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Regulation of the *ALBINO3*-mediated transition to flowering in *Arabidopsis* depends on the expression of *CO* and *GA1*

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Abstract

ALBINO3, a homologue of *PPF1* in *Arabidopsis*, encodes a chloroplast protein, and is essential for chloroplast differentiation. In the present study, *ALBINO3(-)* transgenic plants exhibited a significant decrease in both the number of rosette leaves at bolting and the days before bolting, suggesting the important roles of ALBINO3 in regulating flowering during non-inductive short-day photoperiods. *ALBINO3* mRNA was apparently accumulated in shoot apical meristem and floral meristems around the shoot apical meristem in wild-type plants. *ALBINO3* might be predominantly involved in inducing the floral repression pathway by activating the expression of *TFL1,* and by suppressing the expression of *LFY*, respectively, in the shoot apical meristem. Moreover, the function of *ALBINO3* in regulating flowering transition depended on the expression of *CO* and *GA1*, because *ALBINO3* might function in the downstream integration of the photoperiod-dependent and the photoperiod-independent pathways. These results suggest that *ALBINO3* may have an important integrative function in the flowering process in *Arabidopsis*.

Additional key words: *LFY*, photoperiod, shoot apical meristem, *TFL1*.

Introduction

Flowering in higher plants is the point of transition from the vegetative to the reproductive stage of growth. It is one of the major developmental switches, and is believed to occur due to the sequential action of two gene groups. Floral meristem identity genes convert the vegetative apex into a floral meristem, and organ identity genes govern the formation of the flower organ (Levy and Dean 1998, Mouradov *et al*. 2002). In *Arabidopsis*, the photoperiodic dependent pathway is only responsible for floral induction on long days (LDs), while other genes required for the day-length response encode components of light signal transduction pathways or the circadian clock (Harmer *et al*. 2000, Suarez-Lopez *et al*. 2001, Simpson and Dean 2002). The vernalization promotion pathway enables flower initiation following exposure to low temperatures for several weeks, and *FLOWERING LOCUS C* (*FLC*) determines the winter-annual versus summer-annul habit as multiple floral regulatory pathways converge (Kim *et al*. 2004). Antagonistic floral repression pathways monitor the internal status of development, target *LFY* and floral meristem genes, and inhibit their expression (Chen *et al*. 1997, Bradley *et al*. 1997, Wagner *et al*. 1999, Dill *et al*. 2001).

PPF1 was cloned from the G2 pea mutant in which vegetative apical buds grow continuously for a much longer duration on short days (SDs) than on LDs (Wang *et al*. 2003, Li *et al*. 2004). Recent studies suggest that *PPF1* regulates flowering time in higher plants by encoding a putative calcium ion transporter to modulate the Ca^{2+} storage capacity within chloroplasts (Wang *et al*. 2003). Further, over-expression of *PPF1* in transgenic *Arabidopsis* plants was observed to affect the chloroplast

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Abbreviations: LD - long days; SD - short days; *FLC* - *FLOWERING LOCUS C*; LHCP - light-harvesting chlorophyll-binding protein; RT-PCR - reverse transcriptase - polymerase chain reaction.
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development; thus, the function of *PPF1* is similar to that of *ALBINO3* (Sundberg *et al*. 1997, Xu *et al*. 2002). *ALBINO3*, a homologue of *PPF1* in *Arabidopsis*, encodes a chloroplast membrane protein, and is required for posttranslational integration of the light-harvesting chlorophyll-binding protein (LHCP) into the thylakoid membrane (Sundberg *et al*. 1997, Moore *et al*. 2000). The high degree of homology between *PPF1* and *ALBINO3* suggests that *ALBINO3* may have multiple biological functions, and it is also probably involved in controlling flowering in *Arabidopsis*. In the current work, we demonstrate that *ALBINO3* may play an important role in regulating flowering time by integrating a series of floral pathways.

Materials and methods

Plants, transformation and growth conditions: *Arabidopsis thaliana* (cv. Columbia) plants were grown in fully automated growth chambers (*Conviron*, Winnipeg, Canada) at 23°C during the light period and at 21 °C during the dark period. The plants were maintained at a 9-h photoperiod (short day, SD) with an irradiance of 100 μmol m^{-2} s^{-1} using cool-white fluorescent lamps along with incandescent lamps. Some plants maintained under LD (18-h photoperiod) were specially indicated in the present study.

Transgenic *Arabidopsis* plants expressing the *ALBINO3* gene were obtained as described by Wang *et al*. (2003). The full-length *ALBINO3* gene isolated from *Arabidopsis* (cv. Columbia) was amplified by polymerase chain reaction (PCR) using primers that contain specific restriction sites and subsequently cloned into the binary vector pKF111 containing a glufosinate resistance gene in an antisense orientation. *Arabidopsis* transformation was performed by using the normal floral dip method. For the antisense construct, we selected a segment of the *ALBINO3* cDNA comprising nucleotides 279-1316. The surfaced-sterilized transgenic and wild-type cv. Columbia *Arabidopsis* seeds were grown as described above. Glufosinate ammonium (5 mg dm^{-3}, *Riededel-de Haen*, **town??**, Germany) was used for screening the transgenic plants. Transgenic lines bearing *ALBINO3(-)* constructs were selected by performing semiquantitative reverse transcriptase (RT)-PCR assays, and were confirmed to be of the T_2 generation. Plants exhibiting homology at the transgene locus were analyzed for single-copy segregation before their flowering phenotypes were characterized.

RT-PCR assay: Total RNA was extracted from wild-type Columbia *Arabidopsis* plants, harvested at noon-time, for cDNA synthesis using a *Qiagen* RNeasy kit (Hilden, Germany). The cDNA synthesis and RT-PCR were performed according to the protocol supplied by the cDNA Synthesis Kit (*Gibco BRL*, Carlsbad, USA). RNA (μg) was used as a template in each reaction. Gene-specific primers were designed for *CO* (*COa*, 5'-CGGGATAAAGTGGAGAGAACAACAGGGC-3'; *COb*, 5'-CCGGTACCCTGTTGGTTATGGCACTGGTG-3'), *LFY* (*LFYa*, 5'-ATTGGTTCAAGCACCTC-3'; *LFYb*, 5'-TTTTTCGCCACGGTCTTTAG-3'), for *TFL1* (*TFL1a*, 5'-CGGGATCCATGGGGAGA-GTGGTAGGAGAT-3'; *TFL1b*, 5'-CCGGTACCGATTCAACTCATCTTTGG CAG-3'), for *FLC* (*FLCa*, 5'-CCCCTC GAGCTTAGTA TCTCCGGCG-3'; *FLCb*, 5'-GGACTA GTCGCCCTT ATCAGCGGA-3'), and for *ALBINO3* (*ALBINO3a*, 5'-CCGATGCTATGGAATCGGTT-3'; *ALBINO3b*, 5'-TCGTCAGGCTGAGCAATAGA-3'). *UBQ* was used to determine the blotting amount for each sample, and primers specific for *UBQ* were used in the control reactions (*UBQa*, 5'-GGTGCTAAGAAGAGGAAG AAT-3'; *UBQb*, 5'-CTCCTTCTTTCTGGTAAACGT-3'). Amplification of all cDNA fragments for RT-PCR or probes was performed for 30 cycles in a *Perkin-Elmer 480* thermal cycler (Waltham, USA) using a 55 °C annealing temperature (60 °C for *ALBINO3*) and a 1-min extension. The gels were transferred onto a *Nytran* membrane (*Promega*, Madison, USA) with a *Turboblotter* (*Schleicher and Schuell*, Dassel, USA), followed by membrane baking at 80 °C for 2 h and auto-cross link in a *UV Stratalinker* (*Merck*, Darmstadt, Germany). The amplified probe fragments (~50 ng) were labelled with (α-^{32}P) dCTP using a *Primer-it II* kit (*Stratagene*, Santa Clara, USA). Members were probed with labelled DNA fragments in 0.3 M sodium phosphate buffer, pH 7.2, containing 7 % (m/v) sodiumdodecyl sulphate (SDS), 1 mM EDTA, pH 8.0, and 2 % (m/v) bovine serum albumin (BSA) at 65 °C for 16 - 24 h. Filters were washed at 65 °C twice with 2× SSC and 0.1 % (m/v) SDS for 30 min, once with 1× SSC and 0.1 % SDS for 20 min, and once with 0.5× SSC and 0.1 % SDS for 10 min. The membranes were exposed to a *Kodak XAR* film at -80 °C for autoradiography.

RNA *in situ* hybridization: The method was performed as described by Kardailsky *et al*. (1999). The samples were collected from apical inflorescences of different lines. Sections were digested by 10 μg cm^{-3} proteinase K at 37 °C for 30 min, and the reaction was blocked by 2 mg cm^{-3} glycine. Anti-sense RNA of *ALBINO3* and *LFY* labelled by digoxigenin was performed as described from *DIG* RNA labeling kit (SP6/T7) (*Boehringer-Mannheim*, Mannheim, Germany). DNA templates were linearized with restriction enzyme (*ALBINO3*: PvuII, *LFY*: Bam HI & Sal I), and then purified by phenol/chloroform, followed by ethanol precipitation. The DNA was cloned into the polylinker sites of transcription vector pSPT19, and RNA probes were

generated using T7 polymerase. Terminal concentration of probe is about 5 μg cm^{-3}. Immunological detection procedure was performed following the protocol of *Boehringer-Mannheim DIG* nucleic acid detection kit and TNM-50 buffer (100 mM Tris, pH 9.5; 100 mM NaCl, 50 mM $MgCl_2$) was selected for not using polyvinyl alcohol in the color reaction. *Anti-DIG-AP* conjugate diluted into 1:1000 with BSA washing solution (1 % BSA, 0.3 % Triton X-100, 100 mM Tris, pH 7.5, 150 mM NaCl) was added onto the slides and incubated at 37 °C for 2h. The sections were incubated with colour-substrate solution (NBT/BCIP) in darkness at room temperature. The signal was detected and analyzed under an *Olympus* (Tokyo, Japan) light microscope.

Strains constructions: Double mutants were constructed by crossing transgenic plants that were homozygous for *ALBINO3(-)* with those that were homozygous for a *co-1* or *ga1-3* or *tfl1-1* mutation. We fertilized mutant plants for *co-1* or *ga1-3* or *tfl1-1* with pollen from the *ALBINO3(-)* transgenic plants, and collected seeds from individual F1 plants. Further, we performed screening for expressing the *co-1* or *ga1-3* or *tfl1-1* phenotype among the F2 progeny. By performing RT-PCR, all double-mutant phenotypes were confirmed in the F3 generation by analyzing the segregation of double mutants obtained from F2 parents that were homozygous for only one of the mutations and expressed the *ALBINO3(-)* phenotype.

Gibberellin (GA) treatment: GA-treated plants were sprayed once a week with 0.1 mM gibberellic acid (*Sigma Chemical Co*., St. Louis, USA) and 0.02 % Tween-20 from day 30 after planting. Wild-type Columbia *Arabidopsis* plants were sprayed with 0.02 % Tween-20 only.

Statistical analysis: All data in this article were expressed as means ± SD. Graphs were generated using *Microsoft Excel* (*Microsoft Corp.*, Redmond, USA). Paired-sample *t* test were performed. Probability levels of 0.05 and 0.01 were explored to reflect the statistically significant state.

Results and discussion

Analysis of flowering time in transgenic *Arabidopsis* plants that underexpress *ALBINO3* cDNA: An *alb3*-null mutation causes seedling lethality, and the mutant plants do not develop beyond the cotyledon stage (Sundberg *et al*. 1997). Although *alb3* mutants may produce > 12 leaves and occasionally flower when cultured on specific media, they are generally infertile (Sundberg *et al*. 1997). To examine the possible roles played by *ALBINO3* in regulating the flowering time, we generated *Arabidopsis* plants transgenic for *ALBINO3* (*ALBINO3(-)*) by expressing *ALBINO3* cDNA driven by the CaMV 35S promoter in the anti-sense orientation. The number of rosette leaves at bolting and the days before bolting were determined and used to evaluate the defects in flowering time in the transgenic *Arabidopsis* plants. We used five independent *ALBINO3(-)* transgenic lines for these assays. As compared to the wild-type plants, the *ALBINO3(-)* transgenic plants exhibited a significant decrease in both the number of rosette leaves at bolting and the days before bolting (Fig. 1), suggesting that *ALBINO3* plays an important role in regulating flowering time during non-inductive SD photoperiods. However, no pronounced differences in flowering time were observed between the *ALBINO3(-)* and wild-type plants during the inductive LD photoperiods (data not shown).

Expression patterns of *ALBINO3* in *Arabidopsis*: We analyzed the expression patterns of *ALBINO3* in order to

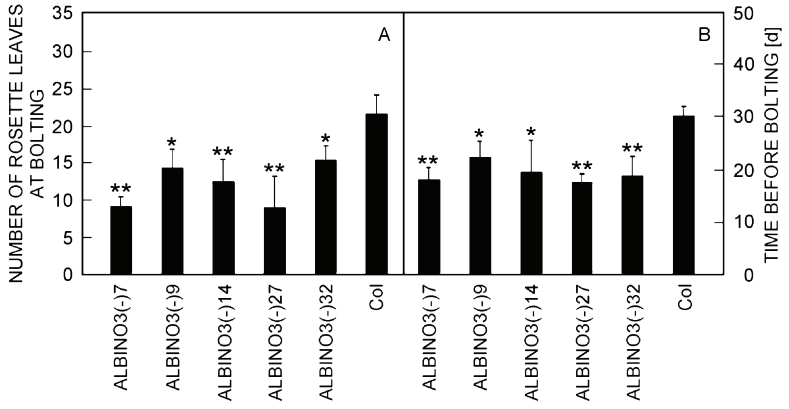

Fig. 1. Flowering time analysis using data obtained from five of independent *ALBINO3(-)* transgenic *Arabidopsis* lines, and one wild-type control, grown in short-day (SD) photoperiod (Col). *A* - Number of rosette leaves at bolting. *B* - The days before bolting. Means ± SD, $n = 30$, * - $P < 0.05$, ** - $P < 0.01$.

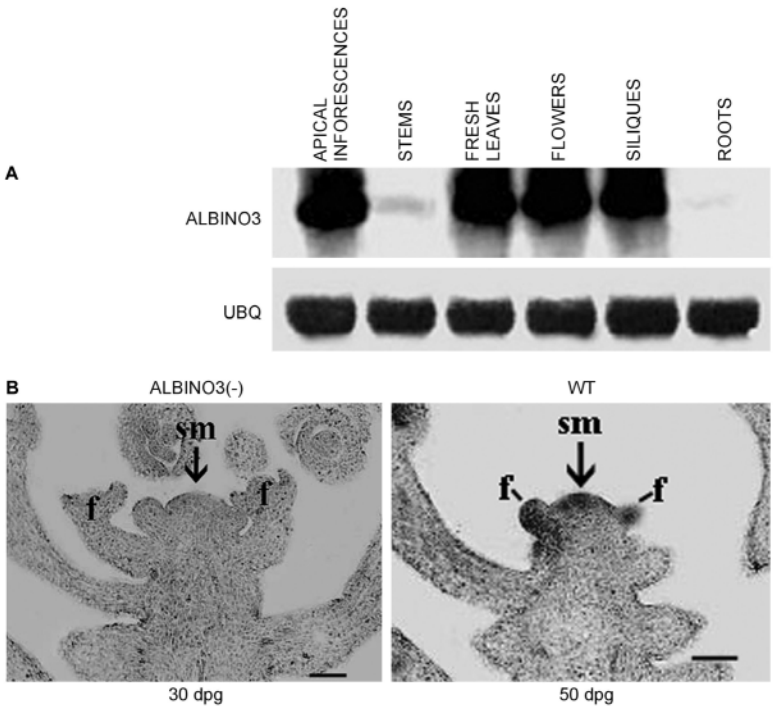

Fig. 2. Expression pattern of *ALBINO3* in *Arabidopsis*. *A* - Reverse transcription polymerase chain reaction analysis. A fragment of *UBQ* gene was amplified as a control. Samples were collected from wild-type Columbia *Arabidopsis* plants at 60 d post-germination. *B* - Expression of *ALBINO3* in apical meristem of transgenic and wild-type Columbia *Arabidopsis* plants determined by *in situ* hybridization. WT - wild-type, dpg - day post-germination, sm - shoot apical meristem, f - flower primordia. *Scale bars* = 50 μm.

investigate its role in flowering. In wild-type *Arabidopsis*, *ALBINO3* was highly expressed in the apical inflorescence, fresh leaves, flowers, and siliques, but was almost undetectable in the stems and roots, as revealed by its transcription patterns (Fig. 2*A*). The *ALBINO3* expression patterns detected in the leaves, flowers, siliques, roots, and stems were consistent with the gel blot analysis results obtained in a study by Sundberg *et al*. (1997). Moreover, we observed that *ALBINO3* was also highly expressed in the apical inflorescence, suggesting that the function of *ALBINO3* in regulating flowering time may be associated with its expression in the apical inflorescence meristem. *In situ* hybridization analysis performed using an anti-sense probe for *ALBINO3* indicated that *ALBINO3* mRNA was apparently accumulated in the shoot apical meristem as well as in the floral meristems around the shoot apical meristem in the wild-type plants (Fig. 2*B*). In contrast to this, no distinct signals of *ALBINO3* expression were observed in the apical inflorescence meristems of the *ALBINO3(-)* transgenic plants (Fig. 2*B*).

Expression patterns of flowering-related genes in the *ALBINO3(-)* transgenic and wild-type plants: To examine the role of *ALBINO3* in the flowering pathways, we used *ALBINO3(-)* transgenic lines (9.1 ± 1.5 rosette leaves at bolting, 18.1 ± 2.3 d before bolting) to further analyze the expression pattern of the flowering-related genes (Fig. 3). We observed a distinct decrease in the levels of *TFL1* transcripts in the *ALBINO3(-)* plants during development (Fig. 3*A*), indicating that *ALBINO3* may positively upstream regulate the expression of *TFL1*, a key component of the floral repression pathway (Putterill *et al*. 1995). No differences were observed between the *ALBINO3(-)* transgenic and wild-type plants at 15 d post-germination with regard to the expression of *CO*, which encodes a protein similar to the zinc finger transcription factor to induce the photoperiod-dependent pathway (Ruiz-Garcia *et al*. 1997). On the other hand, *CO* expression was almost undetectable early at 30 d post-germination in the *ALBINO3(-)* plants, suggesting that *ALBINO3* does not regulate the upstream expression of *CO* but may be required for the maintenance of its expression during development (Fig. 3*A*). In addition, we did not detect marked differences between the *ALBINO3(-)* and wild-type plants with regard to *FLC* expression (Fig. 3*A*), suggesting that *ALBINO3* may not be involved in controlling the vernalization promotion pathway (Noh and Amasino 2003).

Moreover, we observed increased levels of *LFY* transcripts in the *ALBINO3(-)* plants as compared to the wild-type plants (Fig. 3*A*). *LFY*, a floral meristem gene, regulates both the photoperiod-dependent and photoperiod-independent pathways downstream to activate the flowering process (Levy and Dean 1998, Wagner *et al*. 1999). The *LFY* gene expression detected by *in situ* hybridization indicated that *LFY* mRNA was largely accumulated in the lateral flower primordia and shoot

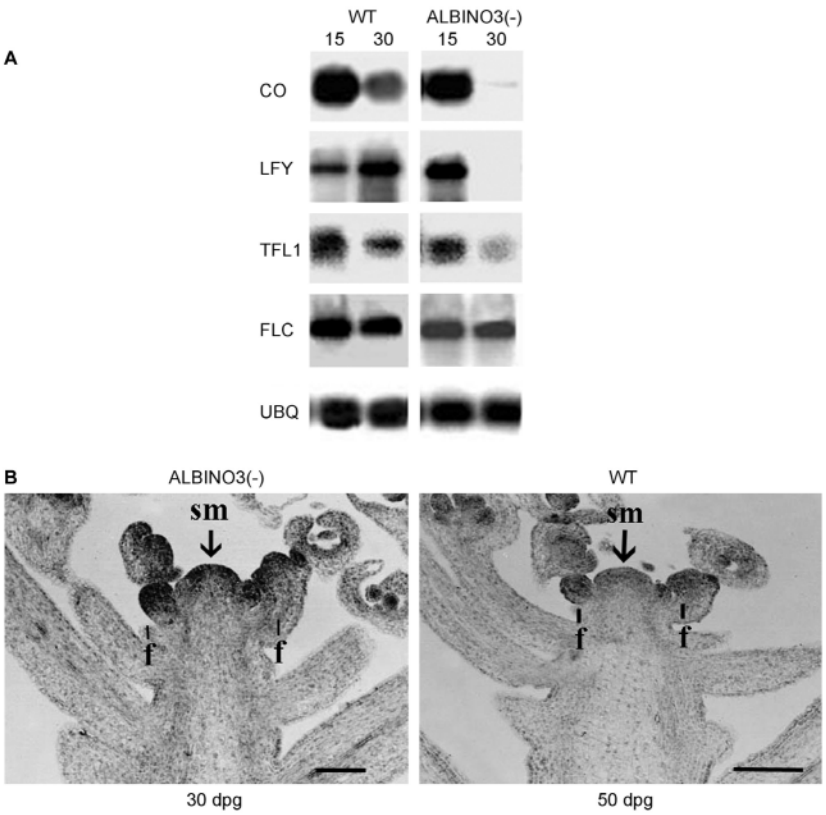

Fig. 3. Floral related genes expression in transgenic and wild-type *Arabidopsis* plants. *A* - Floral related genes expression by reverse transcription polymerase chain reaction assay. A fragment of *UBQ* gene was amplified as a control. *B* - Expression of *LFY* in apical meristem of transgenic and wild-type Columbia *Arabidopsis* plants determined by *in situ* hybridization. WT - wild-type, dpg - day post-germination, sm - shoot apical meristem, f - flower primordia. *Scale bars* = 50 μm.

apical meristems in the *ALBINO3(-)* transgenic plants as compared to control plants, indicating a potential transition from the shoot apical meristem to the floral meristem in the *ALBINO3(-)* plants (Fig. 3*B*). These data demonstrate that *ALBINO3* may inhibit or even block *LFY* expression in apical inflorescence meristems as well as in newly formed floral meristems around the apical meristems.

Interaction between *ALBINO3* and *TFL1*: Since the results described above indicates that *ALBINO3* may positively regulate *TFL1* expression, we investigated whether *TFL1* may be the downstream target of *ALBINO3* to mediate its function in negatively regulating the flowering process. *TFL1* induces the floral repression pathway to directly suppress expression of the floral meristem gene *LFY* (Kobayashi *et al*. 1999). Two lines of evidence indicated that *TFL1* may be regulated by *ALBINO3* upstream in the floral repression pathway. First, the flowering phenotypes of the *ALBINO3(-) tfl1-1* double mutant plants (8.9 ± 1.1 rosette leaves at bolting, 18.4 ± 1.5 d before bolting) were similar to those of the *ALBINO3(-)* single mutant plants but dissimilar to those of the *tfl1-1* single mutant plants (6.5 ± 2.7 rosette leaves at bolting, 15.9 ± 3.7 d before bolting) (Fig. 4*A,B*). Second, the *LFY* mRNA expression pattern in the *ALBINO3(-) tfl1-1* double mutant plants was similar to that in the *ALBINO3(-)* single mutant plants and contrasting to that in the *tfl1-1* single mutant plants (Fig. 4*C*). In addition, we observed an increased expression of *TFL1* in *ALBINO3*-overexpressing transgenic *Arabidopsis* plants (data not shown). Thus, we conclude that *ALBINO3* may participate in regulating the floral repression pathway and may inhibit *LFY* expression by positively upstream regulating the expression of *TFL1*.

Interaction between *ALBINO3* and *CO*: In *Arabidopsis*, the transition to flowering is regulated *via* the integration of various signaling pathways. To investigate the possible roles played by *ALBINO3* in the photoperiod-dependent pathway, we further analyzed the genetic interaction between *ALBINO3* and *CO* by constructing *ALBINO3(-) co-1* double mutants. The flowering phenotypes of the *ALBINO3(-) co-1* double mutant plants during SD (20.2 ± 3.2 rosette leaves at bolting, 30.8 ± 2.7 d before bolting) were similar to those of the *co-1* single mutant plants (21.9 ± 3.1 rosette leaves at bolting, 31.2 ± 2.2 d before bolting) but dissimilar to those of the *ALBINO3(-)* single mutant plants (Fig. 5*A,B*); this suggests that *ALBINO3* may be regulated by *CO* upstream in the

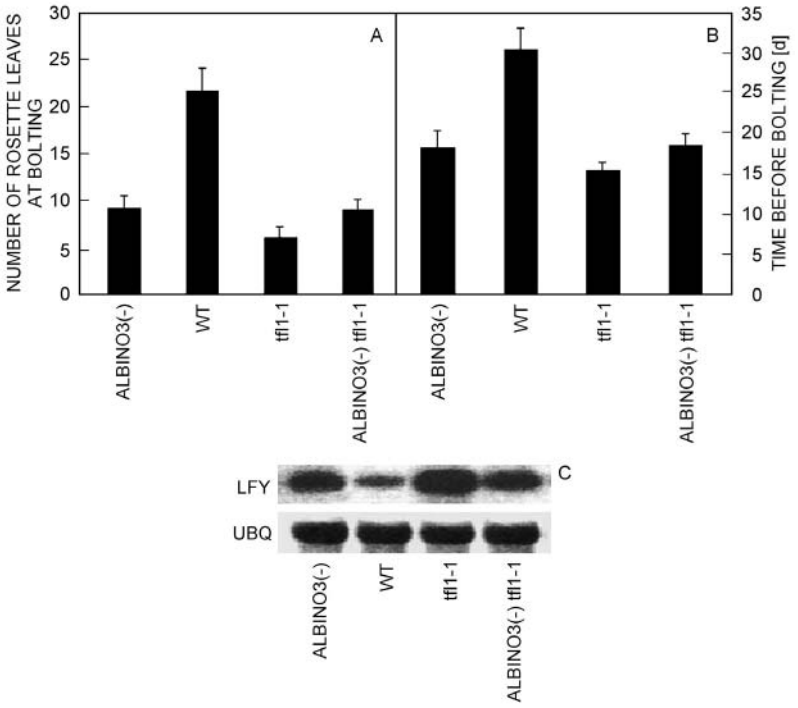

Fig. 4. Genetic interaction between *ALBINO3* and *TFL1*. WT - wild-type Columbia *Arabidopsis* plants. Number of rosette leaves at bolting (*A*) and the days before bolting (*B*) for the *ALBINO3(-) tfl1-1* double mutants. Means ± SD, $n = 30$. *C* - Reverse transcription polymerase chain reaction analysis for the effects of *ALBINO3(-) tfl1-1* double mutation on *LFY* mRNA expression. A fragment of *UBQ* gene was amplified as a control. Samples were collected at 25 d post-germination.

photoperiod-dependent pathway and that *CO* is essential for the *ALBINO3*-mediated flowering phenotype. Further, the *LFY* mRNA expression in the *ALBINO3(-) co-1* double mutant plants was similar to that in the SD-grown *co-1* single mutant plants, and its level was not as high as that in the *ALBINO3(-)* single mutant plants (Fig. 5*C*).

Interaction between *ALBINO3* and *GA1*: Expression of *PPF1* can be stimulated in G2 pea mutant plants under both LD and SD conditions (Wang *et al*. 2003, Zhu *et al*. 1998). In *Arabidopsis*, gibberellic acid (GA_3) promotes flowering *via* coordinated interaction with *cis* elements on the *LFY* promoter (Blazquaz and Weigel 2000). In the present study, exogenous application of GA_3 was observed to enhance the expression of both *ALBINO3* and *LFY* in the wild-type *Arabidopsis* plants (Fig. 6*C*); this suggests the possible involvement of *ALBINO3* in the photoperiod-independent pathway. To verify this possibility, we constructed *ALBINO3(-) ga1-3* double mutants. *GA1*, which encodes cyclase ent-kaurene synthetase A, defines the photoperiod-independent pathway that negatively regulates the response to GA_3 (Sun and Kamiya 1994, Peng *et al*. 1997). The flowering phenotypes of the *ALBINO3(-) ga1-3* double mutant plants (23.7 ± 2.4 rosette leaves at bolting, 47.5 ± 1.6 d before bolting) were similar to those of the *ga1-3* single mutant plants 25.7 ± 1.8 rosette leaves at bolting, 50.7 ± 1.2 d before bolting) but dissimilar to those of the *ALBINO3(-)* single mutant plants (Fig. 6*A,B*), suggesting that *ALBINO3* may also be regulated by *GA1* upstream in the photoperiod-independent pathway. Furthermore, the *LFY* mRNA expression pattern in the *ALBINO3(-) ga1-3* double mutant plants was similar to that in the *ga1-3* single mutant plants but dissimilar to that in the *ALBINO3(-)* single mutant plants (Fig. 6*D*). Thus, *ALBINO3* may be regulated by *GA1* upstream in the photoperiod-independent pathway, and its function in regulating the transition to flowering may depend on *GA1* expression. In other words, *ALBINO3* may play an important role in the downstream integration of both the photoperiod-dependent and -independent pathways to further negatively regulate the expression of floral meristem genes such as *LFY* and the flowering.

Flowering is controlled by developmental regulation and environmental conditions. *Arabidopsis* is an excellent model organism to approach this complexity, which flowers earlier under LDs but eventually flowers under SDs. Studies in *Arabidopsis* have led to the identification of components within individual signaling pathways involved in flowering control. Our results suggest that *ALBINO3*-mediated signaling is involved in regulating the transition to flowering; it plays an important role in integrating the photoperiod-dependent and -independent pathways as well as in the floral repression pathway. In other words, *ALBINO3* functions as a suppressor during the flowering process in *Arabidopsis*. Thus, in this study,

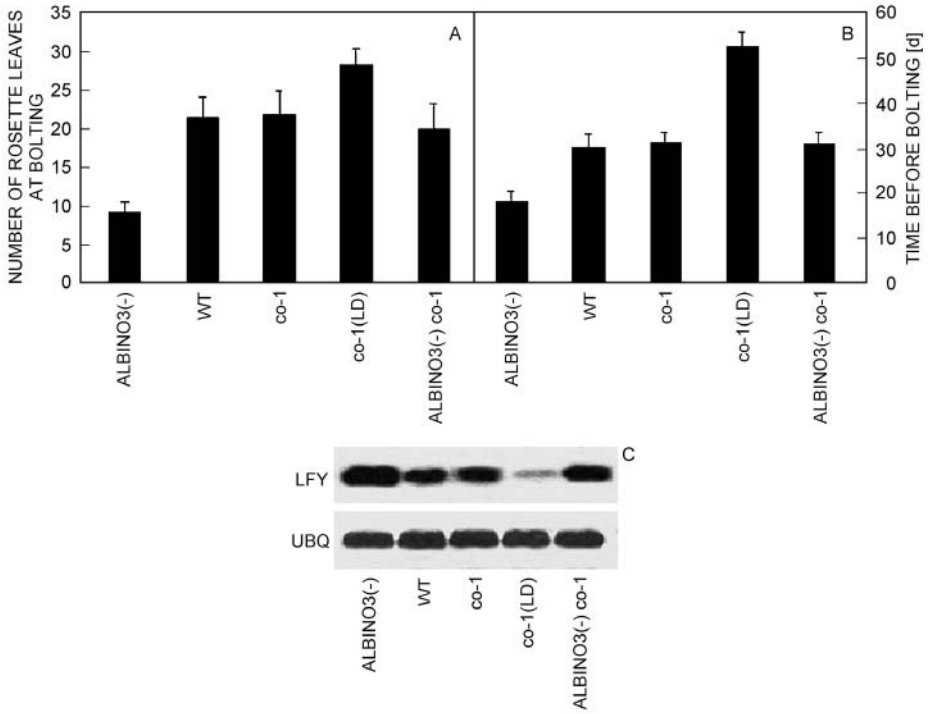

Fig. 5. Genetic interaction between *ALBINO3* and *CO*. WT - wild-type *Arabidopsis* Columbia plants. Number of rosette leaves at bolting (*A*) and the days before bolting (*B*) for the *ALBINO3(-) co-1* double mutants. Means ± SD, $n = 30$. *C* - Reverse transcription polymerase chain reaction analysis for the effects of *ALBINO3(-) co-1* double mutation on *LFY* mRNA expression. A fragment of *UBQ* gene was amplified as a control. Samples were collected at 25 d post-germination.

we have identified a new participant in the floral repression pathway, facilitating a more detailed understanding of the complex regulation network involved in plant flowering. Moreover, we observed that a feedback mechanism is involved in the regulation of *ALBINO3* and *TFL1* (Wang *et al*. personal communication). Thus, the function of *ALBINO3* may be coupled with that of *TFL1* in *Arabidopsis*.

ALBINO3 was originally observed to function in regulating the chloroplast differentiation in *Arabidopsis*. Chloroplasts of the *alb3* mutants are abnormal, and contain reduced levels of chlorophyll (Sundberg *et al*. 1997). Because *alb3* does not prevent expression of the genes encoding the chlorophyll *a*/*b* binding protein or the small subunit of ribulose-bisphosphate carboxylase, the chloroplasts of *alb3* mutants are not entirely nonfunctional (Sundberg *et al*. 1997). *ALBINO3* functioning is required for posttranslational integration of the LHCP into the thylakoid membranes; a model that was created based on this indicated that LHCP can be integrated into the thylakoid membrane using a specific translocase that contains ALBINO3 (Moore *et al*. 2000). In addition, *ALBINO3*, when expressed in bacteria, is essential for the insertion of the chloroplast cpSecE protein into the inner bacterial membrane (Jiang *et al.* 2002). However, based on the fact that a majority of the thylakoid proteins in plants do not require *ALBINO3* or any other known type of translocation apparatus (Woolhead *et al*. 2001, Mant *et al*. 2001), *ALBINO3* may have multiple biological functions in plant development. In the present study, we provided evidence that *ALBINO3* plays an important role in regulating flowering.

Moreover, our data suggest that the function of *ALBINO3* depends on the expression of *CO* and *GA1* in *Arabidopsis*. *CO* is the latest acting of the known genes that is reported to be specific to the photoperiod-dependent pathway in response to LDs (Ruiz-Garcia *et al*. 1997)**.** *GA1* encodes an enzyme that catalyzes the first committed step in GA biosynthesis and defines the photoperiod-independent pathway (Sun and Kamiya in the integration between these two pathways simultaneously in *Arabidopsis*. The levels of *ALBINO3* expression in wild-type plants taken at 14-h different time points during a 24-h cycle (12-h photoperiod) did not change significantly, and exposure to light after several days of dark treatment did not increase the abundance of the *ALBINO3* mRNA (Sundberg *et al*. 1997). In the current work, our data suggest that the expression of *CO* in *Arabidopsis* plants under SD conditions might be also very important for the flowering transition control, because mutation of *CO* could obviously suppress the early-flowering phenotype of *ALBINO3(-)* transgenic strain (Fig. 5). In addition, the level of *ALBINO3* expression in seedlings cultured on media supplemented with GA_3 was the same as for seedlings cultured without hormones (Sundberg *et al*. 1997), whereas exogenous

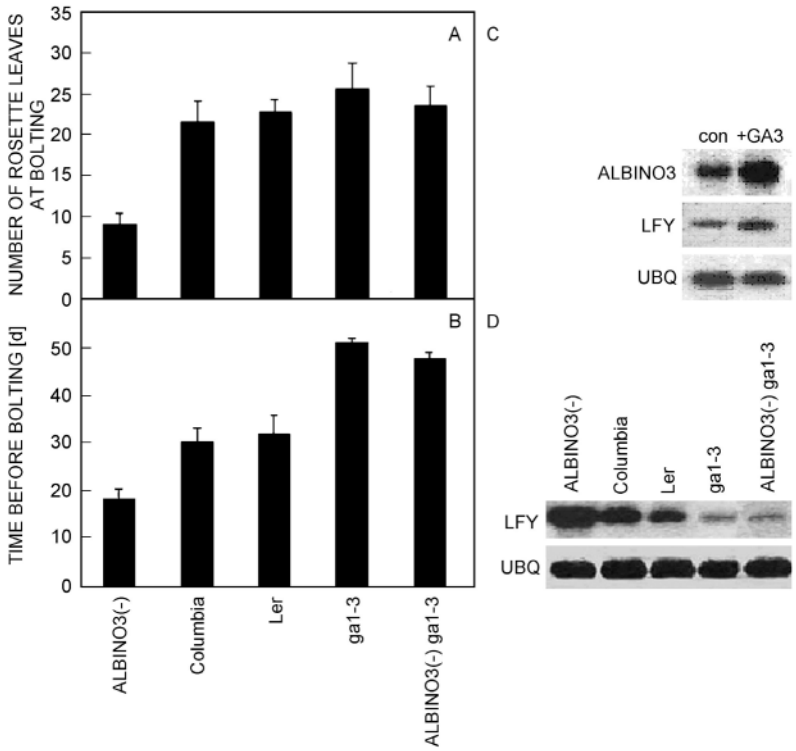

Fig. 6. Genetic interaction between *ALBINO3* and *GA1*. Number of rosette leaves at bolting (*A*) and the days before bolting (*B*) for the *ALBINO3(-) gal-3* double mutants. Means ± SD, n = 30. *C* - Reverse transcription polymerase chain reaction analysis of *ALBINO3* and *LFY* expression after exogenous GA_3 application. A fragment of *UBQ* gene was amplified as a control. Apical inflorescences of wild-type *Arabidopsis* Columbia were sprayed with 0.1 mM GA_3 solution from 30 d post-germination and treated for 5 d. con - control. *D* - Reverse transcription polymerase chain reaction analysis for the effects of *ALBINO3(-) ga1-3* double mutation of on *LFY* mRNA expression. A fragment of *UBQ* gene was amplified as a control. Samples were collected at 25 d post-germination.

application of GA_3 enhanced the expression of both *ALBINO3* and *LFY* in the wild-type Columbia *Arabidopsis* plants at 30 d post-germination, suggesting the function of *ALBINO3* in regulating flowering may be stage-specific. Several studies also described the genetic interactions between the GA_3 pathway and other flowering-time pathways in *Arabidopsis* (Mouradov *et al*. 2002).

In addition, *PPF1* is a homologue of *ALBINO3* in pea that possibly encodes a putative calcium transporter in order to control the calcium storage capacity within the chloroplasts in plant cells (Wang *et al*. 2003). Ca^{2+} is considered to function as a secondary messenger in signal transduction pathways (Bush 1995, Sanders *et al*. 2002). Thus, the calcium storage capacity or the cellular dynamics of Ca^{2+} may play a role in regulating or inducing flowering. In this case, what are the possible biochemical functions of *ALBINO3* in Ca^{2+} signaling, and what is the relationship between posttranslational integration of the LHCP into the thylakoid membranes and Ca^{2+} signaling in *Arabidopsis*? The relationship between Ca^{2+} signaling and flowering pathways is an interesting issue that should be further elucidated. Research regarding the functions of *ALBINO3* and its homologues could aid in solving this issue.

In conclusion, in this project, we identified a new component for the repressing pathway in the flowering transition control. In addition, we investigated the possible genetic interaction of *ALBINO3* with genes involved in the flowering control in *Arabidopsis*. Recently, many more genes have been identified and found to be involved in the control of flowering transition or floral development in *Arabidopsis* and other plants (Chen *et al*. 2007). Systematic analysis of the functions of *ALBINO3* and other genes in accelerating or suppressing the flowering and senescence will make for better understanding of the molecular mechanisms of flowering transition and senescence inhibition in plants.

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