Arabidopsis thaliana Contains Two Differentially Expressed Farnesyl-Diphosphate Synthase Genes*

(Received for publication, January 3, 1996)

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The enzyme farnesyl-diphosphate synthase (FPS; EC 2.5.1.1/EC 2.5.1.10) catalyzes the synthesis of farnesyl diphosphate (FPP) from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). This reaction is considered to be a rate-limiting step in isoprenoid biosynthesis. Southern blot analysis indicates that Arabidopsis thaliana contains at least 2 genes (FPS1 and FPS2) encoding FPS. The FPS1 and FPS2 genes have been cloned and characterized. The two genes have a very similar organization with regard to intron positions and exon sizes and share a high level of sequence similarity, not only in the coding region but also in the intronic sequences. Northern blot analysis showed that FPS1 mRNA accumulates preferentially in roots and inflorescences, whereas FPS2 mRNA is predominantly expressed in inflorescences. The cDNA corresponding to the FPS1 gene was isolated by functional complementation of a mutant yeast strain defective in FPS activity (Delourme, D., Lacroute, F., and Karst, F. (1994) Plant Mol. Biol. 26, 1867-1873). By using a reverse transcription-polymerase chain reaction strategy we have cloned the cDNA corresponding to the FPS2 gene. Analysis of the FPS2 cDNA sequence revealed an open reading frame encoding a protein of 342 amino acid residues with a predicted molecular mass of 39,825 Da. FPS1 and FPS2 isoforms share an overall amino acid identity of 90.6%. Arabidopsis FPS2 was able to rescue the lethal phenotype of an ERG20-disrupted yeast strain. We demonstrate that FPS2 catalyzes the two successive condensations of IPP with both DMAPP and geranyl diphosphate leading to FPP. The significance of the occurrence of different FPS isoforms in plants is discussed in the context of the complex organization of the plant isoprenoid pathway.

Higher plants synthesize a great variety of isoprenoid products that are required not only for their normal growth and development, but also for their adaptive responses to environmental challenges (1). Plant isoprenoid biosynthesis involves a complex multibranched pathway. The ramifications leading to the specific isoprenoid products emerge from a central pathway in which acetyl-CoA is converted, via mevalonic acid and isopentenyl diphosphate (IPP), to a series of prenyl diphosphates of increasing size. These polypropylene diphosphates serve as donors or intermediates in the synthesis of the wide range of isoprenoid end products (1, 2). It is generally accepted that this metabolic pathway must be stringently regulated to maintain the appropriate cellular balance of isoprenoids under changing physiological conditions. In spite of this, the major rate-limiting steps in the pathway have not yet been clearly identified. It is likely that the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase, which catalyzes the synthesis of mevalonic acid, plays a relevant role in the overall control of the isoprenoid biosynthetic pathway (3–7). However, there is also general agreement that additional key enzymes are involved in the control of the pathway to ensure the synthesis of the necessary isoprenoid compounds required for many different purposes in different parts of the plant at different stages of growth and development (1).

Farnesyl-diphosphate synthase (FPS; EC 2.5.1.1/EC 2.5.1.10) catalyzes the sequential 1–4 condensation of two molecules of IPP with both dimethylallyl diphosphate (DMAPP) and the resultant 10-carbon compound geranyl diphosphate (GPP), to produce the 15-carbon compound FPP (8). In plants, FPP serves as a substrate for the first committed reactions of several branched pathways leading to the synthesis of compounds that are required for growth and development, such as phytosterols (membrane structure and function), dolichols (glycoprotein synthesis), ubiquinones, and heme (electron transport), abscisic acid (growth regulator), or sesquiterpenoid phytoalexins (defense against pathogen attack). FPP is also a prenyl donor in protein prenylation, a mechanism that promotes membrane interactions and biological activities of a va-
riety of cellular proteins involved in signal transduction, membrane biogenesis, and cell growth control (9, 10). Therefore, changes in FPS activity could alter the flux of isoprenoid compounds down the various branches of the pathway and, hence, play a central role in the regulation of a number of essential functions in plant cells. The role of FPS in the control of the plant isoprenoid pathway is further supported by the observation that in mammals FPS is a regulated enzyme known to have an important role in the overall control of the sterol biosynthetic pathway (11–14).

Plant FPS has been purified and characterized from different species (1, 15, 16) and, recently, cDNA sequences encoding this enzyme have been cloned from Arabidopsis thaliana (17) and Lupinus albus (18, 19). Comparison of the amino acid sequences of FPS from a variety of organisms, ranging from bacteria to higher eukaryotes, has shown that all the FPS known so far contain five distinct regions with high similarity at the amino acid level (19, 20). These regions are also conserved in other prenyltransferases, including geranylgeranyl- (C20), hexaprenyl-(C30), and heptaprenyl-(C35) diphosphate synthases (20, 21). Two of these regions are the aptase-rich domains that have been shown to play a role in the catalytic reactions of the enzyme, most likely acting as binding sites for the metal ion-complexed pyrophosphate moieties of IPP and the allylic substrates (22, 23).

As a first step toward a better understanding of the role of FPS in the biosynthesis of isoprenoids in plants, we have undertaken the characterization of the genes encoding Arabidopsis FPS. In this paper we report the isolation and characterization of the Arabidopsis FPS1 and FPS2 genes. The FPS1 gene encodes the FPS isoform previously described (17). We have also isolated the cDNA corresponding to the FPS2 gene and shown that it encodes a functional FPS.

**EXPERIMENTAL PROCEDURES**

Enzymes and Biochemicals—Restriction endonucleases and DNA modifying enzymes were purchased from Boehringer Mannheim and Promega. [α-32P]dCTP (3000 Ci/mmol), [35S]Met (1000 Ci/mmol), and [35S]cysteine (2000 Ci/mmol), (w/v) yeast nitrogen base without amino acids and (NH4)2SO4 were from Difco Laboratories. All other chemicals were of the highest commercial grade available.

Plant Material—A. thaliana plants (ecotype Columbia) were grown under a 16 h light/8 h dark illumination regime at 22°C on a perlite/vermiculite/sphagnum (1:1:1) mixture irrigated with mineral nutrients under a 16-h light/8-h dark illumination regime at 22°C on a perlite/vermiculite/sphagnum (1:1:1) mixture irrigated with mineral nutrients.

Strains, Media and Plasmids—Saccharomyces cerevisiae TRP1, Escherichia coli /proABlacI (Stratagene) were used. Recombinant DNA was produced using a commercially available system (Life Technologies, Inc.), and 400 units of Moloney murine leukemia virus-reverse transcriptase (Life Technologies, Inc.). The reaction mixture was incubated for 2 h at 42°C and rapidly cooled in ice. Two μl of the single-stranded cDNA pool was denatured for 5 min at 95°C in a
50-μl reaction mixture containing 25 pmol each of an upstream primer specific for the leader region of the FPS2 mRNA (5'-GGTTCCACATT-TGGCCTTGGCA-3', nucleotides -41 to -20 in Fig. 4), and the adaptor d[10g]nucleotide as a downstream primer (5'-GACTCGAGTCA-GATCCATCGG-3'), 1.5 mM MgCl\textsubscript{2}, 0.2 mM each dATP, dCTP, dGTP, and dTTP, and PCR buffer (Amersham). After cooling to 72°C, 1 unit of Taq polymerase (Pharmacia) was added and the mixture was annealed for 1 min at 58°C. The cDNA was amplified by incubation of the mixture for 40 min at 72°C, followed by 40 cycles of 40 s at 94°C, 1 min at 58°C, and 2 min at 72°C, with a 15-min final extension at 72°C. The resulting PCR product (approximately 1.3 kilobases) was gel-purified and ligated into plasmid pGEM-T (Promega) prior to sequencing. The resulting plasmid was named pCHC2.

Mapping of the 5'-end of FPS2 mRNA-The 5'-end of the Arabidopsis FPS2 mRNA was determined by the 5' RACE technique using the 5'-Amplifier RACE kit (Clontech Laboratories). Five μg of poly(A)RNA from Arabidopsis inflorescences was reverse transcribed according to the manufacturer's recommendations, using an antisense gene-specific primer (5'-CTTGAGGATGGATGCGAA-3') complementary to the nucleotide sequence +373 to +393 in the Arabidopsis FPS2 cDNA (Fig. 4). An anchor di[10g]nucleotide (provided in the kit) was then ligated to the 3'-end of the single-stranded cDNA using T4 RNA ligase. The 5'-end of the FPS2 cDNA was amplified by PCR using a forward primer complementary to the anchor di[10g]nucleotide and a reverse nested FPS2-specific primer (5'-GGCTTCTAACCAAAAGGCTGG-3') complementary to the nucleotide sequence +312 to +335 in the Arabidopsis FPS2 cDNA (Fig. 4). PCR was performed under the same conditions described above for 35 cycles of 35 s at 94°C, 45 s at 60°C, and 2 min at 72°C, with a 15-min final extension at 72°C. The resulting PCR product was gel-purified, digested with EcoRI, and cloned into the corresponding site of plasmid pUC19 prior to sequencing.

In vitro transcription/Translation- A SacI-Sall fragment of plasmid pcNC2, containing the FPS2 cDNA, was cloned into the corresponding sites of pcBluescript. The resulting plasmid was named pcBNC2. The FPS2 cDNA was cut out as a SacI-Sall fragment from plasmid pBNC2 and cloned into the corresponding sites of plasmid pSP65 (Promega). The resulting plasmid, pSPNC2, was used as a template for in vitro transcription/translation using [-35S]Met and the TNT coupled Wheat Germ Extract System (Promega), according to the manufacturer. The 35S-labeled protein was separated by SDS-polyacrylamide gel electrophoresis (12% acrylamide) and detected by fluorography.

Assay for FPS Activity—Yeast strains were grown in minimal medium containing ergosterol and/or the amino acids required to-supplement auxotrophies. The cell-free extracts (105,000 × g) were prepared in 50 mM phosphate buffer, pH 7.0 (25), and incubated for 6 min in the-Amplifinder RACE kit (Clontech Laboratories). Five 5 μg of poly(A)RNA from Arabidopsis inflorescences was reverse transcribed according to the manufacturer's recommendations, using an antisense gene-specific primer (5'-CTTGAGGATGGATGCGAA-3') complementary to the nucleotide sequence +373 to +393 in the Arabidopsis FPS2 cDNA (Fig. 4). An anchor di[10g]nucleotide (provided in the kit) was then ligated to the 3'-end of the single-stranded cDNA using T4 RNA ligase. The 5'-end of the FPS2 cDNA was amplified by PCR using a forward primer complementary to the anchor di[10g]nucleotide and a reverse nested FPS2-specific primer (5'-GGCTTCTAACCAAAAGGCTGG-3') complementary to the nucleotide sequence +312 to +335 in the Arabidopsis FPS2 cDNA (Fig. 4). PCR was performed under the same conditions described above for 35 cycles of 35 s at 94°C, 45 s at 60°C, and 2 min at 72°C, with a 15-min final extension at 72°C. The resulting PCR product was gel-purified, digested with EcoRI, and cloned into the corresponding site of plasmid pUC19 prior to sequencing.

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RESULTS

Isolation and Characterization of Genomic Clones Corresponding to Arabidopsis FPS1 and FPS2 Genes—Southern blot analysis of Arabidopsis genomic DNA digested with different restriction enzymes was performed using as a probe a 340-bp NotI-HindIII cDNA fragment from the recombinant clone pDD71, which contains the Arabidopsis FPS2 cDNA previously isolated (17), and herein referred to as FPS1 cDNA. The simple pattern of bands obtained under high stringency hybridization conditions (Fig. 1A) suggested that the fragments detected correspond to the gene that encodes the FPS1 isoform previously reported (17). This gene is referred to as FPS1 gene. However, additional bands were observed when hybridization was performed using the same probe under low stringency conditions (Fig. 1C). These results indicated that the Arabidopsis genome contains sequences related to the FPS1 gene, thus revealing that in this plant FPS might be encoded by a small gene family.

To clone the Arabidopsis FPS genes, a 730-bp EcoRI-PstI cDNA fragment from clone pDD71 was used to screen an Arabidopsis genomic library under low stringency conditions. Eleven positive clones were isolated. These clones were classified in two distinct groups since restriction endonuclease mapping and Southern hybridization analyses showed that they contained DNA inserts corresponding to two different genomic regions. Clones pgNC10 and pgNC24 were selected for further characterization as representatives of each group. Two genomic fragments from each clone hybridizing to the cDNA probe were subcloned. Sequence analysis revealed that plasmids pgNC241 and pgNC242 (Fig. 2A) contained overlapping inserts including the entire coding region of the FPS1 gene as well as 5'- and 3'-flanking regions. Plasmids pgNC101 and pgNC102 (Fig. 2A) contained overlapping fragments with a sequence different although highly similar to that of the FPS1 gene, which corresponds to a second FPS gene (FPS2), as was later verified.

Southern blot analysis of Arabidopsis genomic DNA, performed under high stringency conditions using as a probe a 800-bp XhoI-HindIII fragment from the FPS2 gene (Fig. 2), revealed a simple pattern of bands (Fig. 1B) which accounted for a subset of genomic fragments previously detected at low stringency by the FPS1 probe (Fig. 1C). It was concluded that these fragments derived from the FPS2 gene. Interestingly, the bands specifically detected by the FPS1 and FPS2 probes (Fig. 1, A and B) accounted for most of the bands identified by the FPS1 probe under low stringency conditions (Fig. 1C). However, one additional weakly hybridizing fragment was detected in each lane. Taken together, these results indicated that Arabidopsis contains two genes encoding FPS (FPS1 and FPS2) and a genomic sequence that might correspond to a gene encoding either an additional FPS isoform or a closely related prenyltransferase. The nucleotide sequences of the FPS1 and FPS2 genes (data not shown) have been deposited in the Gen-
The alignment of the nucleotide sequence of the FPS1 gene with that of the FPS1 cDNA showed that the gene consists of 12 exons and 11 introns (Fig. 2B). Comparison of these two sequences revealed several single-base differences. Because of two of these changes, Ser-177 (TCC) and Thr-283 (ACC) in the predicted amino acid sequence of the FPS1 protein previously reported (17) are converted to Ala (GCC) and Pro (CCC), respectively, in the protein encoded by the FPS1 gene. These changes presumably represent DNA polymorphisms associated with the different Arabidopsis ecotypes used. The organization of exons and introns of the FPS2 gene was initially deduced by comparing its sequence with that of the FPS1 gene, and further confirmed after alignment with the sequence of the FPS2 cDNA (see below). The FPS2 gene consists of 11 exons and 10 introns. The two genes have a very similar structure, although it is worth noting that exon 4 in the FPS2 gene corresponds to exons 4 and 5 in the FPS1 gene (Fig. 2B). In both genes, introns are located at equivalent positions relative to the coding sequences. All exon-intron junctions follow the GT/AG rule (36). The alignment of the sequences of the FPS1 and FPS2 genes revealed that they share a high level of similarity not only in the coding region (87% overall identity) but also in the intronic sequences (identity higher than 57%).

Expression Analysis of FPS1 and FPS2 Genes—Northern blot analysis of total RNA from different Arabidopsis tissues using FPS1 and FPS2 gene-specific probes revealed that each probe detected a transcript of approximately 1.3 kilobases (Fig. 3). The two genes were expressed in all tissues analyzed although they had a different pattern of expression. The highest level of expression of FPS1 mRNA was found in roots and inflorescences whereas FPS2 mRNA was expressed at a lower level and accumulated preferentially in inflorescences. No significant change in the levels of FPS1 or FPS2 mRNA was detected when RNA samples were prepared from light- or dark-grown seedlings (Fig. 3A). Equal amounts of RNA were present in each lane, as confirmed by hybridization of the filters with a fragment of the wheat 25 S rRNA gene (data not shown).

Isolation and Characterization of a cDNA Encoding Arabidopsis FPS2—Attempts to isolate cDNA clones corresponding to the FPS2 gene from different Arabidopsis cDNA libraries were unsuccessful. To clone an FPS2 cDNA, a reverse transcription-PCR strategy was developed (for details see “Experimental Procedures”). A cDNA fragment of approximately 1.3 kilobases, obtained in PCR experiments using poly(A+) RNA from Arabidopsis inflorescences, was cloned (pcNC2) and sequenced. The cDNA insert was found to have a nucleotide sequence of 1300 bp (Fig. 4) which, excluding a polyadenylate tail of 39 bases, was identical to the sequence of the predicted fragment of the wheat 25 S rRNA gene (data not shown).

Fig. 2. Restriction and structural maps of Arabidopsis FPS1 and FPS2 genomic clones. A, restriction map of the genomic regions containing the FPS1 and FPS2 genes. FPS1 and FPS2 transcription units are represented by solid boxes. The donoted regions contained in recombinant plasmids are indicated below the restriction maps. The 800-bp XhoI-HindIII probe from pgNC102 used in genomic Southern blot analysis is indicated by a double arrowhead line. Restriction sites are as follows: B, BamHI; E, EcoRI; EV, EcoRV; H, HindIII. B, structural organization of the FPS1 and FPS2 genes. Exons are represented by boxes and are numbered from the 5' end of the genes. Lines between boxes correspond to introns. Coding regions are represented by solid boxes.

Bank data base with accession numbers L46367 and L46350, respectively.

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Fig. 3. Northern blot analysis of Arabidopsis FPS1 and FPS2 mRNA. A, total RNA samples from different tissues of Arabidopsis (30 μg/ lane) was electrophoresed in 1% agarose-formaldehyde gels and transferred onto nylon membranes. Filters were hybridized with the FPS1 and FPS2 gene-specific probes shown in B. Exposure times were 9 days for FPS1 and 21 days for FPS2. B, map of the 3' -region of the FPS1 and FPS2 genes. The last exon of each gene is represented by a box. The 3' -untranslated regions are represented by open boxes. Lines correspond to the genomic regions flanking the 3' -end of the genes. The FPS1 (570-bp BglII-HindIII fragment) and FPS2 (450-bp BglII-KpnI fragment) gene-specific probes are indicated by double arrowhead lines.
ATCAATGGC) fit the consensus reported for functional start codons in plants (AACAATGGC) (38), except that a Ti s found at position 23 relative to the ATG codon. The clone also contained a 41-bp non-coding sequence preceding the ATG start codon and a 191-bp 3'-untranslated region, including a consensuspolyadenylation motif (AATAAA) located 16 bp upstream of the polyadenylate tail. The 5'-end of the FPS2 mRNA was determined by the RACE technique and found to have 5 additional nucleotides with respect to the FPS2 cDNA (Fig. 4). This additional sequence corresponds exactly with the sequence of the FPS2 gene.

To check the size of the protein encoded by the FPS2 cDNA, the FPS2 transcript was synthesized in vitro from plasmid pcSPNC2 and translated in a wheat germ cell-free system. A single protein migrating with an apparent molecular mass of about 41 kDa was generated from FPS2 mRNA (data not shown). The apparent molecular mass of this protein is in good agreement with the predicted molecular mass of FPS2 (39,825 Da).

The Arabidopsis FPS1 and FPS2 isoforms are composed of 343 and 342 amino acid residues, respectively. The alignment of the amino acid sequence of FPS1 and FPS2 is shown in Fig. 4. The two proteins are highly conserved throughout their sequence, showing an overall amino acid identity of 90.6% and a similarity of 94.5%. Both enzymes contain the five conserved regions, designated I to V (Fig. 4), which appear to be common not only to all the FPS isoforms previously reported (19) but also to other prenyltransferases (20, 21). Regions II and V correspond to the two aspartate-rich domains that have been shown to be involved in enzyme catalysis (22, 23).

Confirmation of the FPS Activity of the Arabidopsis FPS2—To check that the Arabidopsis FPS2 cDNA encoded a functional enzyme, the cDNA was expressed in the mutant yeast strain CC25, which is defective in FPS activity. The strain CC25 is a thermosensitive mutant strain that carries the leaky mutation erg20–2 affecting the ability of FPS to catalyze the condensation of GPP with DMAPP. The reaction products obtained were analyzed by TLC after enzymatic hydrolysis. The radioactivity was detected only in the geraniol and farnesol fractions, and was measured as described under “Experimental Procedures.” The amount of GPP and FPP produced is expressed as percentage with respect to the sum counts in the geraniol and farnesol fractions, which was considered as 100%. Results are the average of three experiments. Variation between measurements was between 5 and 12%.

 FIG. 5. Confirmation of the FPS activity of the Arabidopsis FPS2 isoform. A, functional complementation of the mutant yeast strain CC25 with plasmid pNCFPS2. Strain CC25 and strain CC25[pNCFPS2] were streaked onto YPD plates or YPD plates supplemented with 80 μg/ml ergosterol and incubated at 36 °C for 3 days. B, identification of the FPS reaction products in CC25, CC25[pNCFPS2], and NC1 strains. Cell-free extracts from each strain were incubated in the presence of [3H]IPP and DMAPP. The reaction products obtained were analyzed by TLC after enzymatic hydrolysis. The radioactivity was detected only in the geraniol and farnesol fractions, and was measured as described under “Experimental Procedures.” The amount of GPP and FPP produced is expressed as percentage with respect to the sum counts in the geraniol and farnesol fractions, which was considered as 100%. Results are the average of three experiments. Variation between measurements was between 5 and 12%.

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Confirmation of the FPS Activity of the Arabidopsis FPS2—To check that the Arabidopsis FPS2 cDNA encoded a functional enzyme, the cDNA was expressed in the mutant yeast strain CC25, which is defective in FPS activity. The strain CC25 is a thermosensitive mutant strain that carries the leaky mutation erg20–2 affecting the ability of FPS to catalyze the condensation of GPP with IPP to yield FPP. As a consequence this strain is auxotrophic for ergosterol at a nonpermissive temperature (36 °C) (25). Strain CC25 was transformed with plasmid pNCFPS2, carrying the Arabidopsis FPS2 cDNA under the control of the PGK promoter. The results, shown in Fig. 5A, demonstrate that plasmid pNCFPS2 complements the ergosterol auxotrophy of strain CC25 at 36 °C. The presence of FPS activity in the transformed yeast mutant was checked by an in vitro assay using cell free extracts obtained from the CC25[pNCFPS2] strain. The major reaction product was found to be FPP (Fig. 5B). In contrast, strain CC25 synthesized GPP
Because the FPS activity in CC25 strain is impaired in the condensation step of GPP with IPP to produce FPP, it was not possible to ascertain whether FPS2 could actually catalyze the two sequential reactions involved in the synthesis of FPP from IPP and DMAPP. To address this question, we checked whether plasmid pNCFPS2 also complemented a disrupted FPS gene. A haploid yeast strain bearing a disrupted FPS gene copy is not viable, even in the presence of ergosterol (26). Haploid strain NC1, constructed as described under “Experimental Procedures,” having a disrupted copy of the yeast FPS and harboring plasmid pNCFPS2, showed a wild type phenotype whatever the growth conditions tested. When cell-free extracts from strain NC1 were assayed for FPS activity the major reaction product was FPP (Fig. 5B). Strain NC1 also synthesized FPP when GPP was used instead of DMAPP as allylic primer (data not shown), thus confirming the ability of FPS to use either C9 or C10 allylic primers. Taken together, these results unequivocally demonstrate that the Arabidopsis FPS2 cDNA encodes a functional FPS isofrom which is able to catalyze the two successive condensations of IPP with both DMAPP and GPP leading to FPP formation.

**DISCUSSION**

The multibranch isoprenoid biosynthetic pathway in plants represents one of the most complex metabolic pathways known (1, 2). One of the most challenging aspects of plant isoprenoid biosynthesis is the identification of the enzymes that catalyze the rate-limiting steps in the pathway. It is widely assumed that 3-hydroxy-3-methylglutaryl-CoA reductase, the enzyme that synthesizes mevalonic acid, plays a relevant role in the overall control of plant isoprenoid biosynthesis (3–7). However, it is also accepted that mevalonic acid synthesis is not the only limiting step in isoprenoid biosynthesis, and that additional key enzymes are involved in the control of the flux through the pathway to maintain the appropriate cellular balance of isoprenoids under different physiological conditions (1). FPS is considered to play a relevant role in the control of plant isoprenoid biosynthesis, since FPP is the starting point of different branched pathways leading to the synthesis of key isoprenoid end products. As a first step to study the role of FPS in the control of plant isoprenoid biosynthesis, we have undertaken the molecular characterization of FPS in A. thaliana.

The results presented here demonstrate that Arabidopsis contains a small FPS gene family consisting of at least two genes (FPS1 and FPS2) that encode closely similar FPS isoforms. The Arabidopsis FPS1 and FPS2 genes have been cloned and characterized. The two genes have a very similar organization with regard to intron positions and exons sizes, and share a high level of sequence similarity not only in the coding region but also in the intronic sequences. These observations indicate that these two genes have arisen from a recent duplication of an ancestral FPS gene. In spite of this, FPS1 and FPS2 have a different pattern of expression. By using gene-specific probes we have shown that, although the two genes are expressed in all the tissues analyzed, FPS1 mRNA is present mainly in roots and inflorescences, whereas FPS2 mRNA is detected at a lower level and accumulates preferentially in inflorescences. It is worth noting that the 3′-untranslated region of the Arabidopsis FPS2 transcript contains one copy of the AUUUA motif (position +1068 in the FPS2 cDNA sequence). This sequence has been shown to act as an mRNA instability determinant (for review, see Ref. 39). However, it remains to be determined whether this motif actually participates in modulating the Arabidopsis FPS2 transcript levels.

It has been previously shown that FPS1 is an active form of the enzyme (17). At the protein level, Arabidopsis FPS1 and FPS2 are very similar (90.6% identity), with amino acid changes distributed throughout their sequence (Fig. 4). This suggested that FPS2 might represent an active form of the enzyme. This was demonstrated by the complementation of the yeast strain CC25 with plasmid pNCFPS2, which carries the Arabidopsis FPS2 cDNA under the control of the yeast PGK promoter. Strain CC25 is auxotrophic for ergosterol at 36°C since it carries the leaky mutation erg20-2 in the FPS gene that impairs the C10 to C15 elongation step. This results in a concomitant accumulation of GPP, which is dephosphorylated by endogenous phosphatases and excreted to the growth medium as geraniol (26). Strain CC25 was initially chosen because it allowed a rapid assay of the functionality of the FPS2. However, due to the nature of the erg20-2 mutation, it remained formally possible that the Arabidopsis FPS2 could catalyze the synthesis of FPP from IPP and GPP, but not the preceding condensation of IPP with DMAPP to form GPP. To rule out this possibility, we generated the haploid strain NC1, which has a disrupted copy of the FPS gene (erg20 mutation) and harbors plasmid pNCFPS2. It has been shown that the disruption of the FPS gene is lethal for yeast even in the presence of exogenously supplied ergosterol (26). However, strain NC1 showed a wild type phenotype, thus indicating that plasmid pNCFPS2 encodes an enzyme which is able to catalyze the two successive condensations of IPP with both DMAPP and GPP leading to FPP formation. The presence of FPS activity was further confirmed by an in vitro assay using cell-free extracts obtained from strain NC1.

In contrast to the controversy surrounding the subcellular location of the enzymes involved in the synthesis of IPP in plants, there is general agreement that the enzymes utilizing IPP are distributed in three subcellular compartments, namely cytosol, mitochondria, and plastids (1, 40). The cytosol is the only cell compartment where plant FPP has been detected (1, 15, 40). In animal cells, the major site of FPP synthesis is also the cytosol. However, it has recently been reported that in mammals FPS activity is also present in mitochondria (41) and peroxisomes (42). This raises the question that in plants FPS might be present in cell compartments other than the cytosol. The alignment of the primary sequence of Arabidopsis FPS1 and FPS2 with that of the known FPS from other organisms (bacteria, fungi, plant, and animals) (19, 20) shows that the two Arabidopsis FPS isoforms lack amino-terminal extensions that could represent transit peptides to plastids and mitochondria. Furthermore, the N-terminal sequence of Arabidopsis FPS1 and FPS2 has no features of transit peptides for targeting into these organelles (43). However, it cannot be ruled out that other forms of the enzyme, resulting from the use of alternative promoters or from alternative splicing processes, might be targeted to different subcellular locations. In addition, we cannot exclude that organellar forms of FPS could be encoded by additional genes not yet characterized.

One of the more intriguing findings arising out of the molecular biology studies of plant isoprenoid biosynthesis is the occurrence of gene families encoding key enzymes of this metabolic pathway. For example, the number of genes encoding 3-hydroxy-3-methylglutaryl-CoA reductase varies from the two genes described in Arabidopsis (44, 45) to at least 11 genes found in potato (5, 46). At least five geranylgeranyl diphosphate synthase genes have been reported to occur in Arabidopsis (47). It has been described that vetispiradiene synthase, a sesquiterpene cyclase found in Hyoscyamus muticus, is encoded by a gene family of six to eight members (48). Our results indicate that Arabidopsis also contains a small FPS gene family consisting of at least two genes. Although the complexity of the FPS gene family in plants has only been studied in Arabi-
dopiosis, it is tempting to speculate that FPS gene families with similar or even greater complexity may also be found in other plant species. The occurrence of FPS isozymes raises the question about the role of each individual FPS isozyme in the isoprenoid biosynthetic pathway. The differential expression of FPS1 and FPS2 might be indicative of an specialized function of each FPS isozyme in directing the flux of pathway intermediates to specific isoprenoid end products. We are currently applying different molecular and cellular approaches to identify the specific control mechanisms. We are currently applying different molecular and cellular approaches to identify the specific function of each FPS isozyme in the organization of the plant isoprenoid pathway.

Acknowledgments—We thank Dr. A. Bachmair for the genomic library and Robin Rycroft for editorial help.

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J. Biol. Chem. 1996, 271:7774-7780.
doi: 10.1074/jbc.271.13.7774

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