Plant Molecular Biology **26**: 1867–1873, 1994.

Cloning of an *Arabidopsis thaliana* cDNA coding for farnesyl diphosphate synthase by functional complementation in yeast

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Received 28 June 1994; accepted in revised form 22 September 1994

Key words: *Arabidopsis thaliana*, cDNA, complementation, *erg20-2* yeast mutant, farnesyl diphosphate synthase, *Saccharomyces cerevisiae*

Abstract

A cDNA encoding farnesyl diphosphate synthase, an enzyme that synthesizes C15 isoprenoid diphosphate from isopentenyl diphosphate and dimethylallyl diphosphate, was cloned from an *Arabidopsis thaliana* cDNA library by complementation of a mutant of *Saccharomyces cerevisiae* deficient in this enzyme. The *A. thaliana* cDNA was also able to complement the lethal phenotype of the *erg20* deletion yeast mutant. As deduced from the full-length 1.22 kb cDNA nucleotide sequence, the polypeptide contains 343 amino acids and has a relative molecular mass of 39689. The predicted amino acid sequence presents about 50% identity with the yeast, rat and human FPP synthases. Southern blot analyses indicate that *A. thaliana* probably contains a single gene for farnesyl diphosphate synthase.

Introduction

Farnesyl diphosphate synthase (EC 2.5.1.1/EC 2.5.1.10) is a key enzyme in isoprenoid biosynthesis. It catalyses the sequential 1′-4 condensation of the 5-carbon isoprenoid compounds isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) to form the 10-carbon geranyl diphosphate (GPP); GPP is then condensed with another IPP molecule to form the 15-carbon product farnesyl diphosphate (FPP) [16]. FPP is a precursor of sterols through head-head condensation catalysed by squalene synthase. In addition three other prenyl transferase activities are found in living cells; *cis*-prenyl transferase involved in dolichol formation [7], *trans*-prenyl transferase involved in ubiquinone side chain synthesis [15] and geranylgeranyl diphosphate (GGPP) synthase catalysing GGPP formation for protein prenylation [10] and carotenoid biosynthesis [9]. One question concerns the substrates of the different prenyl transferases *in vivo*; is FPP a precursor of sterols only or is it a common precursor of all the cellular isoprenoids, in-

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number X75789.

cluding dolichol and carotenoids? In yeast, it has been shown that FPP synthase is an essential gene, even when sterols are exogenously supplied [1, 3] suggesting the FPP does not act only as a precursor of ergosterol. In plants, the model of Kleinig [11] supposes that prenyl transferases are localized in specific compartments; GGPP synthase in plastids, *trans*-prenyl transferase in mitochondria, the FPP synthase in the cytosol. In this model IPP is formed only in cytosol and transported into the different cell compartments. A protein involved in IPP uptake in plastids has indeed been recently characterized in *Vitis viniferas* cells [20].

The identification of FPP synthase gene(s) in plants could thus be helpful to gain a better insight into isoprenoid formation in plants and compartmentation of substrates. We describe here the isolation of an *Arabidopsis thaliana* cDNA encoding FPP synthase and the characteristics of the predicted protein.

Materials and methods

Strains, media and plasmids

The following yeast strains were used: the wild type strain FL100 (ATCC 28383, MAT a), CC25 (MAT a, *erg12-2, erg20-2, ura3-1, trp1-1* [4]). LB311 (*erg20::URA3/ERG20, ura3-1/ura3-1, trp1-1/trp1-1* [3]). DD163 (MAT a, *erg20::URA3, ura3-1, trp1-1* [pDD63]), is a haploid Ura^+, Trp^+ segregant isolated from diploid strain LB331 transformed by plasmid pDD63 carrying the *Arabidopsis thaliana* FPP synthase cDNA and the selectable marker *TRP1*. *Escherichia coli* strain was DH5α. *A. thaliana* was ecotype Landsberg erecta.

Yeast strains were grown at 28 °C. The complete medium used consisted of 2% yeast extract (Biokar), 1% peptic digest of meat (Biokar) and 2% glucose. The minimal medium used consisted of 0.16% of Yeast Nitrogen Base without amino acids and $(NH_4)_2SO_4$ (Difco), 0.5% $(NH_4)_2SO_4$ and 1% glucose. For auxotrophic strains, ergosterol was supplied by dilution of stock solution (4 mg/ml) in a mixture of Tergitol NP40/ethanol (1:1 v/v) and tryptophane was supplied at 50 μg/ml. *E. coli* was grown at 37 °C in LB medium composed of 1% tryptone (Biokar), 0.5% yeast extract (Biokar) and 1% NaCl [12]. Ampicillin (100 μg/ml) was added as required. Plants grown in soil were kept in 16 h light and 8 h of darkness.

The *A. thaliana* cDNA library was constructed in pFL61; in this vector, the cDNAs are under the control of the strong yeast phosphoglycerate kinase (PGK) promoter [14]. The plasmid Bluescript SK + (Stratagene) was used for subcloning and sequencing. Plasmid pDD71 is a plasmid from the *A. thaliana* library containing the FPP synthase cDNA in *Not* I site under the control of the PGK promoter and terminator sequences. Plasmid pDD63 was derived from pDD71; it contains the selectable marker TRP1 instead of URA3 in the *Bgl* II site.

Library screening

The yeast ergosterol auxotrophic strain CC25 was transformed with *A. thaliana* cDNA library by the lithium acetate procedure [8]. The transformants were first selected on minimal medium supplemented with tryptophane (50 μg/ml) and ergosterol (80 μg/ml). A second selection was done by plating the Ura + transformants on minimal medium with tryptophane without ergosterol.

Southern blot and sequence analysis

Genomic DNA was prepared from mature soil-grown plants [17], digested with different restriction enzymes, electrophoresed in agarose gel 0.8%, blotted onto nitrocellulose membrane (Amersham Hybond-C extra) and hybridized with radioactive probes. Probes were labelled with [α-^{32}P]dCTP and random hexamer primers (Pharmacia). Blots were hybridized overnight at 65 °C as described [12] and were washed in 2 × SSC at 65 °C. *Not* I/*Not* I, *Not* I/*Hin*d III, *Hin*d III/*Not* I, *Not* I/*Pst* I and *Pst* I/*Not* I subclones of pDD71 were constructed in the plasmid

Bluescript SK +. The *Not* I/*Not* I subclone was used to construct other subclones with the Erase-a-Base System (Promega). The sequence of both strands of the FPP synthase gene was determined by the dideoxynucleotide method [18] with reverse primer and universal primer of the Autoread Sequencing Kit (Pharmacia) with the A.L.F. DNA sequencer (Pharmacia). Sequences were assembled and analysed using Dnasis (Hitachi). The GCG program PILEUP was used to align the translated nucleotide sequence with protein sequences extracted from the SWISS-PROT data bank (accession numbers P08524, P15495, P14324 and P05369).

Ergosterol level and FPP synthase assay

The amount of sterols with 5–7 dienoic system (sum of ergosterol 75% and ergosta-5,7-dienol 25%) was determined from the maximum absorbance at 281.5 nm [19] and expressed as % of cell dry weight. FPP synthase assay was performed as described previously [4]. Yeast cells were grown in minimal medium. The cell-free extract (105 000 × g) was prepared in 50 mM phosphate buffer pH 7, 5 mM iodoacetamine. Incubation mixture (final volume 100 µl) contained 5 to 60 µg of proteins, 120 µM DMAPP, 60 µM [^{14}C] IPP (2.1 mCi/mmol), 1 mM $MgCl_2$. Incubation (5 min) was performed at 35 °C and initiated by addition of the 105 000 × g supernatant. The sample was ice-chilled, 0.5 ml water was added, followed by 1 ml hexane and 0.2 ml 1 M HCl. After 10 min incubation at 37 °C, the mixture was shaken vigorously and an aliquot of the hexane layer was counted for radioactivity by liquid scintillation.

Results

Isolation of cDNA encoding FPP synthase

Predicted primary sequences of eucaryotic FPP synthases present strong conserved domains proximal to the C-terminus [2]. In a first attempt to clone the plant gene by hybridization we used the *Eco* RV/*Eco* RV fragment (318 bp) of the yeast FPP synthase gene encoding the conserved regions as probe [1]. No hybridization could be observed, even at low stringency with *A. thaliana* and *Nicotiana tabacum* genomic DNA.

We decided therefore to clone the plant gene by functional complementation, using the *A. thaliana* cDNA library constructed in the yeast expression vector pFL61 [14]. Sterol-auxotrophic yeast strain CC25, defective in FPP synthase was used as recipient. This strain, carries two leaky mutations *erg20-2* and *erg12-2* affecting respectively FPP synthase and mevalonate kinase genes [4]. The *erg20-2* mutation alters Lys-197 to Glu; the end product of the defective FPP synthase is GPP subsequently dephosphorylated and excreted. The viability of the strain is depending on the *erg12-2* mutation required to reduce the amount of geraniol being toxic to the cells [3]. In addition strain CC25 carries a *ura3-1*-defective allele. Cells were transformed by the lithium acetate procedure [8]. The frequency of transformation was about 2000 clones per µg DNA. Among 70 000 Ura$^+$ transformants plated on medium without ergosterol, we isolated 14 Erg$^+$ clones. For one clone, plasmid loss was clearly linked to the recovery of Ura$^-$ and Erg$^-$ phenotype, revealing that ergosterol auxotrophy was complemented by a plant cDNA. Plasmid pDD71 was isolated from this clone. A restriction map of the insert is presented in Fig. 1.

Analysis of cDNA and deduced amino acid sequences

The 1.3 kb *Not* I/*Not* I insert was subcloned in plasmid Bluescript SK + and both strands were sequenced. Complete sequence analysis of this cDNA revealed that the insert was 1314 bp long. The nucleotide and predicted amino acid sequences of FPP synthase are shown in Fig. 2. Not shown in the figure are the 15 bp in the 5′ and 3′ regions corresponding to additional nucleotides generated during the library construction [14] and 60 bp in the 3′ region corresponding to

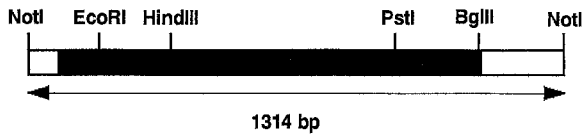

Fig. 1. Restriction map of the cDNA encoding *Arabidopsis thaliana* FPP synthase. Fragment *Not* I/*Not* I of 1314 bp corresponds to the entire cDNA. The *Not* I sites were originate from the vector. The coding region is represented by a solid box.

the poly(A)$^+$. Translation of the longest open reading frame revealed that it encodes a protein of 343 amino acids with a deduced molecular mass of 39689 Da. The 3′-untranslated region contains the putative polyadenylation signal AATAAA. The predicted amino acid sequence of the plant FPP synthase aligned with the rat [5], human [21] and *S. cerevisiae* [1] FPP synthases is shown in Fig. 3. The *A. thaliana* protein presents 51%, 47% and 46% identity with *S. cerevisiae*, rat and human FPP synthases, respectively.

Southern blot analysis

To determine the number of genes related to FPP synthase in the *A. thaliana* genome, Southern blot analysis was performed at high stringency using a 340 bp *Not* I/*Hin*d III probe (Fig. 4). A single band was detected with genomic DNA digested with *Bam* HI. With *Hin*d III two bands were observed, one with a strong hybridization signal, the other weak. With *Eco* RI three bands were observed, two strong and one weak. Two bands were expected with *Eco* RI since *Eco* RI cleaves at the 5′ of the coding region in the cDNA, between *Not* I and *Hin*d III. The Southern blots experiments were repeated with *A. thaliana* genomic

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   1 AGTAATTTAGCTGAGAAGTCTTGGTGGGAGTCTCTATCGTCGTCGTATCCAAAGCTCTTCAATGGAGACCGATCTCAAGT   80
                                                          M  E  T  D  L  K  S

  81 CAACCTTTCTCAACGTTTATTCTGTTCTCAAGTCTGACCTTCTTCATGACCCTTCCTTCGAATTCACCAATGAATCTCGT  160
       T  F  L  N  V  Y  S  V  L  K  S  D  L  L  H  D  P  S  F  E  F  T  N  E  S  R

 161 CTCTGGGTTGATCGGATGCTGGACTACAATGTACGTGGAGGGAAACTCAATCGGGGTCTCTCTGTTGTTGACAGTTTCAA  240
       L  W  V  D  R  M  L  D  Y  N  V  R  G  G  K  L  N  R  G  L  S  V  V  D  S  F  K

 241 ACTTTTGAAGCAAGGCAATGATTTGACTGAGCAAGAGGTCTTCCTCTCTTGTGCTCTCGGTTGGTGCATTGAATGGCTCC  320
       L  L  K  Q  G  N  D  L  T  E  Q  E  V  F  L  S  C  A  L  G  W  C  I  E  W  L  Q

 321 AAGCTTATTTCCTTGTGCTTGATGACATTATGGATAACTCTGTCACCCGCCGTGGTCAACCTTGCTGGTTCAGAGTTCCT  400
       A  Y  F  L  V  L  D  D  I  M  D  N  S  V  T  R  R  G  Q  P  C  W  F  R  V  P

 401 CAGGTTGGTATGGTTGCCATCAATGATGGGATTCTACTTCGCAATCACATCCACAGGATTCTCAAAAAGCATTTCCGTGA  480
       Q  V  G  M  V  A  I  N  D  G  I  L  L  R  N  H  I  H  R  I  L  K  K  H  F  R  D

 481 TAAGCCTTACTATGTTGACCTTGTTGATTTGTTTAATGAGGTTGAGTTGCAAACAGCTTGTGGCCAGATGATAGATTTGA  560
       K  P  Y  Y  V  D  L  V  D  L  F  N  E  V  E  L  Q  T  A  C  G  Q  M  I  D  L  I

 561 TCACCACCTTTGAAGGCGAAAAGGATTTGTCCAAGTACTCATTGTCAATCCACCGTCGTATTGTCCAGCACAAAACGGCT  640
       T  T  F  E  G  E  K  D  L  S  K  Y  S  L  S  I  H  R  R  I  V  Q  H  K  T  A

 641 TATTACTCATTTTATCTCCCTGTTGCTTGTGCGTTGCTTATGGCGGGCGAAAATTTGGAAAACCATATTGACGTGAAAAA  720
       Y  Y  S  F  Y  L  P  V  A  C  A  L  L  M  A  G  E  N  L  E  N  H  I  D  V  K  N

 721 TGTTCTTGTTGACATGGGAATCTACTTCCAAGTGCAGGATGATTATCTGGATTGTTTTGCTGATCCCGAGACGCTTGGCA  800
       V  L  V  D  M  G  I  Y  F  Q  V  Q  D  D  Y  L  D  C  F  A  D  P  E  T  L  G  K

 801 AGATAGGAACAGATATAGAAGATTTCAAATGCTCTTGGTTGGTGGTTAAGGCATTAGAACGCTGCAGCGAAGAACAAACT  880
       I  G  T  D  I  E  D  F  K  C  S  W  L  V  V  K  A  L  E  R  C  S  E  E  Q  T

 881 AAGATATTATATGAGAACTATGGTAAAACCGACCCATCGAACGTTGCTAAAGTGAAGGATCTCTACAAAGAGCTGGATCT  960
       K  I  L  Y  E  N  Y  G  K  T  D  P  S  N  V  A  K  V  K  D  L  Y  K  E  L  D  L

 961 TGAGGGAGTGTTCATGGAGTATGAGAGCAAAAGCTACGAGAAGCTGACTGGAGCGATTGAGGGACACCAAAGTAAAGCAA 1040
       E  G  V  F  M  E  Y  E  S  K  S  Y  E  K  L  T  G  A  I  E  G  H  Q  S  K  A  I

1041 TCCAAGCAGTGCTAAAATCCTTCTTGGCTAAGATCTACAAGAGGCAGAAGTAGTAGAGACAGACAAACATAAGTCTCAGC 1120
       Q  A  V  L  K  S  F  L  A  K  I  Y  K  R  Q  K  *

1121 CCTCAAAAATTTCCTGTTATGTCTTTGATTCTTGGTTGGTGATTTGTGTAATTCTGTTAAGTGCTCTGATTTTCAGGGGG 1200

1201 AATAATAAACCTGCCTCACTCTGT
```

Fig. 2. Nucleotide and predicted amino acid sequences of *Arabidopsis thaliana* FPP synthase. The typical polyadenylation signal of AATAAA is underlined.

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                1                                                    50
       Rat FPPS MNGDQKLDVH NQEKQNFIQH FSQIVKVLTE DELGHPEKGD AITRIKEVLE
     Human FPPS MNGDQNSDVY AQEKQDFVQH FSQIVRVLTE DEMGHPEIGD AIARLKEVLE
Arabidopsis FPPS .......MET DLKST.FLNV YSVLKSDLLH D.PSFEFTNE SRLWVDRMLD
     Yeast FPPS .....MASEK EIRRERFLNV FPKLVEELNA SLLAYGMPKE ACDWYAHSLN
     consensus  ---------- ------F--- -------L-- ---------- --------L-

               51                                                   100
       Rat FPPS YNTVGGKYNR GLTVVQTFQE LVEPRKQD.. AESLQRALTV GWCVELLQAF
     Human FPPS YNAIGGKYNR GLTVVVAFRE LVEPRKQD.. ADSLQRAWTV GWCVELLQAF
Arabidopsis FPPS YNVRGGKLNR GLSVVDSFKL L..KQGNDLT EQEVFLSCAL GWCIEWLQAY
     Yeast FPPS YNTPGGKLNR GLSVVDTYAI LSNKTVEQLG QEEYEKVAIL GWCIELLQAY
      Consensus YN--GGK-NR GL-VV----- L--------- ---------- GWC-E-LQA-

               101                                                  150
       Rat FPPS FLVLDDIMDS SYTRRGQICW YQKPGIGLDA INDALLLEAA IYRLLKFYCR
     Human FPPS FLVADDIMDS SLTRRGQTCW YQKPGVGLDA INDANLLEAC IYRLLKLYCR
Arabidopsis FPPS FLVLDDIMDN SVTRRGQPCW FRVPQVGMVA INDGILLRNH IHRILKKHFR
     Yeast FPPS FLVADDMMDK SITRRGQPCW YKVPEVGEIA INDAFMLEAA IYKLLKSHFR
      Consensus FLV-DD-MD- S-TRRGQ-CW ---P--G--A IND---L--- I---LK---R
           DOMAIN I
               151                                                  200
       Rat FPPS EQPYYLNLLE LFLQSSYQTE IGQTLDLITA PQGQVDLGRY TEKRYKSIVK
     Human FPPS EQPYYLNLIE LFLQSSYQTE IGQTLDLLTA PQGNVDLVRF TEKRYKSIVK
Arabidopsis FPPS DKPYYVDLVD LFNEVELQTA CGQMIDLITT FEGEKDLSKY SLSIHRRIVQ
     Yeast FPPS NEKYYIDITE LFHEVTFQTE LGQLMDLITA PEDKVDLSKF SLKKHSFIVT
      consensus ---YY----- LF-----QT- -GQ--DL-T- -----DL--- -------IV-

               201                                                  250
       Rat FPPS YKTAFYSFYL PIAAAMYMAG IDGEKEHANA LKILLEMGEF FQIQDDYLDL
     Human FPPS YKTAFYSFYL PIAAAMYMAG IDGEKEHANA KKILLEMGEF FQIQDDYLDL
Arabidopsis FPPS HKTAYYSFYL PVACALLMAG ENLENHI.DV KNVLVDMGIY FQVQDDYLDC
     Yeast FPPS FKTAYYSFYL PVALAMYVAG ITDEKDLKQA RDVLIPLGEY FQIQDDYLDC
      Consensus -KTA-YSFYL P-A-A---AG ---E------ ---L---G-- FQ-QDDYLD-
                                                    DOMAIN II
               251                                                  300
       Rat FPPS FGDPSVTGKV GTDIQDNKCS WLVVQCLLRA TPQQRQILEE NYGQKDPEKV
     Human FPPS FGDPSVTGKI GTDIQDNKCS WLVVQCLQRA TPEQYQILKE NYGQKEAEKV
Arabidopsis FPPS FADPETLGKI GTDIEDFKCS WLVVKALERC SEEQTKILYE NYGKTDPSNV
     Yeast FPPS FGTPEQIGKI GTDIQDNKCS WVINKALELA SAEQRKTLDE NYGKKDSVAE
      Consensus F--P---GK- GTDI-D-KCS W-----L--- ---Q---L-E NYG-------

               301                                                  350
       Rat FPPS ARVKALYEEL DLRSVFFKYE EDSYNRLKSL IEQC..SAPL PPSIFLELAN
     Human FPPS ARVKALYEEL DLPAVFLQYE EDSYSHIMAL IEQY..AAPL PPAVFLGLAR
Arabidopsis FPPS AKVKDLYKEL DLEGVFMEYE SKSYEKLTGA I.EGHQSKAI QA.VLKSFLA
     Yeast FPPS AKCKKIFNDL KIEQLYHEYE ESIAKDLKAK ISQVDESRGF KADVLTAFLN
      Consensus A--K-----L --------YE ---------- I--------- ----------

               351
       Rat FPPS KIYKRRK
     Human FPPS KIYKRRK
Arabidopsis FPPS KIYKRQK
     Yeast FPPS KVYKRSK
      Consensus K-YKR-K
```

Fig. 3. Alignment of the amino acid sequence of *Arabidopsis thaliana* FPP synthase with the proteins encoded by rat, human and yeast genes. The alignment was generated using the GCG program, PILEUP. The consensus line indicates residues that are identical between the sequences. The conserved amino acids in Domain I and II [2] are in bold-face type.

DNA digested with *Eco* RI, *Hin*d III and *Pst* I, using a 720 bp *Eco* RI/*Pst* I probe and a similar result with strong and weak signals was obtained (data not shown). In contrast, for the yeast genomic DNA used as control, no hybridizing bands were detected.

Measurements of plant FPP synthase activity and sterol composition

In order to characterize further the isolated plant gene, we checked first if it was able to complement, not only the *erg20-2*-defective allele in CC25, but also a disrupted FPP synthase gene copy. Strain CC25 excretes geraniol, showing that the defective FPP synthase is affected especially in the second condensation step of GPP with IPP; in contrast a yeast strain carrying a disrupted gene copy is not viable [3]. Haploid strain DD163 bearing a disrupted copy of the yeast FPP synthase gene complemented by the plant cDNA presents a wild-type phenotype whatever the growth conditions tested; in addition no plasmid loss was observed after 4 days subculturing in

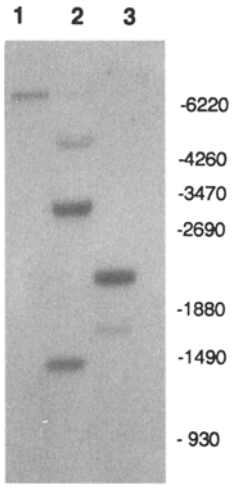

Fig. 4. Southern blot analysis of the FPP synthase gene locus. Lanes 1–3: 3 to 5 μg genomic DNA digested with *Bam* HI, *Eco* RI and *Hind* III, respectively. The molecular size standards are shown to the right.

complete medium supplemented with ergosterol. Therefore, the plant enzyme is able to catalyse the two successive condensations of IPP leading to FPP.

Table 1 shows that FPP synthase specific activity measured in cell free extracts of strain DD163 is more than ten-fold lower than in yeast wild-type strain. Nevertheless, the 5,7 dienols content is only slightly lowered (10%) in comparison to the wild type strain.

Analysis of the reaction products by HPLC of plant FPP synthase *in vitro* revealed that they correspond to 25% GPP and 75% FPP (results not shown), as observed with the yeast enzyme [3].

Discussion

Isolation of genes by functional complementation is a convenient strategy which does not require nucleic acid homology and generally leads to complete open reading frames.

We were able to use the strategy for the isolation of *A. thaliana* cDNA encoding FPP synthase since yeast mutant strains defective in FPP synthase were available. The isolated plant gene

Table 1. Ergosterol level and FPP synthase activity in FL100, CC25 and DD163. Grown in minimal medium (FL100 and DD163), or minimal medium supplemented with 4 mg/l ergosterol (CC25). The amount of 5,7 dienols was expressed as % of cell dry weight ± SD (number of independent experiments). The FPP synthase specific activity was expressed as nmol [^{14}C]IPP converted into acid-labile products per min per mg protein with DMAPP as the second substrate ± SD (number of independent experiments). ND not detected.

Strains	% of 5,7 dienols	FPP synthase specific activity
FL100	0.82 ± 0.01(2)	8.2 ± 0.8(3)
CC25	0.31 ± 0.02(2)	ND
DD163	0.70 ± 0.01(2)	0.6 ± 0.1(3)

complements both the leaky *erg20-2* yeast mutation and the disrupted gene copy. Measurement of FPP synthase specific activity in cell-free extracts of strain DD163 (*erg20::URA3, ura3-1, trp1-1* [pDD63]) revealed a ten-fold lowering in comparison to the wild type strain. This low specific activity of the plant enzyme, expressed from cDNA under the control of the strong PGK promoter can be explained by the presence of the 5′-upstream plant sequence that hinders the translation efficiency [6] or by specific catalytic properties of the plant enzyme.

The predicted plant farnesyl diphosphate synthase is clearly related to the other eucaryotic farnesyl diphosphate synthases previously described, especially in the two conserved domains, domain I ([LIVM][LIVM]XDDXXDXXXXR-RG), and domain II ([LIVMFY]GXXFQ[LIVM]XDD[LIVMFY]X[DN]), present in all polyprenyl synthases [13] including farnesyl diphosphate synthases, geranylgeranyl diphosphate synthases and hexaprenyl diphosphate synthases.

The plant cDNA we have isolated probably encodes a cytoplasmic protein, since no additional transit peptide is predicted at the N-terminus. The Southern blots experiments revealed weak signals, even at high stringency in addition to the strong hybridization bands. Thus, the *A. thaliana* genome might contain a single gene for FPP synthase, and one or several more distantly related genes, possibly coding for other prenyl transferases.

Acknowledgements

We thank Conseil Regional du Poitou-Charentes for a grant to D. Delourme and French Ministry of Research and space and Verneuil Recherche for supporting this work by grant 92T0352. We are grateful to L. Brémaud for helpful advice during this work and to Professor F. Nau for critical reading of this manuscript.

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