Cloning of the *Arabidopsis WIGGUM* gene identifies a role for farnesylation in meristem development

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Control of cellular proliferation in plant meristems is important for maintaining the correct number and position of developing organs. One of the genes identified in the control of floral and apical meristem size and floral organ number in *Arabidopsis thaliana* is *WIGGUM*. In *wiggum* mutants, one of the most striking phenotypes is an increase in floral organ number, particularly in the sepals and petals, correlating with an increase in the width of young floral meristems. Additional phenotypes include reduced and delayed germination, delayed flowering, maturation, and senescence, decreased internode elongation, shortened roots, aberrant phyllotaxy of flowers, aberrant sepal development, floral buds that open precociously, and occasional apical meristem fasciation. As a first step in determining a molecular function for *WIGGUM*, we used positional cloning to identify the gene. DNA sequencing revealed that *WIGGUM* is identical to *ERA1* (enhanced response to abscisic acid), a previously identified farnesyltransferase β-subunit gene of *Arabidopsis*. This finding provides a link between protein modification by farnesylation and the control of meristem size. Using *in situ* hybridization, we examined the expression of *ERA1* throughout development and found it to be nearly ubiquitous. This extensive expression domain is consistent with the pleiotropic nature of *wiggum* mutants and highlights a broad utility for farnesylation in plant growth and development.

The development of multicellular organisms relies on signal transduction for basic cellular functions and intercellular communication. The function of proteins involved in signal transduction pathways often depends on covalent attachment of chemical side groups. Protein prenylation, in which lipophilic isoprenyl groups like the 15-carbon farnesyl and 20-carbon geranylgeranyl moieties are covalently attached to target proteins, has been shown to be one such posttranslational modification (1, 2). The largest group of proteins known to be prenylated is the Ras superfamily of small GTP-binding proteins, with other prenylated proteins falling into the classes of lipopeptide pheromones, nuclear lamins, and trimeric G proteins (1, 2). The functional diversity of known prenylated proteins indicates a broad utility for isoprenylation in the control of cell growth and differentiation, cytokinesis, and membrane trafficking.

Prenylation of proteins is catalyzed by farnesyltransferase (FTase) or one of two types of geranylgeranyltransferases (GGTase-I and GGTase-II). The closely related FTase and GGTase-I enzymes function as heterodimers, sharing a common α-subunit with a distinct β-subunit responsible for providing substrate specificity (2, 3). After isoprenylation by FTase or GGTase-I, the substrate protein then is methylated. Prenylation and subsequent methylation strongly increase the hydrophobicity of proteins, facilitating membrane localization. Despite the observation that most prenylated proteins have been found to be associated with membranes (2), the isoprenoid group *per se* is not the determinant of localization. Prenylated proteins contain a variety of membrane targeting signals allowing for localization to distinct intracellular membranes. Other roles for prenylation are protein–protein interactions and assembly into multisubunit protein complexes (2).

Prenylation, a modification that has profound implications for signal transduction and intracellular trafficking pathways, has been studied extensively over the past two decades in both fungal and mammalian systems. cDNAs for α- and β-subunits of FTase and GGTase-I have been identified from yeast, rat, bovine, and human libraries (2), and numerous substrate proteins also have been identified (4). More recently, the enzymes responsible and pathways affected have begun to be studied in plants as well; FTase α and β genes have been cloned from pea, tomato, tobacco, and *Arabidopsis thaliana* (5).

A connection between prenylation and hormone signaling in plants was made when *ERA1* (enhanced response to abscisic acid) was cloned from *Arabidopsis* (6). Abscisic acid is a plant hormone involved in modulating development, most notably with regard to seed dormancy and drought-induced water retention (7). *era1* mutants were identified based on their increased seed dormancy, even in the absence of abscisic acid (6). *era1* mutants also displayed increased tolerance to drought stress, through a reduction in stomatal apertures (8). The *ERA1* gene encodes the β-subunit for protein FTase, and mutants were found to be devoid of FTase activity in an *in vitro* assay (6). Based on biochemical assays investigating expression, activity, and inhibition, farnesylation has been proposed to play other roles in plant growth and development, including nutrient allocation (9) and cell division (9–11).

The control of cell division and differentiation is critical for the proper development of multicellular organisms. Groups of undifferentiated stem cells at the growing tips of plants comprise meristems, in which cell division control is vital. The size of meristems generally remains constant throughout the lifespan of the plant, indicative of the highly controlled pattern of cell division within meristems, both in number and position (12). Cellular differentiation occurs at the periphery of meristems to produce organs flanking the meristem. In *Arabidopsis*, mutants that are unable to initiate and/or maintain viable shoot apical meristems [e.g., *shoot meristemless* (13) and *wuschel* (14)] and mutants that have enlarged shoot apical and floral meristems [e.g., *clavata1* (*clv1*), *clv2*, and *clv3* (15–17)] and *wiggum* (*wig*; ref. 18) have been identified. The *SHOOTMERISTEMLESS* (19) and *WUSCHEL* (20) genes have been cloned and shown to encode homeodomain proteins that are likely to act as transcription factors in shoot and floral meristem cells. The cloning of all three *CLV* genes also has been reported: *CLV1* encodes a receptor kinase (21), *CLV3* encodes the presumed extracellular protein ligand for CLV1 (22), and *CLV2* encodes a receptor-like

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Abbreviations: *WIG*, *WIGGUM*; *ERA1*, enhanced response to abscisic acid; FTase, farnesyltransferase; GGTase, geranylgeranyltransferase; Ler, Landsberg *erecta*; CAPS, cleaved amplified polymorphic sequences; RACE, rapid amplification of cDNA ends; *CLV*, *CLAVATA*.

Data deposition: The sequence in this paper has been deposited in the GenBank database (accession no. AF214106).

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Table 1. CAPS markers designed for this study

Marker	Size, bp	Polymorphism*		Primers
MYH19L	175	*Ssp*I:	Col=175	5′-CTATATATTTTATACAAGTTTAAAATAAATA-3′
			L*er*=145, 30	5′-CTACCATGTGATGACATG-3′
MYH19VR	880	*Xho*I:	Col=610, 270	5′-CGCGTGGGTACTCATCTAGTG-3′
			L*er*=880	5′-CGGATTAGCAGCTAGGACAG-3′
MSN9L	190	*Afl*III:	Col=190	5′-GACAAGGCTTATTGTTTACGT-3′
			L*er*=170, 20	5′-GAATGTATTATGCGTAGTGAC-3′
MSN9R	200	*Spe*I:	Col=200	5′-TGGCAGTAATTTAATGATAAACTA-3′
			L*er*=180, 20	5′-CGTATTGAGCCTACATAACAA-3′
MPO12VL	180	*Fnu*4HI:	Col=160, 20	5′-AATTAATTTGAGTTTGTTTGGCAG-3′
			L*er*=180	5′-TATGTTTCAAACAATAGTTTAGG-3′
MPO12.2	220	*Hin*fI:	Col=220	5′-GTCTTGTAGACTCTCCAAGACT-3′
			L*er*=200, 20	5′-CTCTTTCTATTATGTTCACCTCT-3′
MPO12M	200	*Hin*fI:	Col=200	5′-GCTCGAGATAAATATTTGAGACT-3′
			L*er*=180, 20	5′-AGGGATTCTTACTCTTTGTAGG-3′
MPO12R	305	*Tth*111I:	Col=285, 20	5′-GTTTCTGATCTGTGGTGACGGA-3′
			L*er*=305	5′-GGCTAATTCTCTCCGGCAC-3′

*Indicated is the restriction enzyme used and the sizes of the digestion products for Col (Columbia) and L*er*.

protein (23). These two classes of *Arabidopsis* meristem genes implicate specific transcription factors and signal transduction components likely to function in cell–cell communication in the control of meristematic growth.

wig mutants, like the *clv* mutants, have enlarged shoot apical and floral meristems, resulting in flowers with extra floral organs in all whorls. *Arabidopsis* flowers are arranged in concentric whorls in which the first and outermost whorl is comprised of sepals, the second of petals, the third of stamens, and the fourth of carpels. *clv* mutants have taller shoot and floral meristems than wild type and show the greatest number of extra floral organs in the inner whorls. *wig* plants, in contrast, have wider meristems and *wig* flowers have more sepals and petals than wild type. *wig* mutants exhibit additional phenotypes including reduced and delayed germination, delayed flowering, longer lifespan, reduced internode elongation, aberrant sepal development, floral buds that open precociously, occasional fasciation, and modest phyllotaxy defects (18).

To expand our understanding of meristematic development, we have cloned the *WIG* gene. Sequencing and complementation experiments indicate that *WIG* is identical to *ERA1* (encoding FTase β). We examined the *ERA1* mRNA expression pattern in wild-type plant tissues by using *in situ* hybridization and found that *ERA1* is broadly expressed throughout development, from embryogenesis through vegetative and reproductive growth. The *wig* alleles therefore have provided genetic and molecular evidence of a role for farnesylation in meristematic growth and development. Furthermore, the pleiotropic *wig* phenotypes indicate that farnesylation also is involved in numerous other aspects of development.

Materials and Methods

Plant Growth. Seeds were sown on a 1:1:1 mixture of soil/vermiculite/perlite, imbibed for 5 days at 4°C, and grown under continuous cool-white fluorescent light at ≈22°C. *wig-1* (renamed *era1–4*, see *Results*), *gl3*, and *tt2* are in the Landsberg *erecta* (L*er*) background. *wig-2* and *wig-3* (renamed *era1–5* and *era1–6*, respectively) originally were identified in the Nossen ecotype and subsequently outcrossed two times to L*er* (18).

Map-Based Cloning and Sequencing of *WIGGUM*. Based on a preliminary map position for *WIG* in the middle of chromosome 5 (18), *wig-1* (*era1–4*) was crossed to closely linked visible markers to obtain *wig-1 gl3* and *wig-1 tt2* double mutants. These lines in the L*er* ecotype were crossed to wild-type plants of the Columbia ecotype to generate a mapping population. F_2 progeny were screened for loss of one of the two phenotypes, indicating that a recombination event between L*er* and Columbia chromosomal DNA in the vicinity of *WIG* had occurred. DNA was prepared from recombinants as described (24). Using chromosome 5 sequence data from the *Arabidopsis* Genome Initiative supplied by the Kazusa group (http://www.kazusa.or.jp/arabi/), polymorphisms were identified to design PCR markers. Cleaved amplified polymorphic sequences (CAPS; ref. 25) and derived CAPS (26) markers were used to map the meiotic recombination breakpoints by PCR and restriction digestion. A list of CAPS markers designed for this study is provided in Table 1.

DNA sequencing was performed by using the ABI PRISM Dye Terminator method (Perkin–Elmer). Two independent PCR amplifications of genomic DNA from the *wig* alleles and the corresponding wild-type ecotype were sequenced. MACVECTOR (Kodak IBI) was used to align our sequencing data with the Columbia sequence from the *Arabidopsis* Genome Initiative database.

A cDNA corresponding to the 3′ two-thirds of *WIG* (renamed *ERA1*, see *Results*) was isolated from a flower cDNA library (27) by using a genomic *ERA1* probe. Rapid amplification of cDNA ends (RACE)–PCR (5′ RACE-PCR; GIBCO/BRL) was performed on RNA isolated from L*er* inflorescences to identify the 5′ end of *ERA1*. PCR generation of a full-length *ERA1* cDNA used high-fidelity Pwo DNA Polymerase (Boehringer Mannheim) and specific primers designed with *Bam*HI ends to amplify from the starting ATG to the poly(A) addition site in the 3′ untranslated region. The PCR product then was cloned into pZero-Blunt (Invitrogen) to create p4B. The entire 1.6-kb insert was sequenced to confirm that no mutations had occurred. An overexpression construct was made by digesting p4B with *Bam*HI and cloning the *ERA1* cDNA into pCGN18 (28), placing *ERA1* under control of the cauliflower mosaic virus 35S promoter. This construct *35S*::*ERA1*, was introduced into plants by *Agrobacterium*-mediated transformation using vacuum infiltration (29).

A genomic *ERA1* clone was constructed by digesting MSN9 with *Hin*dIII and ligating a 6.8-kb DNA fragment into pPZP211 (30). This construct, *ERA1–6.8*, was introduced into plants by *Agrobacterium*-mediated transformation using vacuum infiltration (29).

***In Situ* Hybridization.** Tissues were fixed (31) and radioactive *in situ* hybridization was performed as described (32). Exposure time was ≈6 wk. The riboprobe template was constructed by digesting p4B with *Bam*HI and cloning the *ERA1* cDNA into pGEM7zf(t) (Promega). Antisense and sense probes were transcribed from *Hin*dIII- and *Sac*I-linearized p*ERA1.7* by using SP6 and T7 RNA polymerase, respectively. This clone was demonstrated to be specific as it hybridized to a single band on a genomic Southern blot under low stringency conditions [washed with 2× standard saline phosphate/EDTA (0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA) at 50°C, data not shown].

Results

Cloning and Sequence of *WIGGUM*. To isolate the *WIG* gene, we used PCR-based CAPS markers (Table 1) to map recombination breakpoints between *wig-1* and either *tt2* or *gl3*. Using 38 *wig-1 tt2* recombinants and 21 *wig-1 gl3* recombinants, we were able to localize *WIG* to a region of less than 100 kb about 15 Mb from the top of chromosome 5, between markers MYH19VR and MPO12VL, respectively (Table 1). Genetic recombination frequencies showed that *wig-1* is ≈1–2 centimorgans from *gl3*, and ≈8–10 centimorgans from *tt2*, indicating that the 21 *wig-1 gl3* recombinants were at a higher density than the 38 *wig-1 tt2* recombinants. The *wig-1 gl3* recombinants mapped at a frequency of ≈1 per 15 kb, suggesting that *WIG* was most likely to lie in the right half of MSN9, a fully sequenced 61-kb bacterial artificial chromosome clone.

Annotation of MSN9 identified 15 putative genes (http://www.kazusa.or.jp/arabi/), providing candidate genes to sequence from the three known *wig* alleles to identify mutations. Nucleotide substitutions for all of these alleles were found in the coding region of MSN9.15; MSN9.15 corresponds to the *ERA1* β-subunit FTase gene (Fig. 1). *wig-1* and *wig-2* contain premature stop codons and *wig-3* contains a splice site mutation (Fig. 1). All of these alleles are likely to result in a complete loss of function. The premature stop codon in *wig-1* occurs before the first glycine-rich amino acid repeat conserved in FTase β-subunits (33), the premature stop codon in *wig-2* disrupts the fourth repeat, and *wig-3* is inappropriately spliced (determined by reverse transcriptase–PCR, data not shown) after the first repeat (Fig. 1).

Transgenic Complementation of *wig*. To confirm by complementation that we had identified the correct gene, we transformed a genomic clone into the *wig-1* mutant. This construct, *ERA1–6.8*, contains the 3,389-bp FTase β coding region plus ≈2.4 kb of upstream and ≈1.1 kb of downstream DNA sequences. *wig-1 ERA1–6.8* T_1 plants were examined for complementation of the *wig-1* phenotypes. Eight transgenic lines were examined closely, and all of these lines showed complete rescue of most *wig-1* phenotypes including germination, flowering time, lifespan, internode elongation, phyllotaxy, sepal development, and splayed floral bud defects. The extra floral organ defect was only partially rescued (Fig. 2 *A–C*), suggesting that flower development may be more sensitive to the exact amount of functional FTase (which may vary because of possible position effects in the transgenic lines). Alternatively, not all of the promoter or regulatory elements may be present on the 6.8-kb genomic fragment. The nearly complete complementation of *wig-1* by the genomic *ERA1–6.8* clone provides genetic evidence that *WIG* truly is the FTase β gene and is thus synonymous with *ERA1*. Consequently, *wig-1* has been renamed *era1–4*, *wig-2* renamed *era1–5*, and *wig-3* renamed *era1–6*.

Identification of the 5′ End of *ERA1* and Construction of a Full-Length *ERA1* cDNA. A discrepancy at the 5′ end of the FTase β gene exists between the MSN9.15 annotation and the reported cDNA for *ERA1*. MSN9.15 is predicted to have an additional 5′ exon

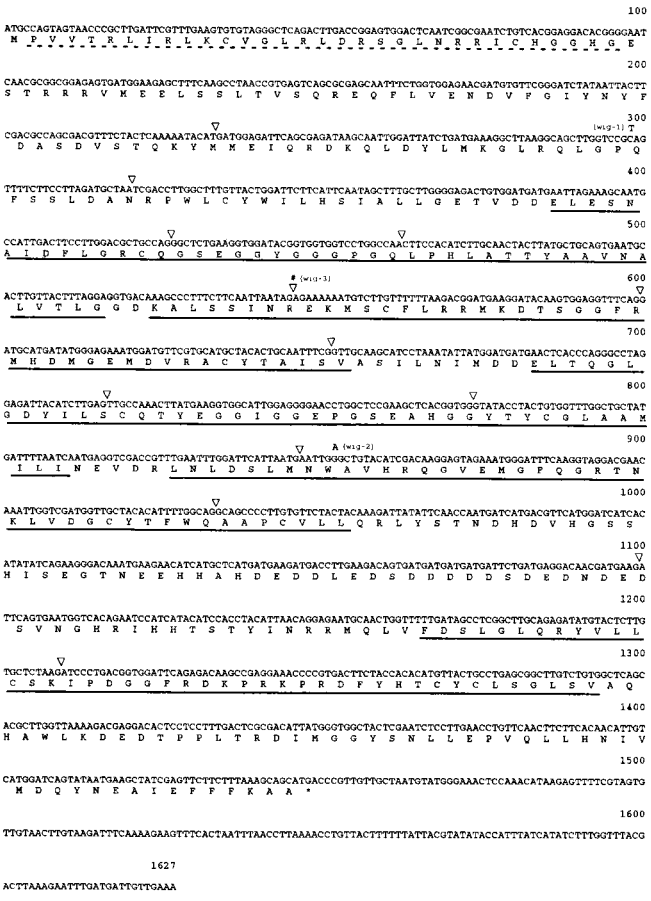


Fig. 1. The *ERA1* gene and protein sequences are shown. Triangles above the cDNA sequence indicate the position of introns. The stop codon at position 1449 is indicated by an *. The C to T and G to A nucleotide substitutions resulting in premature stop codons in *wig-1* (*era1–4*) and *wig-2* (*era1–5*), respectively, are indicated. # denotes the position of the G to A substitution at the 3′ end of the fifth intron in *wig-3* (*era1–6*). Conserved repeats with glycine-rich amino acid cores that are common to FTase β-subunits (33) are underlined. The additional N-terminal amino acid stretch found in *Arabidopsis* but not in pea and tomato is underlined with a dashed line.

encoding 78 aa. Others raised the suspicion that the 1.45-kb cDNA sequence reported for *ERA1* (6) was incomplete because both pea and tomato FTase β genes encode an additional N-terminal sequence of approximately 40 aa (34). To reconcile this discrepancy, we performed 5′ RACE-PCR on RNA isolated from wild-type L*er* inflorescences. The 5′ RACE product included the 14th predicted exon with ≈50 nts of upstream untranslated sequence. A stop codon in-frame and immediately before the start ATG indicated that the 5′ RACE product contains the bona fide 5′ end of the FTase β gene.

We also attempted to isolate a full-length *ERA1* cDNA by screening a cDNA library (27) with an *ERA1*-specific genomic probe. The longest clone identified was ≈1 kb in length and corresponded to more than half of the gene at the 3′ end. To construct the full-length cDNA, PCR was used to combine the 5′ RACE product with the 1-kb partial 3′ cDNA. The resulting cDNA corresponds to an mRNA with 1.45-kb coding sequence and a 3′ untranslated region of ≈180 bp (Fig. 1). A protein alignment of the translated *ERA1* cDNA with other FTase β subunits revealed that *Arabidopsis* FTase β contains an N-terminal stretch of 30–40 aa that is not present in tomato and pea but is present in rat and yeast (data not shown). The function of

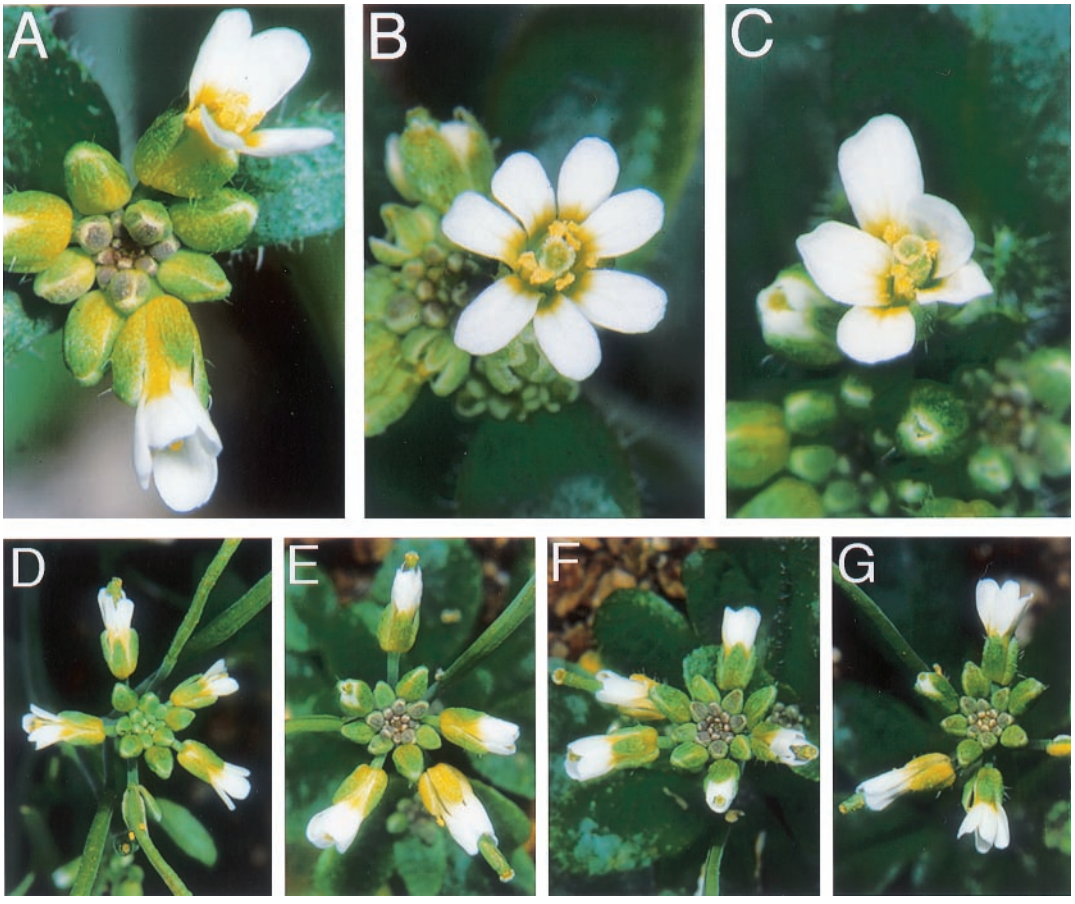


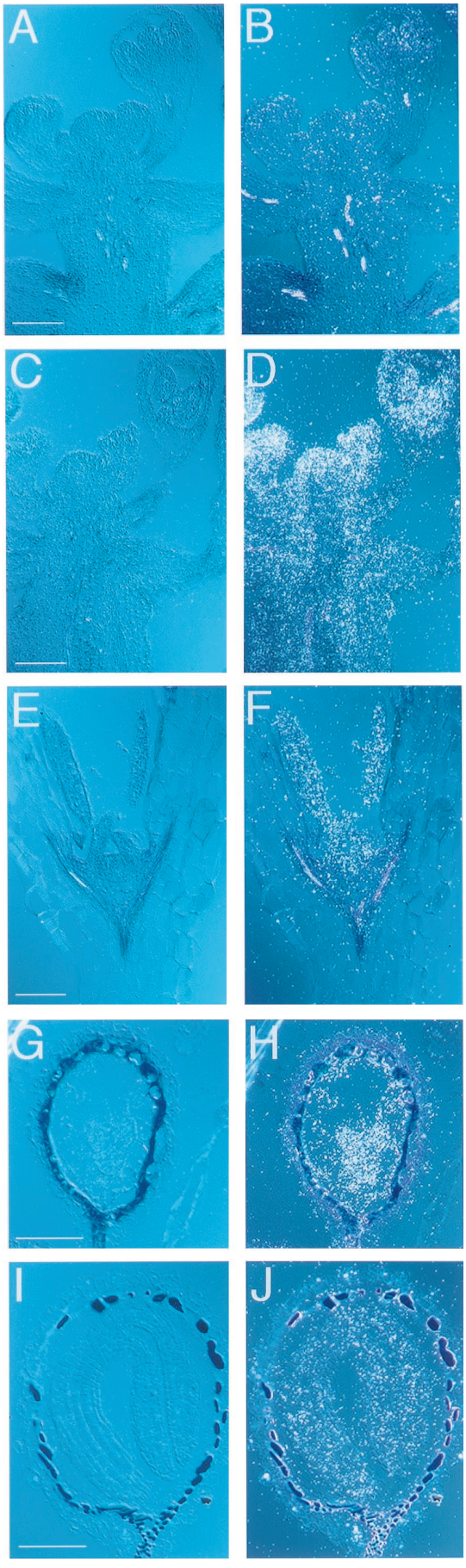
Fig. 2. Transgenic complementation of *era1* mutants. Shown are inflorescences of L*er* (*A*), *era1–4* (*B*), and T_1 plants of the following genotypes: *era1–4 ERA1–6.8* (*C*), *era1–4 35S*::*ERA1 (D), era1–5 35S*::*ERA1 (E), era1–6 35S*::*ERA1 (F),* and L*er* 35S::*ERA1 (G).* Wild-type L*er* flowers consist of four sepals, four petals, six stamens, and two carpels (A). *era1–4* mutants produce floral buds that open precociously and can produce flowers with many more sepals and petals than wild type (B). *ERA1–6.8* partially complements the *era1–4* floral phenotypes, evident by the reduced number of extra floral organs and the more enclosed floral buds. *35S*::*ERA1* fully complements all mutant phenotypes in *era1–4*, *era1–5*, and *era1–6*, and results in no overexpression phenotype in L*er*.

these N-terminal amino acids is unclear, as their identities are not conserved.

Overexpression of *ERA1*. Because *era1–4* through *era1–6* loss-of-function alleles display a variety of developmental defects (18), we were interested in determining the effect of *ERA1* overexpression. An overexpression cDNA clone was made by placing the full-length *ERA1* cDNA under control of the cauliflower mosaic virus 35S promoter. The *35S*::*ERA1* construct was transformed into wild-type L*er* and *era1–4*, *era1–5*, and *era1–6* mutant plants. Thirty-three of 35 transgenic L*er* lines showed no obvious overexpression phenotypes (Fig. 2*G*), whereas two lines appeared to be cosuppressed as they exhibited weak *era1–4*-like phenotypes (data not shown). A total of 64 T_1 lines in *era1–4*, *era1–5*, and *era1–6* showed complete rescue of all mutant phenotypes, including floral organ number (Fig. 2 *D–F*), providing yet another line of evidence that *WIG* and *ERA1* are identical.

***ERA1* Expression Pattern.** In the initial study of *ERA1*, mRNA was detected only in flower buds by Northern blot analysis (6). A subsequent study of transgenic plants expressing an *ERA1* promoter-*GUS* fusion revealed expression in vegetative tissues, most notably in guard cells (8). Given the enlarged meristem phenotypes of *era1–4*, *era1–5*, and *era1–6* (18), we predicted that *ERA1* also would be expressed in the shoot apical meristem. Therefore, we performed a more thorough investigation of the *ERA1* expression pattern by using *in situ* hybridization on various wild-type plant tissues.

We first examined *ERA1* expression in inflorescence tissue. A sense control provided a measure of background (Fig. 3 *A* and *B*). An *ERA1* antisense probe hybridized to wild-type L*er* inflorescences with signal throughout the stem, shoot apical meristem, and developing flowers (Fig. 3 *C* and *D*). *ERA1* was expressed throughout the inflorescence in all stages of development, with levels highest in the shoot apical meristem and in the center of developing flowers. *ERA1* mRNA also was detected throughout the vegetative shoot apical meristem and developing leaves of 7-day-old L*er* seedlings, but not in the cotyledons (Fig. 3 *E* and *F*). In addition, *ERA1* expression was detected throughout embryogenesis in the embryo and the endosperm (Fig. 3 *G–J*), beginning in embryos just undergoing the first division to produce a two-cell embryo and continuing through the fully mature stage, although expression was reduced in the later stages. *ERA1* expression also was detected throughout *era1–4*, *era1–5*, and *era1–6* mutant inflorescences, seedlings, and embryos (data not shown), indicating that none of these alleles is an RNA null. Reverse transcription–PCR of RNA extracted from developing siliques, inflorescences, leaves, roots, and stems of wild-type plants showed comparable levels of *ERA1* in all tissues (data not shown). Taken together, these results led us to conclude that *ERA1* is expressed essentially ubiquitously in all actively growing tissues.



Discussion

***WIG* Encodes FTase β.** We have cloned the *WIGGUM* gene in *Arabidopsis* and discovered that it encodes a protein FTase β-subunit. As this gene was previously isolated as *ERA1*, we have renamed the *wig* alleles (*wig-1*, *wig-2*, and *wig-3*; ref. 18) with *era1* designations (*era1–4*, *era1–5*, and *era1–6*, respectively). Low stringency genomic DNA gel blot analysis and *Arabidopsis* sequence database searches suggest that only one FTase β gene exists in *Arabidopsis* (data not shown).

Farnesylation, and isoprenylation in general, provides a mechanism to increase the hydrophobicity of substrate proteins posttranslationally. Such a lipophilic covalent modification facilitates membrane localization and protein–protein interactions, both important for signal transduction. In yeast, prenyltransferase activity by FTase or GGTase-I has been shown to be essential: *RAM2*, *RAM1*, and *CDC43* encode the common α-subunit and the β-subunits of FTase and GGTase-I, respectively (1, 2). Although cells carrying a *RAM1* deletion are viable, *ram1 cdc43* double mutants are inviable, and deletion of *RAM2* is also lethal (1).

It seems likely that loss-of-function mutants of FTase and GGTase-I α- and β-subunit genes in *Arabidopsis* would yield similar results to those obtained in yeast. Loss-of-function *era1* mutants are viable, indicating that FTase β is not essential in *Arabidopsis*. The *FTA* gene, encoding the FTase and GGTase-I α-subunit, has been identified in *Arabidopsis* (35), but no mutational studies have been reported. Although the functional specificity of FTase and GGTase-I is generally quite strong, cross-specificity between these closely related enzymes has been detected in yeast (2). By analogy, it seems reasonable to speculate that the common α-subunit may be essential in *Arabidopsis*, as it is in yeast, and that GGTase-I likely compensates to some extent for the loss of FTase β in *era1* mutants, although additional mutational studies will be required to test this hypothesis.

***ERA1* Is Expressed Throughout Development.** *In situ* RNA hybridization revealed nearly ubiquitous expression of *ERA1* throughout development in *Arabidopsis*. *ERA1* expression was detected in the earliest stages of embryogenesis, in embryos undergoing the first division to produce two cells, and continues to be detectable throughout embryogenesis, with all tissues exhibiting expression. *ERA1* continues to be expressed in actively growing and dividing tissues throughout vegetative and reproductive growth, with the highest levels detected in shoot and floral meristems. This broad expression domain suggests that *ERA1* is not markedly regulated at the transcriptional level throughout development. The precise regulation of substrate proteins for FTase or availability of farnesyldiphosphate or the α-subunit are likely to be responsible for FTase activity on target proteins. Consistent with this idea is the observation that constitutive

Fig. 3. ERA1 mRNA expression pattern as determined by *in situ* hybridization. (*Left*) Bright-field images photographed by using Nomarski optics. (*Right*) Bright-field/dark-field double exposures in which both the toluidine blue-stained tissue and the silver grains developed from the photographic emulsion are visible. Longitudinal sections of L*er* inflorescences were hybridized with either a sense (*A* and *B*) or antisense (*C* and *D*) *ERA1* probe. The sense control (*B*) showed no signal above background (regions of the slide where no tissue is present). The antisense probe revealed *ERA1*-specific signal throughout inflorescence tissue (*D*), including the inflorescence meristem, developing flowers, and the stem. The antisense probe also was hybridized to longitudinal sections of 7-day-old L*er* seedlings (*E* and *F*) and various stage L*er* embryos including heart (*G* and *H*) and bent cotyledon (*I* and *J*) stages. *ERA1* mRNA is expressed throughout the vegetative shoot apical meristem and developing leaves in seedlings and throughout the embryo in all embryonic stages examined. (Scale bar, 100 μm.)

overexpression of *ERA1* in otherwise wild-type plants results in no detectable phenotype.

Our *ERA1* RNA expression results are consistent with those obtained for *FTA* and *FTB* RNA, protein, and FTase activity in both pea and tomato (10, 12, 36). Furthermore, the broadly distributed *ERA1* expression pattern correlates well with the observation that *era1–4*, *era1–5*, and *era1–6* mutants are defective in multiple aspects of growth and development (18). A role for FTase activity in abscisic acid-mediated responses of seed dormancy and drought resistance has been shown from the initial studies of *era1–1*, *era1–2*, and *era1–3* mutants (6, 8). The pleiotropy exhibited by *era1–4*, *era1–5*, and *era1–6* mutants reveals an additional requirement for farnesylation in controlling multiple, specific developmentally important signaling pathways including flowering time, senescence, internode elongation, phyllotaxy, sepal initiation, and meristem size maintenance (18).

Farnesylation in Plants. Very little is known about the targets of prenylation in plants, although several lines of investigation are providing clues. To date only one plant protein, ANJ1 (the *Atriplex nummularia* DnaJ homolog), has been shown to be farnesylated *in vivo* (37). Additional farnesylated proteins have been identified *in vitro* by expression library screening (38). Several geranylgeranylated proteins also have been isolated in plants, including a subfamily of Rho-related GTPases from plants, or Rop proteins (39, 40). A Rop protein was identified in a CLV1/CLV3 signaling complex (41), implicating geranylgeranylation in meristem control. Furthermore, computer-assisted database searches for potential isoprenylation substrates in plants has led to the identification of numerous candidate proteins involved in diverse biological processes. These include proteins involved in DNA binding and transcriptional regulation, Ras-related GTP-binding and calcium signaling, cell cycle regulation, cell wall modification, and hormone, defense, and stress responses (5, 42). As the *Arabidopsis* genome sequencing project nears completion, additional candidates for protein prenylation will likely be identified for further study.

A large task for the future lies in the characterization of farnesylated proteins in the signal transduction pathways involved in meristem control and in the various other developmental pathways implicated by the *era1* mutants. The pleiotropy of *era1–4*, *era1–5*, and *era1–6* suggests that multiple, specific developmental processes rely on farnesylation. The expression library screening technique for the identification of farnesylated proteins by Biermann *et al.* (38) presents itself as a method of great potential, particularly because expression libraries could be made from specific *Arabidopsis* tissues at specific times during development. For example, using this technique for a vegetative or reproductive meristem-specific library would help to identify possible signaling proteins that require farnesylation to act in the control of meristem size maintenance.

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