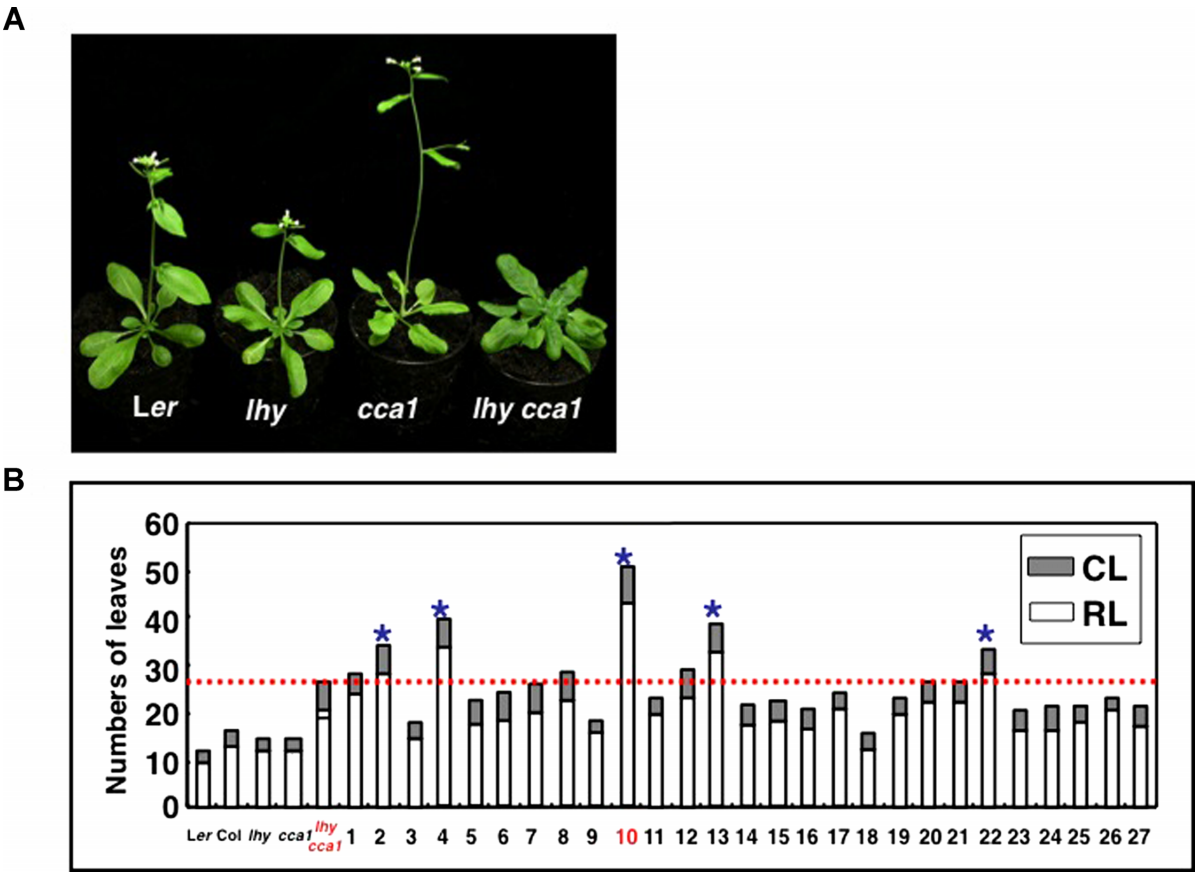

Figure 1. Screening for enhancers and suppressors of the late-flowering phenotype of *lhy cca1* under LL based on natural variation between L*er* and Col. (A) L*er* wild-type, *lhy-11*, *cca1-1*, and *lhy-11 cca1-1* plants in LL. (B) Isolation of candidates for enhancers and suppressors of the late-flowering phenotype of *lhy cca1* in LL. The *lhy-11 cca1-1* (L*er*) was crossed with Col *gl1*, and F_1 plants were self-fertilized. F_2 progeny were screened for plants that produced more or fewer leaves than *lhy-11 cca1-1* plants when they flowered in LL at 24°C. The following three primers were used to detect the presence of the *lhy-11* mutation by PCR: 1) LHY P1, 5′-gtttaaggagaggtaatgagagata-3′; 2) LHY P2, 5′-ttcccatcctactttcatccctg-3′; 3) LHY P3, 5′-cttagctaataattcttctccagat-3′. For *cca1-1*, 1) CCA1 P1, 5′-tgagatttctccatttccgtagcttctgg-3′; 2) CCA1 P2, 5′-gatgcactcgaaatcagccaattttagac-3′; 3) CCA1 P3, 5′-atccgtttgggatctttctgttccacatg-3′ were used. Wild-type *LHY* gene and *lhy-11* were detected with the *LHY* primer sets 1)+3) and 2)+3), respectively. Wild-type *CCA1* gene and *cca1-1* were detected with the *CCA1* primer sets 1)+3) and 1)+2), respectively. Flowering time was measured as described previously (Mizoguchi et al., 2002). Data are presented as means±SE. Differences in flowering times were analyzed for statistical significance using Student's *t*-test ($p<0.05$). CL and RL represent cauline and rosette leaves, respectively. The dotted line indicates the total leaf numbers of *lhy-11 cca1-1* as a control. Asterisks indicate the five candidate lines for enhancers (lines #2, 4, 10, 13, and 22) that produced significantly more leaves than the control. Among these, the phenotype of line #10 was most significant, and this line was used for further analysis.

the late-flowering phenotype of *lhy cca1* under LL by both ethyl methanesulfonate (EMS) mutagenesis and genetic analysis. Based on our results, we proposed novel roles of the oscillator components LHY and CCA1 in the control of flowering time through the ELF3-SVP/FLC pathway in *Arabidopsis* (Mizoguchi and Yoshida 2009).

Some mutations in key clock genes resulted in severe defects in clock function and differences between phenotypes of plants with these mutations under conditions of light/dark cycling compared with under LL. These circadian clock mutants include *lhy cca1* (Fujiwara et al. 2008; Yoshida et al. 2009), *elf3 cry2* (Natsui et al. 2010), and *prr9 prr7 prr5* (Niinuma et al. 2008). Petiole length of *prr9 prr7 prr5* was longer than that of wild-type plants under LD, but shorter under LL (Niinuma et al. 2008). Both *lhy cca1* and *elf3 cry2* produced fewer and more leaves than wild-type plants under SD and LL, respectively.

Nevertheless, isolation of enhancers and suppressors of these mutants would be important to understand the precise molecular mechanism of inversion in the photoperiodic response type. Therefore, we performed mutagenesis of *lhy cca1* seeds with EMS and screening of putative enhancers and suppressors of the clock mutant. Use of natural populations has several advantages for the discovery of genes affecting circadian rhythms of leaf movements, flowering time, and light responses (Koornneef et al. 2004; Maloof et al. 2001; Tajima et al. 2007).

Under LL, *lhy cca1* plants produced more leaves before the transition from vegetative to reproductive phase than did wild-type, *lhy*, and *cca1* plants (Figure 1A; Fujiwara et al. 2008). To identify enhancers of the late-flowering phenotype of *lhy cca1* by using natural

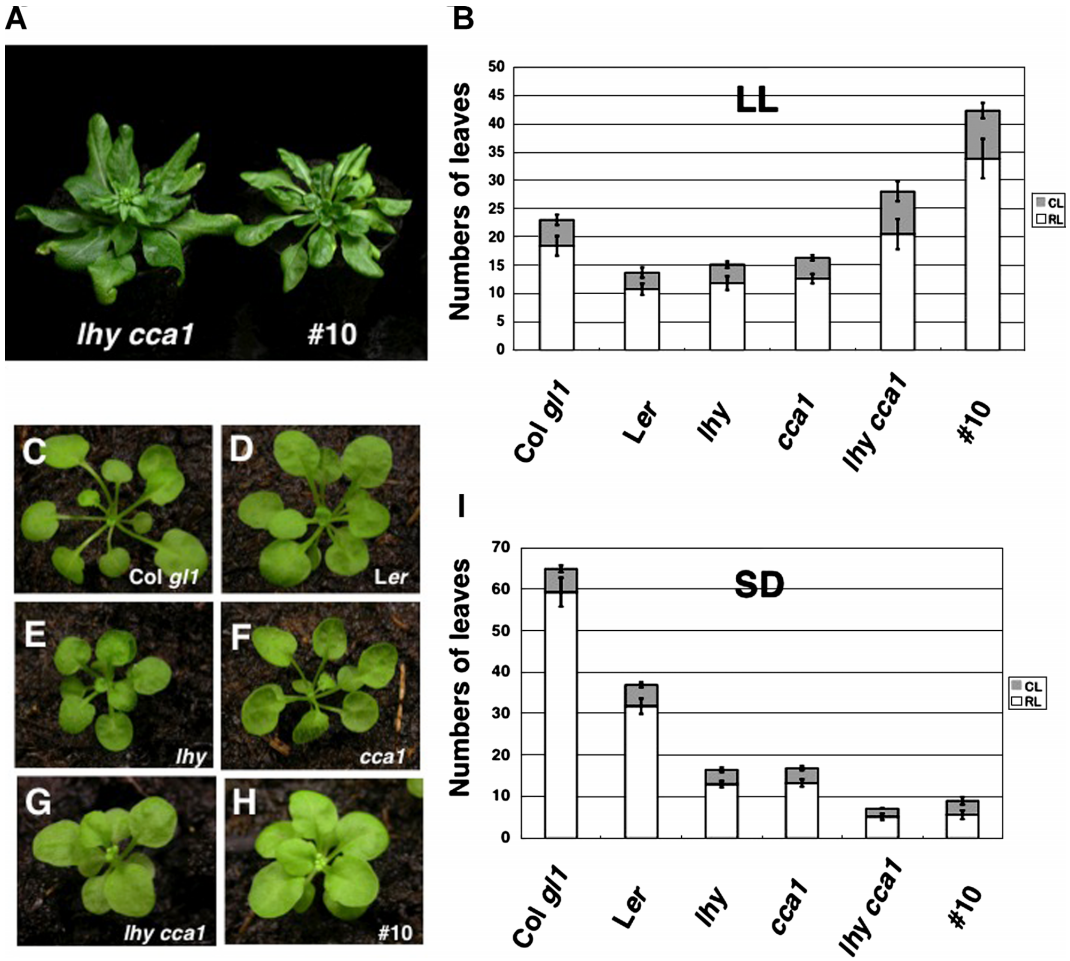

Figure 2. *ELLCL* delayed flowering time of *lhy cca1* in LL but had no significant effect in SD (10 h light/14 h dark). (A) The *lhy-11 cca1-1* (L*er*) and line #10 (F_3) grown in LL for 6 weeks. (B) Flowering times of Col *gl1*, L*er* wild-type, *lhy-11, cca1-1*, *lhy-11 cca1-1*, and line #10 (F_3) plants grown in LL. Numbers of rosettes (open boxes) and cauline leaves (gray boxes) when they flowered were scored; the data are presented as means±SE. (C–H) Col *gl1* (C), L*er* wild type (D), *lhy-11* (E), *cca1-1* (F), *lhy-11 cca1-1* (G) and line #10 (H) grown in SD for 4 weeks. (I) Flowering times of plants shown in (C–H).

populations but not by EMS-mutagenesis, *lhy-11 cca1-1* (Mizoguchi et al. 2002) was crossed with Col *gl1* and F_2 plants with more or fewer leaves than the progenitor line *lhy-11 cca1-1* were screened under LL (Figure 1B). The *gl1* is a mutation in *GLABROUS1* (*GL1*) gene that plays a key role in the trichome development in *Arabidopsis* (Marks and Feldmann 1989). The *gl1* does not affect flowering time. The Col *gl1* is often used for genetic analysis of *Arabidopsis*. In this screen, we isolated five F_2 plants (#2, 4, 10, 13, and 22) with significantly more leaves than the *lhy-11 cca1-1* plants. The enhancement of the late-flowering phenotype of *lhy cca1* was most significant in line #10, and this line was used for further analysis. To test whether how many genes were involved in the late flowering phenotype of the line #10, the line #10 (F_3) plants were crossed with *lhy-11 cca1-1* plants. In our preliminary experiment, approximately 25% of F_2 progeny of crosses between line #10 (F_3) and *lhy-11 cca1-1* exhibited late flowering phenotype similar to that of the line #10 under LL (data not shown). This preliminary experiment suggested that a natural variation between L*er* and Col accessions in the line #10 appeared to be responsible for the enhancement of the late flowering phenotype of *lhy-11 cca1-1* under LL. The putative natural variation in the line #10 was named *enhancer of the late flowering of lhy cca1 under LL* (*ELLCL*). Other potential enhancer and suppressor lines will be characterized and reported elsewhere.

Line #10 was self-fertilized, and F_3 progeny were confirmed to carry both the *lhy-11* and *cca1-1* mutations by PCR. Therefore, line #10 obtained from the F_2 plants that produced significantly more leaves than the *lhy cca1* was derived from the *lhy cca1*. The F_3 progeny exhibited a similar late-flowering phenotype to that of line #10 under LL (Figures 1B, 2A, B), indicating that the enhancer phenotype was heritable. Under SD, the *lhy-11 cca1-1* flowered much earlier, had shorter hypocotyls and petioles, and produced more round leaves than did L*er* and Col wild-type, *lhy*, and *cca1* plants (Figure 2C–G, I). Although *ELLCL* appeared to delay the flowering time of

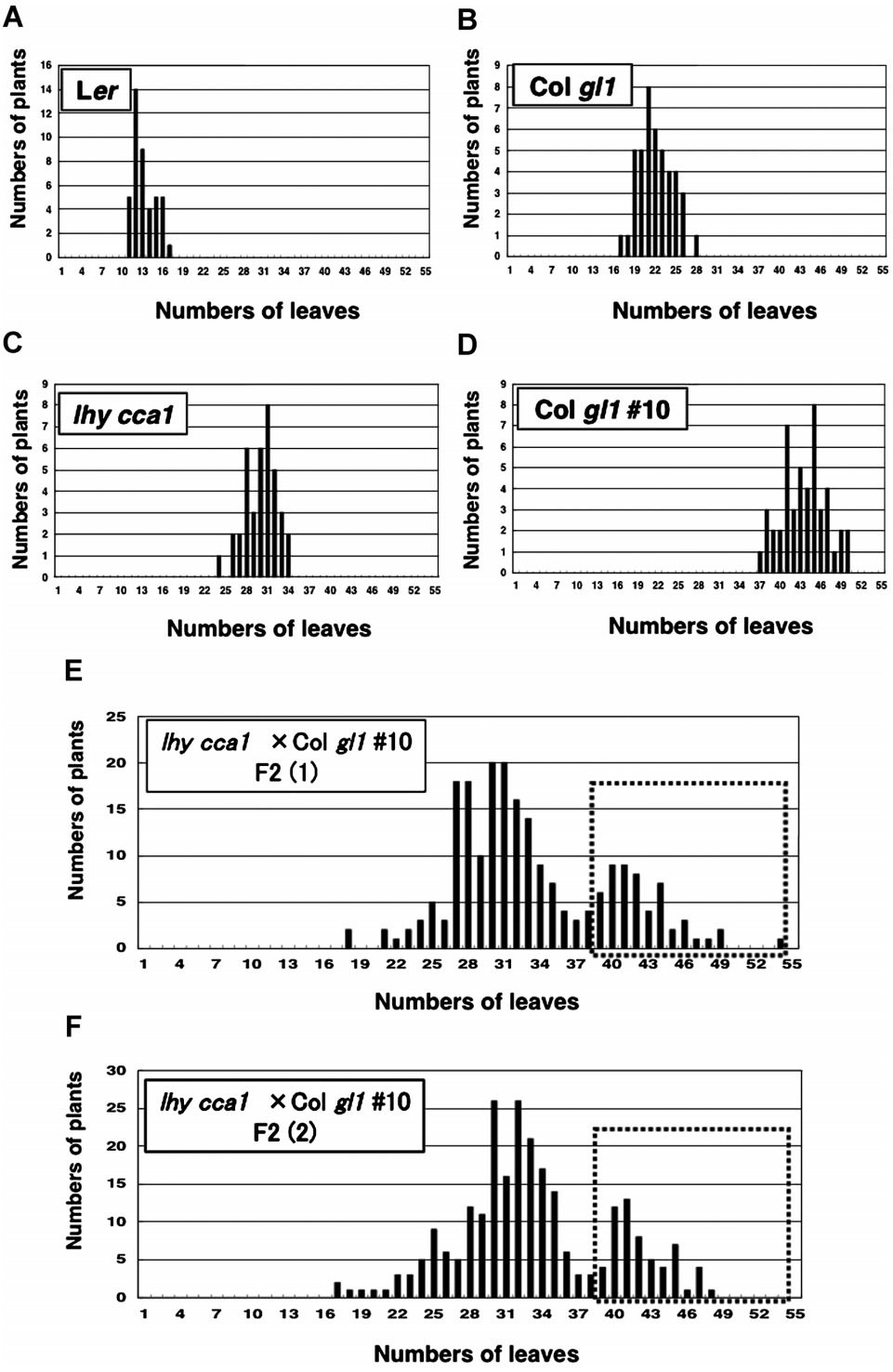

Figure 3. Frequency of distribution of rosette and cauline leaves number at flowering in L*er* wild type (A), Col *gl1* (B), *lhy-11 cca1-1* (C), line #10 (D), and plants from segregating populations derived from two independent crosses between line #10 (F_3) and *lhy-11 cca1-1* (L*er*). (E, F) F_2 populations showed a 3:1 ratio of plants that flowered similarly to *lhy-11 cca1-1*, and these flowered significantly later than *lhy-11 cca1-1* ($p<0.05$). F_2 progeny surrounded by dotted lines in (E, F) were used for mapping of *ELLCL*.

lhy cca1 under LL (Figures 1B, 2A, B), it did not significantly affect early flowering of *lhy cca1* under SD (Figure 2I). These observations suggested that the effect of *ELLCL* on flowering of *lhy cca1* may be specific to LL condition or masked under SD.

To map *ELLCL*, line #10 (F_3) plants were crossed with *lhy-11 cca1-1* plants. All the F_1 plants flowered at almost the same time as *lhy-11 cca1-1* under LL (data not shown). F_2 progeny of crosses between line #10 (F_3) and *lhy-11 cca1-1* were grown under LL, and their flowering times were scored (Figure 3E, F) and compared with those of L*er* (Figure 3A), Col *gl1* (Figure 3B), *lhy-1*

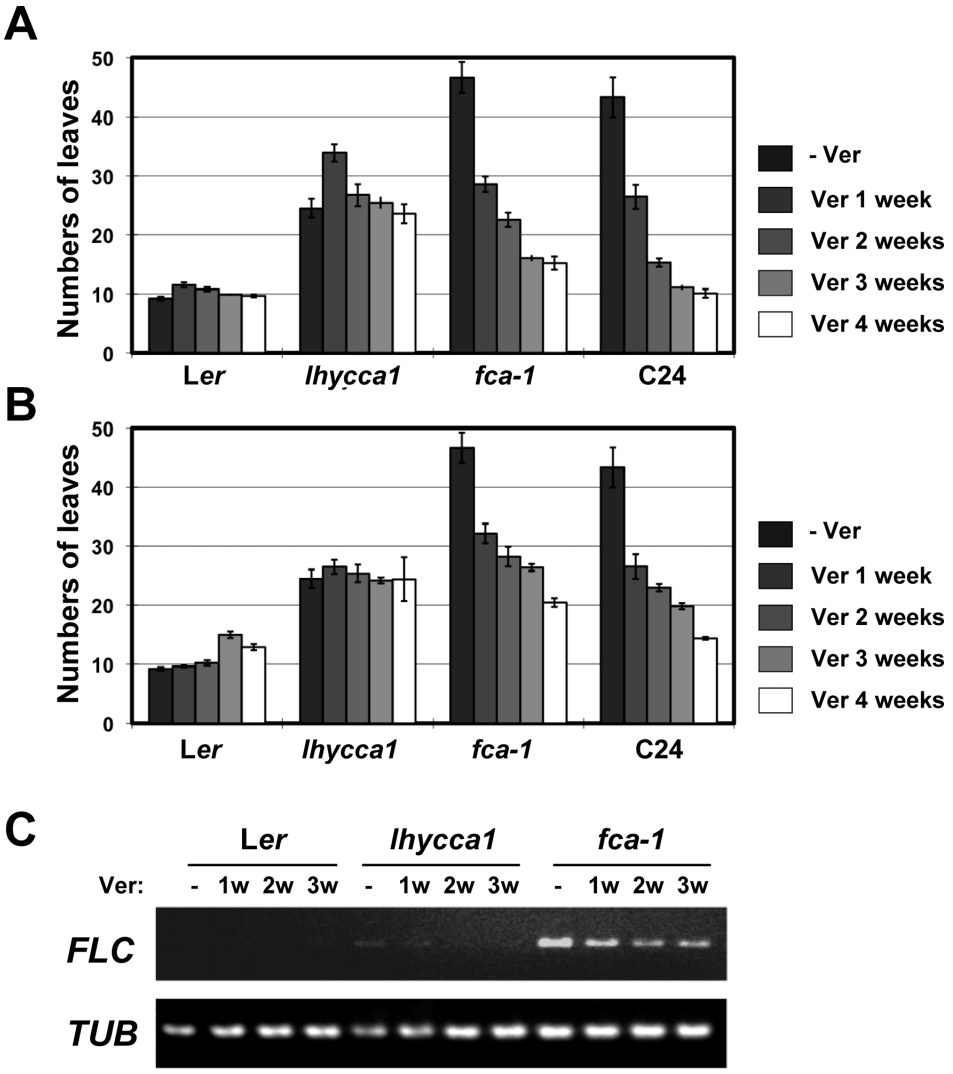

Figure 4. Vernalization did not suppress the late-flowering phenotype of *lhy cca1*. Flowering time of seed (A) and seedling (B) of vernalized L*er* wild-type, *lhy cca1*, *fca-1*, and C24 wild-type plants. For seed vernalization, plants were vernalized by germinating seed on soil in the dark for 1, 2, 3, or 4 weeks at 4ÅãC, and the plants were then grown at 24°C under LL. For seedling vernalization, plants were grown on soil under LL for 10 days and were then vernalized for 1, 2, 3, and 4 weeks at 4°C under LL. After each vernalization period, plants were grown at 24°C under LL. Numbers of rosettes and cauline leaves at flowering were scored; the data are presented as means±SE. (C) RT-PCR analysis of *FLC* gene in seed of vernalized L*er* wild-type, *lhy cca1*, and *fca-1* plants. For RT-PCR analysis, seeds vernalized in the dark for 1, 2, and 3 weeks at 4°C were grown at 24°C under LL for 10 days, and aerial parts were used for RNA preparation. RT-PCR was performed with primers for *TUB* (Fujiwara et al. 2005) and *FLC* (Ratcliffe et al. 2003). RT-PCR procedures were as described previously with some modifications (Fujiwara et al. 2005).

cca1-1 (L*er*; Figure 3C), and line #10 (F_3; Figure 3D). The ratio between the extremely late-flowering plants and others was close to 3:1 ($p<0.05$) in two independent crosses (experiment 1, 161:53 and experiment 2, 192:59) (Figure 3E, F). These results indicated that *ELLCL* behaved as a recessive mutation in *lhy-11 cca1-1* (L*er*) to enhance the late-flowering phenotype under LL. Alternatively, *ELLCL* in Col may have slightly higher activity to enhance the late flowering of *lhy cca1* in LL compared to that in L*er*, but the heterozygotes may not show sufficiently clear enhancement.

The F_2 plants with a late-flowering phenotype were used to map the *ELLCL* in the line #10. SSLP markers were used to map the *ELLCL*. Enhancement of late flowering of *lhy-11 cca1-1* was closely linked to the genetic markers ciw15 and nga151 on the top strand of chromosome 5, including the *FLC* locus. There were no significant linkage between the late flowering phenotype and the SSLP markers on chromosome 1, 2, 3 and 4.

We proposed that FLC was likely to contribute to the late flowering of *lhy cca1* under LL based on the following four findings. First, we found that *FLC*-L*er* appeared to enhance the early flowering of *svp-31* in Col (Fujiwara et al. 2008). Indeed, *flc-102* mutation (Col) enhanced the early flowering of *svp-31* (Col) (Fujiwara et al. 2008). Second, plants showing overexpression of

FLC (*FLC-ox*) had similar phenotypes to *lhy cca1* in LL, such as late flowering, negative regulation of both *FT* and *SUPPRESSOR OF OVEREXPRESION OF CO 1* (*SOC1*) gene expression, dark green/curled leaves, and short hypocotyls (Fujiwara et al. 2008). Third, suppressor mutants of *lhy cca1* that flowered earlier than *lhy cca1* were screened in LL (Fujiwara et al. 2008; Yoshida et al. 2009). By RT-PCR, we identified at least two classes of suppressor mutant. These exhibited markedly reduced levels of either *FLC* or *ELF3* expression. Finally, *lhy-11 cca1-1 flc-102* (Col) plants flowered earlier than *lhy-11 cca1-1* (Col) in LL (Fujiwara et al. 2008).

The *flc* did not affect the flowering time of *co* (Michaelis and Amasino 2001). Therefore, late flowering of *lhy cca1* in LL was unlikely to be explained solely by downregulation of *CO* mRNA levels or destabilization of CO proteins (Valverde et al. 2004). The *FLC-ox* and *lhy cca1* plants had shorter hypocotyls than did wild-type plants under LL (Fujiwara et al. 2008). These findings were consistent with the results of the present study. Our results obtained based on natural variation support the suggestion that FLC may play important roles in the inversion of day-length response by *lhy cca1*.

These findings raise questions regarding the mechanism by which *lhy cca1* delayed flowering in LL. One possibility is that *lhy cca1* may increase the levels of *FLC* and/or *SVP* mRNA. However, this is unlikely because the level of *SVP* mRNA was almost the same in L*er* wild-type and *lhy cca1* plants in LL (Fujiwara et al. 2008). Although *FLC* mRNA level tended to be higher in *lhy cca1*, *lhy*, and *cca1* than in L*er* wild type, the increase did not explain the delay of flowering time in *lhy cca1* (Fujiwara et al. 2008). Neither *lhy* nor *cca1* significantly delayed flowering in LL (Figure 1A). Mutations in the autonomous pathway, such as *fca*, *ld*, and *fpa*, increased *FLC* mRNA level and delayed flowering (Boss et al. 2004). These mutants were sensitive to vernalization, and this long-term cold treatment suppressed the upregulation of *FLC* and late flowering in the mutants. To test the sensitivity of *lhy cca1* to vernalization, seeds and seedlings of *lhy-11 cca1-1* and control plants were exposed to low temperature treatment (4°C) for 1, 2, 3, and 4 weeks, and both flowering times and *FLC* mRNA levels of these plants were investigated. The *fca-1* plants were sensitive to vernalization as reported previously (Figure 4B; Boss et al. 2004). The *FLC* mRNA level was decreased and the late flowering phenotype of the *fca-1* was suppressed by vernalization. In contrast, the late flowering phenotype of *lhy cca1* was unaffected by vernalization in LL (Figure 4). The mRNA level of the *FLC* in *lhy cca1* decreased in response to vernalization. These observations indicated that *lhy cca1* affected a different pathway from *fca-1*. One explanation for the late flowering of *lhy cca1* in LL is that protein stability or complex formation of floral repressors, such as FLC and SVP, may be affected in *lhy cca1* in LL.

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References

Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T (2005) FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex. *Science* 309: 1052–1056

Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: enabling, promoting, and resetting. *Plant Cell* 16 Suppl: S18–31

Calvino M, Kamada H, Mizoguchi T (2005) Is the role of the short-day solely to switch off the CONSTANS in *Arabidopsis*? *Plant Biotechnol* 22: 179–183

Fekih R, Miyata K, Yoshida R, Ezura H, Mizoguchi T (2009a) Isolation of suppressors of late flowering and abnormal flower shape phenotypes caused by overexpression of the *SHORT VEGETATIVE PHASE* gene in *Arabidopsis thaliana*. *Plant Biotechnol* 26: 217–224

Fekih R, Nefissi R, Miyata K, Ezura H, Mizoguchi T (2009b) Roles of circadian clock and histone methylation in the control of floral repressors. *Adv Bot Res* 50: 199–225

Fujiwara S, Nakagawa M, Kamada H, Coupland G, Mizoguchi T (2005a) Circadian clock components in *Arabidopsis* I. The *terminal flower 1* enhances the early flowering phenotype of a circadian clock mutant, *lhy cca1*. *Plant Biotechnol* 22: 311–317

Fujiwara S, Oda A, Kamada H, Coupland G, Mizoguchi T (2005b) Circadian clock components in Arabidopsis II. The circadian clock components LHY/CCA1 regulate the floral integrator gene *SOC1* in both GI-dependent and –independent pathways. *Plant Biotechnol* 22: 319–325

Fujiwara S, Nakagawa M, Kamada H, Mizoguchi T (2005c) Circadian clock components in Arabidopsis III. Roles of the circadian clock components LHY/CCA1/GI in regulating the floral integrator genes *LFY*/*SOC1*/*FT* to control flowering time and shoot architecture. *Plant Biotechnol* 22: 327–331

Fujiwara S, Oda A, Yoshida R, Niinuma K, Miyata K, Tomozoe Y, Tajima T, Nakagawa M, Hayashi K, Coupland G, et al. (2008) Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in Arabidopsis. *Plant Cell* 20: 2960–2971

Garner WW, Allard HA (1920) Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. *J Agric Res* 18: 553–606

Huang T, Bohlenius H, Eriksson S, Parcy F, Nilsson O (2005) The mRNA of the *Arabidopsis* gene *FT* moves from leaf to shoot apex and induces flowering. *Science* 309: 1694–1696

Koornneef M, Alonso-Blanco C, Vreugdenhil D (2004) Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annu Rev*

Plant Biol 55: 141–172

Maloof JN, Borevitz JO, Dabi T, Lutes J, Nehring RB, Redfern JL, Trainer GT, Wilson JM, Asami T, Berry CC, et al. (2001) Natural variation in light sensitivity of *Arabidopsis*. *Nat Genet* 29: 441–446

Marks MD, Feldmann KA (1989) Trichome development in *Arabidopsis thaliana*. I. T-DNA tagging of the *GLABROUS* gene. *Plant Cell* 11: 1043–1050

Michaels SD, Amasino RM (2001) Loss of FLOWERING LOCUS C activity eliminates the late-flowering phenotype of FRIGIDA and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell* 13: 935–941

Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song H-R, Carré IA, Coupland G (2002) *LHY* and *CCA1* are partially redundant genes required to maintain circadian rhythms in *Arabidopsis*. *Dev Cell* 2: 629–641

Mizoguchi T, Wright L, Fujiwara S, Cremer F, Lee K, Onouchi H, Mouradov A, Fowler S, Kamada H, Putterill J, Coupland G (2005) Distinct roles of *GIGANTEA* in promoting flowering and regulating circadian rhythms in Arabidopsis. *Plant Cell* 17: 2255–2270

Mizoguchi T, Putterill J, Ohkoshi Y (2006) Kinase and phosphatase: the cog and spring of the circadian clock. *Int Rev Cytol* 250: 47–72

Mizoguchi T, Niinuma K, Yoshida R (2007) Day-neutral response of photoperiodic flowering in tomatoes: possible implications based on recent molecular genetics of *Arabidopsis* and rice. *Plant Biotechnol* 24: 83–86

Mizoguchi T, Yoshida R (2009) Punctual coordination: switching on and off for flowering during a day. *Plant Signal Behav* 4: 113–115

Natsui Y, Nefissi R, Miyata K, Oda A, Hase Y, Nakagawa M, Mizoguchi T (2010) Isolation and characterization of suppressors of the *early flowering 3* (*elf3*) in *Arabidopsis thaliana*. *Plant Biotechnology* 27: 463–468

Niinuma K, Nakagawa M, Calvino M, Mizoguchi T (2007) Dance of plants with the circadian clock. *Plant Biotechnol* 24: 87–97

Niinuma K, Nakamichi N, Miyata K, Mizuno T, Kamada H, Mizoguchi T (2008) Roles of Arabidopsis *PSEUDO-RESPONSE REGULATOR* (*PRR*) genes in the opposite controls of flowering time and organ elongation under long-day and continuous light conditions. *Plant Biotechnol* 25: 165–172

Oda A, Reeves PH, Tajima T, Nakagawa M, Kamada H, Coupland G, Mizoguchi T (2007) Isolation of novel gain- and loss-of-function alleles of the circadian clock gene *LATE ELONGATED HYPOCOTYL* (*LHY*) in *Arabidopsis. Plant Biotechnol* 5: 457–465

Ratcliffe OJ, Kumimoto RW, Wong BJ, Riechmann JL (2003) Analysis of the Arabidopsis *MADS AFFECTING FLOWERING* gene family: MAF2 prevents vernalization by short periods of cold. *Plant Cell* 15:1159–1169

Schaffer R, Ramsay N, Samach A, Corden S, Putterill J, Carre IA, Coupland G (1998) The *late elongated hypocotyl* mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. *Cell* 93: 1219–1229

Súarez-López P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G (2000) CONSTANS mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature* 410: 1116–1120

Tajima T, Oda A, Nakagawa M, Kamada H, Mizoguchi T (2007) Natural variation of polyglutamine repeats of a circadian clock gene *ELF3* in *Arabidopsis. Plant Biotechnol* 24: 237–240

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

Wang ZY, Tobin EM (1998) Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) gene disrupts circadian rhythms and suppresses its own expression. *Cell* 93: 1207–1217

Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D (2005) Integration of spatial and temporal information during floral induction in *Arabidopsis*. *Science* 309: 1056–1059

Yanovsky MJ, Kay SA (2002) Molecular basis of seasonal time measurement in *Arabidopsis*. *Nature* 419: 308–312

Yoshida R, Fekih R, Fujiwara S, Oda A, Miyata K, Tomozoe Y, Nakagawa M, Niinuma K, Hayashi K, Ezura H, et al. (2009) Possible role of EARLY FLOWERING 3 (ELF3) in the clock-dependent floral regulation by SHORT VEGETATIVE PHASE (SVP) in *Arabidopsis*. *New Phytol* 182: 838–850