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Suppression of Pleiotropic Effects of Functional *CRYPTOCHROME* Genes by *TERMINAL FLOWER 1*

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ABSTRACT

TERMINAL FLOWER 1 (*TFL1*) encodes a protein with similarity to animal phosphatidylethanolamine-binding proteins and is required for normal trafficking to the protein storage vacuole. In *Arabidopsis thaliana* the *tfl1* mutation produces severe developmental abnormalities. Here we show that most aspects of the *tfl1* phenotype are lost in the *cry1 cry2* double-mutant background lacking cryptochromes 1 and 2. The inhibition of hypocotyl growth by light is reduced in the *tfl1* mutant but this effect is absent in the *cry1* or *cry2* mutant background. Although the promotion of flowering under long rather than short days is a key function of cryptochromes, in the *tfl1* background, cryptochromes promoted flowering under short days. Thus, normal *CRY* control of photoperiod-dependent flowering and hypocotyl growth inhibition requires a functional *TFL1* gene.

PLANT development is under the control of endogenous signals that provide cells with positional and temporal cues. Plants also adjust their growth and development in response to exogenous signals such as fluctuations of the environment that correlate with stress conditions (Casal *et al.* 2004). For instance, the control of flowering time by photoperiod is a clear example of a response to an environmental condition that correlates with the occurrence of a favorable season, where stress events are less likely. Arabidopsis plants detect long days (LD) by the coincidence between high levels of mRNA of *CONSTANS* (*CO*) and light (Suarez-Lopez *et al.* 2001; Yanovsky and Kay 2002). This light signal is perceived by cryptochrome 2 (cry2) (Guo *et al.* 1998), cryptochrome 1 (cry1) (Ahmad and Cashmore 1993), and phytochrome A. cry2 is a nuclear-localized photoreceptor (Guo *et al.* 1999), whereas cry1 has both nuclear and cytoplasmic functions (Wu and Spalding 2007). The expression of *CO* is controlled by the circadian clock and modulated by *GIGANTEA* (*GI*), *FLAVIN-BINDING, KELCH REPEAT, F-BOX 1* (*FKF1*), and *CYCLING DOF FACTOR 1* (*CDF1*) (Sawa *et al.* 2007). As a result of these controls, *CO* mRNA levels are low during the light period of short days (SD) but under LD, the rising phase of *CO* expression coincides with the presence of light, which stabilizes the CO protein (Valverde *et al.* 2004). In turn, CO promotes the expression of *FLOWERING LOCUS T* (*FT*) (Suarez-Lopez *et al.* 2001), which is a strong activator of flowering (Kardailsky *et al.* 1999; Kobayashi *et al.* 1999).

TERMINAL FLOWER 1 (*TFL1*) is a homolog of *FT*, but it acts as a repressor of flowering (Kardailsky *et al.* 1999; Kobayashi *et al.* 1999). It encodes a protein with similarity to animal phosphatidylethanolamine-binding proteins (Bradley *et al.* 1997). It is a small protein that accumulates in the apical and axillary meristems, through localized trafficking of the protein (Conti and Bradley 2007). TFL1 localizes to endomembrane compartments and is required for normal trafficking to the protein storage vacuole (Sohn *et al.* 2007). The *tfl1* mutants have a severe phenotype characterized by early flowering, a terminal flower that limits the development of the indeterminate meristem that in the wild type (WT) simply senesces, and a reduced number of paraclades and basal ramifications (Shannon and Meeks-Wagner 1991). TFL1 delays the progression of vegetative and reproductive phases of shoot development and negatively regulates the expression of the meristem identity genes *LEAFY* (*LFY*) and *APETALA1* (*AP1*), which in turn restrict the domain of expression of *TFL1* (Ratcliffe *et al.* 1998, 1999). Ectopic expression of *LFY1* or *AP1* reproduces the early flowering and floral *tfl1* phenotypes (Mandel and Yanofsky 1995; Weigel and Nilsson 1995) and the *tfl1* phenotype is virtually absent in the *lfy ap1* double-mutant background (Shannon and Meeks-Wagner 1993). Thus, to a large extent, the phenotype of the *tfl1* can be accounted for by the increased *LFY* and *AP1* levels.

Previous studies have suggested that the flowering phenotype of *tfl1* is suppressed under SD (Shannon and Meeks-Wagner 1991). The dependency of the *tfl1*

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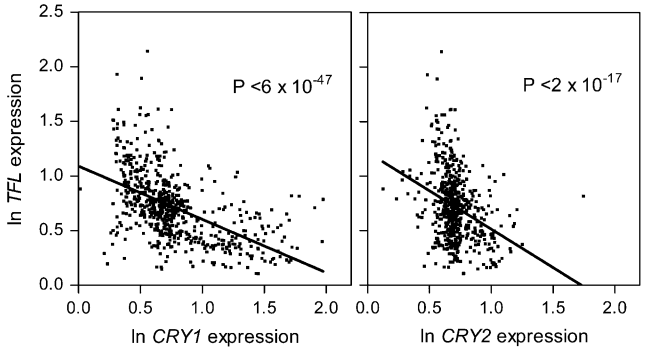

FIGURE 1.—Negative correlation between the expression of *CRY1* or *CRY2* and the expression of *TFL1*. Shown are 633 data points corresponding to different developmental contexts and biotic or abiotic treatments (1–3 biological replicates per point) taken from 46 experiments (1388 microarrays, http://www.arabidopsis.org). Data were normalized to the median of each experiment and transformed as ln $(x + 1)$. The lines show least-square linear fits of the 633 points and the significance is indicated.

phenotype on photoperiod could be the result of changes in sucrose levels that have been shown to alter *TFL1* expression (ZIMMERMANN *et al.* 2004) and the *tfl1* phenotype (OHTO *et al.* 2001). Alternatively, since *TFL1* expression is increased by *CO* (SIMON *et al.* 1996), and LD stabilize CO (VALVERDE *et al.* 2004), LD perceived mainly by cry2 and cry1 could increase *TFL1* expression, while under SD, *TFL1* expression would be weaker and the *tfl1* phenotype less pronounced. To test this hypothesis we investigated the effects of cry1 and cry2 on *TFL1* expression under SD and LD. We also produced double and triple mutants of *tfl1* with *cry1* and *cry2* and characterized the phenotype under SD and LD and under a range of fluence rates of photosynthetically active radiation.

MATERIALS AND METHODS

Plant material: The *tfl-1* (SHANNON and MEEKS-WAGNER 1991), *cry1-hy4-b104* (BRUGGEMAN *et al.* 1996), *cry2-1* (GUO *et al.* 1998, 1999) and their double- and triple-mutant combinations and the WT used in the analysis of growth and development were maintained in the Columbia background. The *cry1 cry2* double mutant (MAZZELLA *et al.* 2001) and the WT used in expression experiments were in the Landsberg *erecta* background.

Growth conditions: Seeds of each genotype were sown on 0.8% (w/v) agar in clear plastic boxes (40 mm × 33 mm × 15 mm height), incubated at 4° for 5 days and 1 day at 22° under either SD or LD to induce germination. Germinating seeds were transferred to pots (110 cm^3) containing equal amounts of perlite (Perlome, Perfiltra, Rosario, Argentina), peat moss (Cuidad Floral, Escobar, Argentina), and vermiculite (Intersum, Aislater, Córdoba, Argentina) and watered as needed with a solution containing 1 g/liter of Hakaphos R (COMPO). In experiments where hypocotyl length was measured, the seedlings remained in the boxes with agar. White light was provided by high-pressure sodium lamps

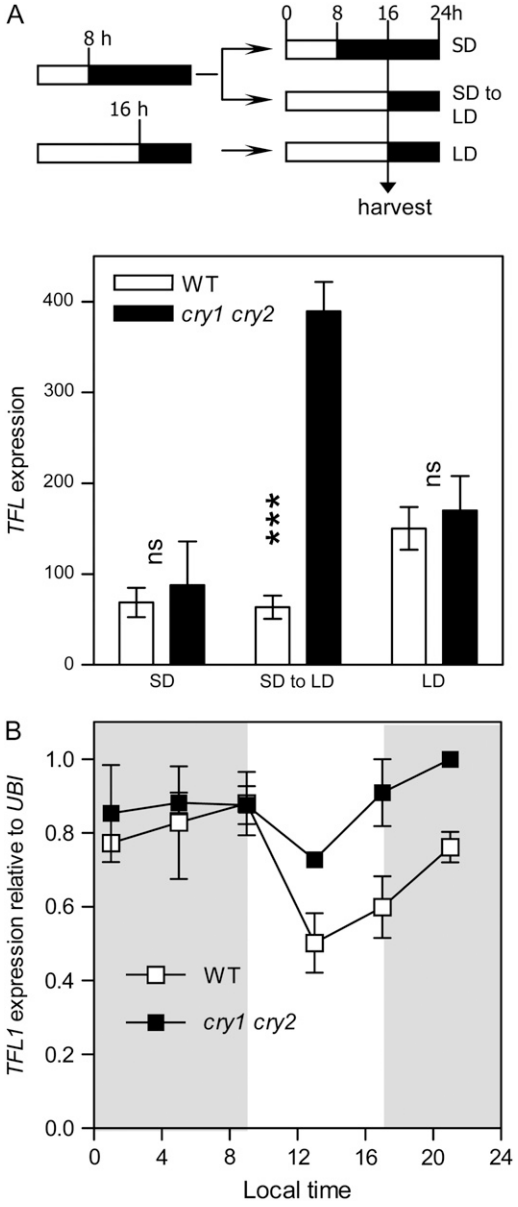

FIGURE 2.—Enhanced expression of *TFL1* in the *cry1 cry2* mutant. (A) Seedlings were grown under SD, under SD followed by LD, or under LD and harvested 16 hr after the beginning of the last photoperiod, when they had nine leaves. Data are means and SE of two biological replicates. Factorial ANOVA indicates significant photoperiod by *cry1 cry2* interaction ($P < 0.005$). ***$P < 0.001$ between *CRY1 CRY2* and *cry1 cry2* in a Bonferroni post test. NS, not significant. (B) Daily time course of *TFL1* expression in seedlings of the WT and of the *cry1 cry2* double mutant grown under SD. Data are means and SE of two biological replicates (data standardized to the maximum of each experiment). The slope of the relationship between expression and time is significantly affected by the *cry1 cry2* mutation ($P < 0.05$).

(400 W Philips SON) at the indicated fluence rates, in growth rooms at 22°.

Measurements: The final number of leaves (rosette plus stem leaves) was used as a measurement of flowering time on a biological scale (KOORNNEEF *et al.* 1991). The number of flowers on the main stem, the number of paraclades, and the length of the main stem were recorded at the end of the plant

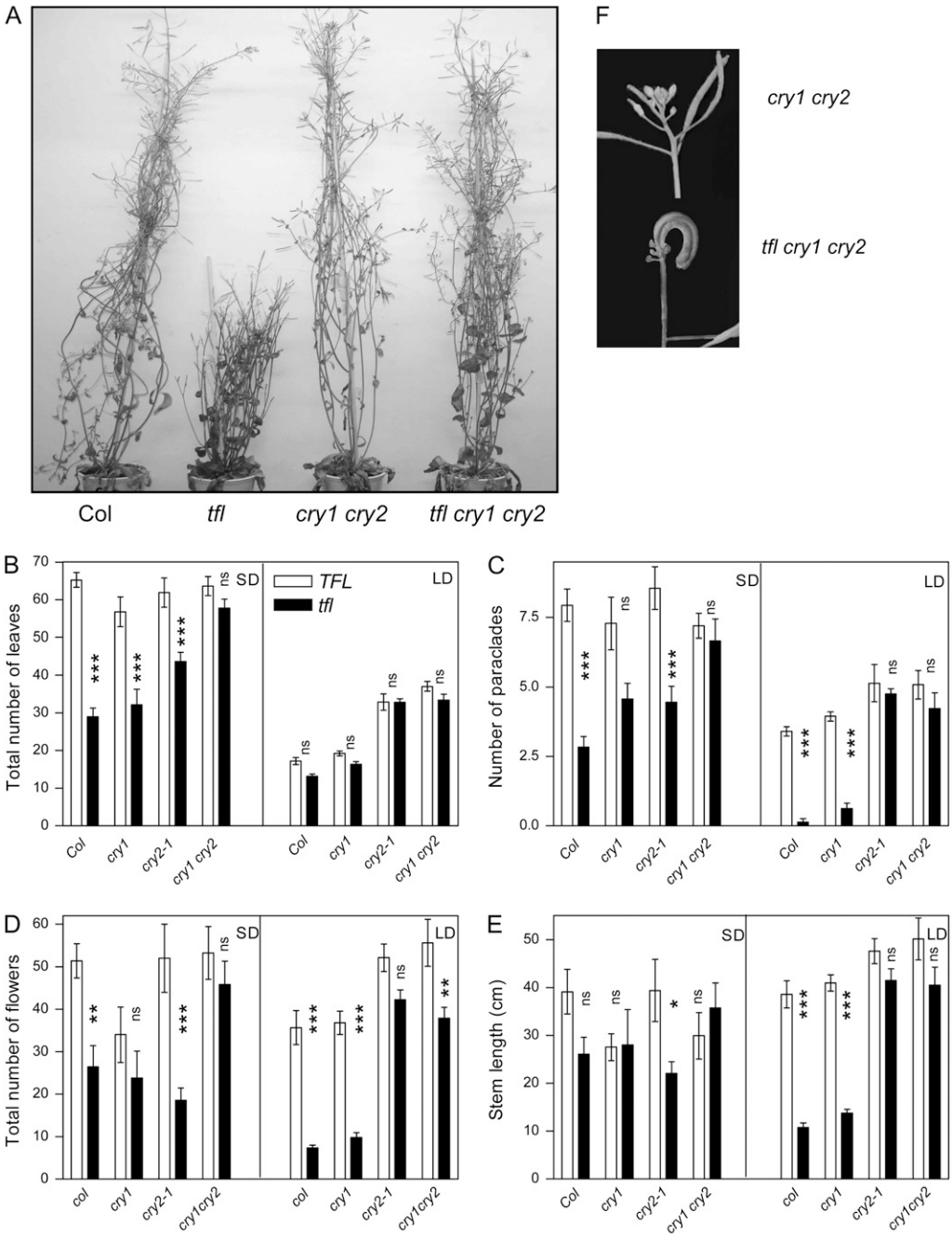

FIGURE 3.—Strong suppression of the *tfl1* phenotype by the *cry1 cry2* mutant background. Plants of the WT and of the *tfl1*, *cry1*, and *cry2* single, double, and triple mutants were grown under SD or LD (irradiance 200 μmol m^{-2} sec^{-1}). (A) Representative plants of the WT, and of the *tfl1*, *cry1 cry2*, and *tfl1 cry1 cry2* mutants grown under LD. (B) Flowering time on a biological scale (leaf number). (C) Number of paraclades. (D) Number of flowers on the main shoot. (E) Length of the main stem. (F) Detail of a terminal flower in *tfl1 cry1 cry2*. Data are means and SE of at least 10 replicate plants. Data were analyzed by factorial ANOVA followed by a Bonferroni post test. The effects of *TFL1 vs. tfl1* are indicated: $***P < 0.001$; $**P < 0.01$. NS, not significant.

cycle. Hypocotyl length was measured to the nearest 0.5 mm with a ruler.

***TFL1* and *FT* expression:** Samples were harvested in liquid nitrogen, and total RNA was extracted using the RNEasy plant mini kit (QIAGEN). In microarray experiments used to measure the expression of *TLF1* in the *cry1 cry2* mutant background, cDNA and cRNA synthesis and hybridization to ATH1 Affymetrix Arabidopsis gene chips were performed according to Affymetrix instructions. Expression data were normalized to the sum of each microarray (CLARKE and ZHU 2006) and analyzed by ANOVA (using Excel). For RT–PCR experiments, 1 μg of RNA was reverse transcribed into cDNA using M-MLV reverse transcriptase (RT, Promega) according to the manufacturer's instructions. PCR was performed in 25 μl final volume. Primers, annealing temperatures, and the size of PCR products expected were as follows:

TFL1: 5′-ATCTCAGATCCTTCTTCACTTTGGTGATGA-3′, 5′-GGAGACCAAGATCATACTCGACCGCAAATT-3′; 55°; 289 bp.
UBQ10: 5′-GGTGTCAGAACTCTCCACCTCAAGAGTA-3′, 5′-TCAATTCTCTCTACCGTGATCAAGATGCA-3′; 63°; 318 bp.
FT: 5′-GCTACAACTGGAACAACCTTTGGCAAT-3′; 61.45°; 365 bp.
FT: 3′-TATAGGCATCATCACCGTTCGTTACTC-5′; 58.6°; 365 bp.
ACT2F: 5′-AGTGGTCGTACAACCGGTATTGTG-3′; 58.8°.
ACT2R: 5′-CCGATCCAGACACTGTACTTCCTT-3′; 58.1°; 593 bp.

In all cases, at least one of the primers used for PCR spanned intron–exon junctions, and amplification of genomic DNA was undetectable in RT controls. PCR products were resolved on 1.5% agarose gels in 1× TBE buffer containing 0.5 μg/ml

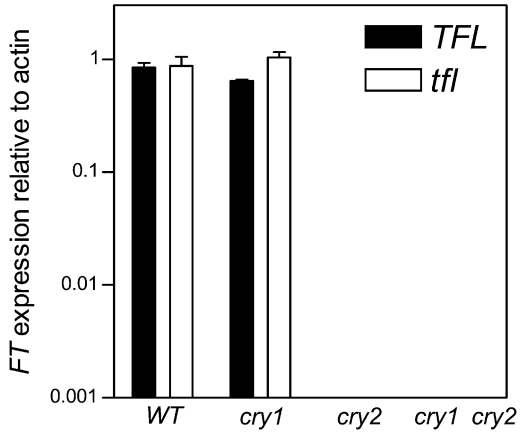

FIGURE 4.—Reduced expression of *FT* in the *cry2* and *cry1 cry2* double-mutant background under SD. Seedlings were grown under SD for 15 days and harvested close to the end of the photoperiod. Data are means and SE of two biological replicates. Factorial ANOVA indicates significant effects of *CRY2 vs. cry2* ($P < 0.0001$) and no effects of *TFL1 vs. tfl1* or interaction. *FT* expression is not detectable in the *cry2* and *cry1 cry2* mutant background under the nonsaturating conditions used here but it was detectable under saturating conditions.

ethidium bromide and quantified using Scion Image software. *FT* PCR products were detected by Southern blot (CERDÁN and CHORY 2003) and quantified using AlkPhos Direct labeling and detection system with CDP-Star, in the exponential range of amplification.

RESULTS

High expression of *TFL1* does not overlap with high expression of *CRY1* or *CRY2*: To investigate the relationship between the expression of *TFL1*, *CRY1*, and *CRY2* we analyzed the correlation of expression between *TFL1* and *CRY1* and between *TFL1* and *CRY2* across 633 conditions representing different tissues, developmental stages, and differentially treated plants (http://www.arabidopsis.org). Publicly available data for each of the three genes were normalized to the median of each experiment and ln $(x + 1)$ transformed to plot *TFL1* expression against *CRY1* or *CRY2* expression (Figure 1). No condition shows simultaneously elevated expression of *TFL1* and either *CRY1* or *CRY2* and there is a significant negative correlation between *TFL1* and *CRY1* and between *TFL1* and *CRY2* expression levels (Figure 1).

Enhanced expression of *TFL1* in the *cry1 cry2* mutant background: The negative correlation between *TFL1* and *CRY1* or *CRY2* expression suggests that *CRY1* and/or *CRY2* could affect *TFL1* expression or vice versa. No effects of the *tfl1-1* mutation on *CRY1* or *CRY2* expression were observed (B. STRASSER and P. D. CERDÁN, unpublished results) and therefore we investigated *TFL1* expression in the *cry1 cry2* mutant background. Plants of the WT and of the *cry1 cry2* double mutant of Arabidopsis were grown under SD (8 hr light, 16 hr darkness) for 24 days. At the end of the last photoperiod, half of the plants began their normal dark period (SD controls) and the rest were exposed to light (SD to LD) (Figure 2A). Eight hours later, *i.e.*, in the middle of the night for SD controls and at the end of the first LD in the SD-to-LD condition, the plants were harvested. A third group of plants was grown under LD (16 hr light, 8 hr darkness) from sowing and harvested at the end of photoperiod 19, when the plants had nine leaves (Figure 2A). This time was chosen to have a comparable developmental stage because SD or SD-to-LD plants had nine leaves at harvest. These samples were used for microarray experiments and we observed that the expression of *TFL1* was unaffected in the *cry1 cry2* double mutant under either continuous SD or LD conditions. *TFL1* expression in the *cry1 cry2* double mutant was significantly higher than in WT by the end of the first LD (Figure 2A). We also investigated the expression of *TFL1* throughout the daily cycle under SD. In accordance with microarray results, *TFL1* expression was not significantly affected in *cry1 cry2* seedlings harvested in the middle of the night (Figure 2B). However, in the *cry1 cry2* double mutant, *TFL1* transcripts accumulated to significantly higher levels during daytime and the first hours of the night relative to WT. These results suggest that cryptochromes are necessary to reduce the levels of *TFL1* gene expression under SD. To explore the interactions between TFL1 and the cryptochromes, all possible mutant combinations between *tfl1*, *cry1*, and *cry2* were generated and analyzed.

Growth under SD does not suppress key features of the *tfl1* phenotype: Plants of the WT and of the *cry1*, *cry2*, *cry1 cry2*, *tfl1*, *tfl1 cry1*, *tfl1 cry2*, and *tfl1 cry1 cry2* mutants were grown under SD or LD. As reported previously (SHANNON and MEEKS-WAGNER 1991), the *tfl1* single mutant is early flowering, has a reduced stature, has a reduced number of paraclades, and has fewer flowers on the main shoot (Figure 3). Under LD growth, the *cry1cry2* double mutant suppressed many of the defects associated with the *tfl1* single mutant. Relative to *tfl1* single mutant, the triple mutants flowered later, had a longer stem, produced more paraclades, and initiated more flowers (Figure 3). Very likely, the latter two responses are consequences of altering flowering time. Early flowering reduces the number of leaves and diminishes the number of buds where paraclades can emerge and the robustness of the plant supporting flower production. Compared to the WT, the *cry1 cry2* mutant showed delayed flowering, an enhanced number of paraclades, and more flowers on the main shoot under LD, a phenotype consistent with the role of cry1 and cry2 as sensors of LD.

We observed that *tfl1* single mutants also had significant effects under SD growth; flowering time, stem length, paraclade number, and flower number were also

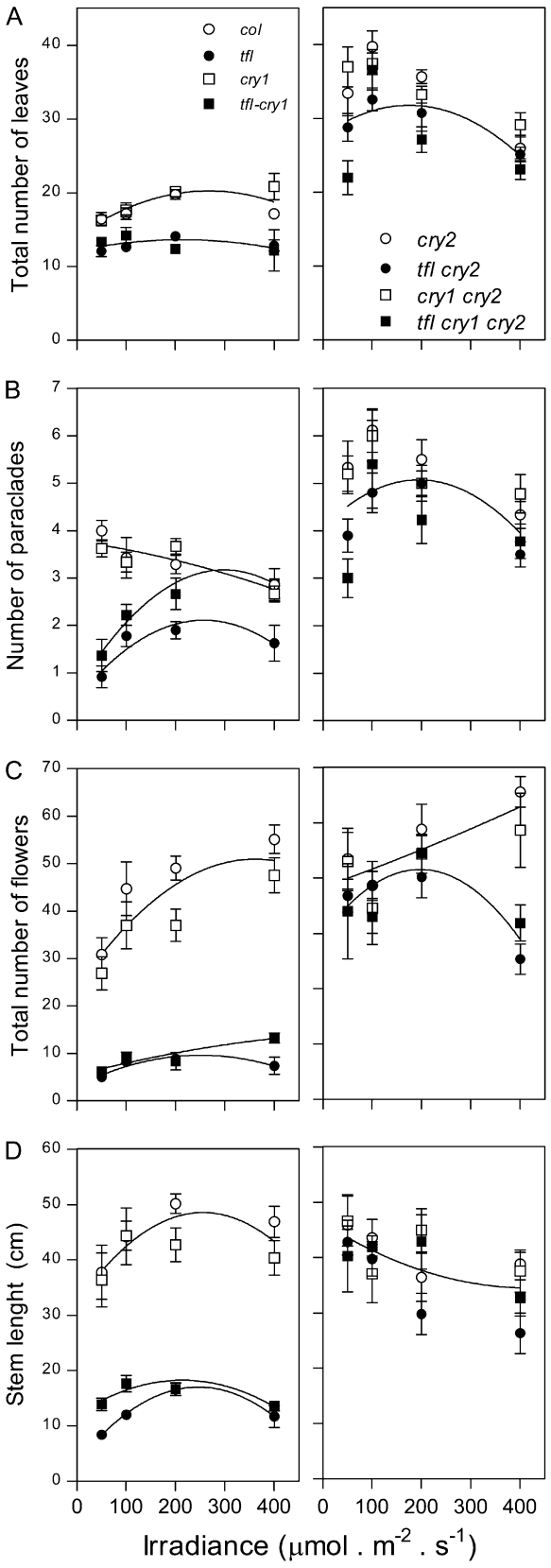

FIGURE 5.—The *tfl1* phenotype persists in plants grown under low irradiances. Plants of the WT and of the *tfl1*, *cry1*, and *cry2* single, double, and triple mutants were grown under LD at the indicated irradiance. (A) Flowering time on a biological scale (leaf number). (B) Number of paraclades. (C) Number of flowers on the main shoot. (D) Length of the main stem. Data are means and SE of at least 10 replicate plants. Different lines indicate significantly different second-order polynomial fits ($P < 0.01$).

significantly different from WT. As discussed previously (SHANNON and MEEKS-WAGNER 1991), SD growth appears to alleviate some of the *tfl1* phenotypes and this may be due to a longer vegetative phase of development. However, if strictly compared to the WT, the absolute impact of *tfl1* on flowering time is stronger under SD than under LD; the impact of *tfl1* on the number of paraclades and on the number of flowers is unaffected and only the impact on stem length is significantly reduced by SD compared to LD (Figure 3).

Significant suppression of the *tfl1* phenotype by *cry1 cry2*: Since cry1 and cry2 are key players in the perception of LD compared to SD (LIN 2002), we expected the *cry1 cry2* background to mimic the effect of SD in plants grown under LD. In contrast, the *cry1 cry2* mutant background significantly reduced or even eliminated the effects of *tfl1* on flowering time, stem length, number of paraclades, and flower number (Figure 3). Noteworthy, these effects were observed both in plants grown under LD and in plants grown under SD, revealing a strong action of cryptochromes under SD.

The relative contribution of *cry1* and *cry2* to the suppression of the *tfl1* phenotypes was complex. In effect, for flowering time and for the number of paraclades the *cry2* mutation was fully epistatic to *tfl1* under LD and only partially epistatic under SD where both cryptochromes showed redundancy (Figure 3). For stem length and flower number, *cry1* was epistatic under SD and *cry2* under LD. Although most aspects of the phenotype were strongly or fully suppressed by the *cry1 cry2* background, the *cry1 cry2 tfl1* triple mutant often retained a morphologically aberrant terminal flower (Figure 3F), which is typical of *tfl1* (SHANNON and MEEKS-WAGNER 1991).

Expression of the flowering activator *FT*: To further characterize the flowering time phenotypes we investigated the expression of the flowering activator *FT*, which acts downstream of cryptochromes. Plants were grown for 15 days under SD and harvested close to the end of the photoperiod. The expression of *FT* was unaffected in the *tfl1* single mutant but it was significantly reduced in the *cry2* mutant and *cry1 cry2* double-mutant background (Figure 4).

Dependency of the *tfl1* phenotype on the growth irradiance: Despite the fact that cry2 and cry1 contribute to photoperiod perception, the *cry1 cry2* background had a much more dramatic impact on the *tfl1* phenotype than the growth under different photoperiods. Since cry1 and cry2 are also sensors of irradiance (LIN 2002), we decided to investigate the *tfl1* phenotype under different irradiances. For this purpose the plants

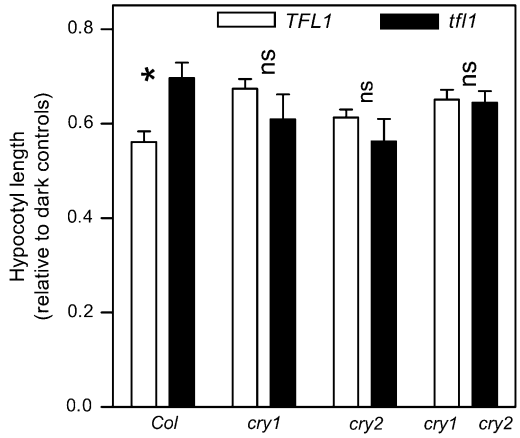

FIGURE 6.—Long hypocotyl in *tfl1* seedlings grown under LD. Chilled seeds were transferred to LD (irradiance 5 μmol m^{-2} sec^{-1}) or to continuous darkness and hypocotyl length was measured 4 days later. Data are means and SE of four replicate boxes (10 seedlings per box). Factorial ANOVA indicated significant interaction between *TFL1* and *CRY* genes ($P < 0.05$). *$P < 0.05$ in Bonferroni post tests. NS, not significant.

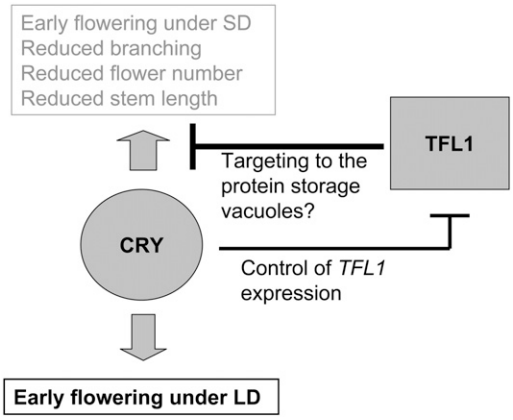

FIGURE 7.—Proposed model of the genetic interactions between *TFL1* and *CRY*. According to this model, in the absence of TFL1, downstream products of cryptochrome signaling cause early flowering under SD and morphological distortions typical of the *tfl1* phenotype.

were grown under LD with a photosynthetic photon fluence rate of 50, 100, 200, or 400 μmol m^{-2} sec^{-1}. In the WT, the number of leaves and stem length moderately increased with fluence rate reaching a maximum at 200 μmol m^{-2} sec^{-1}, while the number of flowers on the main stem increased and the number of paraclades decreased with increasing irradiance (Figure 5). Compared to the WT, in the *cry1 cry2* mutant the responses to irradiance showed some modifications in pattern but they were still present, indicating that although in dark-grown seedlings cry1 and cry2 are sensors of blue light irradiance the responses to irradiance reported here were not largely mediated by cry1 and cry2. The latter conclusion is particularly obvious when the *cry1* mutant, affecting the main sensor of blue light irradiance in young seedlings, is compared to the WT. As described for the aforementioned experiments, the *tfl1* mutant showed early flowering, reduced stature, reduced number of paraclades, and reduced number of flowers on the main shoot. The *tfl1* mutation had little effect on the shape of flowering-time and stem-length responses to irradiance. However, the *tfl1* mutant showed reduced response of the total number of flowers to irradiance and changed the response shape for the number of paraclades. The *cry1 cry2* background largely suppressed the *tfl1* phenotype in plants grown under 200 μmol m^{-2} sec^{-1}, the irradiance used for SD–LD experiments, but a residual *tfl1* phenotype in the *cry1 cry2* background was evident for the total number of flowers at high irradiance and for flowering time and number of paraclades at the lowest fluence rates (Figure 5).

Long hypocotyl of the *tfl1* mutant: Since the *tfl1* phenotype depends strongly on cry1 and cry2, we investigated whether the control of hypocotyl growth by light, which is also controlled by cry1 and cry2 (LIN 2002), was affected in *tfl1*. In seedlings grown under LD, *tfl1* showed longer hypocotyls than the WT. The *cry1* and/or *cry2* mutations were epistatic to this novel *tfl1* phenotype (Figure 6). A similar pattern was observed for seedlings grown under SD (data not shown).

DISCUSSION

In this report we have shown that many of the *tfl1* mutant phenotypes are suppressed in the *cry1 cry2* double mutant. Compared to the *tfl1* single mutant, the *tfl1 cry1 cry2* triple mutant flowered later, had a longer stem, produced more paraclades, and initiated more flowers (Figure 3). Noteworthy, this epistatic effect is equally important under LD and SD (Figure 3), despite the fact that cry1 and cry2 play a major role in the perception of LD, *i.e.*, they are predicted to be active during a larger proportion of the 24-hr cycle under LD than under SD. These photoreceptors are also important for the perception of irradiance. However, the reduction of irradiance did not cause a general weakening of the *tfl1* phenotype (Figure 5). In other words, the *tfl1* mutant does not converge to the phenotype of the *tfl1 cry1 cry2* triple mutant at low irradiances or under SD. Therefore, the *tfl1* phenotype depends on the presence of functional *CRY1* and *CRY2* genes but it does not depend on cry1 and cry2 physiological activity within the (wide) range tested here. Subtle variations in the epistatic relationships between *tfl1* and *cry1/cry2* mutations (Figure 3) might reflect the involvement of different residues of the TFL1 protein in the control of flowering time and in the determinacy of the inflorescence meristem (HANZAWA *et al.* 2005) or different patterns of *CRY1/CRY2* expression.

Previous reports have described the phenotype of mature plants of the *tfl1* mutant (SHANNON and MEEKS-

Wagner 1991). Seedling morphology is also affected, because the inhibition of hypocotyl growth by white light was reduced in the *tfl1* single mutant (Figure 6). As described above for the classical aspects of the *tfl1* phenotype, the *cry1 cry2* mutation was also epistatic to *tfl1* for hypocotyl growth. This suggests that the normal control of hypocotyl growth by cry1 and cry2 requires a functional *TFL1* gene.

In the WT background, the *cry1* and *cry2* mutations cause little or no effects on flowering time under short days (Guo *et al.* 1998; Mockler *et al.* 1999) (Figure 3B). In the absence of a functional *TFL1* allele, cry1 or cry2 cause very early flowering under SD (*cf. tfl1 vs. tfl1 cry1 cry2* in Figure 3B). The Cvi allele of *CRY2* also promotes flowering under SD but this allele represents an adaptation to low latitudes and shows no obvious role in photoperiodic responses (El-Assal *et al.* 2001). A key regulatory function of cryptochromes is to perceive the difference between SD and LD and induce flowering under the appropriate conditions for a LD plant like Arabidopsis (Lin 2002). TFL1 downregulates cry1- and cry2-mediated promotion of flowering under SD allowing adequate adjustment of development to photoperiodic conditions (Figure 3). We propose a model where a player acting downstream of cryptochromes is able to promote flowering if TLF1 is absent but not if TFL1 is present (Figure 7). TFL1 negatively regulates cryptochrome-mediated effects and cryptochromes downregulate *TFL1* expression (Figure 2). This could provide a positive feedback mechanism on cryptochrome signaling (Figure 7).

Although cry2 enhances the expression of *FT* under LD compared to SD (Yanovsky and Kay 2002), a positive regulation of *FT* expression by cry2 occurs even under SD (Figure 4). In the presence of TFL1, the differences in *FT* expression caused by cry2 under SD do not translate into differences in flowering time (Figure 3B). *TFL1* and *FT* are homologous genes with antagonistic action (Kobayashi *et al.* 1999; Hanzawa *et al.* 2005). TFL1 activity could set a minimum threshold of *FT* activity necessary to affect flowering and this threshold would not be reached by cry2-mediated promotion of *FT* expression under SD.

Pleiotropic effects of functional *CRY1* or *CRY2* alleles become evident in the *tfl1* background. The *tfl1* loss-of-function phenotype can therefore be interpreted in terms of cry1 and cry2 gain of function. In the *tfl1* background cry1 and cry2 distort plant body form (*cf. tfl1* and *tfl1 cry1 cry2* in Figure 3A). A key function of cryptocrhomes is to promote flowering under LD compared to SD (Lin 2002) but in the *tfl1* background cry1 and cry2 strongly promote flowering under SD (Figure 3B). In other words, TFL1 negatively regulates cry1- and cry2-mediated misregulation of flowering time and the pleiotropic distortions of plant body development (Figure 7). In genetic canalization, epistatic relationships permit a phenotype to remain relatively invariant in response to mutations (De Visser *et al.* 2003; Flatt 2005). Conversely, in the case reported here, TFL1 provides homeostasis not in the context of *cry1* or *cry2* mutations but in the context of functional *CRY1* and *CRY2* alleles.

On the basis of the recently discovered role of TFL1 in the control of trafficking to the protein storage vacuoles in vegetative tissues, Sohn *et al.* (2007) have proposed that these vacuoles could store factors involved in the control of flowering and meristem maintenance, until the proper combination of developmental and environmental cues trigger their secretion and activate the flowering pathway. An alternative, although not an exclusive proposal, would be that TFL1 could target selected downstream products of cry1 and cry2 signaling to the protein storage vacuoles (Figure 7). Therefore, these products would generate maladaptive misregulations in the absence of TFL1 but not in its presence.

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