Plant Physiology Preview. Published on June 9, 2011, as DOI:10.1104/pp.111.176685

Name: Ilha Lee

Address: School of Biological Sciences, Seoul National University, Seoul 151-742, Korea.

Telephone Number: 82-2-880-8864

E-mail Address: ilhalee@snu.ac.kr

Journal Research Area: Development and Hormone Action

WEREWOLF, a Regulator of Root Hair Pattern Formation, Controls Flowering Time Through the Regulation of *FT* mRNA stability

Eunjoo Seo[a], Jihyeon Yu[a], Kook Hui Ryu[b], Myeong Min Lee[b]and Ilha Lee[a*]

[a]National Research Laboratory of Plant Developmental Genetics, School of Biological Sciences, Seoul National University, Seoul 151-742, Korea.
[b]Department of Biology, Yonsei University, Seoul 120-749, Korea.

[*]For correspondence (fax 822-872-6950; e-mail ilhalee@snu.ac.kr)

Financial source: National Research Foundation of Korea (NRF)
The experiment station: School of Biological Sciences in SNU
Institution paper number:

* To whom correspondence should be addressed (E-mail ilhalee@snu.ac.kr; Fax 82-2-872-1993)

Running Title: *WER* regulates *FT* mRNA stability

Key Words: Root hair patterning, WEREWOLF, FT, flowering, mRNA stability

ABSTRACT

A key floral activator *FT* integrates stimuli from long-day, vernalization, and autonomous pathways, and triggers flowering by directly regulating floral meristem identity genes. Since small amount of *FT* transcript is sufficient for flowering, the *FT* level is strictly regulated by diverse genes. In this study, we show that *WEREWOLF* (*WER*), a MYB transcription factor regulating root-hair pattern, is another regulator of *FT*. The mutant *wer* flowers late in long days but normal in short days, and shows a weak sensitivity to vernalization, which indicates that *WER* controls flowering time through the photoperiod pathway. The expression and double mutant analyses showed that *WER* modulates *FT* transcript level independent of *CO* and *FLC*. The histological analysis of *WER* shows that it is expressed in the epidermis of leaves where *FT* is not expressed. Consistently, *WER* regulates not transcription but stability of *FT* mRNA. Our results reveal a novel regulatory mechanism of *FT* which is non-cell autonomous.

As for other plants, the correct timing of flowering is essential for reproductive success in *Arabidopsis*. The flowering time in *Arabidopsis* is regulated by complex genetic networks monitoring various environmental and endogenous signals. Four major genetic pathways for these signals have been revealed: the photoperiod and the vernalization pathways responding to environmental stimuli; and the autonomous and the gibberellin-dependent pathways monitoring internal conditions (Mouradov et al., 2002; Simpson and Dean, 2002; Boss et al., 2004). These pathways are converged on common downstream target genes, *FT*, *SUPPRESSOR OF OVEREXPRESSION OF CO1* (*SOC1*),and *LEAFY* (*LFY*),so called, flowering pathway integrators (Simpson and Dean, 2002).Flowering time in *Arabidopsis* is quantitatively controlled by the transcript level of these integrators (Kardailsky et al., 1999; Kobayashi et al., 1999; Blazquez and Weigel, 2000; Onouchi et al., 2000; Samach et al., 2000; Moon et al., 2003; Moon et al., 2005).

FT, encoding a small ~20 kDa protein with homology to Raf kinase inhibitor protein (RKIP), is one of the key floral activators integrating multiple floral inductive pathways (Kardailsky et al., 1999; Kobayashi et al., 1999). *FT* promotes the transition to flowering by activating other floral integrator, *SOC1*, and floral meristem identity genes such as *APETALA1* (*AP1*), *FRUITFULL* (*FUL*), *CAULIFLOWER* (*CAL*) and *SEPALLATA3* (*SEP3*) (RuizGarcia et al., 1997; Abe et al., 2005; Teper-Bamnolker and Samach, 2005; Wigge et al., 2005; Yoo et al., 2005). Recently, many laboratory have shown that FT protein produced in the leaf phloem moves to shoot apex, and executes its role through the interaction with the bZIP transcription factor, FD, which is expressed in shoot apex (Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). Consequently, FT protein is considered as a graft-transmissible florigen, or at least a component of the floral stimuli.

Although the abundance of *FT* transcripts in wild type is as low as not to be detected by *in situ* hybridization, *FT* overexpression plants or loss-of function alleles show dramatic changes in flowering time (Koornneef et al., 1991; Kardailsky et al., 1999; Kobayashi et al., 1999). This suggests that a small amount of *FT* is sufficient for flowering in wild-type plants, and inappropriate *FT* expression causes disorder in flowering-time. Hence, *FT* expression is regulated strictly by diverse range of regulators. *CONSTANS* (*CO*) directly activates *FT* through the photoperiod pathway (Kobayashi et

al., 1999; Samach et al., 2000; Suarez-Lopez et al., 2001; Valverde et al., 2004). *CO* expression, of which transcription arises around 12 hours after dawn and stays high until the following dawn, is activated in both long days and short days (Suarez-Lopez et al., 2001). However, *CO* mRNA expressed at night does not cause the activation of *FT* because CO protein is degraded in the dark (Valverde et al., 2004). Therefore, flowering is delayed in short days due to the absence of *FT*. In contrast, the mutations in genes involved in photoperiod pathway, such as *gi*, *co*, and *ft*, delay flowering only in long days but not in short days since the signal from photoperiod pathway is mainly mediated through *FT* (Koornneef et al., 1991; Suarez-Lopez et al., 2001).

The MADS box transcription factor *FLOWERING LOCUS C* (*FLC*), a central floral repressor in autonomous and vernalization pathways, represses the *FT* expression. *FT* expression repressed by *FLC* in winter annual is important because this repression prevents flowering until the following spring (Searle et al., 2006). Another negative flowering repressor, *SHORT VEGETATIVE PHASE* (*SVP*), regulates *FT* expression through ambient temperature signaling in the thermosensory pathway (Lee et al., 2007). Recently, it has been reported that SVP acts in a repressor complex together with FLC protein, and this complex binds directly to the CArG-box DNA motifs in the first intron of the *FT* gene (Helliwell et al., 2006; Lee et al., 2007; Li et al., 2008). Besides, the chromatin-associated proteins, *TERMINAL FLOWER 2* (*TFL2*) and *EARLY BOLTING IN SHORT DAYS* (*EBS*), repress *FT* transcription by direct binding in *FT* chromatin (Pineiro et al., 2003; Takada and Goto, 2003). In addition, *CURLY LEAF* (*CLF*) and *FERTILIZATION INDEPENDENT ENDOSPERM* (*FIE*), the subunits of *Arabidopsis* Polycomb Repressive Complex 2 (PRC2),strongly repress *FT* during vegetative development (Jiang et al., 2008). Many other genes such as *PHYTOCHROME AND FLOWERING TIME1* (*PFT1*),*PHYTOCHROME-INTERACTING FACTOR 3* (*PIF3*) and *TEMPRANILLO* genes (*TEM1* and *TEM2*), which are involved in light signaling and circadian rhythm, are also known to be involved in *FT* regulation (Cerdan and Chory, 2003; Oda et al., 2004; Castillejo and Pelaz, 2008). Although various functions of upstream genes for *FT* regulation are revealed, little is known about post-transcriptional regulation of *FT* mRNA.

In this study, we show that *WEREWOLF* (*WER*), known as a regulator of root hair patterning, is involved in post-transcriptional regulation of *FT*. *WER*, which is classified

in the same subgroup with *GLABROUS1*(*GL1*) and *AtMYB23* (*MYB23*), encodes an R2R3 MYB transcription factor (Lee and Schiefelbein, 1999; Stracke et al., 2001). In *Arabidopsis*, root epidermal cells differentiate into either root hair cells or hairless cells in a position-dependant manner: epidermal cells between two cortical cells differentiate into root hair cells (called H-cells), whereas epidermal cells in contact with a single cortical cell usually become hairless cells (called N-cells) (Dolan et al., 1994). *WER* is highly expressed in N-cells whereas it is suppressed in H-cells (Lee and Schiefelbein, 1999; Kwak et al., 2005; Kwak and Schiefelbein, 2007). In N-cells, WER protein forms a transcriptional complex with a WD40 protein, TRANSPARENT TESTA GLABRA1 (TTG1), and a bHLH transcription factor, GLABRA3 (GL3), which functions redundantly with ENHANCER OF GLABRA3 (EGL3). This complex positively regulates *GLABRA2* (*GL2*), which inhibits the generation of root hair, thus makes cells differentiate into N-cells (Lee and Schiefelbein, 1999; Walker et al., 1999; Payne et al., 2000; Bernhardt et al., 2003; Zhang et al., 2003; Koshino-Kimura et al., 2005).

Here, we report the late-flowering phenotype of the *wer* mutant which has been previously reported to have hairy roots. Delayed flowering occurred in long days but not in short days, thus *wer* can be classified into the photoperiod pathway mutant. The transcript level of *FT* was reduced in *wer* mutant in long days, which was independent of the *CO* and *FLC*. In addition, such decrease of *FT* transcript level is due not to altered transcription but reduced stability of mRNA. This study suggests that *WER* in epidermis modulates *FT* transcript level in phloem through a novel mechanism.

RESULTS

WER Regulates Flowering Time through Photoperiod Pathway

While exploring if *WER* acts in other developmental process, we observed the flowering time of *wer* loss-of-function mutant and *WER* overexpression transgenic plants is changed. The *wer-1* allele in Columbia (Col) background and *wer-3* allele in Wassilewskija(Ws) background have nonsense mutations in the region of the second MYB domain, thus both alleles apparently produce nonfunctional proteins (Lee and Schiefelbein, 1999). Under long-day (16 h light/8 h dark) conditions, both *wer-1* and *wer-3* plants produced more rosette leaves than wild type at the time of bolting (Fig. 1A). In contrast, the transgenic plants containing *WER* genomic DNA under the control of the strong cauliflower mosaic virus 35S promoter (*35S::WER*) flowered earlier than wild type (Fig. 1A). To verify that the late-flowering phenotype of *wer-1* was caused by loss of *WER* function, the *WER* genomic construct containing 5 kb *WER* genomic fragment that includes 2.5 kb upstream sequence was transformed into *wer-1*. Most of the resulting transformants showed comparable flowering time to wild type, indicating that late flowering is caused by the loss of *WER* (Fig. 1B). In addition, the heterozygous lines obtained from the cross between Col and *wer-1* showed similar flowering time with Col, confirming that *wer* is recessive late-flowering mutant (Supplemental Fig. S1).

The flowering time in *Arabidopsis* is regulated by four major pathways, photoperiod, vernalization, autonomous and GA pathways (Boss et al., 2004; Baurle and Dean, 2006; Oh and Lee, 2007). To determine in which pathway *WER* regulates flowering, the flowering characteristics of *wer* were checked in response to photoperiod and vernalization. Unlike in long days, *wer-1* flowered similarly to wild type in short days (Fig. 1C). After 8 weeks of vernalization treatment, all genotypes showed acceleration of flowering compared to non-vernalization treated control. Although *wer-1* showed slight acceleration of flowering, the responsiveness was weaker than wild type (Fig. 1D). These observations demonstrated that loss-of-function in *WER* results in a delay of flowering only under long days and weak sensitivity to vernalization, which is similar flowering characteristics to photoperiod pathway mutants as *co* and *gi* (Koornneef et al., 1991).

WER Expression in Root Does Not Affect Regulation of Flowering Time

Floral evocation occurs in shoot apex by inducing floral initiation genes such as *AP1*and *LFY* (Weigel and Nilsson, 1995; Hempel et al., 1997). However, it was previously reported that *WER* is expressed mainly in roots (Lee and Schiefelbein, 1999). To determine whether a root-derived signal induced by *WER* affects flowering time, graft chimeras among *wer-1*, *35S::WER* and the wild type were produced by a transverse cut grafting method described before (Turnbull et al., 2002). All grafts were denoted as scion/rootstock genotypes. Self-grafted plants, in which the scion and rootstock were from the same genotype, were also produced as controls. These plants appeared to flower slightly earlier than ungrafted plants probably due to mechanical stress (Fig. 2). The results showed that grafting failed to rescue the late flowering of *wer-1*scions whichever Col or *35S::WER* was used as rootstocks. All grafted plants showed similar flowering time as the plants used as scion (Fig. 2). These results suggest that *WER* expressed in root does not affect flowering time.

Expression of *WER*

To examine the tissue expression pattern of *WER*, RT-PCR analysis was performed with total RNA extracted from various tissues. Although *WER* was highly expressed in roots as previously reported (Lee and Schiefelbein, 1999), transcripts were also detectable in young leaves, shoot apices, adult rosette leaves, stems, and inflorescences including floral buds and mature flowers (Fig. 3A).

To inspect the spatial pattern of *WER* expression, *WERp::GUS* transgenic plants, in which *GUS* reporter is driven by the *WER* promoter with a 4 kb DNA fragment upstream of the *WER* coding sequence, were used for histochemical GUS staining. This transgenic line was used before for the spatial expression analysis because GUS staining faithfully followed the endogenous expression in roots (Lee and Schiefelbein, 1999). In 6-day-old seedlings, GUS expression was most notable in the hypocotyl and the shoot apex as well as in the root tip, whereas weak GUS expression was detected in the margin of the cotyledons (Fig. 3B; a). GUS was also detected in petiole, stem, stigma, and siliques (Fig. 3B; b-g). In shoot and root apices, GUS was detected from embryo stage (Fig. 3B; h). Interestingly, GUS expression was gradually disappeared while leaves get mature (Fig. 3B; b and e). The detailed examination in the shoot apex by

longitudinal sectioning of *WERp::GUS* revealed that *WER* is concentrated in the epidermis of leaf primordia (Fig. 3B; i and j). Immunohistochemistry using *WERp::MYC-WER* transgenic plants, in which WER protein fused with MYC epitope is driven by the *WER* promoter, was shown that WER proteins are also expressed along the epidermis (Fig. 3B; k).

The temporal changes in *WER* transcript level were further determined by RT-PCR to investigate how *WER* expression is regulated during the flowering process. *WER* expression in the aerial part of seedlings peaked in 3 days; afterwards it was reduced and became steady under both long days and short days (Fig. 3C). Such temporal expression was not affected by photoperiod. Moreover, *WER* expression did not show any daily rhythm under long days (Fig. 3D).

When vernalization effect on *WER* expression was checked in Col:*FRI*^{SF2} plants, which has strong flowering response to vernalization, there was no change (Fig. 3E). In addition, GA treatment did not change the expression level (Fig. 3F). These results indicate that the vernalization or GA-dependent pathway does not affect *WER* expression.

WER Regulates *FT* Transcript Level Independent of *CO* and *FLC*

WER expression was further studied in various flowering-time mutants to elucidate the possible involvement of *WER* in the previously known flowering pathways. Although *wer* showed the flowering characteristics similar to the mutants of the long day pathway, *WER* expression was unchanged in these mutants as *ft-1*, *co-101*, and *gi-2* (Fig. 4A). In addition, the expression was not affected by the mutations in autonomous pathway or *flc*, indicating that *WER* does not act downstream of the previously known flowering pathways.

To examine the molecular basis of the late flowering of *wer*, we checked the expression of the genes in long day pathway or genes acting on the shoot meristem as *CO, FT, FD,* and *SOC1* (Fig. 4B). The real-time quantitative RT-PCR (qRT-PCR) showed that only *FT* expression was reduced in *wer* mutants under long days whereas the levels of *CO, FD*, and *SOC1* were similar to wild type. In short days, there was no difference between wild type and *wer* in the transcript level of all four genes. Such result is well consistent with the flowering characteristics of *wer*, late flowering only

under long days. It also strongly suggests that *WER* regulates *FT* independent of *CO*. The double mutant analyses also support this hypothesis such that *35S::FT* completely suppresses but *35S::CO* partially suppresses the late-flowering phenotype of *wer-1* (Fig. 4, C and D). Double mutant analysis also shows that *flc* and *wer* are additive (Fig. 4F), suggesting that *WER* acts independent of *FLC*. It is noteworthy that the molecular basis of the early flowering of *35S::WER* was a little complicated because ectopic overexpression of *WER* caused increase of both *FT* and *SOC1* (Fig. 4B). Thus, neither *ft* nor *soc1* mutation was epistatic to *35S::WER* (Fig. 4, C and E).

Because *FT* is known to be regulated by the circadian clock, we wondered whether the daily rhythm of *FT* is affected by *wer*. To address this, *FT* expression was analyzed every 4 h over a 24-h period under long days by RT-PCR. The result showed that the daily rhythm of *FT* expression is not affected by *wer* although the amplitude is lower than wild type (Fig. 4, G and H).

wer Mutation Affects the *FT* mRNA Stability

Although *WER* is expressed in the epidermis (Fig. 3B; i and j), *FT* is expressed in the vasculature (Takada and Goto, 2003). Thus, it is not likely that *WER* directly regulates *FT*. However, it is still possible that *WER* acts non-cell autonomously through mRNA or protein transport. To check if *wer* mutation affects transcription of *FT*, the expression of *GUS* reporter gene driven by *FT* promoter in *wer-1* was analyzed (Fig. 5A). The result showed that *GUS* expression level in *wer-1* was similar to that in Col, whereas endogenous *FT* level in *wer-1* was less than that in Col. Since initiation of transcription is achieved by binding of RNA polymerase II to the promoter DNA in eukaryotes, we checked if *wer* mutation affects the binding capacity of RNA polymerase II to the proximal region of the *FT* promoter by chromatin immunoprecipitation (ChIP) assay. In *wer-1* mutants, the enrichment of RNA polymerase II at the *FT* promoter was similar to that in Col, demonstrating that *wer* mutation does not affects *FT* transcription (Fig. 5B). Unlike at the *FT* promoter, RNA polymerase II enrichment was reduced by *wer-1* approximately 40% at promoters of *GL2* and *AP1*, which are direct target of WER and a downstream gene of *FT*, respectively. It suggests that the transcription rate of these two genes is reduced in *wer-1*. Taken together, these results strongly suggest that *FT* transcript level is reduced in *wer-1* by post-transcriptional regulation such as mRNA

decay.

To directly assess whether reduced *FT* mRNA level in *wer-1* was a result of altered mRNA stability, the half-life of *FT* mRNA was compared between wild type and *wer-1* after actinomycin D, a transcription inhibitor, was treated. Total RNA of 7-day-old seedlings grown under continuous light was isolated after incubation with actinomycin D for 0, 2, 4, or 8 hours. After transcriptional block with the treatment of actinomycin D, *FT* mRNA abundance in *wer-1*was more rapidly reduced than that in wild type (Fig. 5C). However, the half-life of control mRNA, *TUB2,* was not different in wild type and *wer-1*, suggesting that the *wer* mutation does not cause a general RNA instability. Therefore, our results suggest that *WER* regulates the stability of *FT* mRNA.

Mutation Affects the *FT* mRNA Stability

For the root hair pattern formation, WER forms a transcriptional protein complex with TTG1 and GL3/EGL3 and positively regulates the transcription of *GL2* and *CPC* (Lee and Schiefelbein, 2002; Schellmann et al., 2002; Wada et al., 2002; Zhang et al., 2003; Koshino-Kimura et al., 2005; Ryu et al., 2005). We wondered if the genetic tool kit regulating root-hair pattern is also involved in the determination of flowering time. Interestingly, *ttg1-13* and *gl3-2 egl3-1,* mutants of components in the WER protein complex, flowered as late as *wer* mutants (Fig. 6A), suggesting that WER complex regulates flowering time also. In contrast, *gl2* and *cpc* showed similar flowering-time to wild-type (Fig. 6B). Therefore, our results indicate that WER downstream signaling does not regulate flowering although WER protein complex do through the regulation of *FT* mRNA stability.

DISCUSSION

Myriad of genes are reported to regulate transcript level of *FT* since FT turns out to be a 'florigen' as well as a key integrator of flowering signals. However, most of the case is not clear if it is regulated at transcriptional level or posttranscriptional level. In this study, we clearly show that *wer*, producing ectopic root hairs, is a typical photoperiod pathway mutant and *WER* regulates *FT* mRNA stability by non-cell autonomous way. Therefore, our study provides a novel mechanism regulating *FT* transcript level.

WER Regulates *FT* Non-Cell Autonomously

Although *WER* expression was mostly detected in root, grafting analysis showed that flowering time was not affected by *WER* in root (Fig.2). This result indicates that *WER* expressed in aerial parts are involved in the regulation of flowering time. Consistent with this, *WER* expression was observed in diverse ranges of aerial parts as young leaves, stems, flowers, and siliques (Fig. 3, A and B). In addition, it is expressed in young, developing leaves where *FT* is expressed (Fig. 3B; Takada and Goto, 2003). The leaf consists of three distinct tissues as mesophyll, vascular bundle, and epidermis. Histological analysis showed that *WER* is expressed in leaf epidermis whereas *FT* is expressed in vascular bundles (Fig. 3B; Takada and Goto, 2003). Therefore, *WER* is most likely to regulate *FT* non-cell autonomously. Alternatively, WER may be transported to vascular bundles for the regulation of *FT*. However, it is not likely because it produces protein complex as discussed below and our preliminary result showed that WER protein is located in the leaf epidermis.

It is noteworthy that root hair pattern formation shares the same genetic tool kit with trichome formation in the leaves (Schiefelbein, 2003). The many *glabrous* mutants with no trichome have defects in root hair formation, too (Masucci et al., 1996; Payne et al., 2000; Ohashi et al., 2002; Bernhardt et al., 2003; Zhang et al., 2003). In addition, such genes are expressed in both root and leaf epidermis. Therefore, it provides good evidence implicating that trichome and root hair are evolutionarily homologous organs as suggested before (Kellogg, 2001). However, our results also show that how functional divergence occurs in WER activity. Although the same WER protein complex

regulates root hair pattern formation and flowering in roots and leaves, respectively, the downstream factors involved in each process are different (Fig. 6). Thus, the divergence occurs at the downstream target genes. Future analysis searching the factors mediating signals between epidermis and vascular bundles for the *FT* regulation would be interesting.

Non-cell autonomous regulation of *FT* is not unprecedented. It has been reported that phytochrome B located in mesophyll suppresses *FT* expression through a downstream gene *PHYTOCHROME AND FLOWERING TIME 1* (*PFT1*) (Cerdan and Chory, 2003; Endo et al., 2005). Interestingly, the *PFT1* mediating phyB signaling also regulates *FT* independent of CO, similar to *WER*. It may indicate that non-cell autonomous regulation of *FT* in the leaf is a common process. Therefore, it is possible to identify the inter-tissue signals regulating *FT* which is critical to understand the florigen entity.

WER Regulates *FT* Posttranscriptionally

CO is known to directly regulate *FT* transcription by binding to the promoter (Samach et al., 2000; Tiwari et al., 2010). Although CO protein does not have conspicuous domain for transcription factor, many compelling evidences support that it plays as transcriptional coactivator (Samach et al., 2000; Hepworth et al., 2002; Wenkel et al., 2006). In addition, *FT* is transcriptionally regulated by *FLC*, a central flowering repressor. FLC protein binds directly to the first intron of *FT* to prevent the induction of *FT* transcription (Helliwell et al., 2006). Therefore, transcriptional regulation of *FT* is relatively well studied but posttranscriptional regulation is poorly studied. Here, we revealed that *WER* positively regulates *FT* by controlling mRNA stability at the post-transcriptional level (Fig. 5). It implicates that a *FT* mRNA decay pathway is involved in the regulation of flowering time. Transcript abundance is determined by the equilibrium between the rate of mRNA synthesis and the rate of degradation; yet in majority of gene expression analysis, mRNA decay process has not been considered seriously. However, recent advances provide some knowledge about mRNA decay pathways such that the mRNAs involved in the regulatory processes have shorter half-lives than those involved in metabolic pathway in *Arabidopsis* (Gutierrez et al., 2002; Belostotsky and Sieburth, 2009). As was in yeast and human studies, it implies that rapid mRNA decay process is required for strict regulation of developmental process.

This is consistent with the result of which *FT* transcript was less stable than *TUB2* in this study (Fig.5C). Because FT protein is considered as florigen, the FT protein level is directly linked to flowering. Therefore, it is probable that *FT* transcripts must be carefully monitored to produce FT protein inappropriate amount. The regulation of *FT* mRNA stability proposed in this study may provide a new mechanism to control *FT* transcripts. Since *WER* encodes a transcription factor, WER would not be directly involved in the regulation of *FT* mRNA stability. Thus, it is likely that WER activates stabilizing factors or inhibits destabilizing factors for *FT* mRNA. The genetic components regulating *FT* mRNA stability will be pursued.

MATERIALS AND METHODS

Plant Materials and Genotyping

The *wer-1* in Col and *wer-3* in Ws background were used (Lee and Schiefelbein, 1999). The *35S::WER* is a transgenic line with *WER* genomic DNA (inserting from the start to the stop codon) driven by *CaMV35S* promoter in *wer-1* mutants (Lee and Schiefelbein, 1999). To confirm that genomic *WER* rescues flowering phenotype of *wer-1*, 5 kb genomic fragment including 2.5 kb of the upstream sequence of *WER* was cloned into the binary vector *pPZP221* and transformed into *wer-1*. The *WERp::GUS* transcriptional reporter construct was previously reported (Lee and Schiefelbein, 1999). The *gl2-1*mutant is in the Landsberg *erecta* (L*er*) background, and *cpc*, *ttg1-13* are in the Ws background (Masucci and Schiefelbein, 1996; Wada et al., 1997; Walker et al., 1999). The homozygous double mutant of *gl3-2 egl3-1* is generated by crossing two single mutants which are in the L*er* background (Payne et al., 2000; Zhang et al., 2003; Bernhardt et al., 2005). The *35S::CO*, *35S::FT* transgenic lines and *ft-1*, *co-101*, *gi-2*, *fca-9*, *fve-3*, *soc1-2*, and *ld-1* were in the Col background as described before (Lee et al., 1994; Fowler et al., 1999; Kardailsky et al., 1999; Kobayashi et al., 1999; Page et al., 1999; Takada and Goto, 2003; Ausin et al., 2004; Lee et al., 2006). The *flc-3* is originally the line obtained from fast neutron mutagenesis of Col:FRI^{SF2}, a Columbia line with *FRIGIDA* (*FRI*) from San Feliu-2 (SF2) by eight times of backcross (Michaels and Amasino, 1999; Lee et al., 2000). However, the FRI^{SF2} allele has been eliminated from the *flc-3* by backcrossing several times into Col. The *FTp::GUS* Collinecontaining8.9kb upstream sequence from the start codon fused to GUS protein was used (Takada and Goto, 2003). The *FTp::GUS wer-1* was obtained from the cross between *FTp::GUS* Col and *wer-1*, and kanamycin-resistant F2 seedlings were genotyped with CAPS markers. To check the genotype of double mutants, the F2 plants were checked using polymerase chain reaction (PCR)-based markers, SSLP markers, and CAPS and dCAPS markers, of which information is described in Supplemental Table S1.

Growth Conditions

Seeds were sterilized by 75% ethanol with 0.05% Triton X-100, then rinsed twice

using absolute ethanol and dried. They were seeded on 0.85% plant agar (Duchefa) containing 1% sucrose and half strength MS (Duchefa) plates, and incubated for 3 days at 4℃ to break seed dormancy. Afterwards plants were transferred and grown at 22℃ with 60 ± 10% relative humidity in long-days (16 h light/8 h dark) or short-days (8 h light/16 h dark) under cool white fluorescent lights (100 μmol m^{-2} s^{-1}). For vernalization treatment, seeds on the MS plates were incubated for 8 weeks at 4℃ under short-day conditions. For exogenous application of gibberellins (GA), we transferred 7-day-old plants into MS medium with gibberellic acid A_3 (GA_3) and incubated for two days under short day conditions. At least 20 plants were used to measure the flowering time of each genotype. The flowering time was measured as the number of rosette leaves produced when flowering occurs.

Grafting

The transverse cut grafting was performed as a method previously described (Turnbull et al., 2002). Grafting experiment was accomplished under a microscope using 5-day-old seedlings grown on sucrose-free media. Horizontal cuts were made in the upper region of the hypocotyl with small blades (Dorco T-300). For 5 days after grafting, grafts were monitored whether they formed good union without bending or any other growth problems. All successful grafts were transplanted to soil. The final proportion of successful grafts which grow normally until flowering was over 70%. At least 10 plants were used to measure the flowering time of each graft.

Analysis of Gene Expression

Total RNA extraction and RT-PCR were performed as described before (Lee et al., 2008). The RT-PCR analysis was repeated at least three times using separately harvested samples. The information of each primer for PCR is described in Supplemental Table S2. For Semi-Quantitative RT-PCR analysis, RT-PCR products were analyzed using the Image J 1.42 (http://rsbweb.nih.gov/ij/) program to quantify the expression levels of each gene. Real-time quantitative PCR (qRT-PCR) analysis was performed in 96-well format using the 7300 Fast Real-Time PCR System (Applied Biosystems) and iQ SYBR Green supermix (Bio-Rad). Four microliter of cDNA was used in a 20μl reaction. Primers were designed to amplify shorter than 150 bp DNA fragments. The details of

each primer for PCR are described in Supplemental Table S3. Reaction conditions were as follows: 5 min at 95℃, 40 cycles of PCR (30 sec at 95℃, 30 sec at 60℃, 30 sec at 72℃), and a dissociation from 60℃ to 95℃. Data was collected at 72℃ in each cycle, and *TUB2* was used as the reference gene. Exceptionally, half-life of mRNA was referenced by 18S rRNA levels. The qRT-PCR analysis was biologically repeated three times, each consists of three technical replicates.

Plasmid construction

To make the chimeric genes of MYC-tagged WER, the DNA fragment for MYC epitope was inserted in frame into the 5' end of the PCR-amplified coding region of *WER* genomic DNA. The insertion of this chimeric *MYC-WER* between a 2.4 kb 5' flanking region DNA fragment and a 1.1 kb 3' flanking region DNA fragment from the WER gene resulted to generate the *WERp::MYC-WER* construct.

GUS Staining and Histological Analysis

GUS staining and histological analysis were performed following standard methods described before (Choi et al., 2007). Embedded samples in paraffin were sectioned at a thickness of 8 μm with a microtome (Leica, RM2135). Photographs were taken by the digital-microscope (Dimis M) or the digital camera (Photometrics) connected to a microscope (Zeiss, Axioskop 2 Plus).

Immunohistochemistry

Immunohistochemistry was performed as described (Paciorek et al., 2006) with some modifications. Tissue samples were fixed in the methanol:acetic acid (3:1) fixative solution. The fixed tissue was dehydrated in ethanol, cleared in Neo-clear (Merck) and embedded in ParaplastPlus. Tissue sections were made with 10 μm thickness and mounted on SuperfrostPlus slides. The sections were deparaffinized and rehydrated with 1X PBS. Antigen unmasking was performed by heating the slides in 10 mM sodium citrate buffer boiled in a microwave oven and incubating the slides in 3 % H_2O_2 solution. After blocking with MTSB solution [50 mM PIPES (pH 7.0), 5 mM EGTA, 5 mM $MgSO_4$] containing 3 % BSA and 5 % goat serum, the sections were incubated with anti-MYC monoclonal antibodies (CalbioChem) at a 1:3000 dilution in the blocking

solution overnight. The slides were washed and incubated with Alexa Fluor 546 goat anti-mouse IgG secondary antibodies (Invitrogen) at a 1:5000 dilution in MTSB. After washing with MTSB, the fluorescence signal was observed using AxioImager fluorescence microscope (Zeiss) with a DsRed filter.

Analysis of mRNA Stability

Whole seedling of wild type and *wer-1* grown for 7 days in continuous light were harvested and incubated in the liquid MS medium with 200 μM actinomycin D (Sigma-Aldrich) for 0, 2, 4, 8 hours. Prior to this treatment, plants were soaked in actinomycin D for 30 min to allow proper distribution of the antibiotic solution.

Chromatin Immunoprecipitation (ChIP) Assay

The 1 g of Col and *wer-1* seedlings grown under long days for 11 days was used for ChIP. Procedures for ChIP were followed the method described before (Lee et al., 2007; Lee et al., 2008), and antibody for the C terminal domain of the RNA polymerase II (Abcam; AB817) was used. Four microliter of ChIP products resuspended in 100μl of TE was used for real-time quantitative PCR (qPCR). In qPCR analysis, expression levels were normalized against the expression in Col. The information of the primer pairs for ChIP-qPCR is presented in Supplemental Table S4.

Accession Numbers

Sequence data from this article can be found in the GenBank EMBL/GenBank data libraries under the following accession numbers: *WER* (AT5G14750), *FT* (AT1G65480), *CO* (AT5G15840), *SOC1* (AT2G45660), *FD* (AT4G35900), *FLC* (AT5G10140), *AP1* (AT1G69120), *GL1*(AT3G27920), *MYB23* (AT5G40330), *TTG1* (AT5G24520), *GL3* (AT1G11130), *EGL3* (AT1G63650), *GL2* (AT1G79840), *CPC* (AT2G46410), and *TUB2* (AT5G62690)

ACKNOWLEDGEMENTS

We thank Dr. Ahn JH for providing *35S::CO* seeds. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government

(MEST) (No. 2011-0017626). E. Seo was supported by the Brain Korea 21 program.

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

Ausin I, Alonso-Blanco C, Jarillo JA, Ruiz-Garcia L, Martinez-Zapater JM (2004) Regulation of flowering time by FVE, a retinoblastoma-associated protein. Nat Genet **36:** 162-166

Baurle I, Dean C (2006) The timing of developmental transitions in plants. Cell **125:** 655-664

Belostotsky DA, Sieburth LE (2009) Kill the messenger: mRNA decay and plant development. Curr Opin Plant Biol **12:** 96-102

Bernhardt C, Lee MM, Gonzalez A, Zhang F, Lloyd A, Schiefelbein J (2003) The bHLH genes GLABRA3 (GL3) and ENHANCER OF GLABRA3 (EGL3) specify epidermal cell fate in the Arabidopsis root. Development **130:** 6431-6439

Bernhardt C, Zhao MZ, Gonzalez A, Lloyd A, Schiefelbein J (2005) The bHLH genes GL3 and EGL3 participate in an intercellular regulatory circuit that controls cell patterning in the Arabidopsis root epidermis. Development **132:** 291-298

Blazquez MA, Weigel D (2000) Integration of floral inductive signals in Arabidopsis. Nature **404:** 889-892

Boss PK, Bastow RM, Mylne JS, Dean C (2004) Multiple pathways in the decision to flower: Enabling, promoting, and resetting. Plant Cell **16:** S18-S31

Castillejo C, Pelaz S (2008) The balance between CONSTANS and TEMPRANILLO activities determines FT expression to trigger flowering. Curr Biol **18:** 1338-1343

Cerdan PD, Chory J (2003) Regulation of flowering time by light quality. Nature **423:** 881-885

Choi K, Park C, Lee J, Oh M, Noh B, Lee I (2007) Arabidopsis homologs of components of the SWR1 complex regulate flowering and plant development. Development **134:** 1931-1941

Corbesier L, Vincent C, Jang SH, Fornara F, Fan QZ, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science **316:** 1030-1033

Dolan L, Duckett CM, Grierson C, Linstead P, Schneider K, Lawson E, Dean C, Poethig S, Roberts K (1994) Clonal relationships and cell patterning in the root epidermis of Arabidopsis. Development **120:** 2465-2474

Endo M, Nakamura S, Araki T, Mochizuki N, Nagatani A (2005) Phytochrome B in the mesophyll delays flowering by suppressing FLOWERING LOCUS T expression in Arabidopsis vascular bundles. Plant Cell **17:** 1941-1952

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Coupland G, Putterill J (1999) GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. EMBO J **18:** 4679-4688

Gutierrez RA, Ewing RM, Cherry JM, Green PJ (2002) Identification of unstable transcripts in Arabidopsis by cDNA microarray analysis: rapid decay is associated with a group of touch- and specific clock-controlled genes. Proc Natl Acad Sci U S A **99:** 11513-11518

Helliwell CA, Wood CC, Robertson M, Peacock WJ, Dennis ES (2006) The Arabidopsis FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex. Plant J **46:** 183-192

Hempel FD, Weigel D, Mandel MA, Ditta G, Zambryski PC, Feldman LJ, Yanofsky MF (1997) Floral determination and expression of floral regulatory genes in Arabidopsis. Development **124:** 3845-3853

Hepworth SR, Valverde F, Ravenscroft D, Mouradov A, Coupland G (2002) Antagonistic regulation of flowering-time gene SOC1 by CONSTANS and FLC via separate promoter motifs. EMBO J **21:** 4327-4337

Jaeger KE, Wigge PA (2007) FT protein acts as a long-range signal in Arabidopsis. Curr Biol **17:** 1050-1054

Jiang D, 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 VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D (1999) Activation tagging of the floral inducer FT. Science **286:** 1962-1965

Kellogg EA (2001) Root hairs, trichomes and the evolution of duplicate genes. Trends Plant Sci **6:** 550-552

Kim YS, Kim SG, Lee M, Lee I, Park HY, Seo PJ, Jung JH, Kwon EJ, Suh SW, Paek KH, Park CM (2008) HD-ZIP III activity is modulated by competitive inhibitors via a feedback loop in Arabidopsis shoot apical meristem development. Plant Cell **20:** 920-933

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

Koornneef M, Hanhart CJ, Vanderveen JH (1991) A genetic and physiological analysis of late flowering mutants in Arabidopsisthaliana. Mol Gen Genet **229:** 57-66

Koshino-Kimura Y, Wada T, Tachibana T, Tsugeki R, Ishiguro S, Okada K (2005) Regulation of CAPRICE transcription by MYB proteins for root epidermis differentiation in Arabidopsis. Plant Cell Physiol **46:** 817-826

Kwak SH, Schiefelbein J (2007) The role of the SCRAMBLED receptor-like kinase in patterning the Arabidopsis root epidermis. Dev Biol **302:** 118-131

Kwak SH, Shen R, Schiefelbein J (2005) Positional signaling mediated by a receptor-like kinase in Arabidopsis. Science **307:** 1111-1113

Lee H, Suh SS, Park E, Cho E, Ahn JH, Kim SG, Lee JS, Kwon YM, Lee I (2000) The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in Arabidopsis. Genes Dev **14:** 2366-2376

Lee I, Aukerman MJ, Gore SL, Lohman KN, Michaels SD, Weaver LM, John MC, Feldmann KA, Amasino RM (1994) Isolation of LUMINIDEPENDENS: a gene involved in the control of flowering time in Arabidopsis. Plant Cell **6:** 75-83

Lee J, He K, Stolc V, Lee H, Figueroa P, Gao Y, Tongprasit W, Zhao HY, Lee I, Deng X (2007) Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development. Plant Cell **19:** 731-749

Lee J, Oh M, Park H, Lee I (2008) SOC1 translocated to the nucleus by interaction with AGL24 directly regulates LEAFY. Plant J **55:** 832-843

Lee JH, Hong SM, Yoo SJ, Park OK, Lee JS, Ahn JH (2006) Integration of floral inductive signals by FLOWERING LOCUS T and SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1. Physiol Plant **126:** 475-483

Lee JH, Park SH, Lee JS, Ahn JH (2007) A conserved role of SHORT VEGETATIVE PHASE (SVP) in controlling flowering time of Brassica plants. Biochim Biophys Acta **1769:** 455-461

Lee MM, Schiefelbein J (1999) WEREWOLF, a MYB-related protein in arabidopsis, is a position-dependent regulator of epidermal cell patterning. Cell **99:** 473-483

Lee MM, Schiefelbein J (2002) Cell pattern in the Arabidopsis root epidermis determined by lateral inhibition with feedback. Plant Cell **14:** 611-618

Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Helliwell CA, Ito T, Meyerowitz E, Yu H (2008) A repressor complex governs the integration of flowering signals in Arabidopsis. Dev Cell **15:** 110-120

Masucci JD, Rerie WG, Foreman DR, Zhang M, Galway ME, Marks MD, Schiefelbein JW (1996) The homeobox gene GLABRA 2 is required for position-dependent cell differentiation in the root epidermis of Arabidopsis thaliana. Development **122:** 1253-1260

Masucci JD, Schiefelbein JW (1996) Hormones act downstream of TTG and GL2 to promote root hair outgrowth during epidermis development in the Arabidopsis root. Plant Cell **8:** 1505-1517

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

Michaels SD, Amasino RM (1999) FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell **11:** 949-956

Moon J, Lee H, Kim M, Lee I (2005) Analysis of flowering pathway integrators in Arabidopsis. Plant Cell Physiol **46:** 292-299

Moon J, Suh SS, Lee H, Choi KR, Hong CB, Paek NC, Kim SG, Lee I (2003) The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. Plant J **35:** 613-623

Mouradov A, Cremer F, Coupland G (2002) Control of flowering time: Interacting pathways as a basis for diversity. Plant Cell **14:** S111-S130

Oda A, Fujiwara S, Kamada H, Coupland G, Mizoguchi T (2004) Antisense suppression of the Arabidopsis PIF3 gene does not affect circadian rhythms but causes early flowering and increases FT expression. FEBS Lett **557:** 259-264

Oh M, Lee I (2007) Historical perspective on breakthroughs in flowering field. J Plant Biol **50:** 249-256

Ohashi Y, Oka A, Ruberti I, Morelli G, Aoyama T (2002) Entopically additive expression of GLABRA2 alters the frequency and spacing of trichome initiation. Plant J **29:** 359-369

Onouchi H, Igeno MI, Perilleux C, Graves K, Coupland G (2000) Mutagenesis of plants overexpressing CONSTANS demonstrates novel interactions among Arabidopsis flowering-time genes. Plant Cell **12:** 885-900

Paciorek T, Sauer M, Balla J, Wisniewska J, Friml J (2006) Immunocytochemica technique for protein localization in sections of plant tissues. Nat Protoc **1:** 104-107

Page T, Macknight R, Yang CH, Dean C (1999) Genetic interactions of the Arabidopsis flowering time gene FCA, with genes regulating floral initiation. Plant J **17:** 231-239

Payne CT, Zhang F, Lloyd AM (2000) GL3 encodes a bHLH protein that regulates trichome development in arabidopsis through interaction with GL1 and TTG1. Genetics **156:** 1349-1362

Pineiro M, Gomez-Mena C, Schaffer R, Martinez-Zapater JM, Coupland G (2003) EARLY BOLTING IN SHORT DAYS is related to chromatin remodeling factors and regulates flowering in Arabidopsis by repressing FT. Plant Cell **15:** 1552-1562

RuizGarcia L, Madueno F, Wilkinson M, Haughn G, Salinas J, MartinezZapater JM (1997) Different roles of flowering-time genes in the activation of floral initiation genes in Arabidopsis. Plant Cell **9:** 1921-1934

Ryu KH, Kang YH, Park YH, Hwang D, Schiefelbein J, Lee MM (2005) The WEREWOLF MYB protein directly regulates CAPRICE transcription during cell fate specification in the Arabidopsis root epidermis. Development **132:**

4765-4775

Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G (2000) Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science **288:** 1613-1616

Schellmann S, Schnittger A, Kirik V, Wada T, Okada K, Beermann A, Thumfahrt J, Jurgens G, Hulskamp M (2002) TRIPTYCHON and CAPRICE mediate lateral inhibition during trichome and root hair patterning in Arabidopsis. EMBO J **21:** 5036-5046

Schiefelbein J (2003) Cell-fate specification in the epidermis: a common patterning mechanism in the root and shoot. Curr Opin Plant Biol **6:** 74-78

Searle I, He YH, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G (2006) The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes Dev **20:** 898-912

Simpson GG, Dean C (2002) Arabidopsis, the Rosetta stone of flowering time? Science **296:** 285-289

Stracke R, Werber M, Weisshaar B (2001) The R2R3-MYB gene family in Arabidopsis thaliana. Curr Opin Plant Biol **4:** 447-456

Suarez-Lopez 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

Teper-Bamnolker P, Samach A (2005) The flowering integrator FT regulates SEPALLATA3 and FRUITFULL accumulation in Arabidopsis leaves. Plant Cell **17:** 2661-2675

Tiwari SB, Shen Y, Chang HC, Hou YL, Harris A, Ma SF, McPartland M, Hymus GJ, Adam L, Marion C, Belachew A, Repetti PP, Reuber TL, Ratcliffe OJ (2010) The flowering time regulator CONSTANS is recruited to the FLOWERING LOCUS T promoter via a unique cis-element. New Phytologist

187: 57-66

Turnbull CGN, Booker JP, Leyser HMO (2002) Micrografting techniques for testing long-distance signalling in Arabidopsis. Plant J **32:** 255-262

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

Wada T, Kurata T, Tominaga R, Koshino-Kimura Y, Tachibana T, Goto K, Marks MD, Shimura Y, Okada K (2002) Role of a positive regulator of root hair development, CAPRICE, in Arabidopsis root epidermal cell differentiation. Development **129:** 5409-5419

Wada T, Tachibana T, Shimura Y, Okada K (1997) Epidermal cell differentiation in Arabidopsis determined by a Myb homolog, CPC. Science **277:** 1113-1116

Walker AR, Davison PA, Bolognesi-Winfield AC, James CM, Srinivasan N, Blundell TL, Esch JJ, Marks MD, Gray JC (1999) The TRANSPARENT TESTA GLABRA1 locus, which regulates trichome differentiation and anthocyanin biosynthesis in Arabidopsis, encodes a WD40 repeat protein. Plant Cell **11:** 1337-1349

Weigel D, Nilsson O (1995) A developmental switch sufficient for flower initiation in diverse plants. Nature **377:** 495-500

Wenkel S, Turck F, Singer K, Gissot L, Le Gourrierec J, Samach A, Coupland G (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

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

Yoo SK, Chung KS, Kim J, Lee JH, Hong SM, Yoo SJ, Yoo SY, Lee JS, Ahn JH (2005) CONSTANS activates SUPPRESSOR OFOVEREXPRESSION OFCONSTANS 1 through FLOWERING LOCUS T to promote flowering in Arabidopsis. Plant Physiol **139:** 770-778

Zhang F, Gonzalez A, Zhao MZ, Payne CT, Lloyd A (2003) A network of redundant bHLH proteins functions in all TTG1-dependent pathways of Arabidopsis.

Development **130:** 4859-4869

Figure 1. Flowering time of *wer* loss-of-function mutants and *WER* overexpression transgenic plants. A, Comparison of flowering time in wild type, *wer* mutants, and *35S::WER* transgenic plants under long days. White bars show the flowering time of *wer-1* and *35S::WER* compared to that of Col, whereas gray bars show the flowering time of the *wer-3* compared to that of Ws. Twenty-five plants were used to measure the flowering time, and the error bars represent standard deviation (SD). Photograph displays the phenotype of each plant when *wer-1* or *wer-3* initiated its flowering. B, Complementation analysis of *wer-1* inserted *WER* genomic DNA. Thirty-six plants were used to measure the flowering time in long days, and the error bars represent SD. C, Flowering time of *wer-1* mutants compared to Col under long days and short days. At least thirty plants were used to measure the flowering time, and the error bars represent SD. D, Flowering time of Col and *wer-1* grown in long days after 0 (Ver−) or 8 weeks (Ver+) of vernalization treatments. At least twenty-five plants were used to measure the flowering time, and the error bars represent SD.

Figure 2. Flowering Time of Grafts among Col, *wer-1* and *35S::WER*. The graft type is 90° transverse cut graft using 5-day-old seedlings grown in long days, which is photographed on the right side. Plants on the left in each panel are ungrafted controls. Genotype notation for stock is shown below X-axis and that of scion is above.

Figure 3. Expression of *WER*. A, RT-PCR analysis of *WER* in diverse tissue. RNAs of young leaves, shoot apices, and roots were isolated from 11-day-old Col seedlings grown in long days, while RNAs of rosette leaves, stems, and inflorescences were isolated from 28-day-old Col plants. *TUB2* was used as a quantitative control. B, Spatial expression patterns of *WER*. a-i, GUS staining in *WERp::GUS* transgenic plants: a, 6-day-old seedling; b, 12-day-old plant; c, 22-day-old plant; d, an inflorescence with flowers; e, leaves obtained from a 12-day-old plant. The leaves are shown in order of production from cotyledon at left; f, siliques of different stages; g, a mature flower; h, a mature embryo; i, longitudinal section; and j, transverse section through shoot apex of 7-day-old seedlings. k, MYC-WER protein expression in *WERp::MYC-WER* transgenic plants: immunohistochemical data obtained from 6-day-old seedling. C, Temporal expression of *WER* detected by RT-PCR analysis. RNA was isolated from shoot of the

Col plants grown for 3, 6, 9, and 12 days in both long days and short days. *TUB2* was used as a quantitative control. D, RT-PCR analysis of *WER* expression in the Col plants during a 24 h cycle in long days. Shoot of 11-day-old seedlings was harvested every 4 h for RNA isolation. The zero time corresponds to right after dawn, and open or filled boxes indicate the light-on or off, respectively. *TUB2* was used as a quantitative control. E, Comparison of *WER* expression between in Col:*FRI*^{SF2} grown for 11 days in long days with (Ver+) and without (Ver−) vernalization at 4℃ for 8 weeks. *TUB2* was used as a quantitative control. F, RT-PCR analysis of *WER* expression with (GA+) or without (GA−) exogenous GA_3 treatment. *TUB2* was used as a quantitative control. The whole seedling was used for RNA extraction.

Figure 4. Regulation of Flowering-Time Genes by *WER*. A, RT-PCR analysis of *WER* expression in various flowering-time mutants. For RNA isolation, shoot of seedlings grown for 11 days under long days was harvested. *TUB2* was used as a quantitative control. B, The expression of various flowering time genes in Col, *wer-1* and *35S::WER* was detected by qRT-PCR. White bars show gene expression in plants grown for 9 days under long days while gray bars show gene expression in plants grown for 21 days under short days. The values and error bars represent mean value and standard deviation (SD), respectively, from three technical replicates. C, Flowering time of Col, *wer-1*, *35S::FT*, *35S::FT wer-1*, *35S::WER*, *ft-1*, and *35S::WER ft-1* grown in long days. D, Flowering time of Col, *wer-1*, *35S::CO*, *35S::CO wer-1*, *co-101*, *35S::WER*, and *35S::WER co-101* grown in long days. E, Flowering time of *35S::WER*, *soc1-2*, and *35S::WER soc1-2* grown in long days. F, Flowering time of *wer-1*, *flc-3*, and *flc-3 wer-1* grown in long days. G-H, Daily rhythm of *FT* in Col and *wer-1* plants grown under long days was detected by RT-PCR analysis (G) or calculated by Image J program from three independent RT-PCR results (H). 9-d-old seedlings grown long-day conditions were harvested every 4 h for RNA isolation. The zero time corresponds to right after dawn, and open or filled boxes indicate the light-on or off, respectively.

Figure 5. Post-transcriptional Regulation of *FT* mRNA. A, Expression level of *GUS* gene driven by *FT* promoter and endogenous *FT* mRNA in *FTp::GUS* Col (Col) and *FTp::GUS wer-1* (*wer-1*) plants were detected by qRT-PCR. The values and error bars

represent mean value and SD, respectively, from three technical replicates. B, ChIP assay with RNA polymerase II antibody. Enrichment in each promoter was confirmed by ChIP-qPCR analysis. Values are normalized against Col, and means of triplicate experiments are presented with error bars representing SD. C, RNA was isolated from 7-day-old Col and *wer-1* grown in continuous light (24 h light)after 200 μM actinomycin D treatment for0, 2, 4, or 8 hours. Expression of *FT* and *TUB2*in each sample was detected RT-qPCR, and values are normalized against expression level of untreated sample (0 h). Mean value from three technical replicates are shown with error bars representing SD.

Figure 6. Flowering Time of Root Hair Patterning Mutants. A, Mutation in components of the same complex with WER delayed flowering time. Double mutant of *gl3-2 egl3-1* is in L*er* background while *ttg1-13* mutant is in Ws background. At least twenty plants were used to measure the flowering time in long days, and the error bars represent SD. The * and *** denotes statistical significance with $p<0.05$ and $p<0.0001$ (t-test), respectively. B, Mutants of *WER* downstream target genes showed similar flowering time with their wild-type plants. *gl2-1* mutant is in L*er* background while *cpc* mutant is in Ws background. At least twenty plants were used to measure the flowering time in long days, and the error bars represent SD. The ** denotes statistical significance with $p<0.01$ (t-test).

Supplemental Figure S1. Flowering Time of Heterozygous Plants between Col and *wer-1*. Flowering time of heterozygous plants was comparable with flowering time of Col, but distinct from flowering time of *wer-1*. Most plants of Col and heterozygous produced less than 11 leaves at bolting, while most of *wer-1* plants did more than 11 leaves.

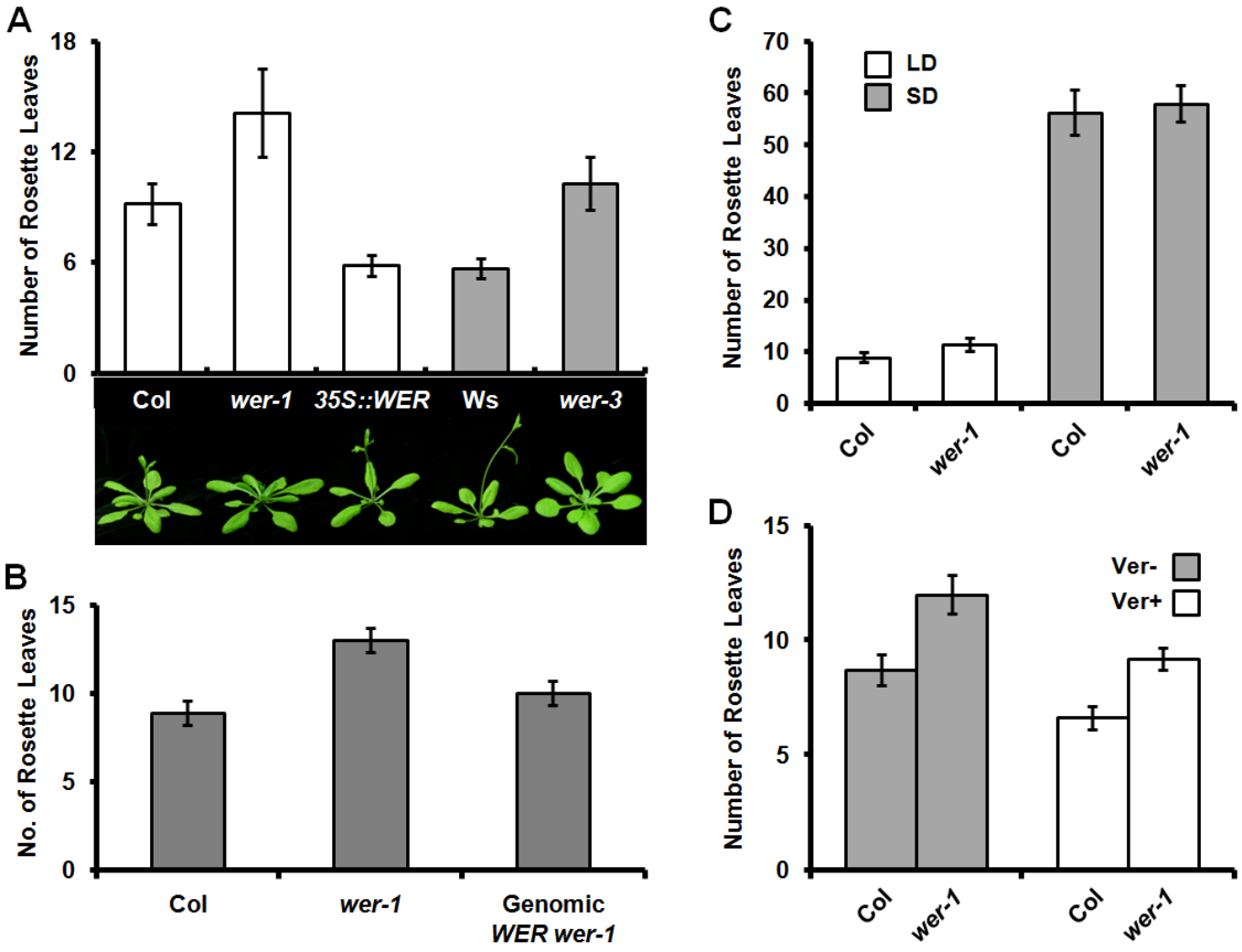


Figure 1. Flowering time of *wer* loss-of-function mutants and *WER* overexpression transgenic plants. A, Comparison of flowering time in wild type, *wer* mutants and *35S::WER* transgenic plants under long days. White bars show the flowering time of *wer-1* and *35S::WER* compared to that of Col, whereas gray bars show the flowering time of the *wer-3* compared to that of Ws. Twenty-five plants were used to measure the flowering time, and the error bars represent standard deviation (SD). Photograph displays the phenotype of each plant when *wer-1* or *wer-3* initiated its flowering. B, Complementation analysis of *wer-1* inserted *WER* genomic DNA. Thirty-six plants were used to measure the flowering time in long days, and the error bars represent SD. C, Flowering time of *wer-1* mutants compared to Col under long days and short days. At least thirty plants were used to measure the flowering time, and the error bars represent SD. D, Flowering time of Col and *wer-1* grown in long days after 0 (Ver-) or 8 weeks (Ver+) of vernalization treatments. At least twenty-five plants were used to measure the flowering time, and the error bars represent SD.

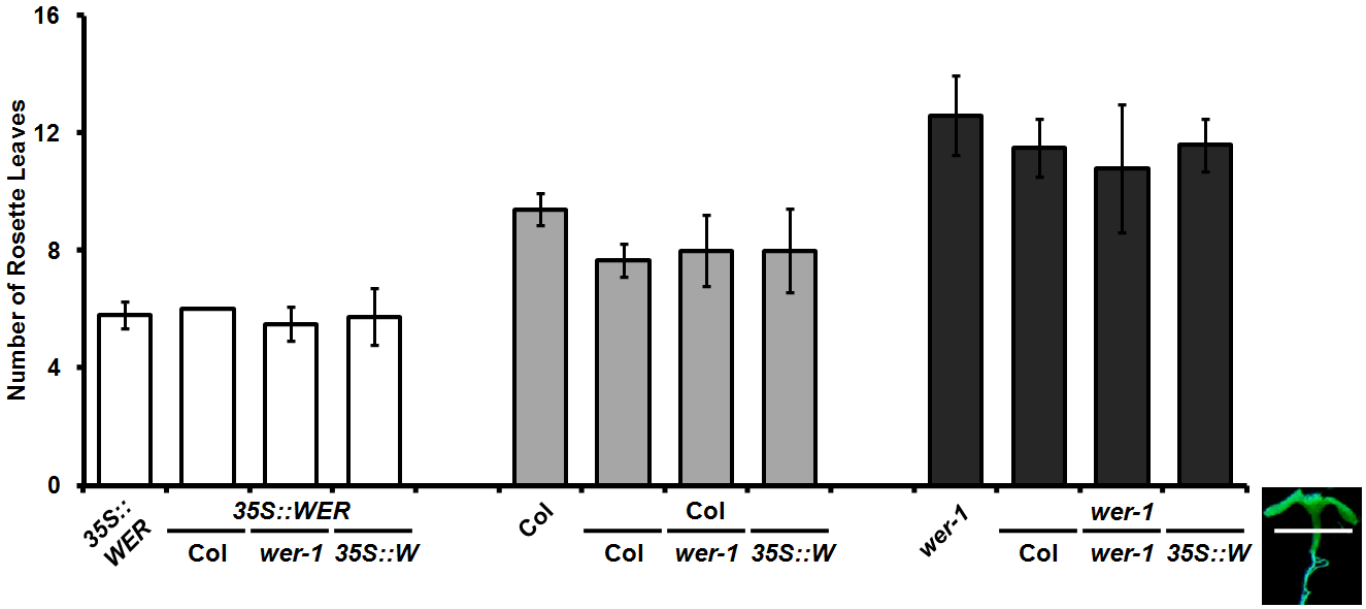


Figure 2. Flowering Time of Grafts among Col, *wer-1* and *35S::WER*. The graft type is 90° transverse cut graft using 5-day-old seedlings grown in long days, which is photographed on the right side. Plants on the left in each panel are ungrafted controls. Genotype notation for stock is shown below X-axis and that of scion is above.

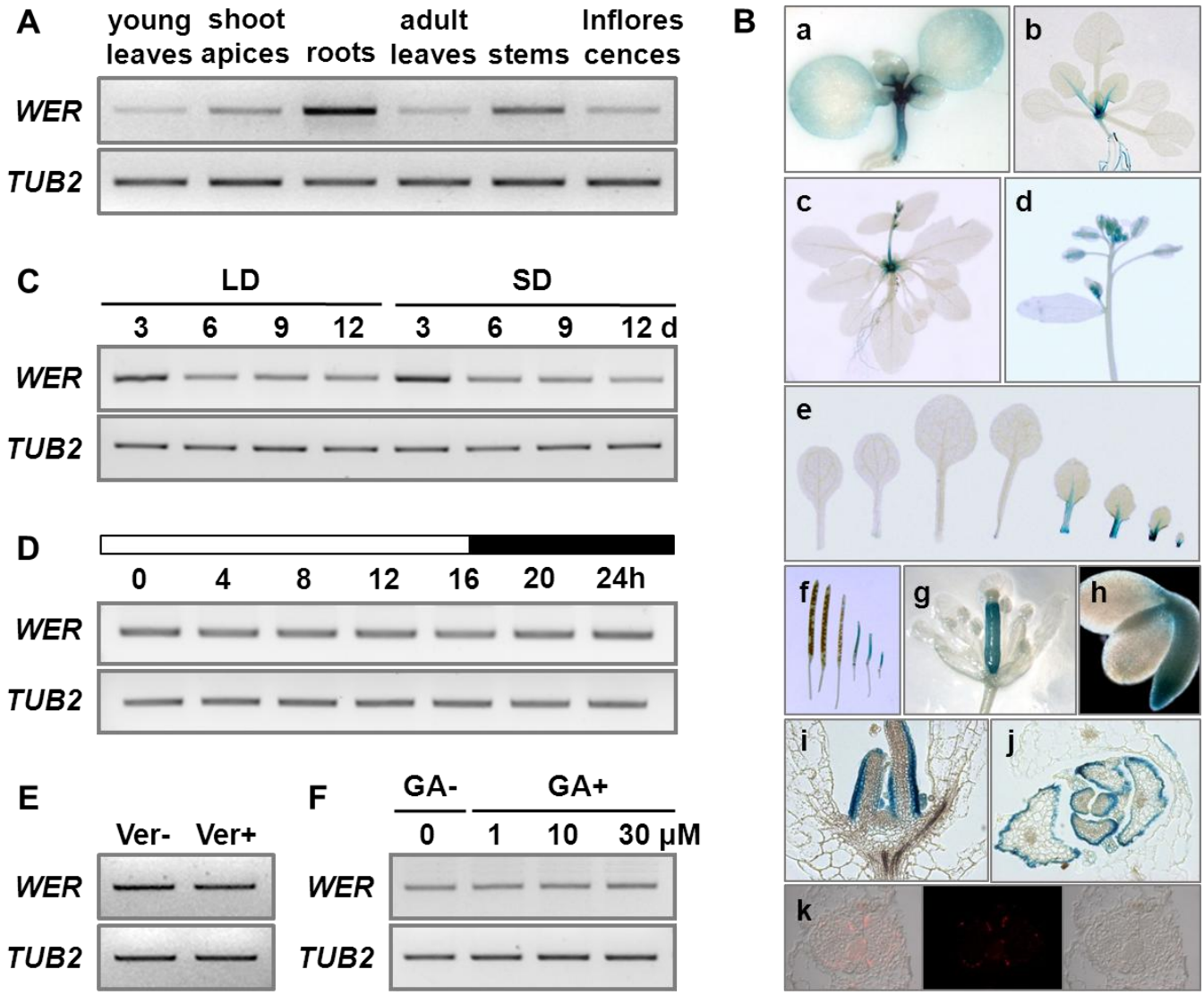


Figure 3. Expression of *WER*. A, RT-PCR analysis of *WER* in diverse tissue. RNAs of young leaves, shoot apices, and roots were isolated from 11-day-old Col seedlings grown in long days, while RNAs of rosette leaves, stems, and inflorescences were isolated from 28-day-old Col plants. *TUB2* was used as a quantitative control. B, Spatial expression patterns of *WER*. a-i, GUS staining in *WERp::GUS* transgenic plants: a, 6-day-old seedling; b, 12-day-old plant; c, 22-day-old plant; d, an inflorescence with flowers; e, leaves obtained from a 12-day-old plant. The leaves are shown in order of production from cotyledon at left; f, siliques of different stages; g, a mature flower; h, a mature embryo; i, longitudinal section; and j, transverse section through shoot apex of 7-day-old seedlings. k, MYC-WER protein expression in *WERp::MYC-WER* transgenic plants: immunohistochemical data obtained from 6-day-old seedling. C, Temporal expression of *WER* detected by RT-PCR analysis. RNA was isolated from shoot of the Col plants grown for 3, 6, 9, and 12 days in both long days and short days. *TUB2* was used as a quantitative control. D, RT-PCR analysis of *WER* expression in the Col plants during a 24 h cycle in long days. Shoot of 11-day-old seedlings was harvested every 4 h for RNA isolation. The zero time corresponds to right after dawn, and open or filled boxes indicate the light-on or off, respectively. *TUB2* was used as a quantitative control. E, Comparison of *WER* expression between in Col:*FRI*^{SF2} grown for 11 days in long days with (Ver+) and without (Ver-) vernalization at 4℃ for 8 weeks. *TUB2* was used as a quantitative control. F, RT-PCR analysis of *WER* expression with (GA+) or without (GA-) exogenous GA_3 treatment. *TUB2* was used as a quantitative control.

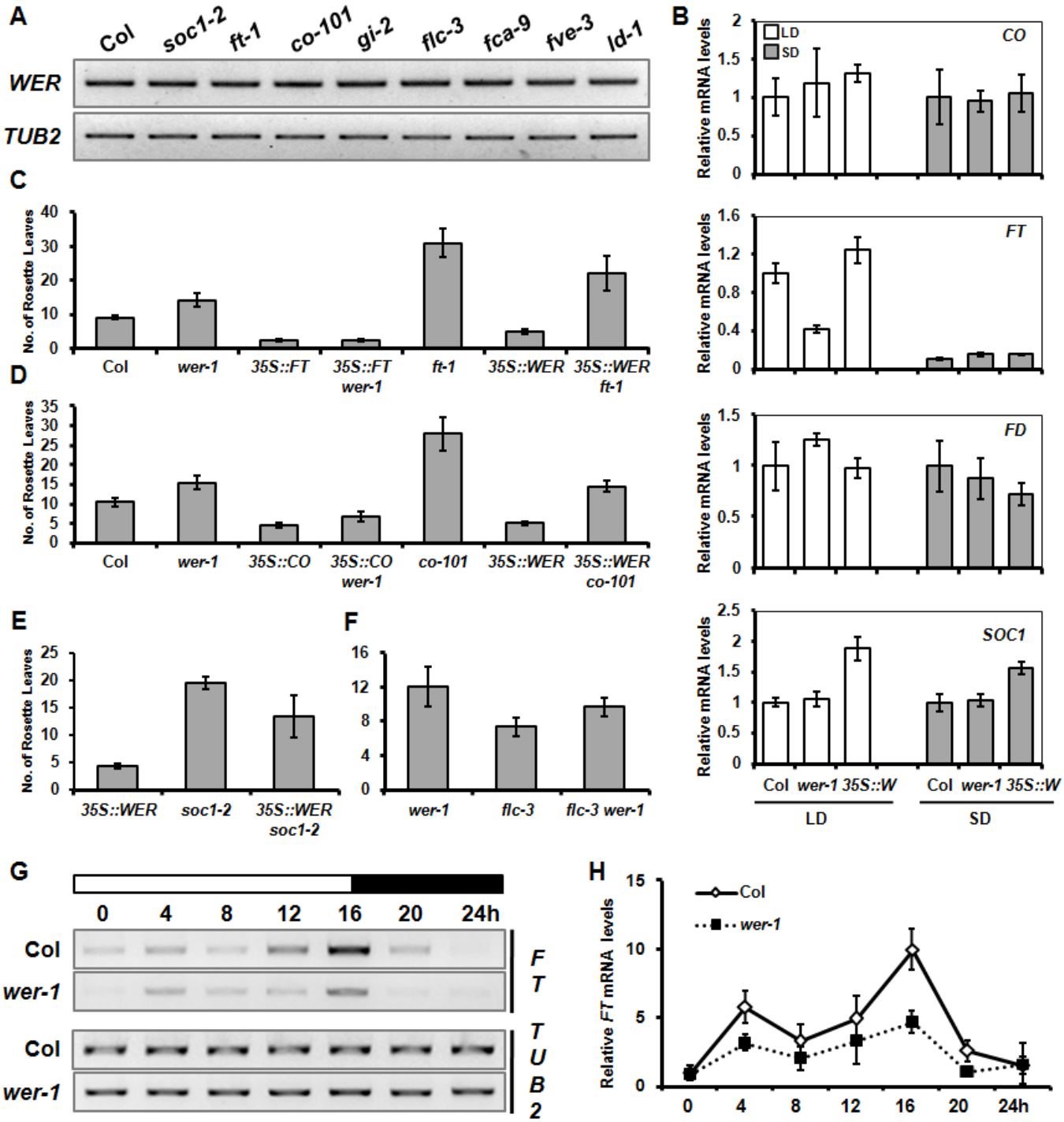


Figure 4. Regulation of Flowering-Time Genes by *WER*. A, RT-PCR analysis of *WER* expression in various flowering-time mutants. For RNA isolation, shoot of seedlings grown for 11 days under long days was harvested. *TUB2* was used as a quantitative control. B, The expression of various flowering time genes in Col, *wer-1* and *35S::WER* was detected by qRT-PCR. White bars show gene expression in plants grown for 9 days under long days while gray bars show gene expression in plants grown for 21 days under short days. The values and error bars represent mean value and standard deviation (SD), respectively, from three technical replicates. C, Flowering time of Col, *wer-1*, *35S::FT*, *35S::FT wer-1*, *35S::WER*, *ft-1*, and *35S::WER ft-1* grown in long days. D, Flowering time of Col, *wer-1*, *35S::CO*, *35S::CO wer-1*, *co-101*, *35S::WER*, and *35S::WER co-101* grown in long days. E, Flowering time of *35S::WER*, *soc1-2*, and *35S::WER soc1-2* grown in long days. F, Flowering time of *wer-1*, *flc-3*, *flc-3 wer-1* grown in long days. G-H, Daily rhythm of *FT* in Col and *wer-1* plants grown under long days was detected by RT-PCR analysis (G) or calculated by Image J program from three independent RT-PCR results (H). 9-d-old seedlings grown long-day conditions were harvested every 4 h for RNA isolation. The zero time corresponds to right after dawn, and open or filled boxes indicate the light-on or off, respectively.

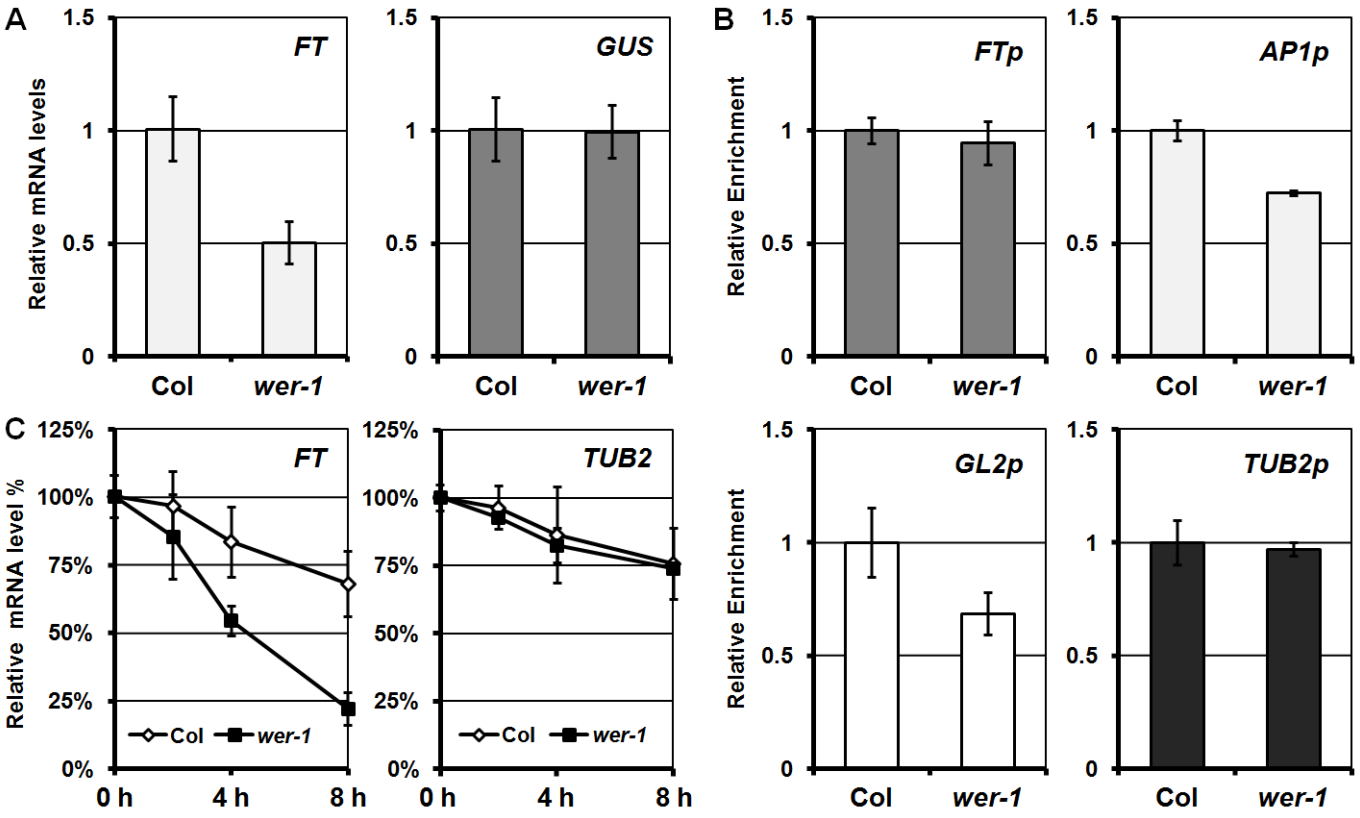


Figure 5. Post-transcriptional Regulation of *FT* mRNA. A, Expression level of *GUS* gene driven by *FT* promoter and endogenous *FT* mRNA in *FTp::GUS* Col (Col) and *FTp::GUS wer-1* (*wer-1*) plants were detected by qRT-PCR. The values and error bars represent mean value and SD, respectively, from three technical replicates. B, ChIP assay with RNA polymerase II antibody. Enrichment in each promoter was confirmed by ChIP-qPCR analysis. Values are normalized against Col, and means of triplicate experiments are presented with error bars representing SD. C, RNA was isolated from 7-day-old Col and *wer-1* grown in continuous light (24 h light) after 200 μM actinomycin D treatment for 0, 2, 4, or 8 hours. Expression of *FT* and *TUB2* in each sample was detected RT-qPCR, and values are normalized against expression level of untreated sample (0 h). Mean value from three technical replicates are shown with error bars representing SD.

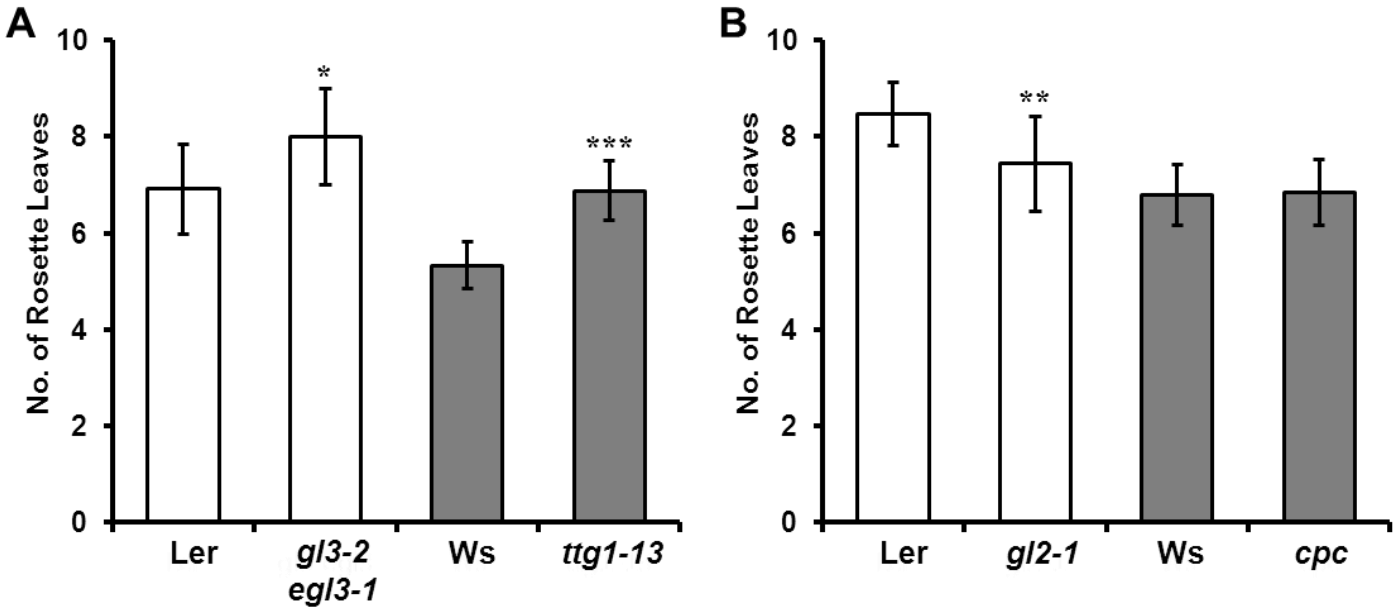
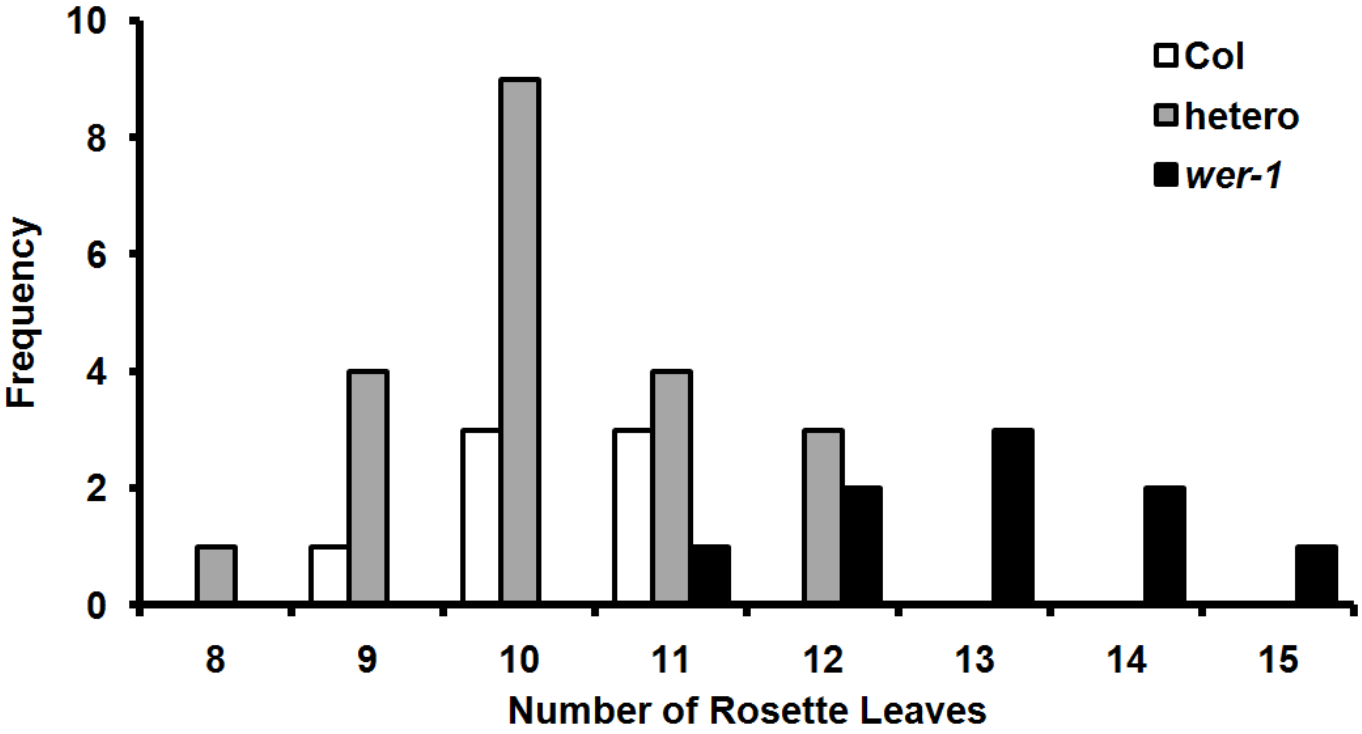


Figure 6. Flowering Time of Root Hair Patterning Mutants. A, Mutation in components of the same complex with WER delayed flowering time. Double mutant of *gl3-2 egl3-1* is in L*er* background while *ttg1-13* mutant is in Ws background. At least twenty plants were used to measure the flowering time in long days, and the error bars represent SD. The * and ** *denotes statistical significance with $p<0.05$ and $p<0.0001$ (t-test), respectively. B, Mutants of *WER* downstream target genes showed similar flowering time with their wild-type plants. *gl2-1* mutant is in L*er* background while *cpc* mutant is in Ws background. At least twenty plants were used to measure the flowering time in long days, and the error bars represent SD. The ** denotes statistical significance with $p<0.01$ (t-test).



Supplemental Figure S1. Flowering Time of Heterozygous Plants between Col and *wer-1*. Flowering time of heterozygous plants was comparable with flowering time of Col, but distinct from flowering time of *wer-1*. Most plants of Col and heterozygous produced less than 11 leaves at bolting, while most of *wer-1* plants did more than 11 leaves.