Arabidopsis era1 was identified as an abscisic acid–hypersensitive mutant caused by disruptions or deletions of the gene for the \( \beta \) 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 \( \beta \) 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 geranylgeranyl transferases (GGTase-I and RabGGTase) have been identified in yeast, animals, and plants. FTase and GGTase-I are heterodimeric enzymes with a common \( \alpha \) subunit but distinct \( \beta \) 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). 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).

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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-Ras1V12) 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 Ras1V12 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.
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 normal inflorescence stems. Sepaloid flowers (S) and filament-like structures (arrowhead) are typical on era1-2 plants grown under short-day conditions. 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 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 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).

**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 ($\chi^2$ 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 ($\chi^2$ 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 ($\chi^2$ 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 so.
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 exhibited similar increases in organ number and size to that observed for plants grown under long-day conditions.

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 develop-
opment was compromised by loss of genetic functions (e.g., ify-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 (era1-2LeFTB). 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 era1-2LeFTB control plants (era1-2 transgenic plants expressing LeFTA) but was present in three independently transformed era1-2LeFTB lines (Figure 8).

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**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.
The development of axillary branches and flowers was no longer blocked in era1-2LeFTB (Figure 7A), and the size and number of organs were restored to wild-type values (Figure 7B). The time to flowering was shorter in era1-2LeFTB 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 era1-2LeFTB 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

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

**(A)** Comparison of a mutant with the weak Ify5 allele (right) and an era1-2 Ify5 double mutant shows several novel phenotypes, including primary and axillary inflorescences that are arrested in the double mutant.

**(B)** Close-up of a Ify5 inflorescence.

**(C) to (J)** Examples of novel phenotypes in era1-2 Ify5 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 fascinated primary inflorescence on an era1-2 Ify5 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 fascinated 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 fascinated meristems.

**(J)** Fasciated floral meristem subtended by a carpeloid leaf on which ovules have formed.
drought resistance of era1-2 plants results from ABA hypersensitivity caused by the lack of FTase, the phenotype of era1-2LeFTB plants suggests that expression of LeFTase was sufficient to restore normal ABA signaling.

The size and structure of 15 inflorescence meristems of era1-2LeFTB 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 era1-2LeFTB 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 era1-2LeFTB (Figure 7F), similar to the 22 μm in the wild type (Figure 7D). Thus, meristem size was restored in the era1-2LeFTB 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, even though 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).
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 fty-5 double mutants (Figure 5). In that case, the lack of FTase most consistently enhanced the loss of cellular differentiation in the weak fty-5 mutant; less frequently, it caused fasciation, the result of a more severe enlargement of meristem size. The synergistic phenotype of era1-2 fty-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 fty-6 (Clark et al., 1993), filamentous flower-5 (fil-5) fty-6 (Chen et al., 1999), ufo-2 fil-5 (Chen et al., 1999), and the ufo-2 fused floral organs (fus) 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 wig gum (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

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 mutants (mgo), other Arabidopsis mutants in which meristem size is increased (Clark, 1997; Laufs et al., 1998a). CLV1 encodes a leucine-rich region 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.

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

Shown are immunoblots of protein extracts prepared from three independent era1-2LeFTB transformants (lanes 1 to 3) and one era1-2LeFTB transformant that served as control (lane 4). Although era1-2LeFTB transformants accumulated different amounts of LeFTB protein, all were fully complemented.
CLV3, CLV1 recruits other proteins into a high molecular mass complex that includes Rop, a Rho-related GTPase (Trotchaud et al., 1999). Two Arabidopsis Rac/Rho–related Rops (ARAaC7 and ARAaC8) 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 reportedly 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 non-prenylated 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 (KanR) seedlings were selected as described previously (Bechtold et al., 1993; Bent et al., 1994). After transfer to soil, KanR 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-41/2 (BB-41/2) (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 for 15 to 60 min, and the BB-41/2 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-41/2 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 conditions, 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 (± 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 LeFTB and LeFTB, we subcloned BamHI-XhoI fragments containing LeFTB or LeFTB cDNAs into the plant binary vector pMD1 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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