Plant Cell Physiol. 45(12): 1882–1888 (2004)

Short Communication

Identification and Subcellular Localization of Two Solanesyl Diphosphate Synthases from *Arabidopsis thaliana*

Luo Jun [1], Ryoichi Saiki [1], Kei Tatsumi [1], Tsuyoshi Nakagawa [2] and Makoto Kawamukai [1, 3]

[1] *Department of Applied Bioscience and Biotechnology, Faculty of Life and Environmental Science, Shimane University, 1060 Nishikawatsu, Matsue, 690-8504 Japan*
[2] *Center for Integrated Research in Science, Shimane University, Matsue, 690-8504 Japan*

Two solanesyl diphosphate synthases, designated SPS1 and SPS2, which are responsible for the synthesis of the isoprenoid side chain of either plastoquinone or ubiquinone in *Arabidopsis thaliana*, were identified. Heterologous expression of either *SPS1* or *SPS2* allowed the generation of UQ-9 in a decaprenyl diphosphate synthase-defective strain of fission yeast and also in wild-type *Escherichia coli.* SPS1–GFP was found to localize in the ER while SPS2–GFP localized in the plastid of tobacco BY-2 cells. These two different subcellular localizations are thought to be the reflection of their roles in solanesyl diphosphate synthesis in two different parts: presumably SPS1 and SPS2 for the side chains of ubiquinone and plastoquinone, respectively.

Keywords: CoenzymeQ — Solanesyl diphosphate synthase — Ubiquinone.

Plastoquinone and ubiquinone are known to be major prenylquinones in plants. Plastoquinone is essential for photosynthetic electron transfer in the chloroplast and ubiquinone is essential for respiratory electron transfer in the mitochondrion (Swiezewska 2004). Both quinones have the isoprenylated tail in the quinone structure moiety and the length of the prenyl side chain differs between species. Most plants possess nine or 10 units of the isoprenoid side chain of plastoquinone (PQ) and ubiquinone (UQ): e.g. *Arabidopsis* contains PQ-9 and UQ-9.

Ubiquinone was initially identified as an essential factor in aerobic growth and oxidative phosphorylation in the electron transport system (Turunen et al. 2004). Recently, however, multiple additional functions of ubiquinone have been proposed, such as a lipid-soluble antioxidant found in many organisms (Turunen et al. 2004), involvement in disulfide bond formation in *Escherichia coli* (Kadokura et al. 2003), sulfide oxidation in the fission yeast *Schizosaccharomyces pombe* (Uchida et al. 2000) and relevance to life span in nematode *Caenorhabditis elegans* (Larsen and Clarke 2002). Thus, it is widely accepted that ubiquinone appears to play multiple roles. However, very little is known about the functions of ubiquinone in plants.

The ubiquinone biosynthetic pathway has been developed mostly from the studies of respiratory-deficient mutants of *E. coli* and *Saccharomyces cerevisiae* (Kawamukai 2002). The length of the isoprenoid side chain of ubiquinone varies between organisms, e.g. *S. cerevisiae*, *E. coli* and *S. pombe* contain UQ-6, UQ-8 and UQ-10, respectively. The length of the side chain is determined by polyprenyl diphosphate synthase, but not by 4-hydroxybenzoate-polyprenyl-diphosphate transferases, which catalyze the condensation of 4-hydroxybenzoate and polyprenyl diphosphate (Okada et al. 1996, Okada et al. 1997b, Okada et al. 1998a, Suzuki et al. 1994).

The genes encoding the long-chain polyprenyl diphosphate synthases have been cloned from various organisms ranging from bacteria to yeast (Okada et al. 1997a, Okada et al. 1998b, Koyama 1999, Liang et al. 2002, Saiki et al. 2003). All long-chain polyprenyl diphosphate synthases that synthesize the side chain of ubiquinone had been thought to be homodimeric enzymes (Kawamukai 2002). But our group found that decaprenyl diphosphate synthase of *S. pombe* formed a heterotetramer (Suzuki et al. 1997, Saiki et al. 2003). This finding prompted us to test the nature of other eukaryotic enzymes. It has been shown very recently that a solanesyl diphosphate synthase (SPS1) from *Arabidopsis* is functional by itself, indicating that this enzyme is a homomeric bacterial type (Hirooka et al. 2003).

In this study, we independently analyzed two solanesyl diphosphate synthases, namely, SPS1 and SPS2, and showed that both solanesyl diphosphate synthases from *Arabidopsis* are functional by themselves, and that SPS1 localized in the ER while SPS2 localized in the plastid (chloroplast). These two different localizations were assumed to be a reflection of the localization of plastoquinone in the chloroplast and ubiquinone in the mitochondrion.

We looked for potential polyprenyl diphosphate synthases by searching the cDNA and genomic DNA sequences of *Arabidopsis* in the National Center for Biotechnology Information database. We picked up three different uncharacterized genes that retain some sequence similarity with prenyl diphosphate synthases. Then, we obtained cDNA clones of GenBank accession numbers T44803, AV524823 and AV440463 from

[3] Corresponding author: E-mail, kawamuka@life.shimane-u.ac.jp; Fax, +81-852-32-6092.

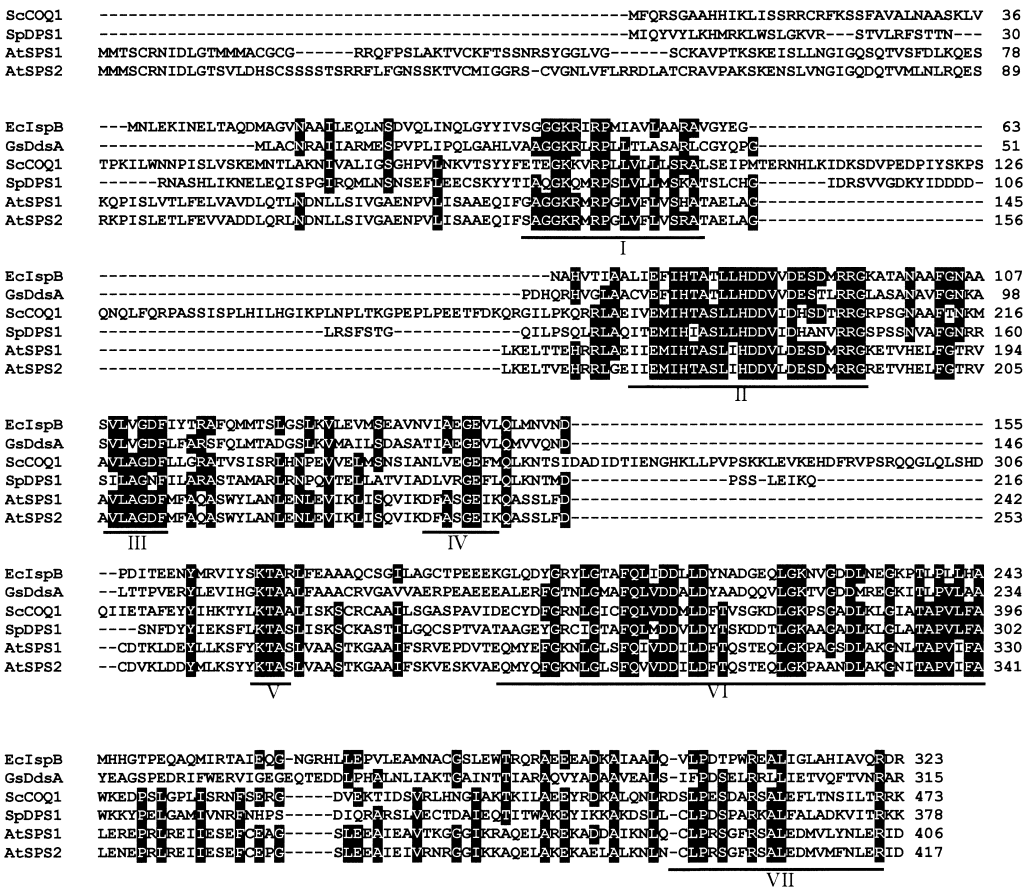

Fig. 1 Alignment of amino acid sequences of long-chain-producing polyprenyl diphosphate synthases from various organisms. From top to bottom they are: (i) octaprenyl diphosphate synthase encoded by *ispB* from *E. coli* (accession no. NP417654); (ii) decaprenyl diphosphate synthase encoded by *ddsA* from *Gluconobacter suboxydans* (accession no. AB006850); (iii) hexaprenyl diphosphate synthase encoded by *COQ1* from *S. cerevisiae* (accession no. J05547); (iv) a component of decaprenyl diphosphate synthase encoded by *dps1* from *S. pombe* (accession no. D84311); (v) solanesyl diphosphate synthase encoded by *SPS1* from *Arabidopsis thaliana* (accession no. AB188497); (vi) solanesyl diphosphate synthase endoded by *SPS2* from *A. thaliana* (accession no. AB188498). Highly conserved amino acids among organisms are indicated by shaded areas. Underlines show the conserved regions. The DDXXD motifs in II and VI are the putative prenyl diphosphate substrate-binding sites.

Michigan State University and the Kazusa Institute. As the full sequences of those clones were not known, we determined the complete sequence of those three cDNAs. The deduced amino acid sequences of those genes possessed the typical conserved domains of polyprenyl diphosphate synthases (Fig. 1). We designated those three genes as *GPS1* (At2g34630), *SPS1* (At1g78510) and *SPS2* (At1g17050). While we were conducting some experiments on *GPS1*, it was reported by Bouvier et al. (2000) that *GPS1* encoded geranyl diphosphate synthase. We then gave up the further characterization of *GPS1* and focused on *SPS1* and *SPS2*. Both SPS1 and SPS2 are highly homologous to the other characterized prenyl diphosphate synthase within the 45–50% range of sequence similarity. SPS1 and SPS2 are 79% identical to each other. During the course of our analysis, one solanesyl diphosphate synthase (SPS1) (Hirooka et al. 2003) was enzymically characterized, but SPS2 has only been assumed to be an enzyme similar to geranyl geranyl diphosphate synthase.

To determine whether *SPS1* and *SPS2* are involved directly in the activity of solanesyl diphosphate synthase, we expressed *SPS1* and *SPS2* in the two fission yeast disruptants, *S. pombe dlp1* and *dps1*. In *S. pombe*, both *dlp1* and *dps1* are necessary to constitute a heteromeric decaprenyl diphosphate synthase, but we know that heterologous expression of monomeric prenyl diphosphate synthase is functional in *S. pombe* in both *dlp1* and *dps1* mutants (Saiki et al. 2003). This system was used to test the functional determination of *SPS1* and *SPS2*. To make prenyl diphosphate synthase functional, it is necessary to localize it in the mitochondria. Thus, a 45-amino-acid mitochondrial targeting signal from Ppt1, which is known to be located in the mitochondria (Uchida et al. 2000), was first added to the *SPS1* and *SPS2* genes. The resulting *ppt1TP–SPS1* and *ppt1TP–SPS2* fusion genes (Fig. 2) were tested for their functional complementation. The result was that they successfully complemented the phenotype of both *dps1* and *dlp1* disruptants, as both cell types were able to grow well on minimal

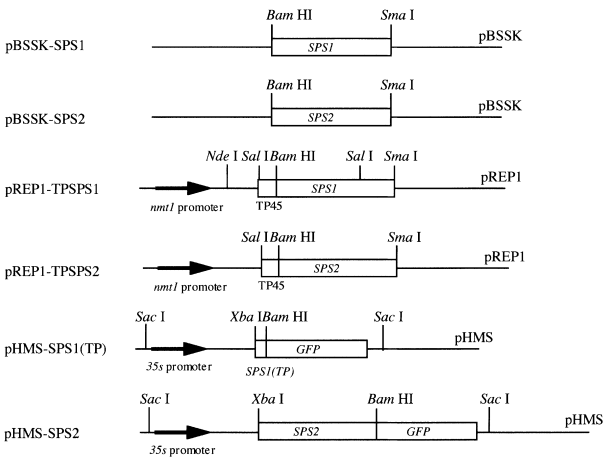

Fig. 2 Plasmid constructs used in this study. pBSSK-SPS1 and pBSSK-SPS2 express the *SPS1* and *SPS2* genes under the control of the *lac* promoter in *E. coli* cells. pREP1-TPSPS1 and pREP1-TPSPS2 contain the entire length of the *SPS1* and *SPS2* genes with mitochondrial targeting sequence of *S. pombe* and they are under the control of the strong *nmt1* promoter. pHMS-SPS1(TP) and pHMS-SPS2 contain the potential targeting sequence of the *SPS1* gene fused with the GFP gene and the entire length of the *SPS2* gene fused with the GFP gene, respectively, in the pHMS vector (Okada et al. 2000), which are under the control of the strong 35S CaMV promoter, which permits expression in plant cells.

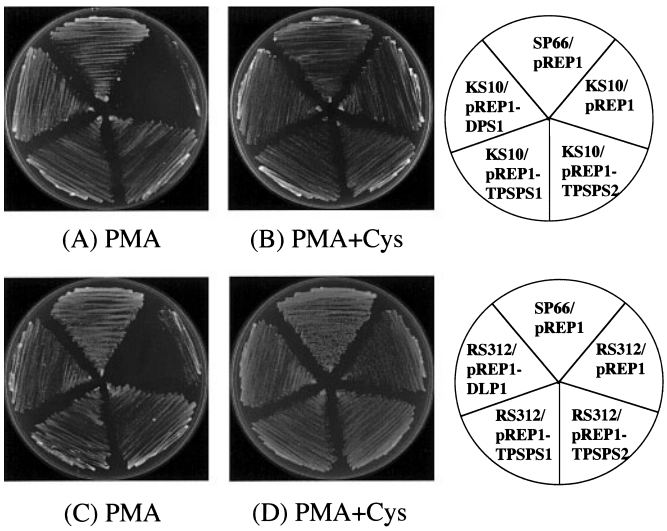

Fig. 3 Recovery of the growth of KS10 or RS312 on minimal medium by expression of TP45-SPS1 or TP45-SPS2. Wild-type SP66 harboring pREP1, and KS10(Δ*dps1::ura4*) harboring pREP1-DPS1, pREP1-TPSPS1, pREP1-TPSPS2 or pREP1 were grown on PM medium supplemented with 75 μg ml^{-1} adenine (A). The same strains were grown on PM medium supplemented with adenine and 400 μg ml^{-1} cysteine (B). Wild-type SP66 harboring pREP1, and RS312(Δ*dlp1::ura4*) harboring pREP1-DLP1, pREP1-TPSPS1, pREP1-TPSPS2 or pREP1 were grown on PM medium supplemented with 75 μg ml^{-1} adenine (C). The same strains were grown on PM medium supplemented with adenine and 400 μg ml^{-1} cysteine (D).

media, which otherwise they would not (Fig. 3). Ubiquinone-less fission yeast cannot grow on minimal medium without cysteine (Saiki et al. 2003), but it grows if fission yeast produces even a small amount of ubiquinone. We next measured the ubiquinone content of the *dps1* and *dlp1* disruptants that express *ppt1TP–SPS1* and *ppt1TP–SPS2* fusion genes. They all synthesized UQ-9 (Fig. 4C, D, G, H), while no ubiquinone was detected in the same strains harboring only the vector (Fig. 4B, F). Thus, both *SPS1* and *SPS2* encode solanesyl diphosphate synthases of *Arabidopsis*.

We speculated that if *SPS1* and *SPS2* are sufficient for solanesyl diphosphate synthase activity, transformation of *E. coli* DH5α with the pBSSK-SPS1 plasmid, which expresses the *SPS1* gene, or the pBSSK-SPS2 plasmid, which expresses the *SPS2* gene (Fig. 2) may result in the formation of a functional enzyme that generates the UQ-9 species. While the cells that were transformed with only the vector produced only UQ-8, which is synthesized by the endogenous *E. coli* octaprenyl diphosphate synthase, the pBSSK-SPS1 or pBSSK-SPS2 transformant produced a small amount of UQ-9 (Fig. 4). This result further supports the idea that both SPS1 and SPS2 encode solanesyl diphosphate synthases and they are functional by themselves.

We next investigated the subcellular localization of SPS1 and SPS2 in tobacco cells. To determine their subcellular localization, SPS1 and SPS2 were fused with GFP (Fig. 2) and expressed in tobacco BY-2 cells. SPS1–GFP was found to be localized in the periphery of the nucleus, typically assumed to be the ER localization (Fig. 5B) (Saito et al. 1999). Then we stained the same tobacco BY-2 cells with ER Tracker (Molecular Probes, Inc., OR, U.S.A.) and found that the same regions were concomitantly stained (Fig. 5C, D). Thus, we concluded that SPS1 was localized in the ER. In contrast, granular fluorescence dispersed throughout the cytosol was observed with expression of SPS2–GFP (Fig. 5F). As this granular fluorescence is assumed to be either plastid or mitochondria, we stained the same BY-2 cells with the mitochondria-staining dye Mito-tracker (Molecular Probes, Inc.), but apparently different granular areas were stained when both images were merged (Fig. 5G, H). We concluded that this localization of SPS2 is in the plastid, judged together with the notion that the N-terminal sequence of SPS2 contains the typical chloroplast targeting signal (http://psort.nibb.ac.jp/). The production of both SPS1–GFP and SPS2–GFP with their expected molecular weights (31K and 73K, respectively) were verified by Western blotting using anti-GFP (data not shown).

In this study, we identified a novel gene named *SPS2* as well as the previously reported *SPS1* as both encoding solanesyl diphosphate synthases in *Arabidopsis*. Both SPS1 and SPS2 proteins contain conserved regions of domains II and VI, which are likely to be the prenyl diphosphate synthase substrate-binding sites. Because the sequence similarity itself does not define which enzymic property they possess, it is absolutely neces-

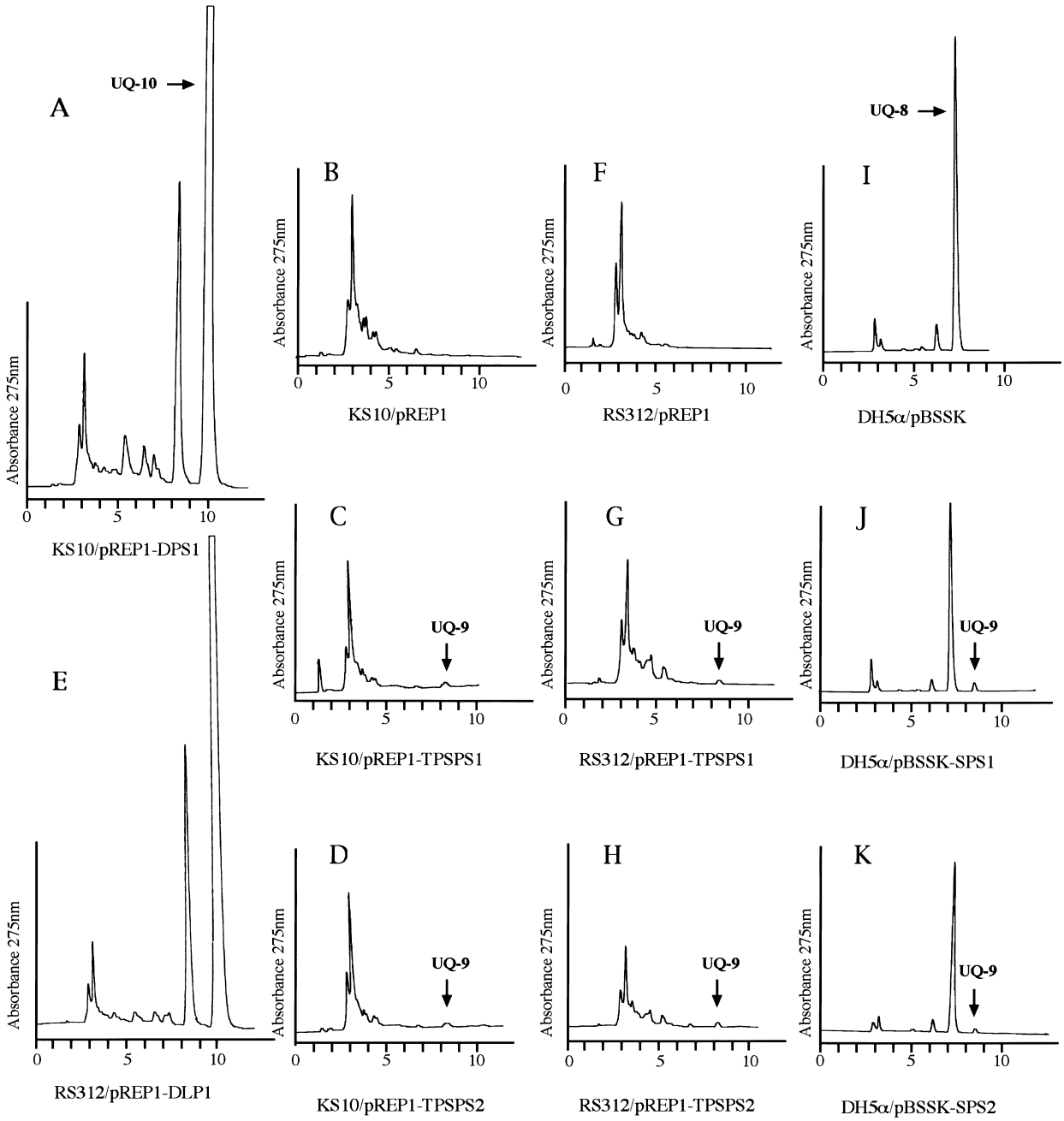

Fig. 4 Detection of UQ-9 in *S. pombe* and *E. coli* strains. Ubiquinone was extracted from KS10(Δ*dps1::ura4*) harboring pREP1-DPS1 (A), pREP1 (B), pREP1-TPSPS1 (C) or pREP1-TPSPS2 (D) and RS312(Δ*dlp1::ura4*) harboring pREP1-DLP1(E), pREP1 (F), pREP1-TPSPS1(G) or pREP1-TPSPS2 (H). Ubiquinone was also extracted from *E. coli* DH5α harboring pBSSK (I), pBSSK-SPS1 (J) or pBSSK-SPS2 (K). Ubiquinone was extracted as described previously (Okada et al. 1996, Saiki et al. 2003).

sary to define the product experimentally. This is especially true for prenyl diphosphate synthases, which share high sequence similarity but where each enzyme synthesizes a different length of isoprenoid. *SPS1* and *SPS2* are functional in both *S. pombe* and *E. coli* in generating UQ-9, indicating that both genes encode a solanesyl diphosphate synthase, although the direct measurement of the activity of SPS1 and SPS2 will provide better evidence. All long-chain-producing polyprenyl diphosphate synthases that synthesize the side chain of ubiquinone appear to exist as homodimers (Kainou et al. 2001) except the one from fission yeast (Saiki et al. 2003). The result that solanesyl diphosphate synthases from *Arabidopsis* are functional in the heterologous system indicates that both enzymes are the typical homomeric type enzymes found in bacteria.

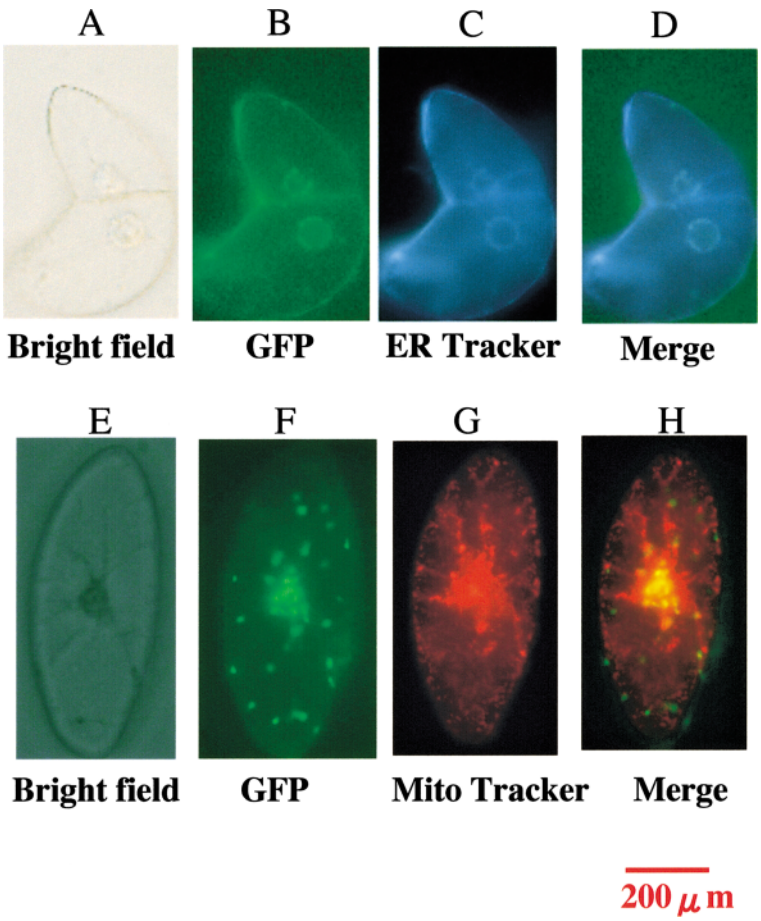

Fig. 5 Subcellular localization of the SPS1(TP)–sGFP or SPS2–sGFP fused protein in tobacco BY-2 cells. The cells of 10-day-old cultures were used for observation of GFP fused proteins by fluorescence microscopy. The tobacco cells harboring pHMS-SPS1(TP) were concomitantly stained with ER-Tracker as observed with a DAPI filter and cells harboring pHMS-SPS2 were stained with red-Mito-Tracker (579–599 nm). (A, E) Normal microscopic pictures under natural light. (B) Fluorescence images of SPS1(TP)–sGFP. (C) Blue ER fluorescence from the cells stained by ER-Tracker. (F) Fluorescence images of SPS2–sGFP. (G) Red mitochondria fluorescence from the cells stained by red Mito-Tracker. (D) and (H) are the merged images of (B) and (C), and (E) and (F), respectively.

The localization of SPS2 in the plastid (chloroplast) in BY-2 is as expected from its N-terminal sequence and the assumption that plastoquinone is synthesized in the chloroplast (Lange and Ghassemian 2003, Swiezewska 2004) where plastoquinone mainly works. The localization of SPS2 in plastid (chloroplast) is consistent with the idea that the enzyme might supply for the side chain of plastoquinone. But localization of SPS1 in the ER is a little unexpected because it has been suggested that ubiquinone is synthesized in the mitochondria from the observation that *Arabidopsis* Ppt1, which catalyzes the first step of ubiquinone synthesis, localizes to the mitochondria (Okada et al. 2004). However, it was reported that in spinach, prenyl diphosphate synthase is localized in the ER (Swiezewska et al. 1993), which is consistent with our result that SPS1 localized in the ER. Thus, the synthesis of the polyprenyl moiety at the ER may help to supply prenyl diphosphate for both ubquinone and plastoquinone. Combined with those results, we speculate that one of the isoprenoid side chains is synthesized in the ER, then transferred to the mitochondria for the synthesis of ubiquinone.

Restriction enzymes and other DNA-modifying enzymes were purchased from Takara Shuzo Co. Ltd., Kyoto, Japan and New England Biolabs, Inc., MA, U.S.A. *E. coli* strain DH5α was used for the general construction of plasmids. Plasmids pBluescript SK+/–, pUC119, pT7Blue-T (Novagen), pREP1 (Maundrell 1993) and pTH121 (Zhu et al. 1997) were used as vectors. Cloning, restriction enzyme analysis and preparation of plasmid DNAs were performed essentially as described previously (Sambrook et al. 1989). The *S. pombe* homothallic haploid wild-type strain SP66 (*h*^{90} *ade6-M210 leu1-32*), RS312 (*h*$^{+}$ *leu1-32 ade-M210 ura4-D18 dlp1::ura4*) and KS10 (*h*$^{+}$ *leu1-32 ade6-M216 ura4-D18 Δdps::ura4*), have been described previously (Kawamukai et al. 1992, Suzuki et al. 1997, Saiki et al. 2003). Yeast cells were grown in YE (0.5% yeast extract, 3% glucose) or PM minimal medium with appropriate supplements as described previously (Kawamukai et al. 1992). YEA and PMA contain 75 μg of adenine per ml in YE and PM, respectively. The concentration of the supplemented amino acids was 100 μg ml^{-1}. *Agrobacterium tumefaciens* (EHA101) and *Nicotiana tabacum* L.cv. Bright yellow (BY-2) were used for the localization study of SPS1 and SPS2.

A microscope (Olympus BX51, DP70) equipped with epi-fluoresence was used for observation of GFP fluoresence. To transform tobacco BY-2 cells, 1 ml of *Agrobacterium* was grown in LB + kanamycin (50 μg ml^{-1}) and hygromycin (50 μg ml^{-1}) at 28°C for 2 d, then 75 μl of the bacteria were added to a Petri dish containing 7.5 ml of 4-day-old BY-2 cells and mixed thoroughly. Plates were further incubated for 2 d at 22°C. The

BY-2 cells were washed by BY-2 liquid medium without selective drugs. Cells were selected on BY-2 plates (kanamycin 200 μg ml^{-1}, carbenicillin 500 μg ml^{-1}), and incubated at 26°C. Transformants that grew large enough were transferred to fresh BY-2 plates in 3–4 weeks.

For construction of pBSSK-SPS1 and pBSSK-SPS2, full length *SPS1* and *SPS2* genes were amplified by PCR with oligonucleotide primers of N-SPS1-*Bam*HI (5′-GCGGATCCTATGATGACGTCATGTC-3′) and C-SPS1-*Sma*I (5′-GTCCCGGGCTAATCAATTCTTTCG-3′) or N-SPS2-*Bam*HI (5′-GGG GATCCCATGTCATGTCGGAATATAG-3′) and C-SPS2-*Sma*I (5′-TACCCGGGCTAA TCAATCCTTTCAAGAT-3′), respectively. The amplified products were cloned into the *Bam*HI–*Sma*I sites of pBluescript SK$^+$, yielding pBSSK-SPS1 and pBSSK-SPS2.

For construction of pREP1-TPSPS1 and pREP1-TPSPS2, two oligonucleotide primers, namely, Mitocon1 (5′-AGGTCGACAGATTAGCATGTAAATAG-3′) and Mitocon2 (5′-ATGGATCCGGGGGTTACAGAGTTTGA-3′) were used to amplify the 345 bp fragment that contains the mitochondrial targeting peptide from *S. pombe* Ppt1 (TP45) (Saiki et al. 2003). The amplified product was digested with *Sal*I and *Bam*HI, and then cloned into the same sites of pUC119 to yield pUC119-TP45. Two other oligonucleotide primers, namely, N-TP-SPS1-B-NEW (5′-TCGGATCCA TGATGACGTCATGTCGG-3′) and C-SPS1-*Sma* I were used to amplify the full length of the *SPS1* gene. The amplified product was digested with *Bam*HI and *Sma*I, and then cloned into the same site of pUC119-TP45 to yield pUC119-TP45-SPS1. The fragment of TP45-SPS1 was amplified with two oligonucleotide primers of PPT–GFP1 and C-SPS1-*Sma*I by LA *Taq*, and then inserted into the pT7Blue-T vector to yield pT7-TP45-SPS1. The *Nde*I and *Sma*I fragment prepared from pT7-TP45-SPS1 was cloned into the same sites of pREP1 vector to yield pREP1-TPSPS1. Similarly, two oligonucleotide primers, namely, SPS2-M3 (5′-TAGGATCCATGTCATGTCGGAATATAG-3′) and C-SPS2-*Sma*I were used to amplify the full length of the *SPS2* gene. The amplified product was digested with *Bam*HI and *Sma*I, and then cloned into the same sites of pUC119-TP45 to yield pUC119-TP45-SPS2. The *Sal*I and *Sma*I fragment prepared from pUC119-TP45-SPS2 was cloned into the same sites of pREP1 vector to yield pREP1-TPSPS2.

For construction of pHMS-SPS1(TP) and pHMS-SPS2, four oligonucleotide primers were used to amplify the predicted targeting sequence of *SPS1* and the full length of *SPS2*: they were SPS1-BY-2-N (5′-GCTCTAGAATGATGACGTCATGTCGG-3′) and SPS1-TP-BY-2-C (5′-CGGGATCCAGTAAACTTACAAACAGTC-3′) or SPS2-BY-2-N (5′-GCTCTAGAATGATGATGTCATGTCGG-3′) and SPS2-BY-2-C (5′-CGGGATCCATCAATCCTTTCAAGATT-3′). The amplified products were digested with *Xba*I and *Bam*HI, and then cloned into the same sites of the pTH121 vector (Zhu et al. 1997) to yield pTH121-SPS1(TP) and pTH121-SPS2, respectively. The SPS1(TP)–GFP and SPS2–GFP fragments prepared from pTH121-SPS1(TP) and pTH121-SPS2 by *Sac*I were cloned into the same site of binary vector pHMS. The resulting constructs pHMS-SPS1(TP) and pHMS-SPS2 were used to transform tobacco BY-2 cells via *Agrobacterium*.

Acknowledgments

We thank Michigan State University and Kazusa Institute for cDNAs. This work is supported by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and Asahi glass foundation.

References

Bouvier, F., Suire, C., d'Harlingue, A., Backhaus, R.A. and Camara, B. (2000) Molecular cloning of geranyl diphosphate synthase and compartmentation of monoterpene synthesis in plant cells. *Plant J.* 24: 241–252.

Hirooka, K., Bamba, T., Fukusaki, E. and Kobayashi, A. (2003) Cloning and kinetic characterization of *Arabidopsis thaliana* solanesyl diphosphate synthase. *Biochem. J.* 370: 679–686.

Kadokura, H., Katzen, F. and Beckwith, J. (2003) Protein disulfide bond formation in prokaryotes. *Annu. Rev. Biochem.* 72: 111–135.

Kainou, T., Okada, K., Suzuki, K., Nakagawa, T., Matsuda, H. and Kawamukai, M. (2001) Dimer formation of octaprenyl diphosphate synthase (IspB) is essential for chain length determination of ubiquinone. *J. Biol. Chem.* 276: 7876–7883.

Kawamukai, M. (2002) Biosynthesis, bioproduction and novel roles of ubiquinone *J. Biosci. Bioeng.* 94: 511–517.

Kawamukai, M., Gerst, J., Field, J., Riggs, M., Rodgers, L., Wigler, M. and Young, D. (1992) Genetic and biochemical analysis of the adenylyl cyclase-associated protein, cap, in *Schizosaccharomyces pombe*. *Mol. Biol. Cell.* 3: 167–180.

Koyama, T. (1999) Molecular analysis of prenyl chain elongating enzymes. *Biosci. Biotechnol. Biochem.* 63: 1671–1676.

Liang, P.H., Ko, T.P. and Wang, A.H. (2002) Structure, mechanism and function of prenyltransferases. *Eur. J. Biochem.* 269: 3339–3354.

Lange, B.M. and Ghassemian, M. (2003) Genome organization in *Arabidopsis thaliana*: a survey for genes involved in isoprenoid and chlorophyll metabolism. *Plant Mol. Biol.* 51: 925–948.

Larsen, P.L. and Clarke, C.F. (2002) Extension of life-span in *Caenorhabditis elegans* by a diet lacking coenzyme Q. *Science* 295: 120–123.

Maundrell, K. (1993) Thiamine-repressible expression vectors pREP and pRIP for fission yeast. *Gene* 123: 127–130.

Okada, K., Kainou, T., Matsuda, H. and Kawamukai, M. (1998a) Biological significance of the side chain length of ubiquinone in *Saccharomyces cerevisiae*. *FEBS Lett.* 431: 241–244.

Okada, K., Kainou, T., Tanaka, K., Nakagawa, T., Matsuda, H. and Kawamukai, M. (1998b) Molecular cloning and mutational analysis of the *ddsA* gene encoding decaprenyl diphosphate synthase from *Gluconobacter suboxydans*. *Eur. J. Biochem.* 255: 52–59.

Okada, K., Kamiya, Y., Zhu, X., Suzuki, K., Tanaka, K., Nakagawa, T., Matsuda, H. and Kawamukai, M. (1997a) Cloning of the *sdsA* gene encoding solanesyl diphosphate synthase from *Rhodobacter capsulatus* and its functional expression in *Escherichia coli* and *Saccharomyces cerevisiae*. *J. Bacteriol.* 179: 5992–5998.

Okada, K., Minehira, M., Zhu, X., Suzuki, K., Nakagawa, T., Matsuda, H. and Kawamukai, M. (1997b) The *ispB* gene encoding diphosphate synthase is essential for growth of *Escherichia coli*. *J. Bacteriol.* 179: 3058–3060.

Okada, K., Ohara, K., Yazaki, K., Nozaki, K., Uchida, N., Kawamukai, M., Nojiri, N. and Yamane, H. (2004) The *AtPPT1* gene encoding 4-hydroxybenzoate polyprenyl diphosphate transferase in ubiquinone biosynthesis is required for embryo development in *Arabidopsis thaliana*. *Plant Mol. Biol.* 55: 567–577.

Okada, K., Saito, T., Nakagawa, T., Kawamukai, M. and Kamiya, Y. (2000) Five geranylgeranyl diphosphate synthases expressed in different organs are localized into three subcellular compartments in *Arabidopsis*. *Plant. Physiol.* 122: 1045–1056.

Okada, K., Suzuki, K., Kamiya, Y., Zhu, X., Fujisaki, S., Nishimura, Y., Nishino, T., Nakagawa, T., Kawamukai, M. and Matsuda, H. (1996) Polyprenyl diphosphate synthase essentially defines the length of the side chain of ubiquinone. *Biochim. Biophys. Acta* 1302: 217–223.

Saiki, R., Nagata, A., Uchida, N., Kainou, T., Matsuda, H. and Kawamukai, M. (2003) Fission yeast decaprenyl diphosphate synthase consists of Dps1 and the newly characterized Dlp1 protein in a novel heterotetrameric structure. *Eur. J. Biochem.* 270: 4113–4121.

Saito, T., Niwa, Y., Ashida, H., Tanaka, K., Kawamukai, M. Matsuda, H. and Nakagawa, T. (1999) Expression of a gene for cyclophilin which contains an amino-terminal endoplasmic reticulum-targeting signal. *Plant Cell Physiol.* 40: 77–87.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, U.S.A.

Suzuki, K., Okada, K., Kamiya, Y., Zhu, X., Tanaka, K., Nakagawa, T., Kawamukai, M. and Matsuda, H. (1997) Analysis of the decaprenyl diphosphate synthase (*dps*) gene in fission yeast suggests a role of ubiquinone as an antioxidant. *J. Biochem.* 121: 496–505.

Suzuki, K., Ueda, M., Yuasa, M., Nakagawa, T., Kawamukai, M. and Matsuda, H. (1994) Evidence that *E. coli ubiA* product is a functional homolog of yeast COQ2, and the regulation of the *ubiA* gene expression. *Biosci. Biotechnol. Biochem.* 58: 1814–1819.

Swiezewska, E. (2004) Ubiquinone and plastoquinone metabolism in plants. *Methods Enzymol.* 378: 124–131.

Swiezewska, E., Dallner, G., Andersson, B. and Ernster, L. (1993) Biosynthesis of ubiquinone and plastoquinone in the endoplasmic reticulum–Golgi membranes of spinach leaves. *J. Biol. Chem.* 268: 1494–1499.

Turunen, M., Olsson, J. and Dallner, G. (2004) Metabolism and function of coenzyme Q. *Biochim. Biophys. Acta* 1660: 171–199.

Uchida, N., Suzuki, K., Saiki, R., Kainou, T., Tanaka, K., Matsuda, H. and Kawamukai, M. (2000) Phenotypes of fission yeast defective in ubiquinone production due to disruption of the gene for *p*-hydroxybenzoate polyprenyl diphosphate transferase. *J. Bacteriol.* 182: 6933–6939.

Zhu, X.F., Suzuki, K., Saito, T., Okada, K., Tanaka, K., Nakagawa, T., Matsuda, H. and Kawamukai, M. (1997) Geranylgeranyl pyrophosphate synthase encoded by the newly isolated gene GGPS6 from *Arabidopsis thaliana* is localized in mitochondria. *Plant Mol. Biol.* 35: 331–341.

(Received August 16, 2004; Accepted October 7, 2004)