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BIOSÍNTESI D'ISOPRENOIDES EN PLANTES: CARACTERITZACIÓ MOLECULAR DE LA FARNESILDIFOSFAT SINTASA D'*Arabidopsis thaliana*.

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CONCLUSIONS

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1. *Arabidopsis thaliana* conté una petita família multigènica que codifica FPS composada com a mínim per dos gens, *FPS1* i *FPS2*, estructuralment molt relacionats. Mitjançant la utilització d'inicis de transcripció alternatius, el gen *FPS1* genera dos mRNAs, FPS1S i FPS1L, que són diferents en el seu extrem 5'. Els tres mRNAs producte de l'expressió dels gens *FPS1* i *FPS2* presenten patrons d'expressió diferents entre si i codifiquen tres isoenzims FPS (FPS1S, FPS1L i FPS2).

2. La isoforma FPS1L presenta una extensió NH_2-terminal de 41 aminoàcids que no té equivalent ni en els isoenzims FPS1S i FPS2 ni en cap de les FPS caracteritzades fins el moment. Aquesta seqüència NH_2-terminal és un pèptid de trànsit que dirigeix la isoforma FPS1L a les mitocòndries, on és processada per donar lloc a la forma madura de la FPS mitocondrial. L'existència d'aquesta FPS mitocondrial reforça la hipòtesi que les mitocòndries, com a mínim les de plantes, utilitzen l'IPP sintetitzat en el citosol-RE com a precursor per a la síntesi de FPP que, posteriorment, pot ser utilitzat per a la síntesi de derivats isoprenoides mitocondrials com les proteïnes prenilades, la ubiquinona o el grup hem a.

3. El gen *FPS1* és un gen bifuncional que pertany al grup de gens eucariotes que codifiquen isoenzims que duen a terme funcions anàlogues en diferents localitzacions subcel.lulars. En el cas del gen *FPS1*, l'estratègia seguida per generar els isoenzims FPS1 localitzats en el citosol-RE i en les mitocòndries és la utilització d'inicis de transcripció alternatius, juntament amb la possible utilització de codons AUG alternatius durant el procés d'inici de traducció. Les característiques del gen *FPS1* confirmen que la biosíntesi d'isoprenoides en plantes és una via metabòlica molt complexa que està regulada a través de mecanismes de control que operen a nivell transcripcional i post-transcripcional.

4. L'anàlisi del patró d'expressió del gen *uid*A d'*Escherichia coli* en *Arabidopsis* transgèniques portadores de gens quimèrics en els que la regió 5'-flanquejant dels gens *FPS1* i *FPS2* s'ha fusionat al gen *uid*A indica que els isoenzims FPS1S i FPS2 s'expressen de forma diferencial. L'expressió generalitzada de l'isoenzim FPS1S suggereix que aquest isoenzim participa en la síntesi d'isoprenoides que duen a terme funcions generals de caràcter bàsic, com per exemple els fitoesterols. En canvi, el gen *FPS2* té un patró d'expressió restringit a òrgans i estadis concrets del desenvolupament que suggereix que l'isoenzim FPS2 participa en la síntesi d'isoprenoides implicats en funcions especialitzades. Aquestes observacions recolzen la hipòtesi que proposa que la via de síntesi d'isoprenoides en plantes està organitzada en canals metabòlics que poden ser regulats de forma independent i que estan dedicats a la síntesi de grups específics de compostos isoprenoides.

5. L'expressió transitòria en protoplastes i l'expressió estable en *Arabidopsis* transgèniques de construccions que contenen fragments progressivament delecionats per l'extrem 5' de les regions 5'-flanquejants dels gens *FPS1* i *FPS2* fusionats al gen *uid*A d'*Escherichia coli* han permès identificar les regions dels promotors implicades en el control de l'expressió dels gens. En el cas del gen *FPS1*, les seqüències involucrades en el control de l'expressió quantitativa de la isoforma FPS1S estan distribuïdes en una àmplia regió que s'extén fins la posició −571 del gen i, en concret, els elements més importants es localitzen entre les posicions −365 i −113. En el cas del gen *FPS2*, les seqüències que participen en el control de l'expressió quantitativa i qualitativa del gen es troben en una petita regió que s'extén fins la posició −111, i en particular, entre les posicions −111 i −61 es localitzen elements clau per dirigir l'expressió del gen *FPS2*.

ANNEXOS

ANNEX I

Aliniament múltiple de les seqüències d'aminoàcids de 14 FPS de plantes.

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A. thaliana 1              M. ETDL.KSTFL NVYSVLKSDL LHDPSFEFTN E.SRLWVDRM
A. thaliana 2              M. .ADL.KSTFL DVYSVLKSDL LQDPSFEFTH E.SRQWLERM
C. annuum                  MS ..DF.KSKFM EAYYVLKSEL LNDPGFEFTD D.SREWVDKM
L. esculentum              MA ..DL.KKKFL DVYSVLKSDL LEDTAFEFTD D.SRKWVDKM
P. argentatum 1            M. SSDL.KSRFL QVYDTLKSEL INDPAFEFDD D.SRQWVEKM
P. argentatum 2            M. STDI.RSKFL QVYDTLKSEL INDPAFEFDD D.SRQWIEKM
A. annua                   MS STDL.KSKFL KVYDTLKSEL INDPAFEFDD D.SRQWIEKM
H. annuus                  MA S.DL.KSKFL HVYQTLKSEL LNDPAFEFHH D.SRQWIDKM
L. albus 1                 MA ..DL.RSTFL NVYSVLKSEL LHDPAFEFSP D.SRQWLDRM
L. albus 2                 MA ..DP.KSSFL NVYSILKSEL LQDPAFEFST D.SRQWVERM
H. brasiliensis            MA ..DL.KSTFL KVYSVLKQEL LEDPAFEWTP D.SRQWVERM
G. arboreum                MA ..DL.RSAFL NVYSQLKSEL LQDPSFELT. DESRQWVERM
Z. mays          MAAG..GNGA GGD.TRAAFA RIYKTLKEEL LTDPAFEFT. EESRQWIDRM
O. sativa        MAAAVVANGA SGDSSKAAFA EIYSRLKEEM LEDPAFEFT. DESLQWIDRM

Consens                    --D-----F- --Y--LK--- --D--FE--- --S--W---M


A. thaliana 1    LDYNVRGGKL NRGLSVVDSF KLLKQGND.L TEQEVFLSCA LGWCIEWLQA
A. thaliana 2    LDYNVRGGKL NRGLSVVDSY KLLKQGQD.L TEKETFLSCA LGWCIEWLQA
C. annuum        LEYNVPGGKL NRGLSVIDSY SLVDDGKE.L TRDEIFKASA LGWCIEWLQA
L. esculentum    LDYNVPGGKL NRGLSVIDSL SLLKDGKE.L TADEIFKASA LGWCIEWLQA
P. argentatum 1  LDYNVPGGKL NRGLSVIDSY QLLK.GGE.L TDNEIFLAAA LGWCIEWLQA
P. argentatum 2  LDYNVPGGKL NRGLSVIDSY QLLK.GGK.L TDDEIFHASA LGWCVEWLQA
A. annua         LDYNVPGGKL NRGLSVVDSY QLLK.GGE.L SDDEIFLSSA LGWCIEWLQA
H. annuus        LDYNVPGGKL NRGLSVVDSY QLLK.GAE.L TDDEIFLASA LGWCIEWLQA
L. albus 1       LDYNVPGGKL NRGLSVIDSY RLLKDGHE.L NDDEIFLASA LGWCIEWLQA
L. albus 2       LDYNVPGGKL NRGLSVIDSY KLLKDGQE.L NDEEIFLASA LGWCIEWLQA
H. brasiliensis  LDYNVPGGKL NRGLSVIDSY KLLKEGQE.L TEEEIFLASA LGWCIEWLQA
G. arboreum      LDYNVPGGKL NRGLSVIDSY RLLKDGKE.L TQDEIFLTSA LGWCIEWLQA
Z. mays          LDYNVLGGKC NRGLSVIDSY KLLK.GADAL GEEETFLACT LGWCIEWLQA
O. sativa        LDYNVLGGKC NRGISVIDSF KMLK.GTDVL NKEETFLACT LGWCIEWLQA

Consens          --YNV-GGK- NRG-SV-DS- -----G---L ---E-F---- LGWC-EWLQA
                       GGK   R                                    E
                 ___________                                      _____

                 Domini I
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A. thaliana 1      YFLVLDDIMD NSVTRRGQPC WFRVPQVGMV AINDGILLRN HIHRILKKHF
A. thaliana 2      YFLVLDDIMD NSVTRRGQPC WFRKPKVGMI AINDGILLRN HIHRILKKHF
C. annuum          YFLVLDDIMD NSHTRRGQPC WYKVEKVGMI AVNDGILLRN HISRILKNHF
L. esculentum      YFLVLDDIMD GSHTRRGQPC WYNLEKVGMI AINDGILLRN HITRILKKYF
P. argentatum 1    YFLVLDDIMD ESHTRRGQPC WFRLPKVGMI AANDGIILRN NVPRILKKHF
P. argentatum 2    YFLVLDDIMD ESHTRRGQPC WFRLPKVGMI AANDGIILRN HVPRILKKHF
A. annua           YFLVLDDIMD ESHTRRGQPC WFRLPKVGMI AANDGILLRN HVPRILKKHF
H. annuus          YFLVLDDIMD GSHTRRGQPC WFRLPKVGMI AANDGLILRN HVPRILKKHF
L. albus 1         YFLVLDDIMD NSHTRRGQPC WFRVPKVGMI AANDGVLLRN HIPRILKKHF
L. albus 2         YFLVLDDIMD NSHTRRGHPC WFRVPKVGMI APNDGVVLRN HIPRILKKHF
H. brasiliensis    YFLVLDDIMD SSHTRRGQPC WFRVPKVGLI AANDGILLRN HIPRILKKHF
G. arboreum        YFLVLDDIMD SSHTRRGQPC WFRLPKVGMI AVNDGVILRN HITRILKNHF
Z. mays            FFLVLDDIMD DSHTRRGQPC WFRVPQVGLI AANDGIILRN HISRILRRHF
O. sativa          YFLVLDDIMD NSQTRRGQPC WFRVPQVGLI AVNDGIILRN HISRILQRHF

Consens            -FLVLDDIMD -S-TRRG-PC W-----VG-- A-NDG--LRN ---RIL---F
                     L  DD  D     RRG               A  D   L
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                         Domini II                  Domini III

A. thaliana 1      RDKPYYVDLV DLFNEVELQT ACGQMIDLIT TFEGEKDLAK YSLSIHRRIV
A. thaliana 2      REMPYYVDLV DLFNEVEFQT ACGQMIDLIT TFDGEKDLSK YSLQIHRRIV
C. annuum          RPKSYYVDLL DLFNEVEFQT ASGQMIDLIT THVGEKDLSK YSLPIHCRIV
L. esculentum      RPESYYVDLL DLFNEVEFQT ASGQMIDLIT TLVGEKDLSK YSLSIHRRIV
P. argentatum 1    RGKPYYVDLL DLFNEVEFQT ASGQMIDLIT TLVGEKDLSK YSLSIHRRIV
P. argentatum 2    RGKPYYVDLV DLFNEVEFQT ASGQMIDLIT TLVGEKDLSK YSLSIHRRIV
A. annua           RGKPYYVDLV DLFNEVEFQT ASGQMIDLIT TLVGEKDLSK YSLSIHRRIV
H. annuus          RGKPYYVDLV DLFNEVEFQT ASGQMIDLIT TLVGEKDLSK YSLSIHRRIV
L. albus 1         RGKPYYADLL DLFNEVEFQT ASGQMIDLIT TLEGEKDLSK YTLSLHRRIV
L. albus 2         RGKPYYVDLL DLFNEVEFQT ASGQMIDLIT TLEGEKDLSK YTLSLHRRIV
H. brasiliensis    RGKAYYVDLL DLFNEVEFQT ASGQMIDLIT TLEGEKDLSK YTLSLHRRIV
G. arboreum        RGKPYYVDLL DLFNEVEFQT ASGQMIDLIT TLEGEKDLSK YSLQQHRRIV
Z. mays            KGKPYYADLL DLFNEVEFKT ASGQLLDLIT THEGEKDLTK YNITVHGRIV
O. sativa          KGKLYYVDLI DLFNEVEFKT ASGQLLDLIT THEGEKDLTK YNLTVHRRIV

Consens            ----YY-DL- DLFNEVE--T A-GQ--DLIT T--GEKDL-K Y----H-RIV
                                         GQ  DL
                                        ---------
                                        Domini IV

A. thaliana 1      QYKTAYYSFY LPVACALLMA GENLENHIDV KNVLVDMGIY FQVQDDYLDC
A. thaliana 2      EYKTAYYSFY LPVACALLMA GENLENHTDV KTVLVDMGIY FQVQDDYLDC
C. annuum          QYKTAYYSFY LPVACALLMA GENLDNHVDV KNILIEMGIY FQVQDDYLDC
L. esculentum      QYKTAYYSFY LPVACALLMV GENLDKHVDV KKILIDMGIY FQVQDDYLDC
P. argentatum 1    QYKTAYYSFY LPVACALLMF GEDLEKHEEV KNVLVEMGTY FQVQDDYLDC
P. argentatum 2    QYKTAYYSFY LPVACALLMF GEDLEKHVEV KNVLVEMGTY FQVQDDYLDC
A. annua           QYKTAYYSFY LPVACALLMF GEDLDKHVEV KNVLVEMGTY FQVQDDYLDC
H. annuus          QYKTAYYSFY LPVACALLMF GEDLDNHVEV KNVLVEMGTY FQVQDDYLDC
L. albus 1         QYKTAYYSFY LPVACALLMV GENLDNHIDV KNILVDMGTY FQVQDDYLDC
L. albus 2         QYKTAYYSFY LPVACALLMV GENLDNHTDV KNILVEMGTY FQVQDDYLDC
H. brasiliensis    QYKTAYYSFY LPVACALLIA GENLDNHIVV KDILVQMGIY FQVQDDYLDC
G. arboreum        QYKTAYYSFY LPVACALVMC GENLDNHIDV KNILVDMGIY FQVQDDYLDC
Z. mays            QYKTAYYSFY LPVACALLLS GENLDNYGDV ENILVEMGTY FQVQDDYLDC
O. sativa          QYKTAYYSFY LPVACALLLS GENLDNFGDV KNILVEMGTY FQVQDDYLDC

Consens            -YKTAYYSFY LPVACAL--- GE-L-----V ---L--MG-Y FQVQDDYLDC
                     KTA                              G   FQ  DD  D
                   ---------                          ---------------
                   Domini V                           Domini VI
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A. thaliana 1	FADPETLGKI GTDIEDFKCS WLVVKALERC SEEQTKILYE NYGKPDPSN.
A. thaliana 2	FADPETLGKI GTDIEDFKCS WLVVKALERC SEEQTKILYE NYGKAEPSN.
C. annuum	FADPEVLGKI GTDIQDFKCS WLVVKALELC NEEQKKILYE NYGK.DDAAC
L. esculentum	FADPEVLGKI GTDIQDFKCS WLVVKALELC NEEQKKILFE NYGK.DNAAC
P. argentatum 1	FGAPEVIGKI GTDIEDFKCS WLVVKALELS NEEQKKILHE NYGKKDPSS.
P. argentatum 2	FGAPEVIGKI GTDIEDFKCS WLVVKALELA NEEQKKVLHE NYGKKDPSP.
A. annua	FGAPEVIGKI GTDIEDFKCS WLVVKALELP NEEQKKTLHE NYGKKDPAS.
H. annuus	FGAPEVIGKI GTDIEDFS.S WLVVKALELA NEEQKKVLHE NYGKKDPSS.
L. albus 1	FGAPETIGKI GTDIEDFKCS WLVVKALELS NDEQKKVLYD NYGKPDPAN.
L. albus 2	FGAPETIGKI GTDIEDFKCS WLVVKALELS NEEQKKVLYE NYGKPDPAN.
H. brasiliensis	FGDPETIGKI GTDIEDFKCS WLVVKALELC NEEQKKVLYE HYGKADPAS.
G. arboreum	FGNPETIGKI GTDIENFKCS WLVVKALEFC NEEHNKVLYE NYGETRPAN.
Z. mays	YGDPEFIGKI GTDIEDYKCS WLVVQALERA DESQKRILFE NYGKKDPA.C
O. sativa	YGDPEFIGKI GTDIEDYKCS WLVVQALERA DENQKHILFE NYGKPDPE.C
Consens	---PE--GKI GTDI---K-S WLVV-ALE-- -------L-- -YG-------
	GK D K S

Domini VI

A. thaliana 1	VAKVKDLYKE LDLEGVFMEY ES.KSYEKL. TGAIEGHQSK AIQAV.LKSF
A. thaliana 2	VAKVKALYKE LDLEGAFMEY EK.ESYEKL. TKLIEAHQSK AIQAV.LKSF
C. annuum	IAKIKTLYND LKLEEVFLEY EKKT.YEKLA NS.IAAHPSK AVQAVQL.SF
L. esculentum	IAKIKALYND LKLEEVFLEY E.KTSYEKLT TS.IAAHPSK AVQAVLL.SF
P. argentatum 1	VAKVKELYHT LNLQGVFEDY EN.TSYKKLI TS.IEGHPSK AVQAV.LKSF
P. argentatum 2	VAKVKELYNT LNLQGVFEDY EN.TSYKKLI TS.IEGHPSK AVQAV.LKSF
A. annua	VAKVKEVYHT LNLQAVFEDY EA.TSYKKLI TS.IENHPSK AVQAV.LKSF
H. annuus	VAKVKELYNT LNLQGVFEDY ES.TSYKKLI TS.IEGHPSK AVQAV.LKSF
L. albus 1	VAKVKALYDE LNLQGVFTEY ES.KSYEKLV TS.IEAHPSK AVQAL.LKSF
L. albus 2	VAKVKTLYNE LNLEGAYADY ES.KSYEKLV TC.IEGHPSK AVQGV.LKSF
H. brasiliensis	VAKVKVLYNE LKLQGVFTEY EN.ESYKKLV TS.IEAHPSK PVQAV.LKSF
G. arboreum	VAKVKALYNE LNLKGVFEDY ES.KSYERLV TS.IEAHPSK PVQAV.LKSF
Z. mays	VAKVKNLYKE LDLEAVFQEY EN.ESYKKLI .ADIEAQPSI AVQKV.LKSF
O. sativa	VAKVKDLYKE LNLEAVFHEY ER.ESYNKLI .ADIEAHPNK AVQNV.LKSF
Consens	-AK-K--Y-- L-L------Y E----Y--L- ---I------ --Q---L-SF

A. thaliana 1	LAKIYKRQK*
A. thaliana 2	LAKIYKRQK*
C. annuum	LGKIYKRQK*
L. esculentum	LGKIYKRQK*
P. argentatum 1	LGKIYKRQK*
P. argentatum 2	LGKIYRRQK*
A. annua	LGKIYKRQK*
H. annuus	LGKIYKRQK*
L. albus 1	LGKIYKRQK*
L. albus 2	WAKIYKRQK*
H. brasiliensis	LAKIYKRQK*
G. arboreum	LGKIYKRQK*
Z. mays	LHKIYKRQK*
O. sativa	LHKIYKRQK*
Consens	--KIY-RQK
	(+)

Domini VII

Les seqüències d'aminoàcids són les següents: FPS1 i FPS2 d'*Arabidopsis thaliana* (Delourme *et al.*, 1994; aquest treball); *Capsicum anuum* (Hugueney *et al.*, 1996); *Lycopersicon esculentum* (Gaffe *et al.*, 1998, número d'accés al *GenBank™/EMBL Data Bank* AF048747); FPS1 i FPS2 de *Parthenium argentatum* (Pan *et al.*, 1996); *Artemisia annua* (Matsushita *et al.*, 1996); *Helianthus annuus* (Park *et al.*, 1997, número d'accés al *GenBank™/EMBL Data Bank* AF019892); FPS1 i FPS2 de *Lupinus albus* (Attucci *et al.*, 1995a; Attucci *et al.*, 1995b); *Hevea brasiliensis* (Adiwilaga i Kush., 1996); *Gossypium arboreum* (Liu *et al.*, 1997, número d'accés al *GenBank™/EMBL Data Bank* Y12072); *Zea mays* (Li i Larkins, 1996) i *Oryza sativa* (Sanmiya *et al.*, 1997).

ANNEX II

Aliniament múltiple de les seqüències d'aminoàcids de FPS de plantes, mamífers, fongs i bacteris.

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A. thaliana 1              M. ETD..L.... ..KSTF...L NVY.S.V..L .KSDLLHDPS
A. thaliana 2              M. .AD..L.... ..KSTF...L DVY.S.V..L .KSDLLQDPS
C. anuum                   MS ..D..F.... ..KSKF...M EAY.Y.V..L .KSELLNDPG
L. esculentum              MA ..D..L.... ..KKKF...L DVY.S.V..L .KSDLLEDTA
P. argentatum 1            M. SSD..L.... ..KSRF...L QVY.D.T..L .KSELINDPA
P. argentatum 2            M. STD..I.... ..RSKF...L QVY.D.T..L .KSELINDPA
A. annua                   MS STD..L.... ..KSKF...L KVY.D.T..L .KSELINDPA
H. annuus                  MA S.D..L.... ..KSKF...L HVY.Q.T..L .KSELLNDPA
L. albus 1                 MA ..D..L.... ..RSTF...L NVY.S.V..L .KSELLHDPA
L. albus 2                 MA ..D..P.... ..KSSF...L NVY.S.I..L .KSELLQDPA
H. brasiliensis            MA ..D..L.... ..KSTF...L KVY.S.V..L .KQELLEDPA
G. arboreum                MA ..D..L.... ..RSAF...L NVY.S.Q..L .KSELLQDPS
Z. mays              MAAG..GNGA GGD..... .TRAAF...A RIY.K.T..L .KEELLTDPA
O. sativa            MAAAVVANGA SGD..S.... .SKAAF...A EIY.S.R..L .KEEMLEDPA
H. sapiens                 M. NGDQNSDVYA QEKQDF...V QHFSQIVRVL TEDEM.GHP.
R. norvegicus              M. NGDQKLDVHN QEKQNF...I QHFSQIVKVL TEDEL.GHP.
S. cerevisiae              MA S.EKEI.... .RRERF...L NVFPKLVEEL .NASLL...A
K. lactis                  M. S.D..... .NRAQF...L EVFPSLVQEL .R.DIL...A
B. stearotherm.            MA ..Q..L.... SVEQ.F...L N..EQ..KQA V.ETALS...
M. luteus                  ML ..QEKL.... TMNRDF...L N........L INESLLN...
E. coli                    M  .......... ....DFPQQL ...EACVKQA .NQ.ALS...

Consens                       ---------- -----F---- ---------- ----------

A. thaliana 1        .F.E.FTNE. .SRLWVDRML DYN.VR.GGK .LNRGLSVVD SFK.LLKQ.G
A. thaliana 2        .F.E.FTHE. .SRQWLERML DYN.VR.GGK .LNRGLSVVD SYK.LLKQ.G
C. anuum             .F.E.FTDD. .SREWVDKML EYN.VP.GGK .LNRGLSVID SYS.LVDD.G
L. esculentum        .F.E.FTDD. .SRKWVDKML DYN.VP.GGK .LNRGLSVID SLS.LLKD.G
P. argentatum 1      .F.E.FDDD. .SRQWVEKML DYN.VP.GGK .LNRGLSVID SYQ.LLK..G
P. argentatum 2      .F.E.FDDD. .SRQWIEKML DYN.VP.GGK .LNRGLSVID SYQ.LLK..G
A. annua             .F.E.FDDD. .SRQWIEKML DYN.VP.GGK .LNRGLSVVD SYQ.LLK..G
H. annuus            .F.E.FHHD. .SRQWIDKML DYN.VP.GGK .LNRGLSVVD SYQ.LLK..G
L. albus 1           .F.E.FSPD. .SRQWLDRML DYN.VP.GGK .LNRGLSVID SYR.LLKD.G
L. albus 2           .F.E.FSTD. .SRQWVERML DYN.VP.GGK .LNRGLSVID SYK.LLKD.G
H. brasiliensis      .F.E.WTPD. .SRQWVERML DYN.VP.GGK .LNRGLSVID SYK.LLKE.G
G. arboreum          .F.E.LT.DE .SRQWVERML DYN.VP.GGK .LNRGLSVID SYR.LLKD.G
Z. mays              .F.E.FT.EE .SRQWIDRMV DYN.VL.GGK .CNRGLSVVD SYK.LLK..G
O. sativa            .F.E.FT.DE .SLQWIDRML DYN.VL.GGK .CNRGISVID SFK.MLK..G
H. sapiens           ...E..IGDA IAR..LKEVL EYNAI..GGK .YNRGLTVVV AFRELVEP.R
R. norvegicus        ...E..KGDA ITR..IKEVL EYNTV..GGK .YNRGLTVVQ TFQELVEP.R
S. cerevisiae        .Y.G.M.PKE .ACDWYAHSL NYN.TP.GGK .LNRGLSVVD TYA.ILS..N
K. lactis            GY.G.M.PEE .AIEWYEKSL NYN.TP.GGK .LNRGLSVVD TYA.LLK..G
B. stearotherm.      RYIERLEGPA ..K..LKKAM AY.SLEAGGK RI.RPLLLLS T....VRALG
M. luteus            KY....H.PA QSR..LHEAI NY.SLSAGGK RI.RPLLVLT T....LDSLG
E. coli              RFIAPL..PF QNTP.VVETM QYGAL.LGGK RL.RPFLVYA T..GHM..FG

Consens              ---------- ---------- -Y-----GGK ---R------ ----------
                                            GGK    R
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                                            Domini I
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A. thaliana 1      .N..D.LTEQ EVFLSCA..L GWC.IEWLQA YFLVLDDI.. MDNSVTRRGQ
A. thaliana 2      .Q..D.LTEK ETFLSCA..L GWC.IEWLQA YFLVLDDI.. MDNSVTRRGQ
C. anuum           .K..E.LTRD EIFKASA..L GWC.IEWLQA YFLVLDDI.. MDNSHTRRGQ
L. esculentum      .K..E.LTAD EIFKASA..L GWC.IEWLQA YFLVLDDI.. MDGSHTRRGQ
P. argentatum 1    .G..E.LTDN EIFLAAA..L GWC.IEWLQA YFLVLDDI.. MDESHTRRGQ
P. argentatum 2    .G..K.LTDD EIFHASA..L GWC.VEWLQA YFLVLDDI.. MDESHTRRGQ
A. annua           .G..E.LSDD EIFLSSA..L GWC.IEWLQA YFLVLDDI.. MDESHTRRGQ
H. annuus          .A..E.LTDD EIFLASA..L GWC.IEWLQA YFLVLDDI.. MDGSHTRRGQ
L. albus 1         .H..E.LNDD EIFLASA..L GWC.IEWLQA YFLVLDDI.. MDNSHTRRGQ
L. albus 2         .Q..E.LNDE EIFLASA..L GWC.IEWLQA YFLVLDDI.. MDNSHTRRGH
H. brasiliensis    .Q..E.LTEE EIFLASA..L GWC.IEWLQA YFLVLDDI.. MDSSHTRRGQ
G. arboreum        .K..E.LTQD EIFLTSA..L GWC.IEWLQA YFLVLDDI.. MDSSHTRRGQ
Z. mays            .A..DALGEE ETFLACT..L GWC.IEWLQA FFLVLDDI.. MDDSHTRRGQ
O. sativa          .T..DVLNKE ETFLACT..L GWC.IEWLQA YFLVLDDI.. MDNSQTRRGQ
H. sapiens         KQDADSL..Q R.....AWTV GWC.VELLQA FFLVADDI.. MDSSLTRRGQ
R. norvegicus      KQDAESL..Q R.....ALTV GWC.VELLQA FFLVLDDI.. MDSSHTRRGQ
S. cerevisiae      .KTVEQLGQE E.YEKVAI.L GWC.IELLQA YFLVADDM.. MDKSITRRGQ
K. lactis          YKSVSELSAE E.YKKVAI.L GWC.IELLQA YFLVADDM.. MDQSITRRGQ
B. stearotherm.    ........KD P...AVGLPV A.CAIEMIHT YSLIHDDLPS MDNDDLRRGK
M. luteus          ..GN...AHD ......GLPF G.IALEMIHT YSLIHDDLPA MDNDDYRRGK
E. coli            VSTN...TLD ....A...P. A.AAVECIHA YSLIHDDLPA MDDDDLRRGL

Consens            ---------- ---------- -----E----- --L--DD--- MD----RRG-
                                          E       L  DD(XX) D    RRG
```

Domini II

```
A. thaliana 1      P.CWFRV.PQ .VGMVAINDG ILLRNHIHRI LKKHFRDK.. .PYYV.DL..
A. thaliana 2      P.CWFRK.PK .VGMIAINDG ILLRNHIHRI LKKHFREM.. .PYYV.DL..
C. anuum           P.CWYKV.EK .VGMIAVNDG ILLRNHISRI LKNHFRPK.. .SYYV.DL..
L. esculentum      P.CWYNL.EK .VGMIAINDG ILLRNHITRI LKKYFRPE.. .SYYV.DL..
P. argentatum 1    P.CWFRL.PK .VGMIAANDG IILRNNVPRI LKKHFRGK.. .PYYV.DL..
P. argentatum 2    P.CWFRL.PK .VGMIAANDG IILRNHVPRI LKKHFRGK.. .PYYV.DL..
A. annua           P.CWFRL.PK .VGMIAANDG ILLRNHVPRI LKKHFRGK.. .PYYV.DL..
H. annuus          P.CWFRL.PK .VGMIAANDG LILRNHVPRI LKKHFRGK.. .PYYV.DL..
L. albus 1         P.CWFRV.PK .VGMIAANDG VLLRNHIPRI LKKHFRGK.. .PYYA.DL..
L. albus 2         P.CWFRV.PK .VGMIAPNDG VVLRNHIPRI LKKHFRGK.. .PYYV.DL..
H. brasiliensis    P.CWFRV.PK .VGLIAANDG ILLRNHIPRI LKKHFRGK.. .AYYV.DL..
G. arboreum        P.CWFRL.PK .VGMIAVNDG VILRNHITRI LKNHFRGK.. .PYYV.DL..
Z. mays            P.CWFRV.PQ .VGLIAANDG IILRNHISRI LRRHFKGK.. .PYYA.DL..
O. sativa          P.CWFRV.PQ .VGLIAVNDG IILRNHISRI LQRHFKGK.. .LYYV.DL..
H. sapiens         T.CWYQK.P. GVGLDAINDA NLLEACIYRL LKLYCREQ.. .PYYL.NL..
R. norvegicus      I.CWYQK.P. GIGLDAINDA LLLEAAIYRL LKFYCREQ.. .PYYL.NL..
S. cerevisiae      P.CWYKV.PE .VGEIAINDA FMLEAAIYKL LKSHFRNE.. .KYYI.DI..
K. lactis          P.CWYKV..E NVGDIAINDA FMLEGAIYCL LKKHFRTE.. .PYYV.DL..
B. stearotherm.    PTNH.KVFGE AMAILA.GDG .LLTYAFQ.L I.TEIDDERI PP.SV....R
M. luteus          LTNH.KRFDE ATAILA.GDA .LLTDAFQCI LNTQLNAE.. ....I....K
E. coli            PTCHVK.FGE ANAILA.GDA .LQTLAFS.I LS...DAD.M .P.EVSDRDR

Consens            ---------- -----A--D- --L------- ---------- ----------
                                 A  D     L
```

Domini III

```
A. thaliana 1      VDLFNEVELQ TAC..... GQMIDLITTF EGE.K..DLA KYSLS.IHRR
A. thaliana 2      VDLFNEVEFQ TAC..... GQMIDLITTF DGE.K..DLS KYSLQ.IHRR
C. anuum           LDLFNEVEFQ TAS..... GQMIDLITTH VGE.K..DLS KYSLP.IHCR
L. esculentum      LDLFNEVEFQ TAS..... GQMIDLITTL VGE.K..DLS KYSLS.IHRR
P. argentatum 1    LDLFNEVEFQ TAS..... GQMIDLITTL VGE.K..DLS KYSLS.IHRR
P. argentatum 2    VDLFNEVEFQ TAS..... GQMIDLITTL VGE.K..DLS KYSLS.IHRR
A. annua           VDLFNEVEFQ TAS..... GQMIDLITTL VGE.K..DLS KYSLS.IHRR
H. annuus          VDLFNEVEFQ TAS..... GQMIDLITTL VGE.K..DLS KYSLS.IHRR
L. albus 1         LDLFNEVEFQ TAS..... GQMIDLITTL EGE.K..DLS KYTLS.LHRR
L. albus 2         LDLFNEVEFQ TAS..... GQMIDLITTL EGE.K..DLS KYTLS.LHRR
H. brasiliensis    LDLFNEVEFQ TAS..... GQMIDLITTL EGE.K..DLS KYTLS.LHRR
G. arboreum        LDLFNEVEFQ TAS..... GQMIDLITTL EGE.K..DLS KYSLQ.QHRR
Z. mays            LDLFNEVEFK TAS..... GQLLDLITTH EGE.K..DLT KYNIT.VHGR
O. sativa          IDLFNEVEFK TAS..... GQLLDLITTH EGE.K..DLT KYNLT.VHRR
H. sapiens         IELFLQSSYQ TEI..... GQTLDLLTAP QGN...VDLV RFTEK.RYKS
R. norvegicus      LELFLQSSYQ TEI..... GQTLDLITAP QGQ...VDLG RYTEK.RYKS
S. cerevisiae      TELFHEVTFQ TEL..... GQLMDLITAP E.D.K.VDLS KFSLK.KHSF
K. lactis          LELFHDVTFQ TEL..... GQLLDLITAP E.D.K.VDLS KFSLE.KHSF
B. stearotherm.    LRLIERLA.. KAAGPEGMVA GQAADM.... EGEGKTLTLS E..LEYIHR.
M. luteus          LSLINLLS.. TASGSNGMVY GQMLDM.... QGEHKTLTLN E..LERIHI.
E. coli            ISMISELA.. SASGIAGMCG GQALDL.... DAEGKHVPLD A..LERIHR.
Consens            ---------- ---------- GQ--DL---- --------L- ----------
                                          GQ  DL
```

Domini IV

```
A. thaliana 1      IVQYKTAYYS FYLPVACALL MAGENL.ENH IDV.K...N. ..VLVD.MGI
A. thaliana 2      IVEYKTAYYS FYLPVACALL MAGENL.ENH TDV.K...T. ..VLVD.MGI
C. anuum           IVQYKTAYYS FYLPVACALL MAGENL.DNH VDV.K...N. ..ILIE.MGI
L. esculentum      IVQYKTAYYS FYLPVACALL MVGENL.DKH VDV.K...K. ..ILID.MGI
P. argentatum 1    IVQYKTAYYS FYLPVACALL MFGEDL.EKH EEV.K...N. ..VLVE.MGT
P. argentatum 2    IVQYKTAYYS FYLPVACALL MFGEDL.EKH VEV.K...N. ..VLVE.MGT
A. annua           IVQYKTAYYS FYLPVACALL MFGEDL.DKH VEV.K...N. ..VLVE.MGT
H. annuus          IVQYKTAYYS FYLPVACALL MFGEDL.DNH VEV.K...N. ..VLVE.MGT
L. albus 1         IVQYKTAYYS FYLPVACALL MVGENL.DNH IDV.K...N. ..ILVD.MGT
L. albus 2         IVQYKTAYYS FYLPVACALL MVGENL.DNH TDV.K...N. ..ILVE.MGT
H. brasiliensis    IVQYKTAYYS FYLPVACALL IAGENL.DNH IVV.K...D. ..ILVQ.MGI
G. arboreum        IVQYKTAYYS FYLPVACALV MCGENL.DNH IDV.K...N. ..ILVD.MGI
Z. mays            IVQYKTAYYS FYLPVACALL LSGENL.DNY GDV.E...N. ..ILVE.MGT
O. sativa          IVQYKTAYYS FYLPVACALL LSGENL.DNF GDV.K...N. ..ILVE.MGT
H. sapiens         IVKYKTAFYS FYLPIAAAMY MAG..I.DG. .E..KEHANA KKILLE.MGE
R. norvegicus      IVKYKTAFYS FYLPIAAAMY MAG..I.DG. .E..KEHANA LKILLE.MGE
S. cerevisiae      IVTFKTAYYS FYLPVALAMY VAG..ITDEK .DL.KQARD. ..VLIP.LGE
K. lactis          IVIFKTAYYS FYLAVALAMF AAG..ITDSK .DL.KQASD. ..VLIP.LGE
B. stearotherm.    ...HKTG..K ..M.LQYS.V HAGA.LIG.G AD.ARQ.TRE LDEFAAHLGL
M. luteus          ...HKTG..E ..L.IRAAIV SAGI.IMNFN .D.A.Q.IEQ LNIIGKNVGL
E. coli            ...HKTG..A ..L.IRAA.V RLGA.L.SAG .DKGRRALPV LDKYAESIGL

Consens            ----KTA/G- ---------- --G------- ---------- --------G-
                       KTA/G                                        G
```

Domini V

```
A. thaliana 1      YFQVQDDYLD CFADPETLGK .IGTDIE.DF KCSW..LVV. KALERCSEEQ
A. thaliana 2      YFQVQDDYLD CFADPETLGK .IGTDIE.DF KCSW..LVV. KALERCSEEQ
C. anuum           YFQVQDDYLD CFADPEVLGK .IGTDIQ.DF KCSW..LVV. KALELCNEEQ
L. esculentum      YFQVQDDYLD CFADPEVLGK .IGTDIQ.DF KCSW..LVV. KALELCNEEQ
P. argentatum 1    YFQVQDDYLD CFGAPEVIGK .IGTDIE.DF KCSW..LVV. KALELSNEEQ
P. argentatum 2    YFQVQDDYLD CFGAPEVIGK .IGTDIE.DF KCSW..LVV. KALELANEEQ
A. annua           YFQVQDDYLD CFGAPEVIGK .IGTDIE.DF KCSW..LVV. KALELPNEEQ
H. annuus          YFQVQDDYLD CFGAPEVIGK .IGTDIE.DF S.SW..LVV. KALELANEEQ
L. albus 1         YFQVQDDYLD CFGAPETIGK .IGTDIE.DF KCSW..LVV. KALELSNDEQ
L. albus 2         YFQVQDDYLD CFGAPETIGK .IGTDIE.DF KCSW..LVV. KALELSNEEQ
H. brasiliensis    YFQVQDDYLD CFGDPETIGK .IGTDIE.DF KCSW..LVV. KALELCNEEQ
G. arboreum        YFQVQDDYLD CFGNPETIGK .IGTDIE.NF KCSW..LVV. KALEFCNEEH
Z. mays            YFQVQDDYLD CYGDPEFIGK .IGTDIE.DY KCSW..LVV. QALERADESQ
O. sativa          YFQVQDDYLD CYGDPEFIGK .IGTDIE.DY KCSW..LVV. QALERADENQ
H. sapiens         FFQIQDDYLD LFGDPSVTGK .IGTDIQ.DN KCSW..LVV. QCLQRATPEQ
R. norvegicus      FFQIQDDYLD LFGDPSVTGK .VGTDIQ.DN KCSW..LVV. QCLLRATPQQ
S. cerevisiae      YFQIQDDYLD CFGTPEQIGK .IGTDIQ.DN KCSW...VIN KALELASAEQ
K. lactis          YFQIQDDFLD CFGKPEDIGK .IGTDIQ.DN KCSW...VIN VALKNATKEQ
B. stearotherm.    AFQIRDDILD IEGAEEKIGK PVGSDQSNN. KATYPALL.. .SL..AG..A
M. luteus          MFQIKDDILD VEGSFENIGK TVGSDLNND. KSTYVSLL.. .GLE.A...S
E. coli            AFQVQDDILD VVGDTATLGK RQGADQQLG. KSTYPALL.. .GLEQA...R

Consens            -FQ--DD-LD --------GK --G-D----- K-S/T------ --L-------
                    FQ  DD  D         GK     D      K S/T
```

Domini VI

```
A. thaliana 1      TKILYENYGK P.DPSN.VA. K.VKDLYK.E LDLE.GVFH. ..EY..ES..
A. thaliana 2      TKILYENYGK A.EPSN.VA. K.VKALYK.E LDLE.GAFH. ..EY..EK..
C. anuum           KKILYENYGK ..DNAACIA. K.IKTLYN.D LKLE.EVFL. ..EY..EKK.
L. esculentum      KKILFENYGK ..DNAACIA. K.IKALYN.D LKLE.EVFL. ..EY..E.K.
P. argentatum 1    KKILHENYGK K.DPSS.VA. K.VKELYH.T LNLQ.GVFE. ..DY..EN..
P. argentatum 2    KKVLHENYGK K.DPSP.VA. K.VKELYN.T LNLQ.GVFE. ..DY..EN..
A. annua           KKTLHENYGK K.DPAS.VA. K.VKEVYH.T LNLQ.AVFE. ..DY..EA..
H. annuus          KKVLHENYGK K.DPSS.VA. K.VKELYN.T LNLQ.GVFE. ..DY..ES..
L. albus 1         KKVLYDNYGK P.DPAN.VA. K.VKALYD.E LNLQ.GVFT. ..EY..ES..
L. albus 2         KKVLYENYGK P.DPAN.VA. K.VKTLYN.E LNLE.GAYA. ..DY..ES..
H. brasiliensis    KKVLYEHYGK A.DPAS.VA. K.VKVLYN.E LKLQ.GVFT. ..EY..EN..
G. arboreum        NKVLYENYGE T.RPAN.VA. K.VKALYN.E LNLK.GVFE. ..DY..ES..
Z. mays            KRILFENYGK K.DPA.CVA. K.VKNLYK.E LDLE.AVFQ. ..EY..EN..
O. sativa          KHILFENYGK P.DPE.CVA. K.VKDLYK.E LNLE.AVFH. ..EY..ES..
H. sapiens         YQILKENYGQ K.EAEK.VA. R.VKALYE.E LDLP.AVFL. ..QY..EED.
R. norvegicus      RQILEENYGQ K.DPEK.VA. R.VKALYE.E LDLR.SVFF. ..KY..EED.
S. cerevisiae      RKTLDENYGK K.D.SVAEA. K.CKKIFN.D LKIE.QLYH. ..EY..EESI
K. lactis          RDILDENYGR K.D.SEKEQ. K.CRAVFN.E LNIQ.DIYH. ..KY..EEET
B. stearotherm.    K....EKLAF HIEA....AQ RHLR...NAD ..VD.GA..A LA.YICELVA
M. luteus          KQLLNDKLT. ..ETYD..AL KTLQPI.N.D .NLK..T..L IT.YIVE...
E. coli            KK.ARD.L.. .ID..D..AR QSLKQL..AE QSLDTSALEA LADYIIQ...

Consens            ---------- ---------- ---------- ---------- ---Y------
```

```
A. thaliana 1      .KSYE.KL.T GAI.E.GHQS KAIQA.V.LK SFLA.KIYKR QK*
A. thaliana 2      .ESYE.KL.T KLI.E.AHQS KAIQA.V.LK SFLA.KIYKR QK*
C. anuum           .T.YE.KLAN S.I.A.AHPS KAVQA.VQL. SFLG.KIYKR QK*
L. esculentum      .TSYE.KLTT S.I.A.AHPS KAVQA.VLL. SFLG.KIYKR QK*
P. argentatum 1    .TSYK.KLIT S.I.E.GHPS KAVQA.V.LK SFLG.KIYKR QK*
P. argentatum 2    .TSYK.KLIT S.I.E.GHPS KAVQA.V.LK SFLG.KIYRR QK*
A. annua           .TSYK.KLIT S.I.E.NHPS KAVQA.V.LK SFLG.KIYKR QK*
H. annuus          .TSYK.KLIT S.I.E.GHPS KAVQA.V.LK SFLG.KIYKR QK*
L. albus 1         .KSYE.KLVT S.I.E.AHPS KAVQA.L.LK SFLG.KIYKR QK*
L. albus 2         .KSYE.KLVT C.I.E.GHPS KAVQG.V.LK SFWA.KIYKR QK*
H. brasiliensis    .ESYK.KLVT S.I.E.AHPS KPVQA.V.LK SFLA.KIYKR QK*
G. arboreum        .KSYE.RLVT S.I.E.AHPS KPVQA.V.LK SFLG.KIYKR QK*
Z. mays            .ESYK.KLI. ADI.E.AQPS IAVQK.V.LK SFLH.KIYKR QK*
O. sativa          .ESYN.KLI. ADI.E.AHPN KAVQN.V.LK SFLH.KIYKR QK*
H. sapiens         ..SYS.HIM. ALIEQYAAP. ..LPPAVFLG ..LARKIYKR RK*
R. norvegicus      ..SYN.RLK. SLIEQCSAP. ..LPPSIFLE ..LANKIYKR RK*
S. cerevisiae      AKDLKAKI.. SQVDE....S RGFKADV.LT AFLN.KVYKR SK*
K. lactis          ASNLREKI.. ANIDE....S RGFKAEV.LT LFLN.KIYHR KK*
B. stearotherm.                                          ARD.H*
M. luteus                                                .RN.K*
E. coli                                                  .RN.K*

Consens            ---------- ---------- ---------- ---------R --
                                                         (+)
                                                    ___________
                                                    Domini VII
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Les seqüències d'aminoàcids són les següents: les 14 seqüències de FPS de plantes presentades en l'Annex I i les seqüències de les FPS de: *Homo sapiens* (Sheares *et al.*, 1989; Wilkin *et al.*, 1990); *Rattus rattus* (Clarke *et al.*, 1987); *Saccharomyces cerevisiae* (Anderson *et al.*, 1989); *Kluyveromyces lactis* (Mulder *et al.*, 1994); *Bacillus stearotermophilus* (Koyama *et al.*, 1993); *Micrococcus luteus* (Shimizu *et al.*, 1998a) i *Escherichia coli* (Fujisaki *et al.*, 1990).

ANNEX III

Publicacions generades a partir d'aquest treball:

- *Arabidopsis thaliana* contains two differentially expressed farnesyl-diphosphate synthase genes.
  Cunillera, N., Arró, M., Delourme, D., Karst, F., Boronat, A. i Ferrer, A.
  J. Biol. Chem. **271**, 7774-7780 (1996).

- The *Arabidopsis thaliana FPS1* gene generates a novel mRNA that encodes a mitochondrial farnesyl-diphosphate synthase isoform.
  Cunillera, N., Boronat, A. i Ferrer, A.
  J. Biol. Chem. **272**, 15381-15388 (1997).

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

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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* and *FPS2* have a different pattern of expression. 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 deffective 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 adaptative 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),[1] to a series of prenyl diphosphates of increasing size. These polyprenyl 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 (FPP) 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 *a* (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-

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EMBL Data Bank with accession number(s) L46349, L46350, and L46367.

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[1] The abbreviations used are: IPP, isopentenyl diphosphate; FPS, farnesyl-diphosphate synthase; FPP, farnesyl diphosphate; DMAPP, dimethylallyl diphosphate; GPP, geranyl diphosphate; PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; bp, base pair(s).

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-(C_{20}), hexaprenyl-(C_{30}), and heptaprenyl-(C_{35}) diphosphate synthases (20, 21). Two of these regions are the aspartate-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-^{32}P]dCTP (3000 Ci/mmol), [^{35}S]Met (1000 Ci/mmol), and [^{14}C]IPP (58.4 mCi/mmol) were obtained from Amersham. Amino acids, ergosterol, geraniol, geranylgeraniol, farnesol, and Tergitol Nonidet P-40 were from Sigma. Yeast extract, bactopeptone, bactotryptone, and yeast nitrogen base without amino acids and $(NH_4)_2SO_4$ 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 (24). Axenic cultures were prepared by surface-sterilizing seeds in 5% (v/v) sodium hypochlorite and germination on Petri dishes containing mineral medium supplemented with 1% (w/v) sucrose and 0.8% (w/v) agar. Roots were obtained from 3-week-old plants grown on filter papers (mineral medium supplemented with 1% (w/v) sucrose and 2% (w/v) agar).

Strains, Media and Plasmids—Saccharomyces cerevisiae strains used in this work derived from the wild type strain FL100 (ATCC28383, MAT a). The following yeast strains were used: CC25 (MAT a, *erg12–2, erg20–2, ura3–1, trp1–1*) (25), LB311 (*erg20::URA3/ERG20, ura3–1/ura3–1, trp1–1/trp1–1*) (26), and NC1 (MAT a, *erg20::URA3, ura3–1, trp1–1* [pNCFPS2]) (this study). The strain NC1 is a haploid Ura$^+$, Trp$^+$ segregant, isolated from diploid strain LB311 transformed by plasmid pNCFPS2 carrying the *Arabidopsis* FPS2 cDNA and the selectable marker *TRP1*. *Escherichia coli* strain XL1-Blue (F′(*proAB lacI*^{q}ZΔ M15, *Tn*10 (tet^r)) *recA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac*) (Stratagene) was used for cloning, maintenance, and propagation of plasmids.

Yeast strains were grown in YPD medium (1% (w/v) yeast extract, 2% (w/v) bactopeptone, and 2% (w/v) glucose) or minimal medium (0.16% (w/v) yeast nitrogen base without amino acids and $(NH_4)_2SO_4$, 0.5% (w/v) $(NH_4)_2SO_4$, and 1% (w/v) glucose). Unless otherwise stated, yeast cells were grown at 28 °C either in liquid culture or on agar plates (media supplemented with 15 g of agar per liter). When required to supplement auxotrophies, uracil (50 μg/ml), tryptophan (50 μg/ml), or ergosterol (4 μg/ml in liquid culture or 80 μg/ml in agar plates) were added to the growth media. Ergosterol was supplied by dilution of a stock solution (4 mg/ml) in a mixture of Tergitol Nonidet P-40, ethanol (1:1). *E. coli* cells were grown in LB medium (1% (w/v) bactotryptone, 0.5% (w/v) yeast extract, and 5% (w/v) NaCl) with tetracycline (15 μg/ml) and with or without ampicillin (100 μg/ml).

Plasmid pNCFPS2 contains the FPS2 cDNA under the control of the strong yeast phosphoglycerate kinase gene (*PGK*) promoter. To construct pNCFPS2, a *Sac*II-*Sac*I fragment from plasmid pcNC2 (see below) was blunt ended with the Klenow fragment of deoxyribonuclease I and cloned into pDD62, cleaved with *Not*I, and blunt ended with the Klenow fragment of deoxyribonuclease I in the presence of deoxynucleotides. The transcription polarity of the insert was examined by restriction analysis. Plasmid pDD62 was derived from plasmid pFL61 (27), and contains the selectable marker *TRP1* instead of *URA3* in the *Bgl*II site. The yeast strains were transformed by the lithium acetate procedure (28).

Isolation of FPS Genomic Clones—2×10^4 recombinant phages of an *Arabidopsis* λEMBL4 genomic library, obtained from Dr. A. Bachmair (Max-Planck Institut für Züchtungsforschung, Köln, Germany), were screened using as a probe a 730-bp *Eco*RI-*Pst*I cDNA fragment from the recombinant clone pDD71, which contains the *Arabidopsis* FPS1 cDNA (17). The probe was ^{32}P-labeled by random priming (29) with [α-^{32}P]dCTP using the Random Primers DNA labeling kit (Boehringer Mannheim). Hybridization of replica filters was for 18 h at 65 °C in 6 × SSC (1 × SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0), 2 × Denhardt's, and 100 μg/ml denatured salmon sperm DNA. Nitrocellulose filters (Millipore) were washed at 45 °C twice in 2 × SSC, 0.1% SDS and twice in 0.2 × SSC, 0.1% SDS. Eleven positive recombinant clones were identified and plaque-purified. The phage DNA of selected clones was isolated and cleaved either with *Eco*RI or *Hin*dIII. The DNA fragments hybridizing to the cDNA probe were subcloned into the appropriate restriction sites of pBluescript (Stratagene) prior to sequencing.

DNA Sequencing—Appropriate restriction fragments were subcloned into pBluescript or pUC19. Both strands of DNA were sequenced by the dideoxynucleotide chain-termination method (30) using an automated fluorescence-based system (Applied Biosystems).

Isolation and Analysis of Nucleic Acids—Genomic DNA from 6-day-old dark-grown *Arabidopsis* seedlings was prepared as described (31). Genomic DNA (8 μg) was digested with the indicated restriction enzymes, size-fractionated by electrophoresis in 0.8% (w/v) agarose gels, and blotted to Hybond-C nitrocellulose membranes (Amersham). Hybridization with the indicated ^{32}P-labeled probes was for 18 h either at 65 °C (high stringency) or at 58 °C (low stringency) in 0.7 M sodium chloride, 40 mM sodium phosphate, pH 7.6, 4 mM EDTA, 0.1% (w/v) SDS, 0.2% (w/v) polyvinylpyrrolidone, 0.2% (w/v) Ficoll, 9% (w/v) dextran sulfate, and 200 μg/ml denatured salmon sperm DNA. High stringency washes were performed at 65 °C twice in 1 × SSC, 0.5% SDS, and twice in 0.2 × SSC, 0.5% SDS. Low stringency washes were done twice in 2 × SSC, 0.5% SDS at 58 °C.

Total RNA from different tissues of *Arabidopsis* was isolated (32), and poly(A)$^+$ RNA was obtained by oligo(dT)-cellulose according to the manufacturer's recommendations (Amresco). For Northern analysis, 30 μg of *Arabidopsis* total RNA from each sample was fractionated by electrophoresis in 1% (w/v) agarose gels containing 2.2 M formaldehyde and blotted to Hybond-N nylon membranes (Amersham). Hybridization with the indicated ^{32}P-labeled probes was for 18 h at 42 °C in 50% (v/v) formamide, 1 M NaCl, 50 mM sodium phosphate, pH 6.5, 7.5 × Denhardt's, 1% SDS, 10% (w/v) dextran sulfate, and 500 μg/ml denatured salmon sperm DNA. Filters were washed twice at room temperature in 2 × SSC, 0.1% SDS and at 40 °C twice in 1 × SSC, 0.1% SDS, once in 0.1 × SSC, 0.1% SDS, and once in 0.1 × SSC. To ascertain that equivalent amounts of RNA were present in each lane, filters were reprobed with a ^{32}P-labeled 900-bp *Bam*HI-*Eco*RI fragment of the gene for the 25 S cytoplasmic rRNA. The probe used was obtained from plasmid pTA250 which contains a wheat rRNA gene repeating unit (33).

Cloning of FPS2 cDNA—The cDNA encoding FPS2 was cloned using a reverse transcription-polymerase chain reaction (PCR) strategy devised for the 3′-end amplification of cDNAs (34). The reverse transcriptase reaction was carried out in a 20-μl reaction mixture containing 5 μg of poly(A)$^+$ RNA from *Arabidopsis* inflorescences, 45 pmol of adaptor-$(dT)_{17}$ primer (5′-GACTCGAGTCGACATCGGGTTTTTTTTTT-TTTTTTTTT-3′), 10 mM dithiothreitol, 1.5 mM each dATP, dCTP, dGTP, and dTTP, 10 units of RNasin (Promega), first strand synthesis buffer (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-TGGCTTTGCAC-3′, nucleotides −41 to −20 in Fig. 4), and the adaptor oligonucleotide as a downstream primer (5′-GACTCGAGTCGA-CATCGGG-3′), 1.5 mM $MgCl_2$, 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 3 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 pcNC2.

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′-Amplifinder 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′-CCTGTGGATATGATTGCGAAG-3′) complementary to the nucleotide sequence +373 to +393 in the *Arabidopsis* FPS2 cDNA (Fig. 4). An anchor oligonucleotide (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 oligonucleotide and a reverse nested FPS2-specific primer (5′-GGCTTTCTAAACCAACAAGGCTGG-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 *Eco*RI, and cloned into the corresponding site of plasmid pUC19 prior to sequencing.

In Vitro Transcription / Translation—A *Sac*II-*Sal*I fragment of plasmid pcNC2, containing the FPS2 cDNA, was cloned into the corresponding sites of pBluescript. The resulting plasmid was named pcBNC2. The FPS2 cDNA was cut out as a *Sac*I-*Sal*I fragment from plasmid pcBNC2 and cloned into the corresponding sites of plasmid pSP65 (Promega). The resulting plasmid, pcSPNC2, was used as a template for *in vitro* transcription/translation using [^{35}S]Met and the TNT™ Coupled Wheat Germ Extract System (Promega), according to the manufacturer. The ^{35}S-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 presence of 10 μM dimethylaminoethyl diphosphate (35) to inhibit the yeast IPP isomerase activity. The reaction mixture (100 μl), containing 60 μM DMAPP, 11 μM [^{14}C]IPP, 1 mM $MgCl_2$, and the 105,000 × *g* supernatant (100 μg of protein), was incubated at 37 °C for 15 min and rapidly ice-chilled. After the addition of 100 μl of 0.15 M Tris glycine, pH 10.5, the reaction products were enzymatically dephosphorylated by incubation at 37 °C for 30 min in the presence of 0.2 units of calf alkaline phosphatase. The sample was then diluted in 0.6 ml of water and the reaction products were extracted with 1 ml of hexane. The hexane extract was concentrated after addition of geraniol, farnesol, and geranylgeraniol (100 ng each) as carriers, and the reaction products were separated on HPTLC RP-18 plates (Merck), using a mixture of methanol/water (95:5) as solvent. The position of the prenyl alcohols was visualized using iodine vapor. The radioactivity was detected only in the geraniol and farnesol fractions, and was quantified using an Automatic TLC linear analyzer Berthold LB2832.

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 *Not*I-*Hin*dIII cDNA fragment from the recombinant clone pDD71, which contains the *Arabidopsis* FPS cDNA previously isolated (17), and herein referred to as FPS1 cDNA. The simple pattern of bands obtained under high stringency hybridization conditions (Fig. 1*A*) 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. 1*C*). 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.

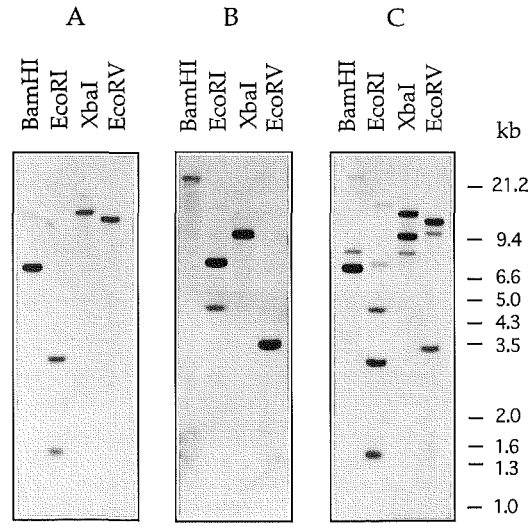

FIG. 1. **Southern blot analysis of *Arabidopsis* FPS genes.** Genomic DNA from *Arabidopsis* (8 μg) was digested with the restriction enzymes indicated at the top, electrophoresed and transferred onto nitrocellulose membranes. Filters were hybridized with a 340-bp *Not*I-*Hin*dIII cDNA fragment from plasmid pDD71, which contains the cDNA encoding the *Arabidopsis* FPS1 isoform (17), under conditions of high (*A*) and low stringency (*C*), or a 800-bp *Xho*I-*Hin*dIII fragment from the *FPS2* gene, shown in Fig. 2, under conditions of high stringency (*B*). *Numbers* on the *right* indicate the mobility of DNA size standards.

To clone the *Arabidopsis* FPS genes, a 730-bp *Eco*RI-*Pst*I 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 λgNC10 and λgNC24 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. 2*A*) 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. 2*A*) 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 *Xho*I-*Hin*dIII fragment from the *FPS2* gene (Fig. 2), revealed a simple pattern of bands (Fig. 1*B*) which accounted for a subset of genomic fragments previously detected at low stringency by the FPS1 probe (Fig. 1*C*). 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. 1*C*). 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-

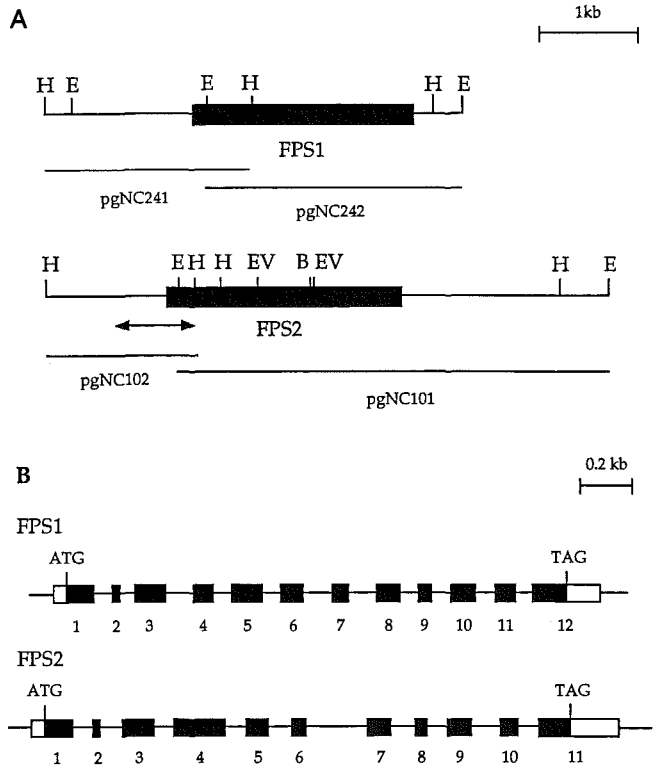

FIG. 2. **Restriction and structural maps of *Arabidopsis FPS1* and *FPS2* genomic clones.** *A*, restriction map of the genomic regions containing the *FPS1* and *FPS2* genes. *FPS1* and *FPS2* transcription units are represented by *solid boxes*. The cloned regions contained in recombinant plasmids are indicated below the restriction maps. The 800-bp *Xho*I-*Hin*dIII probe from pgNC102 used in genomic Southern blot analysis is indicated by a *double arrowhead line*. Restriction sites are as follows: *B*, *Bam*HI; *E*, *Eco*RI; *EV*, *Eco*RV; *H*, *Hin*dIII. *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*.

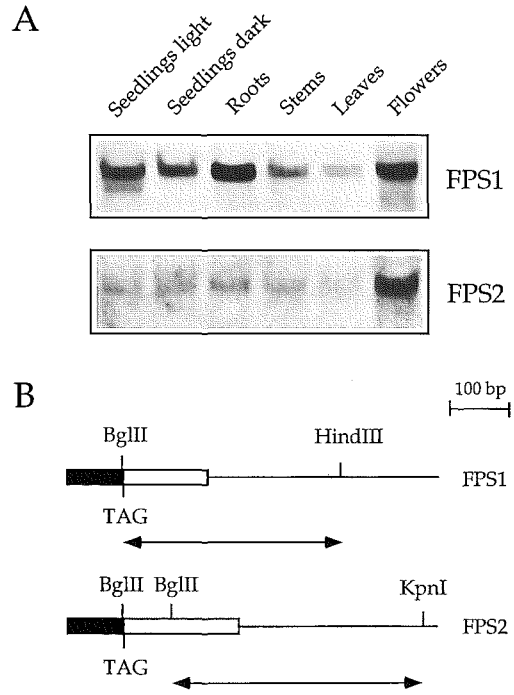

FIG. 3. **Northern blot analysis of *Arabidopsis* FPS1 and FPS2 mRNA.** *A*, total RNA samples from different tissues of *Arabidopsis* (30 μg/lane) was electrophoresed in 1% agarose-formaldehyde gels and transferred onto nylon membranes. Filters were hybridized with the *FPS1* and *FPS2* gene-specific 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* (370-bp *Bgl*II-*Hin*dIII fragment) and *FPS2* (450-bp *Bgl*II-*Kpn*I fragment) gene-specific probes are indicated by *double arrowhead lines*.

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

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. 2*B*). 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. 2*B*). 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. 3*A*). 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 exons of the *FPS2* gene. Analysis of the cDNA sequence indicated the presence of an open reading frame of 1029 nucleotides encoding a protein of 342 amino acid residues (Fig. 4) with a predicted molecular mass of 39,825 Da. The 5′-proximal ATG triplet has been assumed to be the start codon since according to the "first-AUG-rule" it serves as the initiator codon to be used in the translation of about 95% of the eukaryotic mRNAs (37). This assignment is supported by the observation that the nucleotide sequences surrounding the translation start triplet

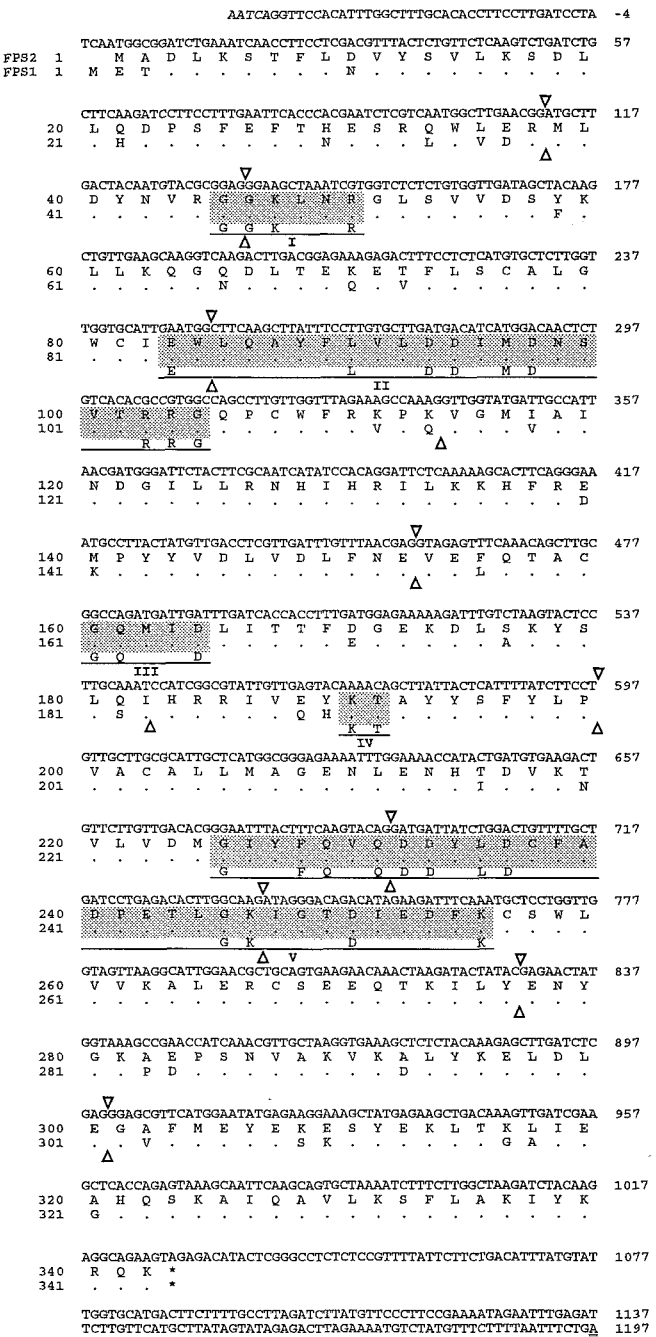

FIG. 4. **Nucleotide sequence of the *Arabidopsis* FPS2 cDNA and amino acid alignment of *Arabidopsis* FPS1 and FPS2.** Nucleotides are numbered (right) by assigning position +1 to the first base of the ATG codon. The 5′-end sequence obtained from the RACE clones is shown in *italic*. A putative polyadenylation signal is *double underlined*. Stop codons are denoted by an *asterisk*. Amino acid positions are indicated on the left. Identical residues are represented by *dots*. The five regions (I to V) that are present in many prenyltransferases are *shaded* and the amino acid residues within these regions that are present in all the FPS known so far are shown below. Intron positions are indicated by *open triangles*.

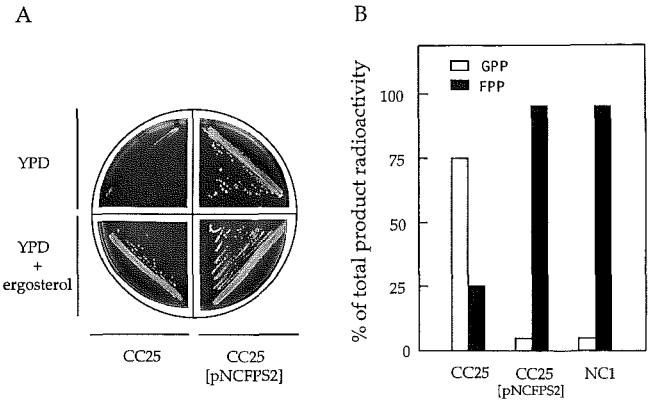

FIG. 5. **Confirmation of the FPS activity of the *Arabidopsis* FPS2 isoform.** *A*, functional complementation of the mutant yeast strain CC25 with plasmid pNCFPS2. Strain CC25 and strain CC25[pNCFPS2] were streaked onto YPD plates or YPD plates supplemented with 80 μg/ml ergosterol and incubated at 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 [^{14}C]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 of 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%.

(ATCAATGGC) fit the consensus reported for functional start codons in plants (AACAATGGC) (38), except that a T is found at position −3 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 consensus polyadenylation 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 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. 5*A*, 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. 5*B*). In contrast, strain CC25 synthesized GPP

as the major product (Fig. 5*B*).

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. 5*B*). 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 C_5 or C_{10} allylic primers. Taken together, these results unequivocally demonstrate that the *Arabidopsis* FPS2 cDNA encodes a functional FPS isoform which is able to catalyze the two successive condensations of IPP with both DMAPP and GPP leading to FPP formation.

DISCUSSION

The multibranched 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 synthetizes 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 mutant 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 C_{10} to C_{15} 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 FPS 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 *Hyosciamus 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-*

dopsis, 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 isoform in the isoprenoid biosynthetic pathway. The differential expression of *FPS1* and *FPS2* might be indicative of an specialized function of each FPS isoform in directing the flux of pathway intermediates into specific isoprenoid end products. This assumption is consistent with the recent hypothesis proposing that specific classes of isoprenoids are synthesized by discrete metabolic channels within the pathway, through the formation of multienzyme complexes (metabolons), which are independently regulated (49, 50). The results presented in this paper lend further support to the view that plant isoprenoid biosynthesis is a complex metabolic pathway which is regulated by sophisticated control mechanisms. We are currently applying different molecular and cellular approaches to identify the specific function of each FPS isoform in the organization of the plant isoprenoid pathway.

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The *Arabidopsis thaliana FPS1* Gene Generates a Novel mRNA That Encodes a Mitochondrial Farnesyl-diphosphate Synthase Isoform*

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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 from isopentenyl diphosphate and dimethylallyl diphosphate. FPS is considered to play a key role in isoprenoid biosynthesis. We have reported previously that *Arabidopsis thaliana* contains two differentially expressed genes, *FPS1* and *FPS2*, encoding two highly similar FPS isoforms, FPS1 and FPS2, (Cunillera, N., Arró, M., Delourme, D., Karst, F., Boronat, A., and Ferrer, A. (1996) *J. Biol. Chem.* 271, 7774–7780). In this paper we report the characterization of a novel *Arabidopsis* FPS mRNA (FPS1L mRNA) derived from the *FPS1* gene. A cDNA corresponding to the FPS1L mRNA was cloned using a reverse transcription-polymerase chain reaction strategy. Northern blot analysis showed that the two *FPS1*-derived mRNAs are differentially expressed. The FPS1L mRNA accumulates preferentially in inflorescences, whereas the previously reported FPS1 mRNA (FPS1S mRNA) is predominantly expressed in roots and inflorescences. FPS1L mRNA contains an in-frame AUG start codon located 123 nucleotides upstream of the AUG codon used in the translation of the FPS1S isoform. Translation of the FPS1L mRNA from the upstream AUG codon generates a novel FPS1 isoform (FPS1L) with an NH_2-terminal extension of 41 amino acid residues, which has all the characteristics of a mitochondrial transit peptide. The functionality of the FPS1L NH_2-terminal extension as a mitochondrial transit peptide was demonstrated by its ability to direct a passenger protein to yeast mitochondria *in vivo* and by *in vitro* import experiments using purified plant mitochondria. The *Arabidopsis* FPS1L isoform is the first FPS reported to contain a mitochondrial transit peptide.

The enzyme farnesyl-diphosphate synthase (FPS[1]; EC 2.5.1.1/EC 2.5.1.10) catalyzes the sequential 1′–4 condensation of two molecules of isopentenyl diphosphate with both dimethylallyl diphosphate and the resultant 10-carbon compound geranyl diphosphate to produce the 15-carbon compound farnesyl diphosphate (1). Because of the central branch point location of farnesyl diphosphate in the isoprenoid pathway, FPS is considered to play a key role in isoprenoid biosynthesis. It has been shown that in mammals FPS is a highly regulated enzyme involved in the control of the sterol biosynthetic pathway (2–5). FPS is also considered to play an important role in the control of plant isoprenoid biosynthesis. In plants, farnesyl diphosphate is the starting point of different branches of the isoprenoid pathway leading to the synthesis of key end products that are required for normal growth and development, such as phytosterols, dolichols, ubiquinone, plastoquinone, sesquiterpenoid phytoalexins, and prenylated proteins. Therefore, changes in FPS activity could alter the flux of isoprenoid compounds down the different branches of the pathway in competition for the available farnesyl diphosphate and, hence, play a central role in the regulation of a number of essential functions in plant cells (6). However, more experimental data are still required before obtaining a clear picture of the regulatory significance of FPS in the overall control of isoprenoid biosynthesis in plants.

Genomic and cDNA sequences encoding FPS have been isolated and characterized from a variety of organisms, ranging from bacteria to higher eukaryotes (7, 8). Recently, the enzyme has been cloned from several plant species such as *Arabidopsis thaliana* (9, 10), *Lupinus albus* (11), *Zea mays* (12), *Artemisia annua* (13), *Hevea brasiliensis* (14), and *Parthenium argentatum* (15). Although the complexity of the FPS gene families has been studied in a limited number of plants, it seems that plant FPS is encoded by multigene families like other key enzymes of the isoprenoid biosynthetic pathway, such as 3-hydroxy-3-methylglutaryl-CoA reductase (16–18), and geranylgeranyl-diphosphate synthase (19). We have recently shown that *Arabidopsis* contains a small FPS gene family consisting of at least two genes, *FPS1* and *FPS2*, which have a very similar structure and share a high level of sequence similarity (10). At least two FPS gene copies have been detected in the maize genome (12), and cDNA sequences encoding two highly similar FPS isoforms have also been reported in *L. albus* (11) and *P. argentatum* (15). The pattern of expression of individual genes encoding FPS has been reported only in *Arabidopsis*, where it has been shown that the two currently characterized FPS genes (*FPS1* and *FPS2*) are expressed differentially at both quantitative and qualitative levels (10). At present, the biological significance of the occurrence of highly similar FPS isoforms in plants is still unclear, although the differential pattern of expression of the two FPS *Arabidopsis* genes suggests that each FPS isoform might have a specialized function in directing the flux of pathway intermediates into specific classes of isoprenoid end products.

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) U80605.

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[1] The abbreviations used are: FPS, farnesyl-diphosphate synthase; PCR, polymerase chain reaction; bp, base pair(s).

FPS has long been considered to be a cytoplasmic enzyme. However, it has recently been demonstrated that in mammals FPS is mainly localized within the peroxisomes (20), although significant levels of FPS activity have also been detected in rat liver mitochondria (21). In plants, the only cell compartment where plant FPS has been detected is the cytosol (6, 22, 23), although it is widely accepted that the enzymes utilizing isopentenyl diphosphate are distributed in three subcellular compartments, namely cytosol, mitochondria, and plastids. All of the FPS reported to date show high amino acid sequence similarity, and all contain several conserved domains, including the two aspartate-rich domains involved in substrate binding and enzyme catalysis (24, 25). However, none of the FPS characterized so far from a number of eukaryotic organisms (including fungi, plants, and animals) contains NH_2-terminal sequences that could represent transit peptides for targeting into plastids or mitochondria. In this paper we report that the expression of the *Arabidopsis FPS1* gene generates a novel mRNA that encodes a mitochondrial FPS isoform.

EXPERIMENTAL PROCEDURES

Enzymes and Biochemicals—Restriction endonucleases and DNA-modifying enzymes were purchased from Boehringer Mannheim and Promega. [α-^{32}P]dCTP (3,000 Ci/mmol), [α-^{32}P]rUTP (3,000 Ci/mmol) and [^{35}S]Met (1,000 Ci/mmol) were obtained from Amersham. Amino acids, glucose, ammonium sulfate, ampicillin, and adenine were from Sigma. Yeast extract, Bacto-peptone, Bacto-tryptone, and yeast nitrogen base without amino acids and ammonium sulfate were from Difco Laboratories. All other chemicals were of the highest commercial grade available.

Plant Material and Strains—*A. thaliana* plants (ecotype Columbia) were grown as described previously (10). *Escherichia coli* strain XL1-Blue (F′(*proAB lacI*^{q}ZD M15, Tn10 (tet^r)) *recA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac*) (Stratagene) was used for cloning, maintenance, and propagation of plasmids. *S. cerevisiae* strain WSR (Mat α, *his3–11, leu2–3, 112, ade2–1, ura3–1, trp1–1, can1–100*, Δ*COXIV::LEU2*) was used for tests of complementation of respiratory deficiency.

Cloning of Arabidopsis FPS1L cDNA—The cDNA encoding FPS1L was cloned by means of a reverse transcription-polymerase chain reaction (PCR) strategy similar to that used previously for the cloning of the *Arabidopsis* FPS2 cDNA (10). The cDNA was amplified from a single-stranded cDNA pool, obtained by reverse transcription of 5 μg of poly(A)$^+$ RNA from *Arabidopsis* inflorescences, using an upstream primer specific for the leader region of the FPS1L mRNA (5′-GGCGTTTTCGGGAGAAGAAGG-3′, nucleotides −97 to −77 in Fig. 1*A*) and an adaptor oligonucleotide (5′-GACTCGAGTCGACATCGGG-3′) as a downstream primer. The resulting PCR product (approximately 1.4 kilobases) was gel purified and ligated into plasmid pGEM-T (Promega) prior to sequencing. The resulting plasmid was designated pcNC3.

DNA Sequencing—Appropriate restriction fragments were subcloned into plasmid pBluescript. Both strands of DNA were sequenced by the dideoxynucleotide chain termination method (26) using an automated fluorescence-based system (Applied Biosystems).

RNase Protection Analysis—To obtain the desired RNase protection probe a DNA fragment extending from nucleotide positions −201 to +141 in the *FPS1* gene was generated by PCR. The amplified fragment was cloned into the *Eco*RI and *Hin*dIII sites of plasmid pSP65 (Promega). To rule out the existence of PCR artifacts, the cloned fragment was sequenced. The resulting plasmid was linearized by digestion with *Hin*dIII and used as a template for *in vitro* transcription using SP6 RNA polymerase (Promega) and [α-^{32}P]rUTP. The 351-nucleotide RNA antisense probe contained 342 nucleotides corresponding to the *FPS1* gene and 9 additional nucleotides derived from plasmid pSP65. RNase protection experiments were performed as described (27), except that the RNA probe was purified on a 5% polyacrylamide, 8 M urea gel before use. The probe was eluted by diffusion at room temperature in 600 μl of 0.5 M ammonium acetate, 1 mM EDTA, and 0.1% SDS. After precipitation, the antisense RNA probe (2.5×10^5 cpm) was hybridized overnight at 42 °C with 8 μg of poly(A)$^+$ RNA from *Arabidopsis* inflorescences or yeast tRNA. Digestion was performed for 2 h with 15 units of RNase ONE (Promega) according to the manufacturer's recommendations. Analysis of the protected fragments was performed by electrophoresis on a 5% polyacrylamide, 8 M urea gel. The gel was dried and exposed to x-ray film. Variation of the quantity of RNA or the digestion conditions did not alter the pattern of protected bands. A known DNA sequencing reaction was included as a marker. The size of the RNA protected fragments was initially calculated according to the sequencing reaction and corrected as described (27).

Northern Blot Analysis—Total RNA from different tissues of *Arabidopsis* was isolated as described (28). Thirty μg of RNA from each sample was fractionated by electrophoresis in 1% (w/v) agarose gels containing 2.2 M formaldehyde and blotted to Hybond-N nylon membranes (Amersham). The ^{32}P-labeled (random primed) probes used were a PCR amplification fragment of 289 bp extending from nucleotides −309 to −20 in the *FPS1* gene, and a 370-bp *Bgl*II-*Hin*dIII fragment from the 3′-flanking region of the *FPS1* gene (10). The conditions of hybridization and washing of the filters were as described previously (10). Filters were reprobed with a ^{32}P-labeled 900-bp *Bam*HI-*Eco*RI fragment of the gene for the wheat 25 S cytoplasmic rRNA (29).

In Vitro Transcription/Translation—A *Sac*II-*Sal*I fragment, containing the FPS1L cDNA, was excised from plasmid pcNC3 and cloned into the corresponding sites of plasmid pBluescript to create plasmid pcBNC3. By using site-directed mutagenesis (30), the ATG start codon of the FPS1S isoform was converted to an ATC codon (encoding Ile). The resulting plasmid was designated pcBNC3Mut. The two cDNA sequences, FPS1L and FPS1LMut, were cut out as *Sac*I-*Sal*I fragments from plasmids pcBNC3 and pcBNC3Mut, respectively, and cloned into the corresponding sites of plasmid pSP65 (Promega). The resulting plasmids, pcSPNC3 and pcSPNC3Mut, were used as templates for *in vitro* transcription/translation using [^{35}S]Met and the TNT™ Coupled Wheat Germ Extract System (Promega). The ^{35}S-labeled proteins were separated by SDS-polyacrylamide gel electrophoresis (12% acrylamide) and detected by fluorography.

Functional Complementation in Yeast—To construct plasmid pFPS1Ltp-YΔCOX, a cDNA fragment encoding the 41 NH_2-terminal amino acid residues of the FPS1L isoform was amplified by PCR using a forward primer (5′-GG*GAATTC*AAAAATG*TC*TGTGAGTTGTT-GTTGTAGG-3′) extending from nucleotide positions −74 to −47 in the *Arabidopsis FPS1* gene (Fig. 1*A*), a reverse primer (5′-AGC*TCTAGA*T-GAAGAGCTTTGGATACG-3′) complementary to the nucleotide sequence +36 to +53 in the *FPS1* gene (Fig. 1*A*), and plasmid pcSPNC3 (see above) as a template. *Eco*RI and *Xba*I sites (shown in italic) were added to the 5′-end of the primers, respectively. The 144-bp PCR product was digested with *Eco*RI and *Xba*I and cloned into the corresponding sites of plasmid pYΔCOX (kindly provided by Ian D. Small; Institut National de la Recherche Agronomique, Versailles, France), which contains the presequence-less yeast *COXIV* gene under the control of the alcohol dehydrogenase gene promoter (31). To optimize the synthesis of the chimeric FPS1Ltp-YΔCOX protein in yeast, four changes (underlined) were introduced in the sequence of the forward primer to convert the nucleotide sequences surrounding the ATG start codon of the *Arabidopsis* FPS1L cDNA (GAA*TATG*AG) into the consensus reported for functional translational start codons in yeast (AAAA*ATG*TC) (32). Changes in the second triplet of the *Arabidopsis* FPS1L cDNA coding sequence did not alter the amino acid residue encoded (Ser). Yeast strain WSR was transformed with plasmids pYCOX, pYΔCOX, or pFPS1Ltp-YΔCOX using the modified lithium acetate procedure described by Gietz *et al.* (33). Ura$^+$ transformants were selected at 28 °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 1% (w/v) glucose) supplemented with histidine (20 μg/ml), tryptophan (40 μg/ml), and adenine (40 μg/ml). After 4 days of growth, selected colonies were subcultured on N3 medium (1% (w/v) yeast extract, 1% (w/v) Bacto-peptone, 2% (v/v) glycerol, and 50 mM potassium phosphate (pH 6.25)) to test for complementation of respiratory deficiency.

Import into Purified Potato Mitochondria—Mitochondria were isolated from potato tubers (*Solanum tuberosum* var. Bintje) as described (34). For *in vitro* import studies the purified mitochondria were washed and resuspended in a buffer containing 400 mM mannitol, 10 mM potassium phosphate (pH 7.2), and 0.1% (w/v) bovine serum albumin. The FPS1L protein was synthesized by *in vitro* transcription/translation of plasmid pcSPNC3 using [^{35}S]Met and the TNT™ Coupled Reticulocyte Lysate System. The import reaction contained 35 μl of purified mitochondria (10 mg of protein/ml), 160 μl of import buffer (250 mM mannitol, 20 mM HEPES (pH 7.5), 80 mM KCl, 1 mM K_2HPO_4, 1 mM ATP, 1 mM malate, 2 mM NADH, and 1 mM dithiothreitol), and 15 μl of the reticulocyte lysate translation mixture. After incubation for 30 min at 20 °C, 70-μl aliquots of the import reaction were treated with proteinase K (20 μg/ml), either in the presence or absence of 0.5% (v/v) Triton X-100. These aliquots and the remainder of the import reaction were then incubated for 20 min at 20 °C. After the addition of 1 mM phenyl-

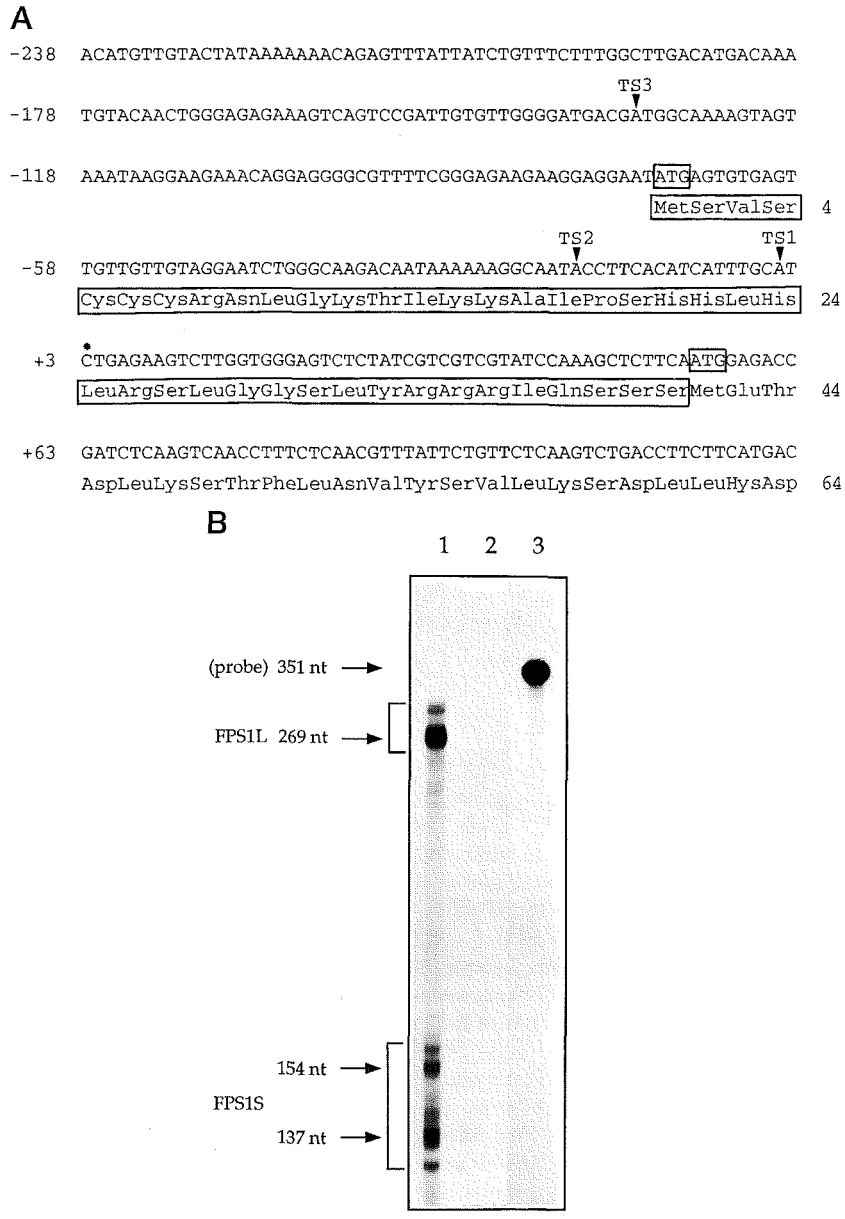


FIG. 1. **Analysis of the 5′-region of the *FPS1* gene.** *Panel A*, nucleotide sequence of the 5′-region of the *FPS1* gene and deduced amino acid sequence of the FPS1L NH_2-terminal region. The transcription start sites of the FPS1S (TS1 and TS2) and the FPS1L (TS3) mRNAs are denoted by *arrowheads*. Nucleotides are numbered (*left*) by assigning position +1 to the most internal transcription start site (TS1) of the *FPS1* gene. The 5′-end of the FPS1S cDNA is indicated by an *asterisk*. ATG start codons are *boxed*. The deduced amino acid sequence (numbered on the *right*) is shown below the nucleotide sequence. The amino acid sequence corresponding to the FPS1L NH_2-terminal extension is *boxed*. *Panel B*, autoradiography of the labeled antisense RNA probe and the protected fragments. *Lane 1*, poly(A)$^+$ RNA from *Arabidopsis* inflorescences; *lane 2*, control yeast tRNA; *lane 3*, undigested probe. The size of the undigested probe and the protected fragments is indicated on the *left* in nucleotides (*nt*).

methylsulfonyl fluoride, samples were incubated for further 15 min. For inhibition of mitochondrial import, purified mitochondria were incubated for 5 min in the presence of 1 μM valinomycin prior to the import reaction. After the different treatments, mitochondria were repurified by centrifugation through a 25% (w/v) sucrose cushion. The resulting pellets were subjected to SDS-polyacrylamide gel electrophoresis (10% acrylamide), and the radiolabeled products were detected by fluorography.

RESULTS

Identification and Cloning of a Novel FPS mRNA Derived from the Arabidopsis FPS1 Gene—We have recently reported that the expression of the *Arabidopsis FPS1* gene generates an mRNA of approximately 1.3 kilobases which encodes the isoform FPS1 (10). A cDNA encoding this isoform had previously been cloned by functional complementation of a mutant yeast strain defective in FPS activity (9). A detailed analysis of the nucleotide sequence of the 5′-flanking region of the *FPS1* gene (Fig. 1A) revealed the presence of an in-frame ATG codon located 123 bp upstream of the ATG start codon used in the translation of the reported FPS1 isoform (9). The utilization of the upstream ATG codon as a translation start site would generate a different FPS1 isoform containing an NH_2-terminal extension of 41 amino acids with respect to the previously described FPS1 isoform.

The occurrence of an mRNA containing the upstream ATG was first detected by using a reverse transcription-PCR strategy (data not shown). To confirm the existence of this novel FPS1 mRNA we isolated the corresponding cDNA by reverse transcription-PCR using poly(A)$^+$ RNA from *Arabidopsis* inflorescences (for details, see "Experimental Procedures"). A cDNA fragment of approximately 1.4 kilobases was cloned and sequenced. The cDNA insert was found to have a nucleotide sequence of 1,396 bp, excluding a polyadenylate tail of 30 bases, which contained the complete sequence of the FPS1 cDNA previously characterized as well as 99 and 89 additional nucleotides at the 5′- and 3′-ends, respectively. The open reading frame starting at the most 5′ ATG triplet encodes a protein of 384 amino acid residues, with a predicted molecular mass of 44,254 Da, which differs from the previously reported FPS1 isoform in having an NH_2-terminal extension of 41 amino acids. The cDNA clone also contains a 5′-untranslated region of 27 bp and a 3′-untranslated region of 214 bp. The polyadenylate tail was located 89 bp downstream of the polyadenylation site of the FPS1 cDNA (9), thus indicating that different polyadenylation sites are used in the *FPS1* gene. These two FPS1

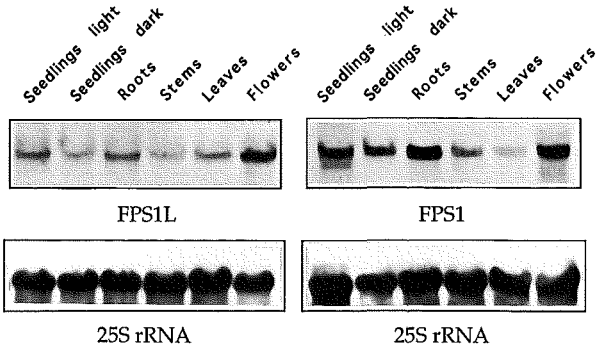


FIG. 2. **Northern blot analysis of *Arabidopsis FPS1*-derived mRNAs.** Total RNA samples from different tissues of *Arabidopsis* (30 μg/lane) were electrophoresed in 1% agarose-formaldehyde gels and transferred onto nylon membranes. Filters were hybridized with the FPS1L mRNA-specific probe and the *FPS1* gene-specific probe described under "Experimental Procedures." The FPS1 probe hybridizes to both FPS1S and FPS1L mRNAs. Exposure time was 9 days. To confirm that equivalent amounts of RNA were present in each lane, filters were reprobed with a fragment of the gene for the wheat 25 S cytoplasmic rRNA.

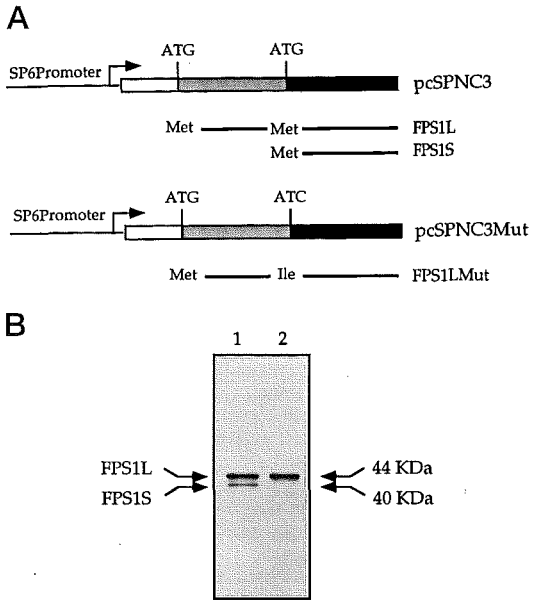


FIG. 3. **Functional *in vitro* analysis of the upstream AUG codon in the FPS1L mRNA.** *Panel A*, schematic representation of the FPS1L and FPS1LMut cDNAs used in the *in vitro* transcription/translation experiments. The regions of the cDNAs corresponding to the 5′-untranslated region (*open*), the NH_2-terminal extension (*hatched*), and part of the coding region common to FPS1L and FPS1S (*black solid*) are shown. *Arrows* indicate the transcription start site of the SP6 RNA polymerase promoter. *Lines* represent the NH_2-terminal region of the proteins resulting from the *in vitro* transcription/translation of plasmids pcSPNC3 and pcSPNC3Mut. *Panel B*, FPS1L (*lane 1*) and FPS1LMut (*lane 2*) transcripts were translated *in vitro* in a wheat germ cell-free system using [^{35}S]Met as labeled precursor. Samples were separated in a SDS, 12% polyacrylamide gel. Bands corresponding to FPS1S and FPS1L are indicated by *arrows*. The estimated molecular masses are also indicated.

mRNAs will be hereafter referred to as FPS1S and FPS1L, with FPS1S mRNA encoding the previously reported FPS1 isoform (9, 10) (FPS1S) and FPS1L mRNA encoding the novel FPS1 isoform (FPS1L) containing the NH_2-terminal extension.

To define the 5′-ends of the two FPS1 mRNAs (FPS1S and FPS1L) we performed RNase protection analysis using poly(A)$^+$ RNA isolated from *Arabidopsis* inflorescences. The results obtained are shown in Fig. 1*B*. The estimated size of the most intense protected bands indicated the occurrence of a major FPS1L transcript (band of 269 nucleotides) with a 5′-end located 62 nucleotides upstream of the FPS1L ATG start codon (TS3, Fig. 1*A*) and two major FPS1S mRNAs (bands of 137 and 154 nucleotides) with 5′-ends located 53 and 70 nucleotides upstream of the FPS1S ATG start codon (TS1 and TS2, Fig. 1*A*). Taken together, these results indicated that the expression of the *Arabidopsis FPS1* gene generates two mRNAs encoding two FPS1 isoforms (FPS1S and FPS1L) that differ only in their NH_2 terminus.

Expression Analysis of the Two FPS1-derived mRNAs—The expression pattern of the *Arabidopsis* FPS1S and FPS1L mRNAs was analyzed by Northern blot analysis using total RNA isolated from roots, stems, leaves and inflorescences (Fig. 2). A PCR fragment of 289 bp extending from nucleotides −309 to −20 in the *FPS1* gene was used as an FPS1L mRNA-specific probe. A 370-bp *Bgl*II-*Kpn*I genomic fragment corresponding to the 3′-end of the *FPS1* gene (10) was used as an *FPS1* gene-specific probe that recognizes both FPS1L and FPS1S mRNAs simultaneously. Similar amounts of RNA were present in each lane, as confirmed by hybridization of the filters with a probe derived from the wheat 25 S rRNA gene, and radiolabeled probes of equivalent specific activity were used. Comparison of the results indicated that FPS1S and FPS1L mRNAs are detected in all tissues analyzed although they have a different pattern of expression. FPS1L mRNA accumulates preferentially in inflorescences, whereas FPS1S mRNA is detected mainly in roots and inflorescences. No significant change in the levels of FPS1L mRNA was detected when RNA samples were prepared from light- or dark-grown seedlings.

Functional in Vitro Analysis of the Upstream AUG Codon in the FPS1L mRNA—To analyze whether the upstream in-frame AUG codon in the FPS1L mRNA is used as translational start codon, the FPS1L transcript was synthesized *in vitro* from plasmid pcSPNC3 (Fig. 3*A*) and translated in a wheat germ cell-free system. The products obtained were separated by SDS-polyacrylamide gel electrophoresis and analyzed by fluorography. Two proteins of 44 and 40 kDa resulted from the translation of the FPS1L mRNA (Fig. 3*B*, *lane 1*). The estimated size of the two proteins is in good agreement with the molecular mass predicted for proteins initiated at the first (44,254 Da) and the second (39,689 Da) AUG codons. When a similar experiment was performed using transcript FPS1LMut (plasmid pcSPNC3Mut, Fig. 3*A*), in which the second AUG codon was converted to an AUC codon (encoding Ile) by site-directed mutagenesis, only the 44-kDa protein was synthesized (Fig. 3*B*, *lane 2*). Taken together, these results show that in the wheat germ lysate system, the most 5′ AUG codon in the FPS1L mRNA is used preferentially to initiate translation, although the second AUG codon is also used, giving rise to significant levels of the FPS1S protein.

Functional in Vivo Analysis of the FPS1L NH_2-terminal Extension—Analysis of the amino acid composition and the predicted secondary structure of the NH_2-terminal extension of the *Arabidopsis* FPS1L isoform (Fig. 4) revealed that it has all of the features characteristic of a mitochondrial transit peptide (35, 36). There is a complete absence of acidic residues and an enrichment in basic (arginine, lysine, and histidine), hydroxylated (serine), and hydrophobic (leucine) residues. It contains the sequence RIQS (amino acid residues 36–39 in Fig. 4*A*), which fits the most commonly reported mitochondrial targeting peptide cleavage motif R*X*/*X*S (where *X* represents any amino acid) (36), preceded by two arginine residues located at positions −2 and −12 relative to the predicted cleavage site RI/QS. Finally, analysis of the FPS1L NH_2-terminal extension using an improved method of protein structure prediction (37) shows that amino acid residues 8–17 can form a positively charged amphiphilic α-helix, in which hydrophobic residues are clustered on one face of the helix while the basic and polar residues

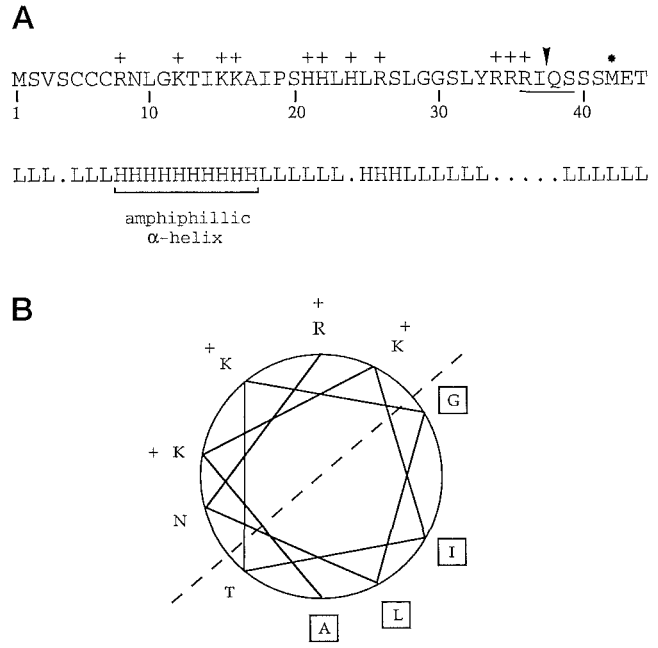
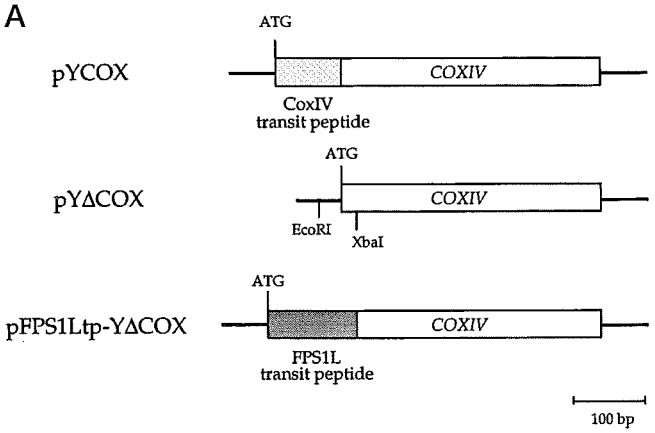


FIG. 4. **Analysis of the *Arabidopsis* FPS1L NH_2-terminal extension.** *Panel A*, amino acid sequence of the FPS1L NH_2-terminal region. Amino acid positions are indicated below the sequence. The potential charge is shown above each residue. The conserved cleavage motif is *underlined*, and the predicted cleavage site is indicated by an *arrowhead*. The NH_2-terminal methionine residue of the FPS1S isoform is indicated by an *asterisk*. Secondary structure prediction for the FPS1L NH_2-terminal region is shown below the amino acid sequence. *H* represents helical structure, *L* represents looped structures, and · represents extended conformation. *Panel B*, helical wheel representation of amino acids 8–17 in the FPS1L sequence. Hydrophobic residues are *boxed*.



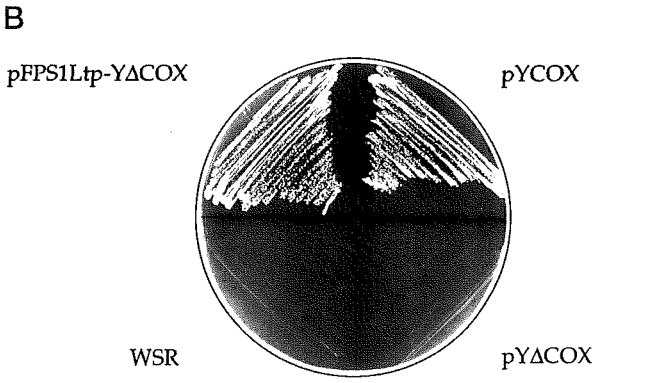


FIG. 5. **Functional complementation of a CoxIV-deficient yeast strain by plasmid pFPS1Ltp-YΔCOX.** *Panel A*, schematic representation of the plasmids used in this study. Plasmid pYCOX contains the wild-type *COXIV* gene from *S. cerevisiae*, including the region encoding its own mitochondrial transit peptide, which has been deleted in plasmid pYΔCOX. Plasmid pFPS1Ltp-YΔCOX contains the region of the *Arabidopsis* FPS1L cDNA coding for the 41 NH_2-terminal amino acid residues of FPS1L ligated in front of the partially deleted *COXIV* gene from yeast. *Panel B*, complementation analysis of yeast strain WSR transformed with the plasmids described in *panel A*. Strains WSR, WSR[pYΔCOX], WSR[pYCOX], and WSR[pFPS1Ltp-YΔCOX] were streaked onto N3 medium, containing glycerol as energy source, and incubated at 28 °C for 2 days.

are on the other face (Fig. 4*B*).

To study the possible role of the FPS1L NH_2-terminal extension as a mitochondrial targeting sequence, we analyzed the ability of this predicted transit peptide to direct the CoxIV subunit of cytochrome *c* oxidase into yeast mitochondria *in vivo*. This experimental approach was based on previous observations demonstrating the interchangeability of mitochondrial targeting sequences between plants and yeast (31, 38–41). For this purpose, an in-frame fusion was made between the fragment of the *Arabidopsis* FPS1L cDNA coding for the 41 NH_2-terminal residues of the FPS1L isoform (FPS1Ltp) and the *S. cerevisiae COXIV* gene lacking the region coding for its own mitochondrial transit peptide (Δ*COXIV*). The resulting plasmid (pFPS1Ltp-YΔCOX, Fig. 5*A*) was used to transform the yeast strain WSR, which contains a disrupted copy of the *COXIV* gene and, consequently, is unable to grow on medium containing glycerol as energy source. The results shown in Fig. 5*B* indicate that plasmid pFPS1Ltp-YΔCOX efficiently complements strain WSR. The same result was obtained when strain WSR was transformed with plasmid pYCOX, which encodes the complete amino acid sequence of the *S. cerevisiae* CoxIV subunit. In contrast, plasmid pYΔCOX, which encodes a truncated form of the CoxIV subunit lacking the mitochondrial transit peptide, does not complement the respiratory-deficient strain WSR. These results clearly demonstrate that the NH_2-terminal extension of the *Arabidopsis* FPS1L isoform is able to replace the mitochondrial targeting sequence of the CoxIV subunit of yeast cytochrome *c* oxidase, thus demonstrating that it is a functional mitochondrial transit peptide.

In Vitro Import of FPS1L into Purified Plant Mitochondria—To confirm further the functional role of the FPS1L NH_2-terminal extension as a mitochondrial targeting sequence we analyzed the import of the *Arabidopsis* FPS1L isoform into plant mitochondria (Fig. 6). The FPS1L mRNA was *in vitro* translated using a rabbit reticulocyte lysate, and the resulting products were analyzed by SDS-polyacrylamide gel electrophoresis and fluorography. The translation mixture contained similar amounts of radiolabeled FPS1L (44 kDa) and FPS1S (40 kDa) isoforms (Fig. 6, *lane 2*), which were completely digested after incubation with proteinase K (Fig. 6, *lane 1*). When the translation mixture was incubated with purified potato mitochondria followed by treatment with proteinase K, a protected polypeptide of approximately 40 kDa was detected (Fig. 6, *lane 4*). The protection was abolished when mitochondria were solubilized with Triton X-100 prior to proteinase K treatment (Fig. 6, *lane 5*). When the import experiment was performed in the presence of valinomycin, which collapses the membrane potential required for protein import into mitochondria, the protected 40-kDa polypeptide was not detected (Fig. 6, *lane 6*). No import into mitochondria was observed when similar experiments were performed using as a precursor the 40-kDa FPS1S isoform generated from the FPS1S mRNA (data not shown). Taken together, these results demonstrate that the 44-kDa *Arabidopsis* FPS1L isoform is targeted into plant mitochondria and processed to a 40-kDa mature FPS1 isoform by cleavage of a transit peptide of approximately 4 kDa.

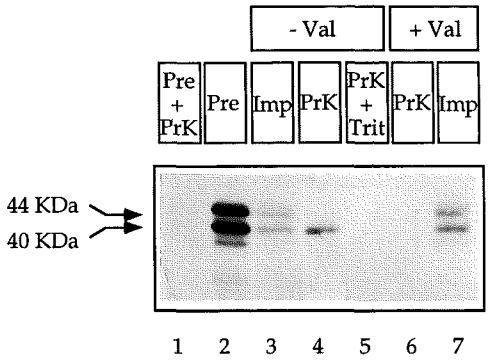


FIG. 6. **Import of *Arabidopsis* FPS1L into purified potato mitochondria.** *Lane 2* shows the radiolabeled FPS1L (44 kDa) and FPS1S (40 kDa) isoforms (precursor, *Pre*), resulting from *in vitro* translation of the FPS1L mRNA. The *in vitro* translated FPS1 isoforms were completely digested after the addition of proteinase K (*lane 1*). For the import reaction, the *in vitro* labeled polypeptides were incubated with purified potato mitochondria. An aliquot of the import reaction was left untreated (*Imp*, *lane 3*). Two aliquots were made from the remainder of the import reaction and were further processed by treatment either with proteinase K (*lane 4*) or proteinase K and Triton X-100 (*lane 5*). The *in vitro* labeled polypeptides were also incubated with mitochondria previously treated with valinomycin. Half of the reaction was left untreated (*Imp*, *lane 7*), and half of the reaction was digested with proteinase K (*lane 6*). Samples were separated in a SDS, 10% polyacrylamide gel and analyzed by fluorography. The estimated molecular masses are indicated on the *left*.

DISCUSSION

The great complexity of the plant isoprenoid biosynthetic pathway has led to the suggestion that its regulation requires the coordinated activity of several key enzymes (6, 42, 43). FPS, a prenyltransferase that catalyzes the synthesis of farnesyl diphosphate from isopentenyl diphosphate and dimethylallyl diphosphate, is considered to play a central role in the overall control of the plant isoprenoid pathway. This assumption has been primarily made on the basis that farnesyl diphosphate is the starting point of different branches of the plant isoprenoid pathway leading to the synthesis of essential end products (6, 42, 43). The proposed regulatory role of plant FPS is further supported by the observation that in mammals FPS is a highly regulated enzyme that plays a relevant role in the control of sterol biosynthesis (2–5). We have recently shown that *Arabidopsis contains a small FPS gene family consisting of at least* two genes, *FPS1* and *FPS2*. These genes are differentially expressed and encode two highly similar FPS isoforms (10). At least two FPS isoforms have also been reported to occur in other plant species (11, 15). Thus, the occurrence of FPS isoforms is a general feature of higher plants, although the specific role of each individual FPS isoform in the isoprenoid biosynthetic pathway is currently unknown.

In this paper we report that the expression of the *Arabidopsis FPS1* gene generates a previously undetected mRNA that encodes a novel FPS1 isoform (FPS1L) with an NH_2-terminal extension of 41 amino acids with respect to the FPS1 isoform (FPS1S) previously characterized (9, 10). The occurrence of the FPS1L mRNA was demonstrated by the cloning of its corresponding cDNA. RNA blot analysis using a FPS1L mRNA-specific probe and an *FPS1*-derived probe that recognizes both FPS1S and FPS1L mRNAs simultaneously showed that FPS1S and FPS1L mRNAs are present in all tissues analyzed, although FPS1L mRNA accumulates preferentially in inflorescences, and FPS1S mRNA is mainly expressed in roots and inflorescences. The fact that the *FPS1* gene generates two mRNAs that are also differentially expressed suggests that the two transcripts are under the control of alternative promoters. The results obtained in the RNase protection analysis show that FPS1S and FPS1L mRNAs have heterogeneous 5′-ends, which correlates with the lack of consensus TATA box located at appropriate distances from the corresponding transcription start sites.

At the protein level, the novel *Arabidopsis* FPS1L isoform is identical in sequence to FPS1S but is extended at its NH_2 terminus by an additional sequence of 41 amino acids. This NH_2-terminal extension has no counterpart among the FPS characterized so far from a number of eukaryotic organisms (7–15). This observation raised the question about its possible function as a transit peptide for targeting the enzyme into subcellular organelles. The accumulation of sequence data on organellar transit peptides has revealed that these targeting sequences share a very low level of sequence conservation, although they have a number of common features in terms of amino acid composition, positional amino acid preferences, and secondary structure (35, 36). Interestingly, the FPS1L NH_2-terminal extension nicely fits all known requirements to be a mitochondrial transit peptide. First, there is a lack of acidic amino acid residues and an enrichment in hydroxylated and hydrophobic residues. Second, the sequence motif RIQS, which fits the consensus mitochondrial targeting peptide cleavage motif R*X*X*S* (where *X* represents any amino acid), is found just two residues upstream of the NH_2-terminal methionine residue of FPS1S isoform. This motif is preceded by two arginine residues located at positions −2 and −12 from the putative cleavage site (RI/QS). It has been proposed that arginine residues located around positions −2 and −10 from the processing site play a role in defining the precise cleavage site of targeting peptides by mitochondrial matrix proteases (35). Third, secondary structure analysis indicates that a sequence of 10 amino acid residues located at the NH_2-terminal part of the FPS1L NH_2-terminal extension can potentially fold into a positively charged amphiphilic α-helix. Taken together, all these observations strongly suggested that the FPS1L NH_2-terminal extension was a mitochondrial transit peptide.

To analyze whether the FPS1L NH_2-terminal extension could function as a mitochondrial transit peptide we first studied its ability to direct a passenger protein into yeast mitochondria *in vivo*. To this end, a construct expressing a chimeric protein containing the 41 NH_2-terminal amino acid residues of FPS1L fused to the CoxIV subunit of yeast cytochrome *c* oxidase lacking its own mitochondrial transit peptide (plasmid pFPS1Ltp-ΔCOXIV) was assayed for its ability to complement the mutant yeast strain WSR. This strain is unable to grow in a medium containing glycerol as energy source because of a disruption of the *COXIV* gene, which encodes the CoxIV subunit of mitochondrial cytochrome *c* oxidase. Plasmid pFPS1Ltp-ΔCOXIV completely restored the respiratory activity of strain WSR, thus indicating that the yeast CoxIV subunit was properly targeted into mitochondria. Since the interchangeability of mitochondrial transit peptides between plants and yeast is well documented (31, 38–41), the observation that the NH_2-terminal extension of the *Arabidopsis* FPS1L isoform acts as a mitochondrial transit peptide in yeast indicates that *Arabidopsis* FPS1L is the precursor of a mitochondrial FPS isoform. This fact was confirmed further by *in vitro* import studies of the *Arabidopsis* FPS1L into plant mitochondria. *In vitro* translated FPS1L protein was imported into purified potato mitochondria and processed to a mature mitochondrial FPS1 isoform of 40 kDa by cleavage of a transit peptide of approximately 4 kDa. From this result and assuming that FPS1L is processed by mitochondrial matrix proteases at the predicted processing site (see above), the mature mitochondrial FPS1 isoform would contain four additional amino acid resi-

dues at its NH_2 terminus (QSSS) with respect to the FPS1S isoform.

Our results indicate that *Arabidopsis FPS1* is a bifunctional gene encoding cytosolic and mitochondrial FPS isoforms. Consequently, *FPS1* belongs to the increasing group of eukaryotic genes encoding isozymes that perform analogous functions at different intracellular locations (44). These genes usually have more than one ATG start codon in their 5′-region and, depending on the translation start codon used to initiate translation of the corresponding mRNAs, have the potential to encode enzymes differing only at their NH_2 terminus. These isozymes can be targeted to different intracellular compartments. Genes of this type have recently been reported in plants. For example, the *Arabidopsis* alanyl-tRNA synthetase gene encodes both mitochondrial and cytosolic forms of the enzyme (31), and the *Arabidopsis* glutathione reductase gene encodes both mitochondrial and chloroplastic isoforms and possibly also a cytosolic isoform (45). In the case of the *Arabidopsis FPS1* gene, the strategy used to generate the cytosolic and the mitochondrial FPS1 isoforms is the use of alternative transcription start sites, resulting in the synthesis of mRNAs that differ in the presence or absence in the 5′-region of the sequence encoding the mitochondrial transit peptide. However, the *in vitro* translation results showed that FPS1L mRNA can give rise not only to the FPS1L isoform but also to significant levels of the FPS1S isoform. It is thus possible that the choice of cytosolic or mitochondrial location of FPS might involve not only the use of alternative transcription start sites but also a mechanism of AUG selection during the translation initiation process. It is remarkable that a similar situation has been reported to occur in the *Arabidopsis HMG1* gene, which encodes another key regulatory enzyme of the isoprenoid pathway (46). This gene contains two alternative promoters that direct the expression of different mRNAs encoding two 3-hydroxy-3-methylglutaryl-CoA reductase isoforms (HMGR1L and HMGR1S), which differ only in the presence or absence of an NH_2-terminal extension of 50 amino acids. In this case both 3-hydroxy-3-methylglutaryl-CoA reductase isoforms are targeted primarily to the endoplasmic reticulum membrane, although it has been proposed that the different NH_2-terminal sequences might direct each 3-hydroxy-3-methylglutaryl-CoA reductase isoform to specific subdomains of the endoplasmic reticulum (46, 47). These observations give further support to the view that the regulation of plant isoprenoid biosynthesis is under the control of complex regulatory mechanisms operating at both transcriptional and post-transcriptional level.

A general feature of the enzymes involved in plant isoprenoid biosynthesis is their occurrence in multiple isoforms (10, 16–19). Although the biological significance of this fact has not yet been fully evaluated, it is likely that the multiplicity of isoforms reflects the great complexity of plant isoprenoid biosynthesis, concerning not only the regulation of the overall pathway but also its subcellular compartmentalization. In contrast to the controversy surrounding the subcellular location of the enzymes involved in the synthesis of isopentenyl diphosphate in plants, it is widely accepted that the enzymes utilizing isopentenyl diphosphate are distributed in at least three cellular compartments: the cytosol, mitochondria, and plastids. However, the cytosol is the only cell compartment in which plant FPS has been detected (6, 22, 23). It is worth noting that a significant level of FPS activity (approximately 13% of the total cellular FPS activity) has been detected in rat liver mitochondria (21). Interestingly, none of the previously reported FPS from a number of eukaryotic organisms (including fungi, plants, and animals) (7–15) contains NH_2-terminal sequences that could represent transit peptides for targeting to mitochondria. The *Arabidopsis* FPS1L isoform described here represents the first reported eukaryotic FPS that contains a mitochondrial transit peptide. This finding reinforces the view that plant mitochondria can use isopentenyl diphosphate as a precursor for the synthesis of farnesyl diphosphate, which in turn could be utilized for the production of mitochondrial isoprenoid compounds such as ubiquinone, heme a, tRNA species, and prenylated proteins. On the basis of these observations, it is reasonable to speculate that FPS isoforms with mitochondrial targeting peptides might be found not only in other plant species but also in eukaryotic organisms other than plants. We are currently applying different molecular and cellular approaches to assess the possible regulatory role of FPS1L in the synthesis of mitochondrial isoprenoid compounds in plants.

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