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The subcellular localization of periwinkle farnesyl diphosphate synthase provides insight into the role of peroxisome in isoprenoid biosynthesis

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ABSTRACT

Farnesyl diphosphate (FPP) synthase (FPS: EC.2.5.1.1, EC.2.5.1.10) catalyzes the formation of FPP from isopentenyl diphosphate and dimethylallyl diphosphate *via* two successive condensation reactions. A cDNA designated *CrFPS*, encoding a protein showing high similarities with *trans*-type short FPS isoforms, was isolated from the Madagascar periwinkle (*Catharanthus roseus*). This cDNA was shown to functionally complement the lethal *FPS* deletion mutant in the yeast *Saccharomyces cerevisiae*. At the subcellular level, while short FPS isoforms are usually described as cytosolic proteins, we showed, using transient transformations of *C. roseus* cells with yellow fluorescent protein-fused constructs, that CrFPS is targeted to peroxisomes. This finding is discussed in relation to the subcellular distribution of FPS isoforms in plants and animals and opens new perspectives towards the understanding of isoprenoid biosynthesis.

Introduction

Two sequential condensations of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DMAPP) lead to the formation of farnesyl diphosphate (FPP). This reaction is catalyzed by FPP synthase (EC 2.5.1.1, EC 2.5.1.10), which belongs to the short-chain prenyltransferase subfamily (Vandermoten et al., 2009). Most known short-chain prenyltransferases are homodimers and catalyze 1′–4 condensation reactions, in which the new double bonds are in *trans* configuration. *Trans*-type prenyltransferases share a common protein fold and display several regions of similarity. In particular, they contain two highly conserved functional aspartate-rich domains [$DD(X)_nD$; where X is any amino acid, $n = 2$ or 4] referred to as first aspartate-rich motif (FARM) and second aspartate-rich motif (SARM). Eukaryotic *trans*-type FPS contain the FARM motif DDXXD and two aromatic amino acid residues at the fourth and fifth positions upstream from the FARM involved in product chain-length determination (Tarshis et al., 1996).

A number of FPSs from organisms of all kingdoms have been identified and it is recognized as one of the most widely studied prenyltransferases due to its key location in isoprenoid biosynthesis (Vandermoten et al., 2009). In plants, FPS plays a pivotal role in isoprenoid formation since FPP serves as a precursor of a variety of isoprenoid end-products such as sesquiterpenes, triterpenes, phytosterols, dolichols, polyprenols, prenyl moieties used for protein prenylation and mitochondrial generated ubiquinones (Bouvier et al., 2005). There is a general agreement that plant prenyltransferases are distributed in cytosol, mitochondria and plastids. However, data concerning the subcellular localization of FPS are sparse. While several indirect experiments have suggested a cytosolic localization of FPS in *Vitis vinifera* (Feron et al., 1990) and *Capsicum annuum* (Hugueney and Camara, 1990), Cunillera et al. (1997) have clearly demonstrated that a long isoform of *Arabidopsis thaliana* FPS, bearing an N-terminal transit peptide, is targeted to mitochondria. In addition, FPS has also been found in the chloroplast of rice mesophyll cells (Sanmiya et al., 1999) and Sallaud et al. (2009) reported the plastidial localization of a tomato *cis*-type FPS involved in sesquiterpene biosynthesis.

Recently, the classical view of the compartmentalization of the plant isoprenoid metabolism has been re-evaluated, following the demonstration of the partial peroxisomal localization of the mevalonic acid (MVA) pathway, generally regarded as cytosolic. Using GFP-tagging approaches, the last two enzymes of this pathway, 5-phosphomevalonate kinase and mevalonate 5-diphosphate decarboxylase from *Arabidopsis thaliana* and *Catharanthus roseus*

Abbreviations: DMAPP, dimethylallyl diphosphate; FOA, 5-fluoroorotic acid; FPP, farnesyl diphosphate; FPS, farnesyl diphosphate synthase; IPP, isopentenyl diphosphate; MVA, mevalonic acid; PTS, peroxisomal targeting signal; YFP, yellow fluorescent protein.

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(Simkin et al., 2011) as well as the *Arabidopsis* short isoforms of isopentenyl diphosphate isomerase (Sapir-Mir et al., 2008) were shown to be targeted to peroxisomes. As FPS is usually related to the MVA pathway and has been reported to be localized within the peroxisome in mammals (Krisans et al., 1994; Olivier et al., 2000; Kovacs et al., 2007), we focused the present study on the cDNA cloning, functional characterization and subcellular localization of the Madagascar periwinkle (*C. roseus*) FPS.

Materials and methods

Plant material and growth conditions

Madagascar periwinkle (*Catharanthus roseus* [L.] G. Don, Apocynaceae) cell suspensions (line C20D) were maintained on a 7-day-growth cycle in the B5 medium of Gamborg et al. (1968) supplemented with 58 mM sucrose and 4.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D).

RNA extraction and cDNA synthesis

Frozen cells were ground to a fine powder in liquid nitrogen. RNA extraction and cDNA synthesis were performed as previously described (Simkin et al., 2008). Total RNAs were extracted with the RNAeasy Plant mini kit (Qiagen, France). The cDNAs were synthesized from total RNA using the oligo-dT abridged primer AP (Supplementary Table S1) according to the protocol in the Superscript II Reverse Transcriptase kit (Invitrogen, France).

Cloning of the C. roseus cDNA encoding farnesyl diphosphate synthase

The full-length periwinkle *FPS* cDNA was recovered by RT-PCR. The sequences of the forward primer FPS1 and the reverse primer FPS4 (Supplementary Table S1) were designed from the two *C. roseus* EST EG554067 (Genbank accession number) and FD660967 (Genbank accession number) matching the 5′ and the 3′ regions of plant FPS amino acid sequences, respectively. Two μL of the RT reaction was used for PCR amplification with PfuTurbo DNA polymerase according to the manufacturer's instructions (Agilent Technologies, France). The thermal profile consisted of denaturation at 95 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 4 min. The 35 cycles were preceded by a denaturation step of 95 °C for 2 min and followed by a final extension of 72 °C for 5 min. The resulting amplicon was cloned into pGEM-T Easy vector (Promega, France) following A-tailing and sequenced.

Database searches for similar protein sequences were performed using NCBI's BLAST network service. Protein sequence alignment was performed using ClustalW from the Mac Vector program (Oxford Molecular Ltd, UK). The full-length cDNA of *C. roseus FPS* (*CrFPS*) has been deposited at NCBI under Genbank accession number: HQ316638.

Yeast complementation assays

The full-length periwinkle *FPS* coding sequence was recovered by RT-PCR using PfuTurbo DNA polymerase (Agilent Technologies, France) and the primer pair FPSf and FPSr (Supplementary Table S1). The thermal profile is described above. The resulting amplicon was digested with *Bam*HI and *Xba*I and cloned into the corresponding restriction sites of plasmid pYES2 (Invitrogen, France) to make the plasmid pYES2-*CrFPS* and sequenced.

Yeast Magic Marker strain YJL167W (*MAT*a/*α ura3*Δ0/*ura3*Δ0 *leu2*Δ0/*leu2*Δ0 *his3*Δ1/*his3*Δ1 *lys2*Δ0/*LYS2 met15*Δ0/*MET15 can1*Δ::*LEU2-MFA1*pr-*HIS3*/*CAN1 erg20*Δ::*KanMX*/*ERG20*) was obtained from Thermo Scientific ABgene (Epsom, UK). Yeast transformation was performed as follow. The equivalent of ten yeast colonies from YJL167W heterozygous diploid strain were recovered with a pipette tips from a 24 h-grown YPD (1% yeast extract, 2% peptone, 2% dextrose, 2% agar) plate and suspended in 100 μL PALD (45% polyethylene glycol 4000, 100 mM lithium acetate, 50 mM DTT). pYES2-*CrFPS* plasmid DNA (1–10 μg in 1–10 μL of sterile water) was added and mixed vigorously. After heat-shock (30 min, 42 °C), cells were directly plated on YCS selective medium (0.67% Yeast Nitrogen Base with ammonium sulphate and without amino acids; 0.08% SC–Ura; 2% dextrose; 2% agar) and incubated at 30 °C for 3 days. Plasmid-transformed heterozygous diploid strain [Sc (*erg20*Δ/*ERG20*) + pYES2-*CrFPS*] was allowed to sporulate by plating 10^9 cells onto ACK medium (1% potassium acetate, 0.25% yeast extract, 2% agar) at 28 °C for 7 days. Haploid transformant cells harboring both the disrupted allele and the plasmid-borne *C. roseus FPS* cDNA [Sc (*erg20*Δ) + pYES2-*CrFPS*] were selected by plating the sporulated culture of heterozygous diploid pools on a modified galactose-containing magic medium (2% galactose, 0.67% Yeast Nitrogen Base with ammonium sulphate and without amino acids, 0.06% SC–Leu–His–Arg–Ura drop out mix, 200 μg/mL G418, 60 μg/mL canavanine, 2% agar) (Pan et al., 2004) and incubated at 30 °C for 3 days. Haploid transformant cells harboring both wild-type allele and the plasmid-borne *C. roseus FPS* cDNA [Sc (*ERG20*) + pYES2-*CrFPS*] were selected by plating the sporulated culture of heterozygous diploid pools on a modified dextrose-containing magic medium (2% dextrose, 0.67% Yeast Nitrogen Base with ammonium sulphate and without amino acids, 0.06% SC–Leu–His–Arg–Ura drop out mix, 60 μg/mL canavanine, 2% agar) and incubated at 30 °C for 3 days. The functional complementation was triggered by spreading 5 μl drops (10^4 cells) of each haploid transformant on YPD or YPG (1% yeast extract, 2% peptone, 2% galactose, 2% agar) and incubated at 30 °C for one day. Plasmid loss ability was analyzed by spreading each haploid transformant on 5-fluoroorotic acid (FOA)–containing plates (0.67% Yeast Nitrogen Base with ammonium sulphate and without amino acids; 0.08% SC–Ura; 2% galactose; 1 mg/mL FOA, 50 μg/mL uracil, 2% agar) and incubated at 30 °C for one day.

Generation of constructs for subcellular localization studies

CrFPS was cloned from *C. roseus* PFuTurbo (Agilent Technologies, France) RT-generated cDNA using primers FPSf′ and FPSr′ (Supplementary Table S1). PCR products were digested with *Spe*I, and cloned into the *Spe*I site of vector pSCA-cassette YFPi to make YFP-cDNA fusion or cloned into the *Nhe*I site of the same vector to make cDNA-YFP fusion (Guirimand et al., 2009).

Biolistic transformation of C. roseus suspension cells and YFP imaging

Transient transformation of *C. roseus* cells by particle bombardment and YFP imaging were performed following the procedures described by Guirimand et al. (2009, 2011). Briefly, *C. roseus* cells plated onto solid culture medium were bombarded with gold DNA coated particles (1 μm) and 1100 psi rupture disk at a stopping-screen-to-target distance of 6 cm, using the Bio-Rad PDS1000/He system. Cells were cultivated during 15–48 h and the protein subcellular localization was determined using an Olympus BX-51 epifluorescence microscope equipped with an Olympus DP-71 digital camera. Two organelle markers were used for co-transformation studies: the "peroxisome"-CFP (CD3-977) marker obtained from the ABRC (http://arabidopsis.org) and the CFP-GUS cytoplasmic marker (Guirimand et al., 2011). The "peroxisome"-CFP marker

has been obtained by addition of a PTS1 (tripeptide SKL) at the C-termini of CFP (Nelson et al., 2007).

Results

Isolation of the C. roseus FPS (CrFPS) full-length cDNA

Using plant FPS amino acid sequences and the online search Blast tool, we identified two EST covering part of the 5′ and 3′ regions of the coding sequence of a putative FPS, among the 20,000 *C. roseus* EST available in Genbank (http://www.ncbi.nlm.nih.gov/genbank). These two EST sequences allowed us to design primers for the amplification and the cloning of the 1238 bp *CrFPS* cDNA. The 1038 bp open reading frame of the *CrFPS* cDNA encoded a protein of 345 amino acids in length with a calculated mass of 39.9 kDa. The CrFPS protein contained the two aspartate-rich motifs (DDxxD) FARM and SARM conserved in the *trans*-type prenyltransferase family (Fig. 1). The amino acid residues Tyr and Phe located at the fifth and fourth positions, respectively, upstream from the CrFPS FARM domain are large residues, commonly present in several FPS and playing a role in product chain length selectivity (Vandermoten et al., 2009). Amino acid sequence comparison revealed that CrFPS showed a high degree of similarity (approximately 80% identity) with other plant FPS (Fig. 1). Furthermore, CrFPS possesses 49% identity with the ortholog present in the yeast *Saccharomyces cerevisiae* and 44% identity with the human (*Homo sapiens*) and rat (*Rattus norvegicus*) orthologs.

In *A. thaliana*, there are two *FPS* genes (Cunillera et al., 1996, 1997). The *AtFPS1* gene (At5g47770) encodes a short isoform AtFPS1S and a long isoform AtFPS1L containing a mitochondrial N-terminal transit peptide. The *AtFPS2* gene (At4g17190) encodes the AtFPS2 protein devoid of the transit peptide and highly similar to the AtFPS1S isoform. As shown in Fig. 1, CrFPS is more closely related to the short isoforms of plant FPS. Indeed, no N-terminal transit peptide was found in the CrFPS enzyme and the analysis of the cDNA sequence revealed the presence of an in-frame stop codon 15 bp upstream of the predicted translation initiation codon.

Functional complementation of CrFPS in S. cerevisiae

FPP represents, in part, the central axis of terpenoids from which more complex terpenoids are generated. It is now well characterized that, in yeast, the FPP is the unique source of the isoprenoid precursor for the biosynthesis of ergosterol, an essential fungal terpenoid. Gene disruption of the enzyme involved in FPP synthesis is lethal in yeast. As a consequence, yeast strains defective in FPS can be used for functional characterization of heterologous FPS (Delourme et al., 1994). A complementation assay was performed using a heterozygous diploid strain lacking a single *FPS* allele to validate the function of the cloned *CrFPS* cDNA. In this study, we used the full-length coding sequences of *CrFPS*.

Diploid heterozygous *S. cerevisiae* colonies carrying the *CrFPS*-plasmid or the empty-vector-pYES2 were plated onto sporulation medium and haploid colonies were recovered. No haploid cells harboring the disrupted allele and the empty control plasmid pYES2 were obtained, suggesting that the empty pYES2 is unable to complement the *Saccharomyces erg20*Δ (*fps*) disrupted allele (data not shown).

A yeast strain carrying both *erg20*Δ (*fps*) disrupted allele and the plasmid pYES2-Cr*FPS* [Sc (*erg20*Δ) + pYES2-Cr*FPS*] could grow on YPG (galactose-containing medium) expression medium, but not on the non-expression medium YPD (dextrose-containing medium) (Fig. 2). Moreover, the haploid transformed strain harboring both the wild-type allele and the plasmid-borne *CrFPS* cDNA was able to grow on both YPG and YPD medium (Fig. 2). This indicated the CrFPS had farnesyl diphosphate synthase activity and could complement the *erg20*Δ (*fps*) disrupted allele.

Finally, haploid transformed cells were plated onto FOA-containing medium to test the ability of each strain to lose their plasmid. The haploid disrupted strain harboring the plasmid-borne *CrFPS* cDNA [Sc (*erg20*Δ) + pYES2-*CrFPS*] could not grow on a FOA-containing medium, confirming that this strain was not able to lose its plasmid (Fig. 2). In contrast, the haploid transformed strain harboring both the wild-type allele and the plasmid-borne *C. roseus* cDNA [Sc (*ERG20*) + pYES2-CrFPS] was able to grow on FOA containing medium, confirming that it was able to lose its plasmid. All of these results clearly indicate that *CrFPS* displays FPS activity.

The CrFPS protein is targeted to the peroxisome

FPS has long been considered a cytosolic enzyme. However, this conventional statement has been progressively challenged by the identification of an organelle targeting for FPS long isoforms (Cunillera et al., 1997; Martin et al., 2007; Sallaud et al., 2009).

Given that in plants, to the best of our knowledge, there are no experimental data directly showing the compartmentalization of short FPS isoforms, we investigated the subcellular localization of CrFPS through a biolistic-mediated transient expression approach in *C. roseus* cells according to Guirimand et al. (2009).

The *CrFPS* full-length sequence was cloned into vector pSCA-cassette YFPi to make CrFPS-YFP and YFP-CrFPS fusion constructs. In transiently transformed *C. roseus* cells, the CrFPS-YFP fusion protein showed a diffuse pattern of fluorescence characteristic of the cytosol, as revealed by the merging with the fluorescence signal of the cytosolic CFP-GUS marker (Fig. 3A–C). However, in the opposite orientation, YFP-CrFPS displayed a punctuated fluorescence pattern, which co-localized with the fluorescence pattern of the peroxisomal marker (Fig. 3E–G). Peroxisomes appear as round or elongated particles distributed randomly in the cytosol (Schrader and Fahimi, 2008). It should also be noted that a significant portion of the protein remained in the cytosol (Fig. 3E) when compared with the specificity of the peroxisomal control (Fig. 3F). Thus, these results clearly show that CrFPS is targeted to the peroxisome without excluding a partial retention of the protein in the cytosol.

Discussion

In this study, we cloned a *C. roseus* cDNA encoding *CrFPS* that displays high similarities with plant *trans*-type FPS. The CrFPS protein was shown to have FPS activity by functional complementation in the yeast *S. cerevisiae*. Due to the lack of transit peptide at the N-terminal end of the protein, CrFPS is related to short FPS isoforms found in plants. In addition, when compared to the three Arabidopsis FPS isoforms (AtFPS1S, AtFPS1L and AtFPS2) encoded by two genes (*AtFPS1* and *AtFPS2*) (Cunillera et al., 1996, 1997), CrFPS was more closely related to AtFPS2. This observation suggests that the *C. roseus* genome likely contains another *FPS* gene encoding a long and short FPS isoforms. Closa et al. (2010) reported that double knockout mutants of Arabidopsis *AtFPS* genes exhibit a lethal phenotype. In contrast, no significant developmental and metabolic differences were observed in single mutants, showing a partial redundancy of FPS1 and FPS2 isozymes in the biosynthesis of FPP-derived products. However, these results are not supported by studies on subcellular compartmentalization and so far, only the long AtFPS1 isoform was clearly localized in the mitochondria (Cunillera et al., 1997). Consequently, due to multiple localizations reported for FPS in plants, without strong evidence about the compartmentalization of short FPS isoforms, we decided to evaluate the subcellular distribution of CrFPS *via* an YFP fusion-protein

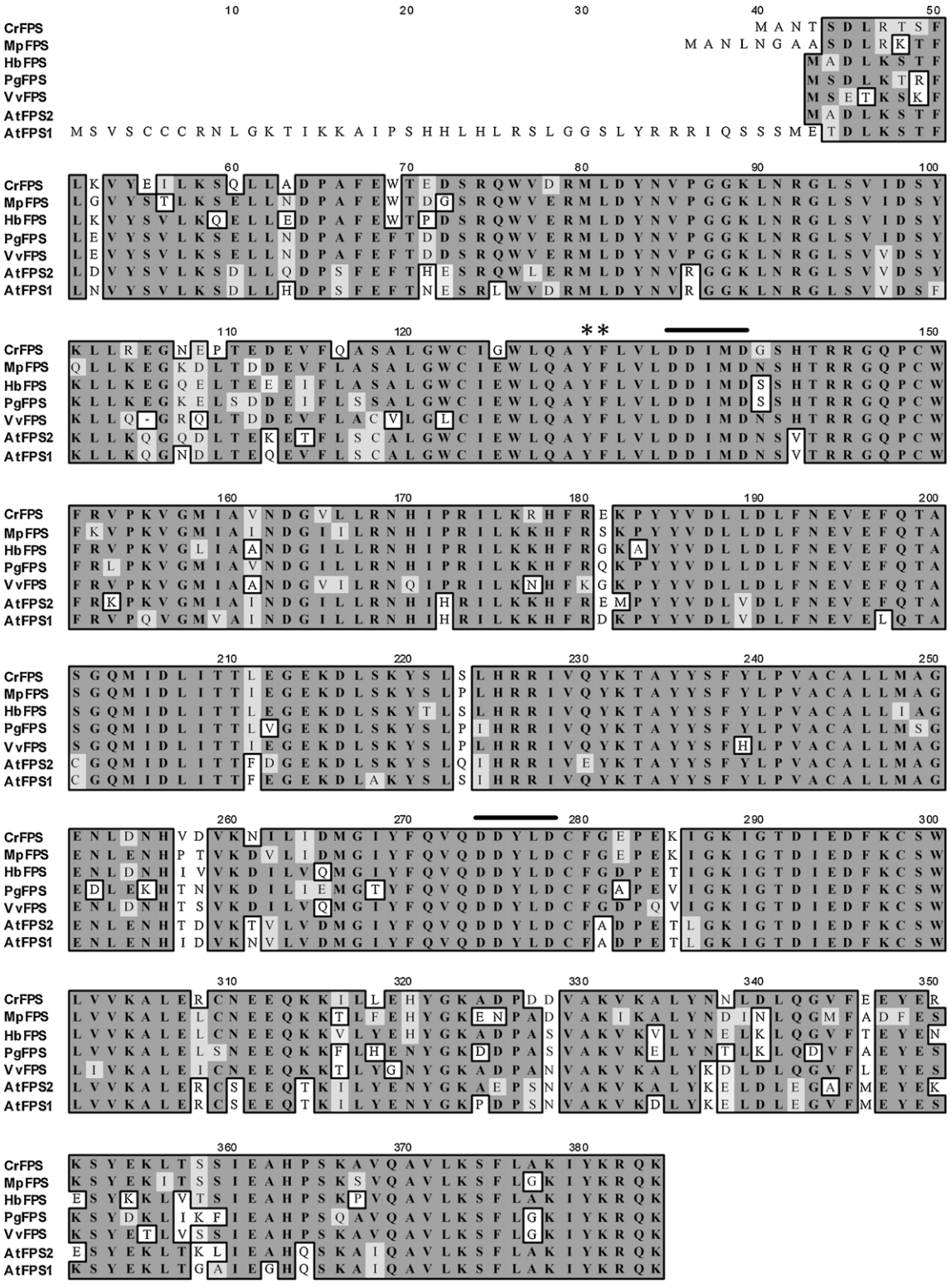

Fig. 1. Multiple sequence alignment of plant FPS. Amino acid comparison of CrFPS from *Catharanthus roseus* (Genbank accession number HQ316638) with FPS from *Arabidopsis thaliana* (the long isoform of AtFPS1, AGI accession number At5g47770 and the short isoform AtFPS2, AGI accession number At4g17190), *Hevea brasiliensis* (HbFPS, Genbank accession number AAM98379), *Mentha* × *piperita* (MpFPS, Genbank accession number AAK63847), *Panax ginseng* (PgFPS, Genbank accession number AAY87903) and *Vitis vinifera* (VvFPS, Genbank accession number AAX76910). Identical and similar residues, common to at least four proteins, are shaded in dark and light grey, respectively. The two upper lines indicate the regions corresponding to the FARM and SARM domains. The two large residues located upstream from the FARM domain and involved in product chain-length determination are indicated by an asterisk. Sequence alignment was performed using the ClustalW program.

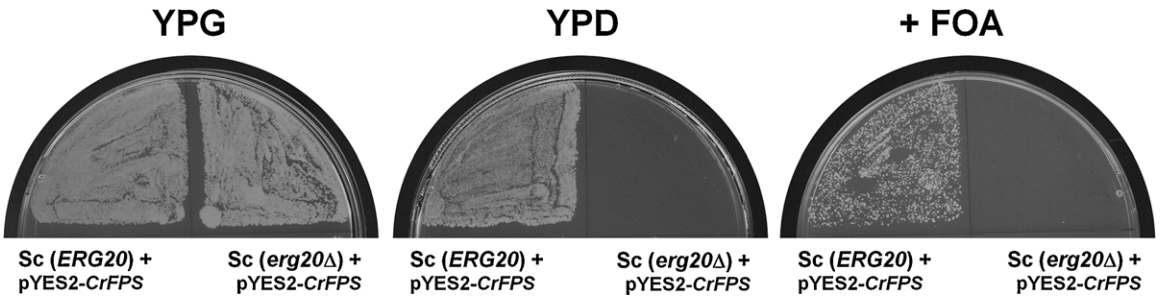

Fig. 2. Yeast complementation assay. Drops containing 10^4 cells from haploid transformed strains harboring both wild-type allele and the plasmid-borne *C. roseus* cDNA [Sc (*ERG20*) + pYES2-Cr*FPS*] and from haploid transformed strains harboring both the disrupted allele and the plasmid-borne *C. roseus* cDNA [Sc (*erg20*Δ) + pYES2-Cr*FPS*] were spread onto YPG, YPD or FOA-containing (+FOA) plates. YPG contains galactose necessary for the induction of the *C. roseus* enzyme CrFPS. Each plate was incubated for 1 day at 30 °C and photographed.

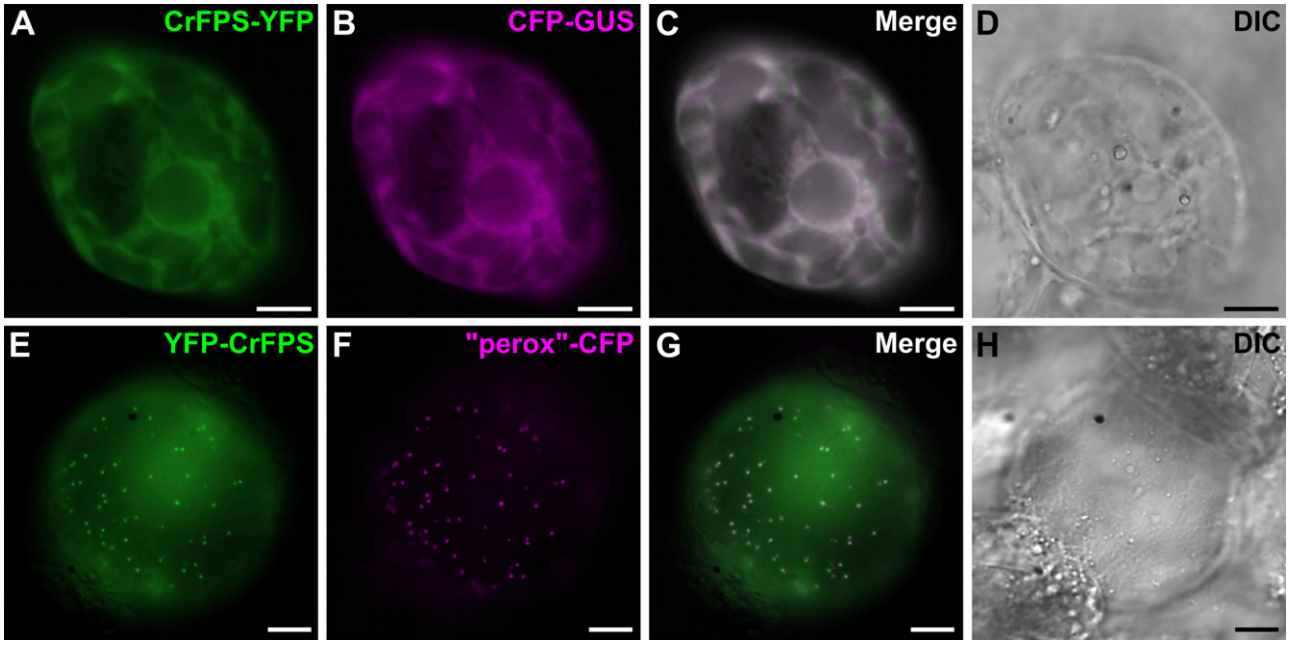

Fig. 3. Subcellular localization of CrFPS. *C. roseus* cells were transiently co-transformed with plasmid expressing either CrFPS-YFP (A) or YFP-CrFPS (E) and the cytosolic CFP-GUS marker (B) or the peroxisomal marker "perox"-CFP (F). Co-localization of two fluorescence signals are shown in the merged image (C, G). The morphology (D, H) is observed with differential interference contrast (DIC). Bars 10 μm.

biolistic approach. This study constitutes the first report of the subcellular localization of a short FPS isoform in plants. Our results showing that CrFPS is targeted to peroxisomes (Fig. 3E–G) are in agreement with recent reports on the subcellular distribution of enzymes involved in the early steps of isoprenoid biosynthesis for both plants and animals.

For instance, in plants, the two short isoforms of *A. thaliana* isopentenyl diphosphate isomerase (IDI) are localized to peroxisomes in agreement with the presence of a peroxisomal targeting signal (PTS) type 1 at their C-terminal end (Sapir-Mir et al., 2008). Two types of PTS (PTS1 and PTS2) mediate the peroxisome import of proteins by the PTS1 receptor PEX5 and the PTS2 receptor PEX7 (Kaur et al., 2009). PTS1 is commonly a C-terminal tripeptide with the consensus sequence (S/C/A)(K/R/H)(L/M) and PTS2 is a non-peptide located internally or near the N-terminus, and having the usual consensus sequence (R/K)(L/V/I/Q)X_5(H/Q)(L/A/F) (Petriv et al., 2004). In addition to the short IDI isoforms, bioinformatic search of PTS motifs and arguments based on substrate channeling lead to a proposed model in which part of the MVA enzymes, IDI and FPS are targeted to the peroxisome in plants (Sapir-Mir et al., 2008). We recently confirmed this prediction for the last two enzymes of the MVA pathway, 5-phosphomevalonate kinase (PMK) and mevalonate 5-diphosphate decarboxylase (MVD) from *C. roseus* and from *A. thaliana*, which were shown to be targeted to peroxisomes (Simkin et al., 2011).

In regards to animals, several studies have reported the peroxisomal localization of FPS (Krisans et al., 1994; Olivier et al., 2000; Kovacs et al., 2007). Transfections of mammalian cells with the rat short FPS isoform followed by immunodetection localized the enzyme to the peroxisome (Olivier et al., 2000; Kovacs et al., 2007). Furthermore, the authors have shown that the rat FPS requires the PTS2 import pathway. They identified, within the N-terminal amino acid sequence, a degenerated PTS2-like motif and demonstrated that this motif is necessary for efficient targeting of rat FPS to the peroxisome (Olivier et al., 2000). By contrast, no PTS1 located at the C-terminal end could be found within the amino acid sequence of CrFPS as well as no canonical PTS2 consensus sequence. However, other proteins that do not contain classic PTS have been shown to be targeted to peroxisomes *via* internal PTS-like motifs or poorly characterized internal targeting signals. Furthermore, proteins have been shown to be targeted to the peroxisome *via* a mechanism known as 'piggy-backing' in which they form a complex with a protein containing a PTS (van der Klei and Veenhuis, 2006; Oshima et al., 2008; Penha et al., 2009). In our experiments, the fact that

only the YFP-CrFPS construct is targeted to peroxisomes could be explained by the presence of a putative non-PTS domain near the CrFPS C-terminus necessary for peroxisome targeting. In turn, the cytosolic localization of the CrFPS-YFP construct could be a consequence of the physical obstruction of the C-terminal domain of CrFPS caused by the fluorescent protein.

By compiling all the fragmentary data on FPS subcellular localization including peroxisomal localization, we can draw the following scheme of FPS distribution in yeast, mammals and plants.

The yeast *S. cerevisiae* contains only one FPS (ERG20) generally regarded as cytosolic, although this was not confirmed by cell imaging studies. Furthermore, no studies have reported a peroxisomal localization of the yeast FPS. The mitochondrial polyprenyl diphosphates, such as the prenyl side chain of ubiquinone, are synthesized by a mitochondrial hexaprenyl diphosphate synthase (*COQ1*), which could accept FPP or longer-chain allelic substrates (Ashby and Edwards, 1990), suggesting that they are transported from the cytosol to the mitochondria. Note that in the above complementation experiment, CrFPS, which is targeted to peroxisome in *C. roseus*, can complement the likely cytosolic deficient FPS from *S. cerevisiae*.

The general view of mammalian FPS is that a single functional gene is present in the genome. This gene encodes more than one isoform, since for example, the Genbank database contains the annotated short (Genbank accession number: EDM00669) and long (Genbank accession number: EDM00670) versions of rat FPS. The long mammalian isoform, bearing an N-terminal transit peptide, is targeted to the mitochondria (Martin et al., 2007) while the short isoform was detected in the peroxisomes (Olivier et al., 2000; Kovacs et al., 2007; Martin et al., 2007) as well as in the cytosol (Martin et al., 2007).

In plants, it appears that there are at least two *FPS* genes (Cunillera et al., 1997; Gaffé et al., 2000) encoding several *trans*-type isoforms. One gene encodes a long isoform targeted to mitochondria (Cunillera et al., 1997) and a short isoform, for which no localization has been reported, thereby raising the question of its cytosolic or peroxisomal nature. The second gene encodes a short isoform potentially targeted to the peroxisome as shown in this study for CrFPS (Fig. 3E–G). Furthermore, in addition to the punctuated peroxisomal pattern, CrFPS-transformed *C. roseus* cells display a diffuse pattern that can be associated with the cytosol (Fig. 3E). Therefore, we cannot totally exclude the possibility that a certain proportion of the enzyme is also localized within the cytosol as in mammals, although such result could also be due to overexpression of the fusion-protein.

In conclusion, the subcellular localization of periwinkle CrFPS gives insight into the role of peroxisome in the isoprenoid metabolism. This result, in combination with the arguments raised above, may suggest that, in plants, semi-independent pools of FPP are generated in peroxisomes, mitochondria and probably in the cytosol. These pools are subsequently available to either peroxisomal, mitochondrial or cytosolic specific downstream enzymatic reactions, leading to the formation of a variety of compartmentalized isoprenoid end-products. The specific role of each individual FPS isoform in their biosynthesis remains to be elucidated in order to apprehend the pivotal position of FPP in isoprenoid metabolism.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jplph.2011.06.017.

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