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Characterization of dehydrodolichyl diphosphate synthase of *Arabidopsis thaliana*, a key enzyme in dolichol biosynthesis[1]

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Abstract The enzyme dehydrodolichyl diphosphate (dedol-PP) synthase is a *cis*-prenyltransferase that catalyzes the synthesis of dedol-PP, the long-chain polyprenyl diphosphate used as a precursor for the synthesis of dolichyl phosphate. Here we report the cloning and characterization of a cDNA from *Arabidopsis thaliana* encoding dedol-PP synthase. The identity of the cloned enzyme was confirmed by functional complementation of a yeast mutant strain defective in dedol-PP synthase activity together with the detection of high levels of dedol-PP synthase activity in the transformed yeast mutant. The *A. thaliana* dedol-PP synthase mRNA was detected at high levels in roots but was hardly detected in flowers, leaves, stems and in *A. thaliana* suspension-cultured cells.

Key words: *cis*-Prenyltransferase; Dehydrodolichyl diphosphate synthase; Isoprenoid; Rer2p; *Arabidopsis thaliana*

1. Introduction

Plant isoprenoids constitute a heterogeneous group of compounds with a great diversity of structures and functions. Some of these compounds play essential roles in plant growth and development. They include phytosterols (membrane structure and function), dolichols (protein glycosylation), carotenoids (photosynthetic pigments), side chains of ubiquinone and plastoquinone (electron carriers), brassinosteroids, abscisic acid, cytokinins and gibberellins (growth regulators), and sesquiterpenoid phytoalexins (defense against pathogen attack). In spite of their structural diversity, all isoprenoids are synthesized with a common building block, isopentenyl diphosphate (IPP) [1,2]. In plants, IPP is synthesized by two different pathways, the acetate/mevalonate pathway, which operates in the cytosol/endoplasmic reticulum (ER) compartment, and the recently discovered 2-*C*-methyl-D-erythritol 4-phosphate pathway, which has been shown to be localized in the plastids [3]. The resulting IPP serves as a substrate for condensation reactions with different allylic prenyl diphosphates leading to the synthesis of a series of linear prenyl diphosphates of increasing size which, in turn, serve as donors or intermediates in the synthesis of the wide range of plant isoprenoid end products [2]. Among these are long-chain polyisoprenoid alcohols (polyprenols and dolichols), a family of compounds which have been reported to occur in a number of plants and shown to have a great heterogeneity in terms of carbon chain length [4–7].

Prenyl diphosphates are synthesized by a family of enzymes known as prenyltransferases. These enzymes have been classified in two different groups, referred to as *trans*- or (*E*)-prenyl diphosphate synthases and *cis*- or (*Z*)-prenyl diphosphate synthases, according to the stereochemistry of the condensation reaction of IPP with the corresponding allylic prenyl diphosphate [8,9]. During the last decade the genes encoding many kinds of prenyltransferases that catalyze *trans*-type prenyl chain elongation have been cloned and characterized [8]. In contrast, very limited information was available about *cis*-prenyl chain elongating enzymes until the recent cloning and characterization of genes encoding *cis*-prenyltransferases from different organisms, including *Micrococcus luteus* B-P 26 [10], *Escherichia coli* [11], *Haemophilus influenzae* [11], *Streptococcus pneumoniae* [11], and *Saccharomyces cerevisiae* [12]. *cis*-Prenyltransferases encoded by the cloned bacterial genes have been identified as undecaprenyl diphosphate synthases (UPS) [10,11], which appear to play an essential role in cell wall biosynthesis [13]. The *S. cerevisiae RER2* gene, which has been cloned as an important gene for protein sorting in the ER, has been recently identified as dehydrodolichyl diphosphate (dedol-PP) synthase. This enzyme catalyzes the synthesis of dedol-PP, the long-chain polyprenyl diphosphate used as a precursor for the synthesis of dolichyl phosphate [12]. Although this isoprenoid end product plays an essential role in protein glycosylation [14], little is known about its biosynthetic pathway. As a first step towards a better understanding of this metabolic pathway in plants, we have undertaken the cloning and characterization of a cDNA from *Arabidopsis thaliana* encoding dedol-PP synthase, a key enzyme of dolichol biosynthesis.

2. Materials and methods

2.1. Plant material

A. thaliana plants (var. Columbia) were grown under a 16 h light/8 h dark illumination regime at 22°C on a 1:1:1 perlite:vermiculite:sphagnum mixture irrigated with mineral nutrients [15]. For axenic cultures, *A. thaliana* seeds were surface-sterilized in 5% (v/v) sodium hypochlorite. Roots were obtained from 3-week-old plants grown on filter papers layered onto petri dishes containing Murashige and Skoog medium supplemented with 1% (w/v) sucrose and 2% (w/v) agar. Suspension-cultured cells of *A. thaliana* (var. Columbia, cell

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[1] The sequence reported in this paper has been deposited in the EMBL database under accession number AJ277136.

Abbreviations: Dedol-PP, dehydrodolichyl diphosphate; ER, endoplasmic reticulum; FPP, farnesyl diphosphate; IPP, isopentenyl diphosphate; PCR, polymerase chain reaction; UPS, undecaprenyl diphosphate synthase

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line T87) were obtained from Michèle Axelos and Bernard Lescure (Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, CNRS-INRA, Castanet-Tolosan, France). Suspension cultures were grown in Murashige and Skoog medium supplemented with 1 μM naphthaleneacetic acid at 22°C in continuous fluorescent light on a rotary shaker, and subcultured as described by Axelos et al. [16]. For RNA isolation cells were collected at the mid-exponential growth phase.

2.2. Cloning of a dedol-PP synthase cDNA from A. thaliana

Restriction enzyme digestions, transformations and other standard molecular biology techniques were carried out as described by Sambrook et al. [17]. A cDNA containing the entire coding sequence of the *A. thaliana* dedol-PP synthase was amplified by RT-PCR using total RNA (6 μg) isolated from axenically grown 12-day-old *A. thaliana* seedlings, primers DPS-1 (forward) and DPS-2 (reverse) (0.2 μM each primer), and the SuperScript™ One-Step™ RT-PCR System (Life Technologies), according to the manufacturer. Primers DPS-1 and DPS-2 were designed from the genomic DNA sequence AC003040 (GenBank) of *A. thaliana.* DPS-1, reverse complement of bp 19 113–19 134 (5′-CGGGATCCGGCAAAGCCAAATTAAGTTGTC-3′); DPS-2, bp 17 594–17 613 (5′-CGGAATTCGAGAGGATGAAAGGTGGATC-3′). To facilitate the cloning of the amplification product, *Bam*HI and *Eco*RI restriction sites (underlined) were added at the 5′ end of the primers, respectively. To synthesize the first strand of cDNA, the reaction mixture was incubated for 50 min at 50°C. The reaction was stopped by heating the mixture for 2 min at 94°C. The cDNA was amplified by 50 cycles of 30 s at 94°C, 40 s at 52°C and 90 s at 72°C, with a 10 min final extension at 72°C. The resulting PCR product (approximately 1.0 kb) was gel-purified, digested with *Bam*HI and *Eco*RI and ligated into plasmid pBluescript KS(+) (Stratagene, La Jolla, CA, USA). After transformation into *Escherichia coli* strain XL1 Blue (Stratagene), the resulting clone, named pBDPS, was sequenced by the dideoxynucleotide chain termination method [18] using an automated fluorescence-based system (Applied Biosystems). The sequence was determined on both strands.

2.3. Expression of A. thaliana dedol-PP synthase in yeast strain SNH23-7D

To express the *A. thaliana* dedol-PP synthase in the yeast mutant strain SNH23-7D (*MATα*, *rer2-2*, *mfα1::ADE2*, *mfα2::TRP1*, *bar1::HIS3*, *ade2*, *trp1*, *his3*, *leu2*, *ura3*, *lys2*) the cloned cDNA was excised from plasmid pBDPS as a *Bam*HI–*Sal*I fragment and cloned into the corresponding sites of plasmid pJR1133, which is identical to pJR1138 [19] but contains the *URA3* instead of the *LEU2* marker. The resulting plasmid, designated pJR1133-DPS, contained the *A. thaliana* dedol-PP synthase cDNA under the control of the strong yeast glyceraldehyde phosphate dehydrogenase (*GPD*) promoter. Yeast strain SNH23-7D was transformed with plasmids pJR1133-DPS and pJR1133 by the lithium acetate procedure [20]. Ura^+ transformants were selected at 23°C on agar plates containing minimal medium (0.16% (w/v) yeast nitrogen base without amino acids and ammonium sulfate, 0.5% (w/v) ammonium sulfate, and 2% (w/v) glucose, supplemented with leucine (60 μg/ml) and lysine (30 μg/ml)). Selected Ura^+ colonies and yeast strain SNY9 (*MATα*, *mfα1::ADE2*, *mfα2::TRP1*, *bar1::HIS3*, *ade2*, *trp1*, *his3*, *leu2*, *ura3*, *lys2*), containing the wild type *RER2* gene, were streaked on agar plates containing YPD medium (1% (w/v) yeast extract, 1% (w/v) bactopeptone, and 2% (w/v) glucose) and incubated at 23°C or 37°C.

2.4. Assay for cis-prenyltransferase activity

Yeast strains were grown at 23°C in minimal medium supplemented appropriately. All subsequent manipulations were performed at 4°C. Yeast cells were harvested by centrifugation at 5000×*g* for 10 min, washed twice in 50 mM potassium phosphate pH 7.5, and resuspended in breakage buffer (50 mM potassium phosphate pH 7.5, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml antipain, 5 μg/ml aprotinin, 5 μg/ml leupeptin, and 5 μg/ml pepstatin). Cells were disrupted by vigorous vortexing in the presence of 0.45 mm glass beads. After centrifugation of the lysates at 300×*g* for 5 min, the resulting supernatant was centrifuged at 13 000×*g* for 15 min. The pellet fraction of the 13 000×*g* centrifugation (membrane fraction) was washed with breakage buffer and resuspended in the same buffer at a final protein concentration of 5 mg/ml. The *cis*-prenyltransferase activity was measured as described by Sato et al. [12] using 50 μM [1-^{14}C]IPP (Amersham, 55 mCi/mmol) and 14 μM farnesyldiphosphate (FPP) (Sigma Chemical Co.) as substrates. After incubation of the mixture for 1 h at 30°C, the reaction was terminated by adding 400 μl of 4 mM $MgCl_2$, and the enzymatic products were extracted with 2.5 ml of chloroform/methanol 3:2 (v/v). After phase separation, the lower phase was washed with 1 ml of the upper phase obtained by mixing water/methanol/chloroform 1:2:3 (v/v), evaporated to dryness, and resuspended in chloroform. An aliquot of the chloroform suspension was mixed with Ecoscint O™ (National Diagnostics) and the radioactivity was measured by liquid scintillation counting. Another aliquot of the chloroform suspension was analyzed by normal phase TLC on a Silica Gel-60 plate (Merck) with a solvent system of benzene/ethyl acetate 95:5 (v/v). The distribution of the ^{14}C-labeled products was analyzed by a Personal Imager® FX (Bio-Rad), and the positions of dolichol standards (Sigma Chemical Co.) were visualized with iodine vapors.

2.5. Northern blot analysis of A. thaliana dedol-PP synthase mRNA

Total RNA from different *A. thaliana* samples was isolated as described by Dean et al. [21]. For Northern analysis, 15 μg of total RNA from each sample was fractionated by electrophoresis in 1% (w/v) agarose gels containing 2.2 M formaldehyde and blotted to a neutral nylon membrane (Nytran®, Schleicher and Schuell). Hybridization with the ^{32}P-labeled *A. thaliana* dedol-PP synthase cDNA was for 18 h at 68°C in ExpressHyb7 hybridization solution (Clontech Laboratories). High stringency washes were performed at 68°C twice in 2×SSC, 0.1% SDS and twice in 0.1×SSC, 0.1% SDS.

3. Results and discussion

3.1. Cloning and sequence analysis of an A. thaliana dedol-PP synthase cDNA

To identify putative plant nucleic acid sequences encoding homologues of the enzyme dedol-PP synthase, the non-redundant database of the National Center for Biotechnology Information (NCBI) was searched with the TBlastN program, using the complete amino acid sequence of the recently cloned dedol-PP synthase (also named Rer2p) from *S. cerevisiae* [12] as a query. A significant level of identity (36%) was found between the query and the amino acid sequence encoded by four putative exons of an *A. thaliana* genomic sequence located in chromosome 2 (reverse complement of bp 17 615–19 106 of the genomic sequence; accession number AC003040) [22].

To demonstrate the existence of mRNA sequences encoding the putative *A. thaliana* dedol-PP synthase, we isolated a cDNA corresponding to the genomic sequence identified in chromosome 2 using a RT-PCR strategy and total RNA from *A. thaliana* seedlings (for details, see Section 2). The cDNA was amplified using a forward primer (DPS-1) corresponding to a sequence located upstream of the ATG start codon of the putative *A. thaliana* dedol-PP synthase, and a reverse primer (DPS-2) complementary to a sequence located downstream of the coding region. A cDNA fragment of approximately 1.0 kb was amplified, cloned, and sequenced. The cDNA insert was found to have a nucleotide sequence of 958 bp which was identical to the four predicted exons (exons I–IV, reverse complement of bp 18 949–19 134, 18 286–18 523, 18 137–18 207, and 17 594–18 056 of the genomic sequence AC003040, respectively) of the *A. thaliana* genomic sequence (further referred to as *DPS* gene) encoding the putative dedol-PP synthase. Analysis of the cDNA sequence indicated the occurrence of an open reading frame encoding a protein of 303 amino acid residues (Fig. 1) with a predicted molecular mass of 34 975 Da. The encoded protein shows an overall amino acid identity of 35–36% (similarity of 53–56%) with both dedol-PP synthase (Rer2p) from *S. cerevisiae* and a

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A. thaliana              MLSLLSSDSS LLSLLFLFLI PCLFITSYIG FPVFLLKLIG LIKIKAARDN EKRDEGTYVV REDGLQRELM   70
S. cerevisiae                                                M ETDSGIPGHS FVLKWTKNIF SRTLRASNCV   31
C. elegans                 MGTEPTNF CSRGERVHHS TTEAMLSPFH HCAVMAANLT ADEEDGWFVA QQEQPWWQWL LRRFIASGPI   68
E. coli                                                                  MLSATQP LSEKLPAHGC   17
M. luteus                                                                MFPIKKRKAI KNNNINAAQI   20

A. thaliana      PRHVAFILDG NRRWAKRAGL TTS.QGHEAG AKRLIDIAEL CFELGVHTVS AFAFSTENWG RDKIEIDNLM SLIQHYRNK.  148
S. cerevisiae    PRHVGFIMDG NRRFARKKEM .DVKEGHEAG FVSMSRILEL CYEAGVDTAT VFAFSIENFK RSSREVESLM TLARERIRQI  110
C. elegans       PRHVAFVMDG NRRFAKTKHL GNVIKGHEKG FTQLAKILDW CNRFGIREIT VYAFSIENFK RSEEEVSGLM RLAEEKFQKL  148
E. coli          .RHVAIIMDG NGRWAKKQGK IRA.FGHKAG AKSVRRAVSF AANNGIEALT LYAFSSENWN RPAQEVSALM ELFVWALD..   93
M. luteus        PKHIAIIMDG NGRWAKQKKM PRI.KGHYEG MQTVKKITRY ASDLGVKYLT LYAFSTENWS RPKDEVNYLM KLPGDFLN..   97
                                  Region I                                    Region II

A. thaliana      .SNIKFFHRS EVRVSVIGNK TKIPESLLKE IHEIEEATKG YKNKHLIMAV DYSGKFDIMH ACK.SLVKKS EKGLIREEDV  226
S. cerevisiae    TERGELACKY GVRIKIIGDL SLLDKSLLED VRVAVETTKN NKRATLNICF PYTGREEILH AMKETIVQHK KGA.....AI  185
C. elegans       LNDSEKLDEK RICFRFYGNR SLLSSRLQKL MSDIEHRTEN FDGGRLNVCM PYTSRDEIAR SF.ETIRKHV KDGKVNVDEI  227
E. coli          .SEVKSLHRH NVRLRIIGDT SRFNSRLQER IRKSEALTAG NTGLTLNIAA NYGGRWDIVQ GVR.QLAEKV QQGNLQPDQI  171
M. luteus        .TFLPELIEK NVKVETIGFI DDLPDHTKKA VLEAKEKTKH NTGLTLVFAL NYGGRKEIIS AVQ.LIAERY KSGEISLDEI  175

A. thaliana      DEALIERELL TNCSDFPSPD LMIRTSGEQR ISNFFLWQLA YSELFF..SP VFWEDFDKDK LLEALASYQR RERRFGCRV   303
S. cerevisiae    DESTLESHLY T..AGVPPLD LLIRTSGVSR LSDFLIWQAS SKGVRIELLD CLWPEFGPIR MAWILLKFS. FHKSFLNKEY  262
C. elegans       NESMIDACLD SGCGGTSP.D LFIRTSGEHR LSDFLMWQAS ETHVYFD..D VLWPEFGYFN LCKAILNYQ. YYRTTVTKMT  303
E. coli          DEEMLNQHVC M..HELAPVD LVIRTGGEHR ISNFLLWQIA YAELYF..TD VLWPDFDEQD FEGALNAFAN RERRFGGTEP  247
M. luteus        SETHFNEYLF T..ANMPDPE LLIRTSGEER LSNFLIWQCS YSEFVF..ID EFWPDFNEES LAQCISIYQN RHRRFGGL    249
                                                  Region III

S. cerevisiae    RLEEGDYDEE T......NGD PIDL.KEKKL N                                                    286
C. elegans       SSKVSD.DNA TSWKMNFSGN DRDLISVKSI RPQGNREK                                              340
E. coli          GDETA                                                                                  252
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Fig. 1. Alignment of the amino acid sequences of the *A. thaliana* dedol-PP synthase with the amino acid sequences of *S. cerevisiae* dedol-PP synthase [12], *C. elegans* T01G1.1 hypothetical protein [12], *E. coli* UPS [11], and *M. luteus* B-P 26 UPS [10]. Dots denote the absence of amino acid residues. Amino acid residues conserved in the five sequences are shaded. The three regions (I–III) conserved in all *cis*-prenyltransferases [9] are shown below. Amino acid residues are numbered on the right.

Rer2p homologue from *Caenorhabditis elegans* [12]. Similar values of identity (37%) and similarity (52–54%) were found with UPS from bacterial species, such as *E. coli* [11] and *M. luteus* [10]. The *A. thaliana* dedol-PP synthase contains the three major conserved regions, designated I–III (Fig. 1), which are common to all *cis*-prenyltransferases characterized so far [9]. This observation strongly suggested that the *A. thaliana* dedol-PP synthase might represent an active form of the enzyme.

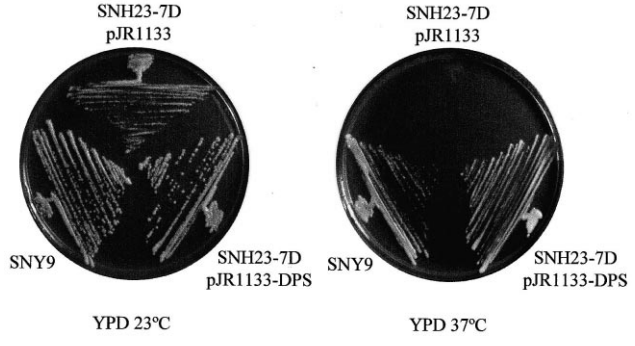

Fig. 2. Functional complementation of the dedol-PP synthase-deficient yeast strain SNH23-7D by plasmid pJR1133-DPS. Strain SNY9 and strain SNH23-7D transformed with plasmids pJR1133 and pJR1133-DPS were streaked onto YPD plates and incubated at 23°C (4 days) or 37°C (2 days).

3.2. Functional analysis of the A. thaliana dedol-PP synthase

To confirm that the cloned *A. thaliana* dedol-PP synthase cDNA encoded a functional enzyme, the cDNA was expressed in the yeast mutant strain SNH23-7D, which is deficient in the activity of dedol-PP synthase [12]. Strain SNH23-7D is a thermosensitive mutant that carries the mutation *rer2-2* affecting the ability of dedol-PP synthase to synthesize dedol-PP. As a consequence, this strain is able to grow at 23°C but not at 37°C [12]. Strain SNH23-7D was transformed with plasmid pJR1133-DPS, carrying the *A. thaliana* dedol-PP synthase cDNA under the control of the *GPD* promoter, and also with control plasmid pJR1133. The results shown in Fig. 2 indicated that overexpression of *A. thaliana* dedol-PP synthase suppresses the temperature-sensitive growth of strain SNH23-7D. The presence of dedol-PP synthase activity in the transformed yeast mutant was checked by an in vitro assay using membrane fractions from strain SNH23-7D[pJR1133-DPS] and ^{14}C-labeled IPP and FPP as substrates. As shown in Table 1, membranes prepared from strain SNH23-7D[pJR1133-DPS] showed 5.7- and 11-fold higher *cis*-prenyltransferase activity than that measured in membrane fractions from strains SNY9 (wild type) and SNH23-7D[pJR1133], respectively. Moreover, analysis of the corresponding reaction products by normal phase TLC (Fig. 3) indicated that membranes from strain SNH23-7D[pJR1133-DPS] synthesized high levels of a ^{14}C-labeled product(s) (Fig. 3, lane 2) which was identified as dolichol(s), since it cochromatographed with authentic dolichol with a chain length of C_{90}, and also with ^{14}C-labeled dolichols synthesized by membranes from strain

Table 1
Activity of *cis*-prenyltransferase in membrane fractions of SNY9, SNH23-7D[pJR1133-DPS], and SNH23-7D[pJR1133] cells

Strain	*cis*-Prenyltransferase activity[a]	
	pmol/min/mg of protein	% of wild type
SNY9	44	100
SNH23-7D[pJR1133-DPS]	251	570
SNH23-7D[pJR1133]	23	52

[a]*cis*-Prenyltransferase activity was measured as described in Section 2. Specific activities are reported in pmol of IPP incorporated into polyprenols/min/mg of protein.

SNY9 (Fig. 3, lane 1). The synthesis of dolichols was barely detectable in strain SNH23-7D[pJR1133] (Fig. 3, lane 3). Taken together these results demonstrated that the cloned *A. thaliana* cDNA encodes a functional dedol-PP synthase. Analysis of the reaction products by reverse phase TLC [23] (data not shown) revealed that membranes from strain SNH23-7D[pJR1133-DPS] synthesized a mixture of dolichols ranging in chain length from C_{75} to C_{95}, C_{85} and C_{90} being the predominant species, whereas membranes from strain SNY9 synthesized dolichols with major chain lengths of C_{80} and C_{85}. These results indicated that in the yeast background the *A. thaliana* enzyme catalyzes the synthesis of dedol-PPs with chain lengths similar to those found in yeast. Nevertheless, the distribution of chain lengths synthesized by the *A. thaliana* dedol-PP synthase in the plant might differ from

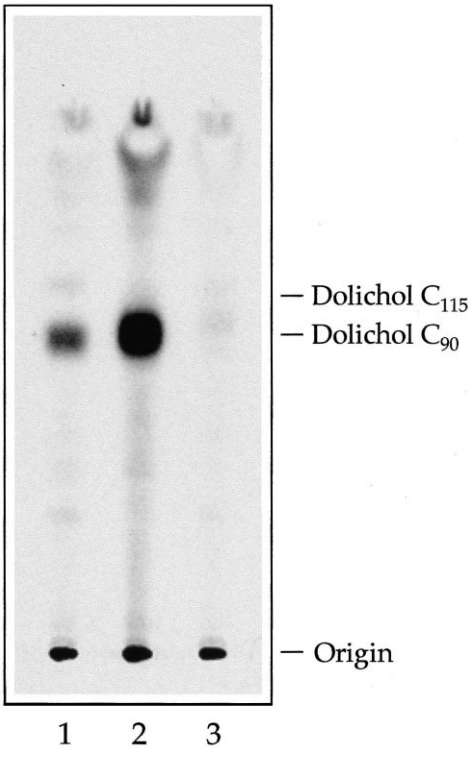

Fig. 3. Analysis of the *cis*-prenyltransferase reaction products synthesized by membrane fractions of strains SNY9 (lane 1), SNH23-7D[pJR1133-DPS] (lane 2), and SNH23-7D[pJR1133] (lane 3) in the presence of [1-^{14}C]IPP and FPP as substrates. The lipidic products were extracted with chloroform/methanol and subjected to TLC on a silica gel-60 plate with a benzene/ethyl acetate 95:5 (v/v) solvent system. Porcine liver dolichols of 18 (C_{90}) and 23 (C_{115}) isoprene units were used as standards.

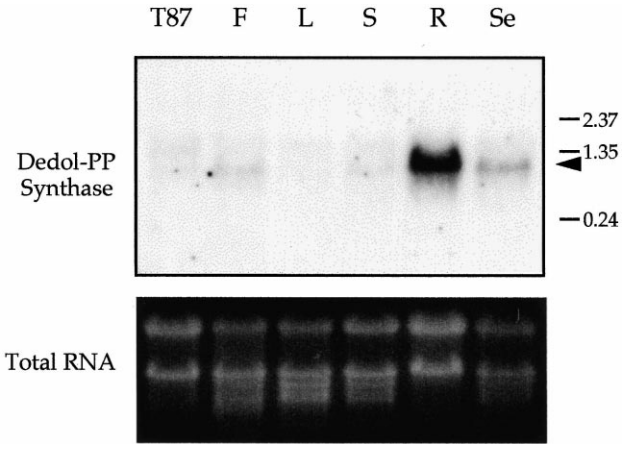

Fig. 4. Northern blot analysis of *A. thaliana* dedol-PP synthase mRNA. Total RNA samples (15 μg) from *A. thaliana* suspension-cultured cells (T87), flowers (F), leaves (L), stems (S), roots (R), and 12-day-old seedlings (Se) were electrophoresed in 1% agarose–formaldehyde gels and transferred onto a nylon membrane. The filter was hybridized with the entire *A. thaliana* dedol-PP synthase cDNA. Exposure time was 15 days. Ethidium bromide staining of the gel before transfer is shown below. The 1.0 kb hybridizing band corresponding to the dedol-PP synthase mRNA is indicated by an arrowhead. The positions of RNA size standards are indicated on the right.

that observed in the transformed yeast mutant strain. In this regard, it has been proposed that in the dolichol biosynthetic pathway, when the chain length of dedol-PP characteristic for a given organism is achieved, it is dephosphorylated to produce the substrate for reduction to dolichol [23]. It is worth noting that the chain length distribution of *A. thaliana* dolichols is yet to be determined. The synthesis of dolichyl phosphate appears to be associated with microsomal membranes [24,25]. This is consistent with the finding that *S. cerevisiae* dedol-PP synthase is a peripheral but tightly associated ER membrane protein [12]. From these observations it has been proposed that the enzymes in dolichol synthesis might form a complex in the ER membranes to increase the efficiency of the sequence of reactions [12]. In this context, our results indicate that the plant enzyme is properly targeted to the yeast ER membranes, and led us to speculate that in plants dedol-PP synthase also might be associated with the ER membranes.

3.3. Expression analysis of the *A. thaliana DPS* gene

As a first approach to define the pattern of expression of the *A. thaliana DPS* gene, we performed Northern blot analysis of total RNA isolated from *A. thaliana* flowers, leaves, stems, roots, seedlings, and the cell line T87 using as a probe the *A. thaliana* dedol-PP synthase cDNA. This analysis revealed the occurrence of a transcript of approximately 1.0 kb (Fig. 4), in good agreement with the size of the cloned cDNA, which accumulated preferentially in roots. In contrast, hardly detectable levels of dedol-PP synthase mRNA were found in flowers, stems, leaves, and suspension-cultured cells of line T87. *A. thaliana* seedlings showed low albeit clearly detectable levels of dedol-PP synthase mRNA which might be primarily attributed to the expression of the *DPS* gene in roots. These results indicate that the *A. thaliana DPS* gene has a specialized pattern of expression and suggest that root tissues need an abundant supply of dedol-PP to sustain an active synthesis of dolichol. Moreover, the extremely low level of

expression of dedol-PP synthase in suspension-cultured cells suggests that the physiological role of this enzyme is not associated with cell division. Close inspection of the results obtained in Northern blot analysis revealed that, in addition to the 1.0 kb mRNA, in all samples analyzed the cDNA probe detected very low levels of a transcript of approximately 1.5 kb (Fig. 4) that might correspond to an additional dedol-PP synthase isoform. One of the most remarkable features of plant isoprenoid biosynthesis is the occurrence of gene families encoding multiple isoforms of key enzymes of this metabolic pathway [26], including *A. thaliana* prenyltransferases such as FPP synthase [27,28] and geranylgeranyl diphosphate synthase [29,30]. Interestingly, genomic Southern blot analysis revealed a complex pattern of bands (data not shown) consistent with the existence of a small *A. thaliana* dedol-PP synthase gene family. Work is currently in progress to investigate whether *A. thaliana* contains additional genomic sequences corresponding to functional *DPS* genes.

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