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Functional Requirement of Plant Farnesyltransferase during Development in Arabidopsis

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Arabidopsis *era1* was identified as an abscisic acid–hypersensitive mutant caused by disruptions or deletions of the gene for the β subunit (*AtFTB*) of farnesyltransferase (FTase). The heterodimeric enzyme catalyzes the covalent attachment of the 15-carbon farnesyl diphosphate to the C terminus of regulatory proteins and is essential for growth in yeast. The first disruption of *FTB* in a multicellular context revealed several developmental and growth regulatory processes that require the function of FTase. The lack of FTase activity in the Arabidopsis *era1-2 FTB* deletion mutant resulted in enlarged meristems and organs, supernumerary organs in floral whorls, arrested development of axillary meristems, late flowering, and homeotic transformations of flowers. Complementation of *era1-2* with *LeFTB*, the tomato gene for the β subunit of FTase, restored a normal phenotype and confirmed that the lesion is in *AtFTB* alone. The effect of this lesion on control of meristem size and on developmental processes suggests the involvement of regulatory proteins that require farnesylation for their function. At least three distinct processes that require the function of FTase were identified: regulation of cellular differentiation in the meristems, meristem maintenance, and regulation of flower development. Together, these results provide a basis for future studies on the involvement of FTase in specific developmental processes and for structure–function analysis of FTase in vivo.

INTRODUCTION

Protein prenyltransferases catalyze the covalent attachment of the 15-carbon farnesyl diphosphate (FPP) and the 20-carbon geranylgeranyl diphosphate (GGPP), two early intermediates of the mevalonate pathway, to the C terminus of a small group of proteins. This unique protein modification mechanism is conserved in all eukaryotic cells. Most of the known prenylated proteins have key regulatory roles in signaling, cell cycle regulation, and vesicle transport, suggesting a functional link between cellular growth control and cytoplasmic isoprenoid biosynthesis (Zhang and Casey, 1996; Rodríguez-Concepción et al., 1999a; Yalovsky et al., 1999). Since the demonstration that inhibition of prenylation could reverse oncogenic proliferation of mammalian cells transformed with activated Ras mutants (James et al., 1993; Kohl et al., 1993, 1995), research has focused on the structure and enzymatic mechanisms of prenyltransferases in animals and yeast. As a result, comparatively less information is available on the function of prenyltransferases during development and the role of protein prenylation in plants (Yalovsky et al., 1999).

A single farnesyltransferase (FTase) and two geranylgeranyltransferases (GGTase-I and RabGGTase) have been identified in yeast, animals, and plants. FTase and GGTase-I are heterodimeric enzymes with a common α subunit but distinct β subunits that determine substrate specificity. Both enzymes recognize a conserved C-terminal amino acid sequence motif known as the CaaX box (Schafer and Rine, 1992; Zhang and Casey, 1996; Rodríguez-Concepción et al., 1999a; Yalovsky et al., 1999). Mutants of *Saccharomyces cerevisiae* in which the β subunit of FTase is deleted show a temperature-dependent inhibition of cell cycle activity (Trueblood et al., 1993) that can be fully complemented by expression of the plant enzyme (Yalovsky et al., 1997). In synchronized tobacco tissue culture cells, an increase in FTase activity is coincident with the initiation of cell division. Moreover, addition of manumycin, a specific inhibitor of FTase, blocks cell division (Morehead et al., 1995; Qian et al., 1996). These experiments suggest that as with yeast, FTase activity in plants is required during entry into the cell cycle. Although several candidate plant protein substrates

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of FTase and GGTase-I have been identified (Rodríguez-Concepción et al., 1999a; Yalovsky et al., 1999), the proteins that require FTase activity during the cell cycle are still unknown.

Unlike results obtained from yeast and tissue culture cells, less information is available on the function of FTase and GGTase-I in a multicellular context. A screen in Drosophila for mutations that suppress the rough eye phenotype induced by activated Ras1 (*sev-Ras1^{V12}*) identified the gene for the β subunit of GGTase-I (*GGT-Iβ*; Therrien et al., 1995). Drosophila GGTase-I is required for prenylation and membrane localization of Ras1, thus explaining the reduction in Ras1^{V12} activity. Several recessive mutations in *GGT-Iβ* are lethal, however, suggesting that GGTase-I activity is also required during development. In contrast to the growth defects of FTase and GGTase-I mutants in yeast and Drosophila, mutations in Arabidopsis that affect FTase activity are not lethal. The mutant *era1* (for enhanced response to abscisic acid [ABA]) was isolated in a screen for plants that showed a hypersensitive response to the plant hormone ABA (Cutler et al., 1996). To germinate, *era1* seeds require at least 4 days of cold treatment (vernalization); however, germination is blocked in the presence of exogenous ABA at 0.3 μM or higher. Activation of anion channels in *era1* stomatal guard cells is also hypersensitive to ABA, and the lack of FTase activity can suppress the ABA-insensitive mutants *abi1* and *abi2* (Pei et al., 1998). Together, these results indicate that FTase function is required for ABA signaling, but the proteins likely to be farnesylated by the enzyme have not been identified.

Because the lack of FTase in Arabidopsis does not cause lethality, *era1* provides a unique opportunity to examine a requirement for FTase activity in a multicellular organism. Closer examination of *era1* plants reveals several developmental abnormalities that can be explained only partially by the ABA-hypersensitive phenotype. Here, we present results from a detailed analysis of *era1-2*, an allele in which the entire *ERA1* gene (*AtFTB*) is deleted (Cutler et al., 1996). We have identified at least three regulatory processes in Arabidopsis shoot development that require a functional FTase. These should lay the basis for future studies in which specific functions can be attributed to the protein substrates of FTase in plants.

RESULTS

Loss of FTase Alters Growth and Development in Arabidopsis *era1-2*

Arabidopsis *era1-2* was produced by fast-neutron bombardment and contains a deletion of ~7.5 kb encompassing *AtFTB*, which encodes the β subunit of FTase. Mutant plants thus have no detectable FTase activity and show an enhanced response to exogenous ABA, suggesting that in Arabidopsis, FTase can act as a negative regulator of ABA sensitivity (Cutler et al., 1996). We investigated other functions that require FTase activity and that therefore might be compromised in *era1-2* by growing plants in long-day (16-hr-light/8-hr-dark) and short-day (8-hr-light/16-hr-dark) regimes. Figure 1 shows that *era1-2* differed from wild-type plants, with phenotypes being more pronounced when the mutants were grown in short-day conditions (see Figure 2). Most strikingly, rosette leaves (Figure 1A), petals, and sepals (Figure 1C) were markedly larger and increased in number, suggesting that FTase acts on a negative regulator of leaf growth and that loss of enzyme activity results in increased cell division or cell elongation. The number of stamens was normal (six), but carpel number occasionally increased from two to three (see also Figure 3).

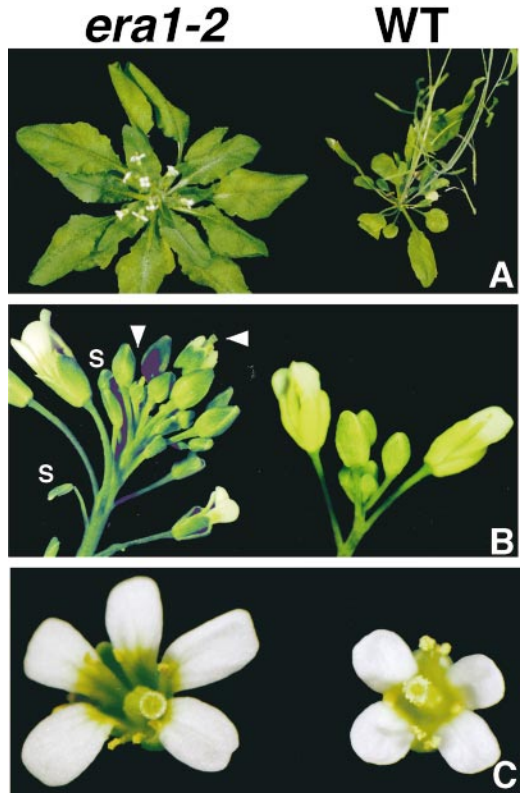

Figure 1. Developmental Phenotypes in *era1-2*.

(A) Representative *era1-2* and wild-type (WT) plants grown under long-day (16-hr-light/8-hr-dark) conditions. The inflorescence and rosette leaves are markedly larger in *era1-2*.

(B) A close-up of the inflorescences shows that the *era1-2* inflorescence is much enlarged and has an altered phylotaxy. Often flowers develop that consist only of sepaloid structures (S). The arrowheads indicate elongated gynoecia that protrude from immature flowers. Note that the *era1-2* inflorescence stem is fasciated.

(C) Flowers with increased numbers of petals and sepals (not shown) are typical on long-day-grown *era1-2* plants.

Figure 2. Developmental Alterations Are Enhanced in *era1-2* Plants Grown in Short-Day Conditions.

(A) Cauline leaves on *era1-2* plants are markedly enlarged, and axillary shoot development is partially blocked. Arrowheads indicate sepaloid flowers (upper) and a leaf that developed instead of an axillary inflorescence (lower).
(B) Sepaloid flowers (S) and filament-like structures (arrowhead) often develop on the fasciated inflorescence stem instead of normal flowers.
(C) The arrowhead points to an abnormal flower that terminates an axillary shoot. These altered floral structures are typical on *era1-2* plants grown under short-day conditions.
(D) A close-up of the flower in **(C)** shows that it has lost the regular organization into four whorls and is composed entirely of carpelloid leaves on which ovules have formed (arrowhead).

In addition to the size differences between *era1-2* and the wild type, other developmental phenotypes were apparent. Figure 1B presents a close-up of an *era1-2* inflorescence stem showing altered phylotaxy, increased inflorescence size, and the sepaloid flowers that frequently develop instead of normal flowers. The arrows point to elongated gynoecia that typically protrude from immature flowers. In short-day-grown *era1-2* plants (Figure 2A), cauline leaves are also enlarged, the stem is often fasciated, and axillary shoot development is partially blocked. The arrow in Figure 2B points to a filament-like structure that often develops instead of flowers. Abnormal flowers that terminated the axillary shoot (Figure 2C, arrow) are sometimes found in the population of mutant plants grown under short-day conditions. Instead of the regular organization into four whorls, these flowers consist of carpelloid leaves on which ovules have formed (Figure 2D, arrowhead).

In summary, several aspects of the normal developmental program are altered in *era1-2*, including control of organ size and numbers during the vegetative phase and in the outer two whorls of flowers, restriction of inflorescence stem expansion, and phylotaxy of axillary branches and flowers. Moreover, formation of flowers and axillary branches is partially blocked, and flowers frequently fail to produce reproductive organs and occasionally even undergo homeotic transformations. Together, these complex phenotypic changes observed in *era1-2* suggest that loss of FTase activity affects specific developmental controls, most probably by compromising regulatory proteins that require farnesylation for their function.

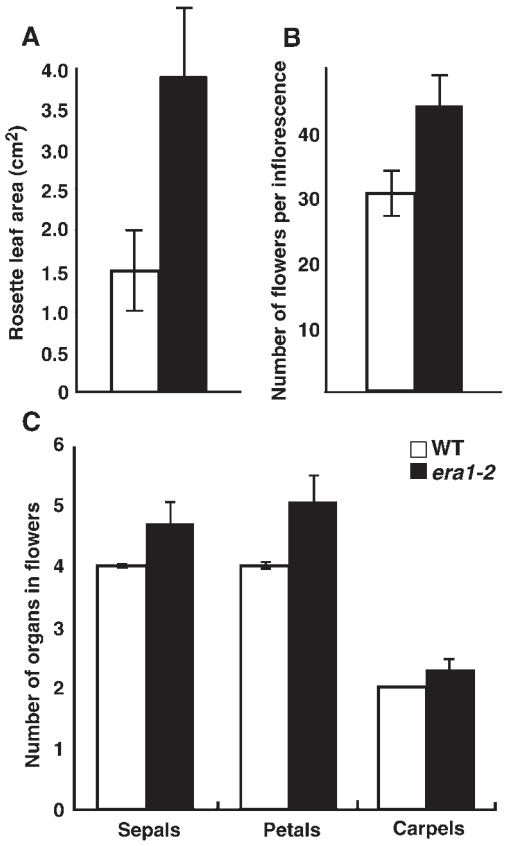

Figure 3. Organ Numbers and Size Are Increased in *era1-2* Plants in Long-Day Growing Conditions.

(A) The sizes of rosette leaves of the same developmental stage from 25 wild-type (WT) and *era1-2* plants, measured as described in Methods, differed significantly (χ^2 test, $P < 0.001$).
(B) The number of flowers on the primary inflorescence stem, determined in 25 wild-type and *era1-2* plants, also differed significantly (χ^2 test, $P < 0.001$).
(C) The numbers of sepals, petals, and carpels determined in 100 flowers of wild-type and *era1-2* plants differed significantly (χ^2 values; sepals, $P < 0.05$; petals, $P < 0.001$; carpels, $P < 0.05$). The differences in stamen numbers were insignificant ($P > 0.95$).
Error bars indicate SD.

Organ Size and Numbers Are Significantly Increased in *era1-2*

A detailed quantitative analysis was performed to establish that the developmental changes in *era1-2* plants were not simply the result of stochastic deviations within a given plant population. Figure 3 confirms that *era1-2* plants had larger rosette leaves, more flowers, and more sepals, petals, and carpels per flower. The average size of *era1-2* rosette leaves was 2.5-fold that of comparable wild-type leaves (Figure 3A). The number of flowers in *era1-2* inflorescences was increased 1.5-fold (Figure 3B), and ~50% of the flowers had increased numbers of organs in whorls 1, 2, and 4 (Figure 3C). The average number of petals increased from four to five, with flowers occasionally containing eight petals, and the number of sepals increased to an average of 4.5 per flower. Carpel number increased only slightly, averaging 2.3 per flower. In contrast, the average number of stamens was identical in *era1-2* and wild-type plants. Chi-square analysis (Figure 3) confirmed that the differences in leaf size, flower number, and the numbers of organs within the flowers were significant and not a result of stochastic deviations within the plant population. Unlike some of the morphological changes, the differences in organ size and numbers between *era1-2* and wild-type plant populations were independent of growth conditions because *era1-2* plants that were grown under short-day conditions deviated from a wild-type population in a similar manner.

era1-2 Has Enlarged Meristems

Increase in organ number and size is often related to expansion of meristems in Arabidopsis (Clark, 1997), and lack of FTase in *era1-2* may also affect control of meristem size. Meristems isolated from *era1-2* and wild-type plants were analyzed using confocal laser scanning microscopy to determine whether meristem size was increased in *era1-2*. To provide a reference, we established a line in vegetative and inflorescence meristems, which originated at the youngest primordium, P1, and traversed the meristem in the broadest plane of a median section (Figure 4). In wild-type inflorescence meristems, this line was ~65 μm long and traversed 11 to 13 cells (Figure 4A). In the *era1-2* inflorescence meristems, the length of this line ranged from 85 to 120 μm and traversed between 14 and 21 cells (Figure 4B). A similar line was established to determine the longitudinal size of inflorescence meristems between the tip of the meristem and the line from P1. This longitudinal line in wild-type inflorescence meristems was ~20 μm long and traversed three or four cells (Figure 4A). The comparable line in *era1-2* ranged between 30 and 40 μm long and traversed four to six cells (Figure 4B). Figures 4C and 4D show an additional example of the size differences between *era1-2* and wild-type inflorescence meristems. Consistent with these observations, the number of optical sections required to reconstruct a three-dimensional image of the inflorescence meristem (Figures 4E and 4F) was greater in *era1-2* than in the wild type at the same magnification (data not shown). Similar size differences were found between vegetative meristems in *era1-2* and wild-type plants (Figures 4G and 4H). Together, these findings demonstrate that the lack of FTase activity in *era1-2* results in enlargement of vegetative and inflorescence meristems. This enlargement correlates with an increase in cell number but not in cell size, a situation similar to that previously described for *clavata* (*clv*) meristems.

Comparison of stage 4 flowers (Smyth et al., 1990) revealed a similar increase in the size of *era1-2* floral meristems relative to wild-type floral meristems (data not shown). This enlargement of the floral meristems in *era1-2* could explain the increased number of flower organs in whorls 1 and 2 (Figure 1C).

Interactions between *ERA1* and *LEAFY*, a Gene Controlling Flower Meristem Identity

Under short-day growth conditions, *era1-2* flowers occasionally showed homeotic transformations (Figure 2D), which resembled the phenotype of flowers in *leafy* (*lfy*) mutants. To determine whether these homeotic transformations resulted from the lack of FTase regardless of growth conditions, we crossed *era1-2* with a weak mutant allele of *LFY*, *lfy-5*. *LFY* encodes a transcription factor required for floral meristem identity and for activation of floral organ identity genes (Weigel et al., 1992; Parcy et al., 1998). When grown at 25°C, *lfy-5* flowers developed only one or two petals and had petal/sepal mosaics and carpeloid sepals (Weigel et al., 1992; see also Figures 5A and 5B). The *lfy-5* phenotype was enhanced at 16°C, a condition in which secondary flowers developed, but petals did not form and most of the flower organs were transformed into leaves, carpels, or carpeloid leaves (Weigel et al., 1992).

In the *era1-2 lfy-5* double mutant, the phenotype of *lfy-5* at 16°C was enhanced at 25°C. Developing flowers formed only leaves, carpels, and carpeloid leaves (Figures 5C, 5F, and 5G). Secondary flower formation was observed on some of the flowers, indicating an incomplete conversion of the inflorescence meristem to a floral meristem (Figures 5F and 5G). In extreme cases, the primary inflorescence was arrested after the first flower developed (Figures 5C and 5D). Interestingly, in two plants, the inflorescence meristem became fasciated, giving rise to numerous filaments (Figures 5D, 5E, and 5H to 5J). Similar fasciation was observed occasionally in *era1-2* plants that experienced stress from adverse environmental conditions such as high light or aphid attack (data not shown), but that was not the case for the *era1-2 lfy-5* double mutants. The enhancement of the weak *lfy-5* phenotype in the *era1-2* background suggests that Arabidopsis FTase plays a critical role during the initiation of flower development. The requirement for FTase activity was most apparent in plants in which the initiation of floral devel-

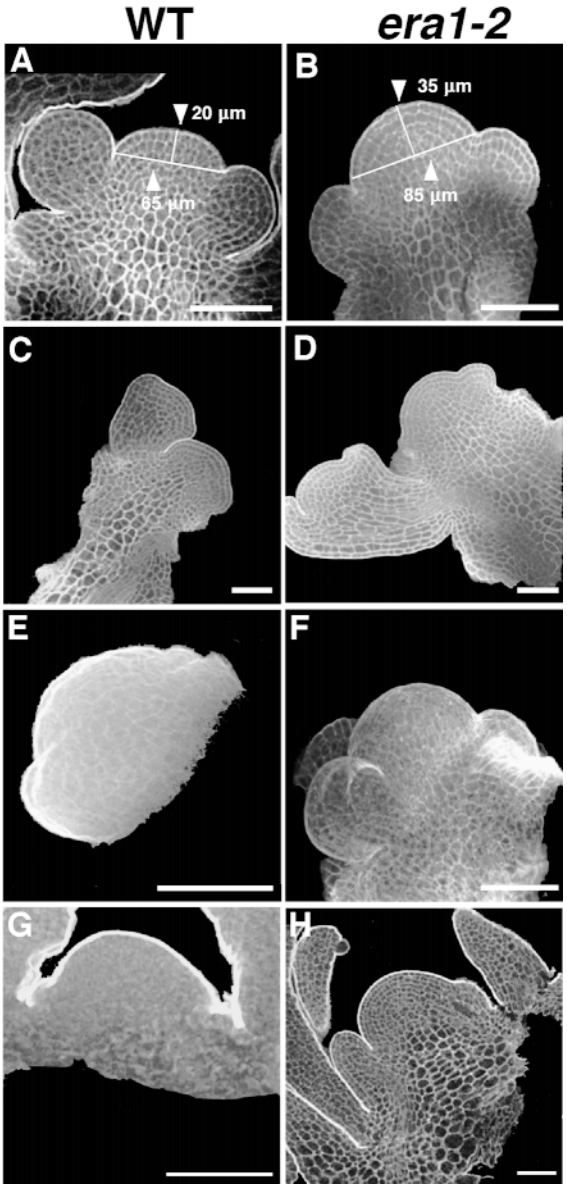

Figure 4. *era1-2* Has Enlarged Meristems.

Fifteen vegetative and inflorescence meristems and 30 floral meristems from either wild-type (WT) or *era1-2* plants were examined with a confocal laser scanning microscope. Examples are shown of optical sections through vegetative meristems of wild-type and *era1-2* plants grown under long-day (16-hr-light/8-hr-dark) growth conditions. Meristems were stained with acryflavine and viewed with a Zeiss R510 confocal laser scanning microscope, as described in Methods.

(A) and **(B)** Lines were established in inflorescence meristems as reference points (see Results) to illustrate and measure the increased size of *era1-2* meristems.

(C) and **(D)** An additional example of size difference between *era1-2* and wild-type inflorescence meristems.

(E) and **(F)** Three-dimensional reconstruction of inflorescence meristems clearly demonstrates the larger size of the *era1-2* inflorescence meristem.

(G) and **(H)** Examples of vegetative meristems in wild-type and *era1-2* plants.

Bars in **(A)** to **(H)** = 50 μM.

opment was compromised by loss of genetic functions (e.g., *lfy-5*) or adverse environmental growth conditions.

Transition from Vegetative to Reproductive Growth Is Delayed in *era1-2*

To further understand the role of FTase during induction of flower development, we measured the time needed to transition from vegetative to reproductive growth in *era1-2* and wild-type plants. Time to flowering was determined by counting the number of days from germination to either bolting or development of the first flower. Under long-day growth conditions, *era1-2* plants bolted after 25 to 26 days (Figure 6). Wild-type plants bolted after ~20 to 21 days. Chi-square analysis confirmed that these differences were statistically significant ($P < 0.05$). In short-day growth, *era1-2* plants bolted after an average of 83 to 84 days, whereas wild-type plants bolted after an average of ~58 days. These results support the conclusion that FTase acts on positive regulators during the normal transition to flowering in Arabidopsis but is not essential for the initiation of flowering.

Complementation of *era1-2* with *LeFTB* Confirms FTase as the Genetic Lesion

The genetic lesion in *era1-2* mutant results from a 7.5-kb deletion encompassing the *AtFTB* gene (Cutler et al., 1996). Although the developmental and physiological changes observed in *era1-2* are most likely caused by the lack of FTase alone, we cannot exclude the possibility that the large genomic deletion affected other genetic functions as well. To exclude this possibility and to confirm that the genetic lesion resided only in FTase, we transformed the gene for the tomato FTase β subunit (*LeFTB*) into *era1-2* plants and ectopically expressed the gene under the control of the cauliflower mosaic virus 35S promoter (*era1-2*^{LeFTB}). Figures 7 and 8 show that the expression of *LeFTB* alone was sufficient to complement all phenotypes associated with the *era1-2* mutation. Immunoblot analysis with polyclonal anti-LeFTB antibodies confirmed that AtFTB was absent in *era1-2* and *era1-2*^{LeFTA} control plants (*era1-2* transgenic plants expressing *LeFTA*) but was present in three independently transformed *era1-2*^{LeFTB} lines (Figure 8).

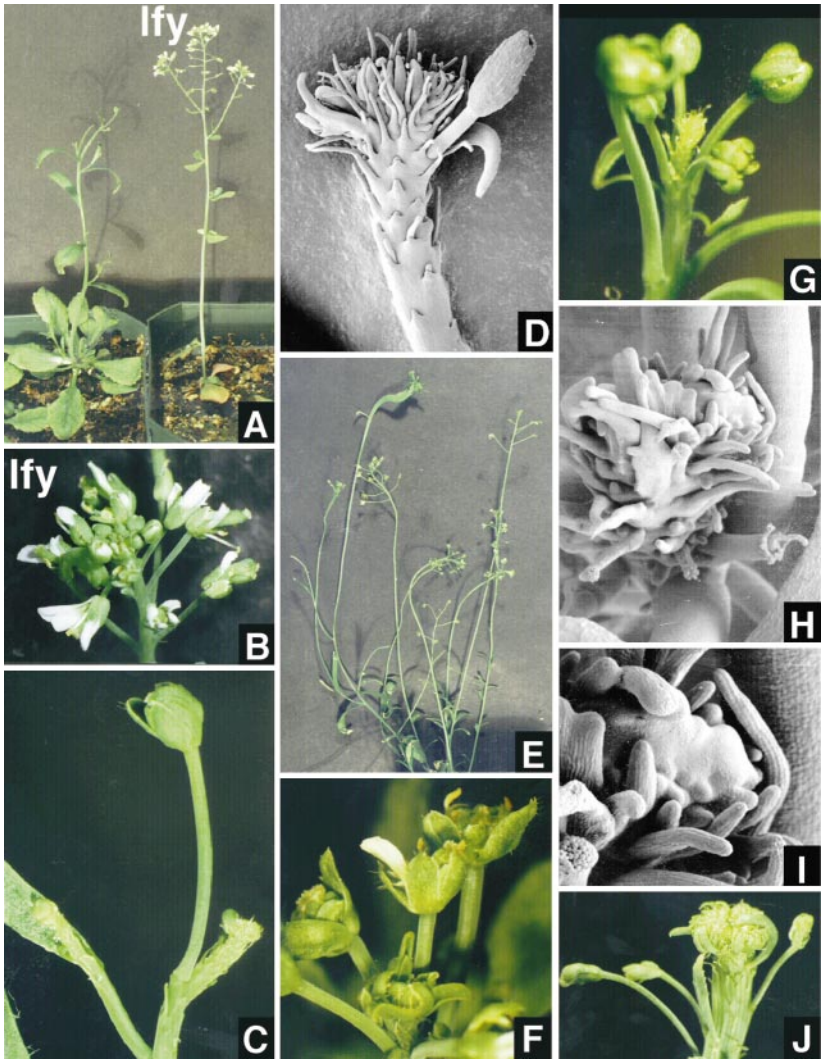

Figure 5. Phenotypes of *era1-2 lfy-5* Double Mutants.

(A) Comparison of a mutant with the weak *lfy5* allele (right) and an *era1-2 lfy5* double mutant shows several novel phenotypes, including primary and axillary inflorescences that are arrested in the double mutant.
(B) Close-up of a *lfy5* inflorescence.
(C) to **(J)** Examples of novel phenotypes in *era1-2 lfy5* double mutants.
(C) Close-up of the arrested primary and axillary inflorescences on the double mutant shown in **(A)**.
(D), **(H)**, and **(I)** Scanning electron microscopy images of a fasciated primary inflorescence on an *era1-2 lfy5* double mutant, viewed from three different angles. Numerous filaments are visible, some of which are subtended by a knob visible in **(D)**.
(E) Double mutant plant with fasciated stems forming late in development.
(F) New floral structures developing in axils of the first and second whorl flower organs (sepals and petals).
(G) Inflorescence-developing flowers with secondary axillary flowers and arrested fasciated meristems.
(J) Fasciated floral meristem subtended by a carpeloid leaf on which ovules have formed.

The development of axillary branches and flowers was no longer blocked in *era1-2*^{LeFTB} (Figure 7A), and the size and number of organs were restored to wild-type values (Figure 7B). The time to flowering was shorter in *era1-2*^{LeFTB} plants but was still longer than in wild-type plants. No flowers showed homeotic transformations under short-day growth conditions (data not shown). In addition, when *era1-2*^{LeFTB} or wild-type plants were not watered for 40 days, they senesced and dried, whereas *era1-2* plants remained green (Figure 7C), as was shown by Pei et al. (1998). Because the

drought resistance of *era1-2* plants results from ABA hypersensitivity caused by the lack of FTase, the phenotype of *era1-2*^{LeFTB} plants suggests that expression of LeFTase was sufficient to restore normal ABA signaling.

The size and structure of 15 inflorescence meristems of *era1-2*^{LeFTB} plants were examined to confirm that normal meristem morphology had been restored in the complemented plants (Figures 7D to 7F). The line that originates at P1 and traverses the meristem through the broadest plane of a median section (see Figures 4A and 4B) was ~62 μm long in *era1-2*^{LeFTB} plants (Figure 7F), similar to the length in wild-type plants (60 μm; Figure 7D). The longitudinal line from the tip of the meristem measured ~25 μm in *era1-2*^{LeFTB} (Figure 7F), similar to the 22 μm in the wild type (Figure 7D). Thus, meristem size was restored in the *era1-2*^{LeFTB} plants, confirming that in Arabidopsis, FTase activity is required for the control of regulatory pathways that maintain meristem size.

DISCUSSION

The work reported here supports the conclusion that plant protein FTase is required for the regulation of plant growth and development. The developmental and growth phenotypes observed in the Arabidopsis *era1-2* mutant suggest that FTase acts positively or negatively on proteins that control specific processes such as meristem cell division activity and size, organ size and numbers, and time to flowering, although most of the proteins that are probably farnesylated by FTase are unknown (Rodríguez-Concepción et al., 1999b). Previous studies established that in Arabidopsis, FTase also acts negatively on proteins that are involved in ABA signal transduction (Cutler et al., 1996) and that lack of FTase in *era1-2* can suppress mutations in *abi1* and *abi2*, which affect ABA signaling in stomatal guard cells (Pei et al., 1998).

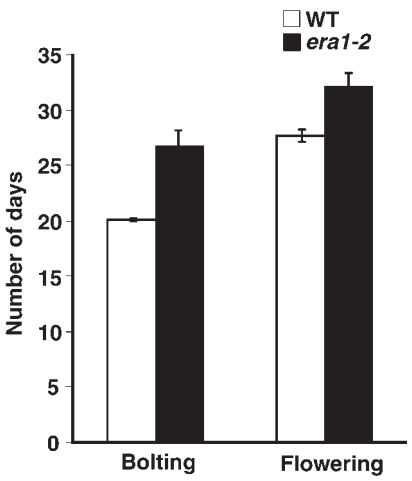

Figure 6. Flowering Time of Wild-Type and *era1-2* Plants in Long-Day Growth Conditions.

Results are the number of days from germination until bolting was detected or until the first flower was visible. Wild-type (WT) and *era1-2* patterns differ significantly (χ^2 test; bolting, $P < 0.001$; flowering, $P < 0.01$). Error bars indicate SD.

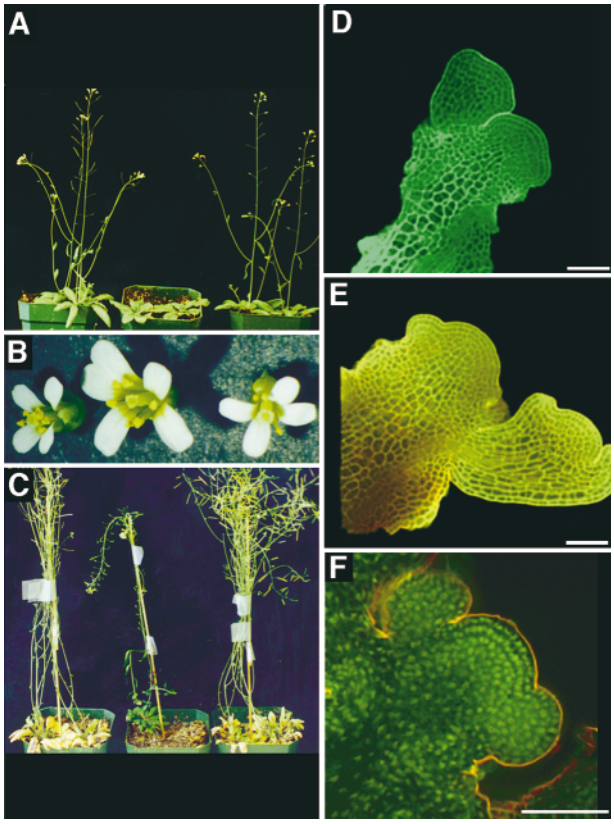

Figure 7. Complementation of *era1-2* by Ectopic Expression of *LeFTB*.

era1-2 plants were transformed with DNA constructs to express the tomato *LeFTB* or *LeFTA* genes as described in Methods.
(A) to **(C)** The wild-type plant is at left, *era1-2* plant is at center, and *era1-2*^{LeFTB} plant is at right.
(A) At 1 month, *era1-2* had just initiated bolting, whereas wild-type and *era1-2*^{LeFTB} plants were flowering already.
(B) Examination of individual flowers shows that expression of *LeFTB* in *era1-2* is sufficient to restore normal organ size and number.
(C) Wild-type and *era1-2*^{LeFTB} plants grown in short-day (8-hr-light/16-hr-dark) conditions had senesced after watering was stopped, whereas the *era1-2* plant was still green after 40 days without watering. This phenomenon is related to the ABA-hypersensitive phenotype of *era1-2* as reported by Pei et al. (1998).
(D) to **(F)** Optical sections through inflorescence meristems of wild-type **(D)**, *era1-2* **(E)**, and *era1-2*^{LeFTB} **(F)** plants. Cell walls (**[D]** and **[E]**) and nuclei **(F)** were stained with acryflavine. Bars = 50 μm.

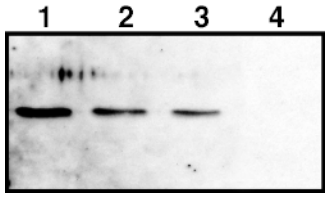

Figure 8. Protein Immunoblot–Confirmed Expression of LeFTB in *era1-2* Plants.

Shown are immunoblots of protein extracts prepared from three independent *era1-2*^{LeFTB} transformants (lanes 1 to 3) and one *era1-2*^{LeFTA} transformant that served as control (lane 4). Although *era1-2*^{LeFTB} transformants accumulated different amounts of LeFTB protein, all were fully complemented.

Our work provides insights into other regulatory processes that probably also require farnesylated proteins.

Requirement of FTase Activity Is Most Pronounced in Control of Shoot Apical Meristem Function

The enlargement of meristems and partial arrest of axillary shoot formation in *era1-2* plants suggest that FTase has an important function in controlling the size and cell division activity of meristems during development. Expansion of primary and axillary meristems in *era1-2* results in an increased number of flowers and floral organs. The larger meristem size is correlated with an increased cell number (Figure 4), which could be explained by a shortening of the cell cycle and thus more cell divisions in the central zone (CZ) or peripheral zone (PZ), or delayed differentiation of cells moving from CZ to PZ or from PZ into the differentiation zone. Similar models were considered for *clv* or *mgoun* (*mgo*), other Arabidopsis mutants in which meristem size is increased (Clark, 1997; Laufs et al., 1998a). *CLV1* encodes a leucine-rich region receptor kinase–like protein, and *CLV3* encodes a small protein that, based on genetic interactions, may function as the ligand for CLV1 (Clark et al., 1997; Fletcher et al., 1999). In *clv1* and *clv3* mutants, additional floral organs may arise in all or any of the floral whorls, but whorl 4 (carpel whorl) is most consistently affected (Leyser and Furner, 1992; Clark et al., 1993, 1995). In contrast, the most consistently affected whorls in *era1-2* are 1 and 2 (Figure 1), suggesting that FTase is required during flower development to restrict the number of cells that give rise to sepals and petals in the outer two whorls. In addition, both vegetative and inflorescence shoot apical meristems in *clv* mutants are substantially taller than and differ in structure from the corresponding meristems in *era1-2* (Figure 4; Clark et al., 1993, 1995). These results suggest that ERA1 and CLV1 (and CLV3) may act in different signaling pathways or that additional signaling pathways are compromised in *era1-2*.

Analyses of cellular parameters in wild-type and *clv3* meristems have now established that cell division rates throughout the enlarged inflorescence dome of *clv3* are less than the mean value for the wild-type meristem (Laufs et al., 1998b). These observations do not support a model of increased cell division rates to explain the increased *clv3* meristem size but indicate that cells accumulate in the CZ (Laufs et al., 1998b, 1998c), confirming earlier results that the CZ is expanded in *clv1* (and *clv3*) (Clark et al., 1993, 1995). Thus, *CLV3* (and *CLV1*) promote transition of cells from CZ to PZ. The extra cells needed for the increase in *era1-2* meristem size could come from additional cell divisions in CZ and accumulation of cells in PZ; additional cell divisions in PZ and delayed transition of cells into primordia or differentiation; or like the *clv* mutants, expansion of the CZ because of delayed transition of cells from CZ to PZ. A delayed cellular differentiation, rather than additional cell divisions, would be consistent with the phenotype observed in *era1-2 lfy-5* double mutants (Figure 5). In that case, the lack of FTase most consistently enhanced the loss of cellular differentiation in the weak *lfy-5* mutant; less frequently, it caused fasciation, the result of a more severe enlargement of meristem size. The synergistic phenotype of *era1-2 lfy-5* double mutants does not necessarily indicate that *LFY* and *ERA1* have closely related roles, because other mutants, when combined with mutations at either *LFY* or *UNUSUAL FLORAL ORGANS* (*UFO*) loci, produce similar synergistic phenotypes. Examples of such double mutants include *clv1-4 lfy6* (Clark et al., 1993), *filamentous flower-5* (*fil-5*) *lfy-6* (Chen et al., 1999), *ufo-2 fil-5* (Chen et al., 1999), and the *ufo-2 fused floral organs* (*ffo*) loci *ffo1-1*, *ffo2-1,* and *ffo3-1* (Levin et al., 1998). In summary, farnesylation is required for the correct function of proteins that regulate meristem size, meristem differentiation processes, or both.

ERA1/*WIGGUM* May Be Required for Signaling through the *CLV* Pathway

The *wiggum* (*wig*) mutant was identified in a screen for meristem mutants (Running et al., 1998) in which *era1-2* and *wig* were found to have striking similarities in their floral morphology: they map to the same position on chromosome 5; and they share many phenotypic alterations, including the enlarged floral meristem phenotype. Recent cloning and sequencing of the *wig* locus confirmed that it encodes *AtFTB* (*ERA1*; Ziegelhoffer et al., 2000).

Genetic analysis of *wig* mutants did not reveal convincing epistatic relationships between *WIG* and several other genes that affect meristem function, including *CLV1* (Running et al., 1998). The phenotypic differences between *wig*/*era1* and *clv* mutants and the additive phenotype of *clv1 wig* double mutants initially led to the conclusion that WIG acts in a unique pathway (Running et al., 1998). Biochemical analysis of proteins that interact with CLV1, however, identified a potential FTase substrate in the CLV pathway. Once bound to

CLV3, CLV1 recruits other proteins into a high molecular mass complex that includes Rop, a Rho-related GTPase (Trotochaud et al., 1999). Two Arabidopsis Rac/Rho–related Rops (ARaC7 and ARaC8) have CaaX motifs ending in Ala and Asn, respectively, indicating that they are preferred substrates of FTase. RhoB, which has a growth-promoting function in mammalian cells, is either farnesylated or geranylgeranylated. In this case, the different prenylated forms of the protein have distinct cellular functions (Lebowitz et al., 1997). Thus, similar to other small GTPases, the CLV1-associated Rop protein is likely farnesylated, and this modification is required for Rop function in the CLV signaling pathway. In *wig/era1-2*, Rop would not be farnesylated or would be prenylated only partially by GGTase-I. This could explain a loss of meristem size control that is less severe in *era1-2* than in *clv1* or *clv3*, in which the putative receptor or ligand is affected. Partial complementation of loss of FTase activity in *era1-2* by GGTase-I would not be unexpected, considering the promiscuity between FTase and GGTase in plants (Rodríguez-Concepción et al., 1999a, 1999b; Yalovsky et al., 1999) and yeast (Trueblood et al., 1993). Additionally, the loss of FTase activity in Arabidopsis probably affects other functions in the meristem as well, which could mask effects on the CLV signaling pathway and thus explain the lack of a strong epistatic relationship between *wig/era1* and *clv* mutants.

FTase Is Required for Control of Developmental Processes

The analysis of *era1-2* presented here has revealed several additional phenotypes not previously reported for *wig* mutant alleles (Running et al., 1998). These phenotypes, which become apparent when plants are grown in short-day conditions, include enlargement of leaf and floral organ size, partial suppression of axillary branching, and homeotic conversions within flowers. The most simple explanation for the marked size increase of rosette leaves, cauline leaves, and floral organs in *era1-2* (Figures 1, 2, and 7) could be a partial loss-of-function of various regulatory proteins that require farnesylation for controlling the rate or number of cell division. Organ enlargement, development of filaments instead of flowers, the suppression of axillary branching, and development of axillary leaves—all observed in *era1-2* plants (Figure 2)—may point to a more complex role for FTase and farnesylated proteins in regulating the balance between meristematic activity and cell differentiation (Talbert et al., 1995). For example, instead of an axillary inflorescence, occasionally a cauline leaf developed in *era1-2* (Figure 2A), and axillary inflorescence meristems were arrested in an aborted structure (Figures 2B and 2D). Moreover, almost every inflorescence stem contained filaments or sepal-like structures that developed instead of flowers (Figures 2C and 2E). In Arabidopsis, axillary meristems are clonally related to their subtending leaves (Furner and Pumfrey, 1992; Irish and Sussex, 1992). Thus, farnesylation may be required of proteins that have a role in controlling the fate of cells in the transition from meristematic activity to organ differentiation. If this control is compromised in *era1-2*, developmental decisions would be affected and organs would grow larger because they are initiated from more cells rather than because the rate of division of meristematic cells is increased. Protein farnesylation may thus be involved in the regulation of both cellular differentiation and cell division. These phenomena may be two facets of the same signaling pathway or may represent independent processes regulated by different signaling pathways. A similar dual role was not previously considered for *wig* alleles (Running et al., 1998), because their analysis focused initially on the enlargement in flower meristem size and the phenotypes of double mutants with other meristem identity genes.

Role of FTase in the Transition from Vegetative to Reproductive Growth

Farnesylated proteins also appear to participate in signaling pathways that induce the transition from vegetative to reproductive growth, as indicated by the late-flowering phenotype of *era1-2* (Figure 6). A direct role of farnesylated protein in flower formation is suggested by the homeotic transformation of flowers in some *era1-2* plants (Figures 2D and 2E) and by the more complete reversion of flowers into shoots in the *era1-2 lfy-5* double mutants (Figures 5F and 5G).

Summary

era1-2 is providing interesting and novel insights into the role of protein farnesylation during plant growth and development. Our study has uncovered at least three distinct processes that require the function of FTase, namely, regulation of cellular differentiation in the meristems, meristem maintenance, and regulation of flower development. These processes are likely to involve several prenylated proteins that remain to be discovered. The Arabidopsis *era1-2* mutant and molecular tools now available provide a unique opportunity to clarify the role of protein prenylation in linking developmental processes with the function of certain proteins and signaling cascades.

Interestingly, the flower meristem identity protein APETALA1 (AP1) terminates in a CFAA CaaX box, which is a typical substrate of FTase. *wig1 ap1-1* double mutants reportly have an additive phenotype (Running et al., 1998), which is to be expected because *ap1-1* is a mutant allele that cannot be farnesylated (Mandel et al., 1992). In a companion article (Yalovsky et al., 2000, this issue), we show that AP1 can be farnesylated both in vitro and in vivo and that a mutant nonprenylated form of AP1 can induce novel phenotypes when ectopically expressed in Arabidopsis. These results suggest

that protein farnesylation also plays a role in flower development by regulating the activity of a protein that determines flower meristem identity.

METHODS

Plant Material and Growth Conditions

Arabidopsis thaliana seeds were sown in Sunshine mix (Fison Horticulture, Bellevue, WA) and stored for 5 days at 4°C before transfer to the greenhouse or growth chambers. Plants were grown at 22°C under long-day (16-hr-light/8-hr-dark) or short-day (8-hr-light/16-hr-dark) conditions. To ensure uniformity for the quantitative analysis (Figures 4 and 7), we grew two groups of 25 wild-type and *era1-2* plants in single pots in the same tray or two adjacent trays. Uniform flowering time for the analysis of inflorescence and floral meristems (Figure 4) was achieved by growing plants for 25 to 30 days under short-day conditions followed by long-day conditions to induce flowering. Transgenic Arabidopsis seeds were surface-sterilized, and kanamycin-resistant (Kan^R) seedlings were selected as described previously (Bechtold et al., 1993; Bent et al., 1994). After transfer to soil, Kan^R seedlings were grown in long-day conditions in the greenhouse or growth chamber.

Preparation of Shoot Apical Meristems and Analysis by Scanning Laser Confocal Microscopy

Arabidopsis meristems were isolated as described by Gisel et al. (1999). Isolated meristems were fixed in either 1 M HCl or formaldehyde fixer (FAA) (Ruzin, 1999), stained with 0.1% acryflavine, and cleared with benzyl benzoate-$4^{1/2}$ (BB-$4^{1/2}$) (Ruzin, 1999). The entire procedure was performed according to Ruzin (1999), with the following modifications. FAA was used as a fixative, incubation in the acryflavine solution was for 15 to 60 min, and the BB-$4^{1/2}$ clearing solution contained methyl salicylate instead of clove oil. The acryflavine solution was prepared by first dissolving the acryflavine in water and then adding the other materials. In Arabidopsis, the technique was used successfully to stain either cell walls or nuclei by slightly changing the staining procedure (Ruzin, 1999). Double staining for cell walls and nuclei was successful; however, the staining in the meristem was too dense because of the small cell size and created a dark shadow over the image. Stained and cleared tissues were mounted on depressed slides in BB-$4^{1/2}$ clearing solution and viewed with a Zeiss (Zeiss, Inc., Thornwood, NY) R510 confocal laser scanning microscope equipped with an argon laser, a dichroic filter at 488 nm, and a long-path filter at 585 nm. Under these condition, acryflavine-stained cell walls and nuclei appeared yellow. Differential views of nuclei and cell walls were obtained using a 488/568-nm dichroic filter, with a 530-nm ($\pm$20 nm) band path and 585-nm long-path filters. Under these conditions, the nuclei appeared green and the cell walls orange.

Image Analysis

All the images were transferred from the scanning laser confocal microscope to a Personal Iris 4D/35 (Silicon Graphics, Mountain View, CA) computer and analyzed using ImageSpace software (Molecular Dynamics, Sunnyvale, CA).

Measurement of Leaf Size

Individual leaves were scanned, and the images were opened as National Institutes of Health (NIH) Image files (NIH Image software). The resulting scanned images were analyzed with NIH Image software.

DNA Constructs

For sense expression constructs for *LeFTA* and *LeFTB*, we subcloned BamHI-XhoI fragments containing *LeFTA* or *LeFTB* cDNAs into the plant binary vector pMDI between the cauliflower mosaic virus 35S promoter and the nopaline synthase terminator to create pSY207 and pSY109, respectively.

Plant Transformation Procedures

Arabidopsis *era1-2* plants were transformed using *Agrobacterium tumefaciens* and vacuum infiltration as described previously (Bechtold et al., 1993; Bent et al., 1994).

Preparation and Analysis of Proteins

Protein extracts were prepared as described by Yalovsky et al. (1996). For immunoblot analysis, proteins were resolved using 10% SDS–polyacrylamide gels (Laemmli, 1970) and transferred to nitrocellulose membranes. Membranes were probed with an anti-LeFTB polyclonal antibody at 1:10,000 dilution (Yalovsky et al., 1996). Secondary antibodies were blotting-grade goat–rabbit IgG conjugated to horseradish peroxidase (Bio-Rad) and diluted 1:20,000. Immunoblots were developed using the Super Signal kit (Pierce Chemical).

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