Chorismate Pyruvate-Lyase and 4-Hydroxy-3-solanesylbenzoate Decarboxylase Are Required for Plastoquinone Biosynthesis in the Cyanobacterium Synechocystis sp. PCC6803

Christian Pfaff1, Niels Glindemann1, Jens Gruber1, Margrit Frentzen1, and Radin Sadre1,2

From the 1Institute for Biology I, Botany, RWTH Aachen University, Worringerweg 1, 52056, Aachen, Germany and the 2Department of Biochemistry and Molecular Biology, Michigan State University, East Lansing, Michigan 48824

Background: Plastoquinone biosynthesis in cyanobacteria differs from that in plants.

Results: Chorismate pyruvate-lyase and 4-hydroxy-3-solanesylbenzoate decarboxylase single-gene knock-out mutants are severely affected in plastoquinone synthesis, cell size/structure, and growth.

Conclusion: The plastoquinone biosynthetic pathway likely evolved from a pre-existing ubiquinone pathway in cyanobacterial ancestors.

Significance: Cyanobacterial mutants deficient in plastoquinone biosynthesis allow deeper understanding of processes related to energy transformation.

Plastoquinone is a redox active lipid that serves as electron transporter in the bifunctional photosynthetic–respiratory transport chain of cyanobacteria. To examine the role of genes potentially involved in cyanobacterial plastoquinone biosynthesis, we have focused on three Synechocystis sp. PCC 6803 genes likely encoding a chorismate pyruvate-lyase (sll1797) and two 4-hydroxy-3-solanesylbenzoate decarboxylases (slr1099 and sll0936). The functions of the encoded proteins were investigated by complementation experiments with Escherichia coli mutants, by the in vitro enzyme assays with the recombinant proteins, and by the development of Synechocystis sp. single-gene knock-out mutants. Our results demonstrate that sll1797 encodes a chorismate pyruvate-lyase. In the respective knock-out mutant, plastoquinone was hardly detectable, and the mutant required 4-hydroxybenzoate for growth underlining the importance of chorismate pyruvate-lyase to initiate plastoquinone biosynthesis in cyanobacteria. The recombinant Slr1099 protein displayed decarboxylase activity and catalyzed in vitro the decarboxylation of 4-hydroxy-3-prenylbenzoate with different prenyl side chain lengths. In contrast to Slr1099, the recombinant Sll0936 protein did not show decarboxylase activity regardless of the conditions used. Inactivation of the sll0936 gene in Synechocystis sp., however, caused a drastic reduction in the plastoquinone content to levels very similar to those determined in the slr1099 knock-out mutant. This proves that not only slr1099 but also sll0936 is required for plastoquinone synthesis in the cyanobacterium. In summary, our data demonstrate that cyanobacteria produce plastoquinone exclusively via a pathway that is in the first reaction steps almost identical to ubiquinone biosynthesis in E. coli with conversion of chorismate to 4-hydroxybenzoate, which is then prenylated and decarboxylated.

Plastoquinone (PQ)2 is synthesized in oxygenic phototrophic organisms only. This prenylquinone plays a central role in photosynthesis. It functions primarily as an electron carrier of the photosynthetic electron transport chain in thylakoid membranes of plants and cyanobacteria where it transfers electrons from the photosystem II reaction center to the cytochrome b6f complex. Similarly to PQ in thylakoid membranes, ubiquinone functions as a mobile electron carrier in respiratory electron transport chains located in the plasma membrane of most aerobic bacteria and in the inner mitochondrial membranes of eukaryotes. Cyanobacteria, however, possess respiratory electron transport chains in both the plasma and the thylakoid membrane but do not synthesize ubiquinone (1–3). Rather, PQ seems to be the only diffusible prenylquinone in cyanobacterial electron transfer pathways.

The biosynthesis of PQ in plants occurs in the inner envelope membrane of plastids, where a homogentisate solanesyltransferase catalyzes the decarboxylation and the prenylation of homogentisate (4). The reaction product is subsequently methylated at position 3 so that PQ with a solanesyl (nonaprenyl) side chain is formed (5). PQ synthesis in Synechocystis sp. PCC 6803 was, in contrast, found to proceed independently of hydroxyphenylpyruvate dioxygenase activity for homogentisate production (6). This suggested that the cyanobacterium uses an alternative or additional pathway for the formation of PQ in comparison to plants. Interestingly, the Synechocystis sp. genome was predicted to encode homologs to some of the Escherichia coli proteins involved in ubiquinone biosynthesis, which probably contribute to PQ biosynthesis (6). Studies on the biosynthesis and role of PQ in cyanobacteria were, however, prevented by difficulties in generating complete loss of function mutants in candidate genes (1, 7). An in vivo labeling approach allowed us to show that Synechocystis sp. can use 4-hydroxybenzoate, or a metabolite derived from it, as an aromatic pre-

1 To whom correspondence should be addressed: Dept. of Biochemistry and Molecular Biology, Michigan State University, East Lansing, MI 48824. Tel.: 517-432-9283; Fax: 517-884-6965; E-mail: sadre@msu.edu.

2 The abbreviation used is: PQ, plastoquinone.
cursor for PQ. Moreover, we provided evidence for the existence of a membrane-bound aromatic prenyltransferase (Slr0926) in *Synechocystis* sp. that likely catalyzes the prenylation reaction in the PQ biosynthetic pathway similar to respective enzymes involved in ubiquinone biosynthesis (Fig. 1) (8).

The present study is focused on three *Synechocystis* sp. genes, *sll1797*, *slr1099*, and *sll0936*. We show by expression studies in *E. coli*, in vitro enzyme assays, and insertional mutagenesis in *Synechocystis* sp. that these genes are required for PQ biosynthesis, where they are involved in the reaction steps before and after the prenylation reaction.

**EXPERIMENTAL PROCEDURES**

**Chemicals**—Farnesyl diphosphate and geranylgeranyl diphosphate were obtained from Sigma-Aldrich. [U-14C]4-Hydroxybenzoate with a radiochemical purity of 98% and a specific activity of ~850 dpm/pmol was synthesized by alkaline fusion of [U-14C]-tyrosine (Hartman Analytis) essentially as described (9).

**Bacterial Strains and Growth Conditions**—Wild type *Synechocystis* sp. PCC6803 was obtained from the Pasteur Culture Collection. The *Synechocystis* sp. cultures were grown photoautotrophically in BG11 liquid medium at 30 °C on an orbital shaker at an photon flux density of 50 μmol m^{-2}s^{-1} using a 16-h light/8-h dark cycle (10). *Synechocystis* sp. mutant strains were propagated on BG11 agar plates containing up to 75 μg/ml kanamycin. The optical density of cyanobacterial suspensions was determined at 730 nm (D_{730}) with a photometer.

To express *sll1797*, *slr1099*, and *sll0936* as N-terminal GST fusion proteins in *E. coli* BL21AI, the respective coding sequences were amplified with the primers *sll1797* forward, 5′-AGG ATC CAT GAA GCT TTC TCC GCC CTG TTC C-3′; *sll1797* reverse, 5′-CTA GAT CTT CAC TCC CCT TCC ATA CCC CCT TG-3′; *slr1099* forward, 5′-CTA GAT CTT TAC ACG TCA TAG CCA AAC AAG TTG-3′; and *ubiD* forward was amplified with the primers *sll1797* forward GW, 5′-CAC CAT GAA GCT TTC TCC GCC CTG TTC C-3′; *sll1797* reverse, 5′-CTA GAT CTT CAC TCC CCT TCC ATA CCC CCT TG-3′; *slr1099* forward, 5′-CTA GAT CTT TAC ACG TCA TAG CCA AAC AAG TTG-3′; and *ubiD* reverse, 5′-CTA GAT CTT TAC ACG TCA TAG CCA AAC AAG TTG-3′ and *ubiD* was amplified from genomic DNA of *E. coli* with the primers ubiD forward GW, 5′-AGG ATC CAT GAA GCT TTC TCC GCC CTG TTC C-3′; *ubiD* reverse, 5′-CTA GAT CTT CAC TCC CCT TCC ATA CCC CCT TG-3′; and *ubiD* was amplified from genomic DNA of *E. coli* with the primers.

For the determination of the ubiquinone-8 content in *E. coli* strains, the respective wild type *E. coli* strains were cultivated in 200 ml of LB medium supplemented with 30 mM succinate and 50 μg/ml carbenicillin in a final concentration of 1 μM, 2-solanesylphenol; 5, 2-solanesyl-1,4-benzoquinol; 6, 3-methyl-5-solanesyl-1,4-benzoquinol; 7, plastoquinol; R, solanesyl.

medium or in M56 minimal medium (13) supplemented with 30 mM succinate as the sole carbon source. Whenever required, suitable antibiotics were added to the media (50 μg/ml kanamycin, 50 μg/ml carbenicillin, 30 μg/ml chloramphenicol). Growth in *E. coli* liquid cultures was monitored by measuring the increase in optical density at 600 nm (D_{600}). Efforts were made to generate an *E. coli* BW25113 *ubiD* knock-out mutant using essentially the methods described (14, 15).

**E. coli Expression Constructs**—The open reading frames *sll1797*, *slr1099*, and *sll0936* (16) were amplified by PCR from genomic DNA of *Synechocystis* sp. with the primers *sll1797* forward, 5′-AGG ATC CAT GAA GCT TTC TCC GCC CTG TTC C-3′; *sll1797* reverse, 5′-AGG ATC CAT GAA GCT TTC TCC GCC CTG TTC C-3′; *slr1099* forward, 5′-CTA GAT CTT TAC ACG TCA TAG CCA AAC AAG TTG-3′; and *ubiD* was amplified from genomic DNA of *E. coli* with the primers ubiD forward GW, 5′-AGG ATC CAT GAA GCT TTC TCC GCC CTG TTC C-3′; *ubiD* reverse, 5′-CTA GAT CTT TAC ACG TCA TAG CCA AAC AAG TTG-3′ and *ubiD* was amplified from genomic DNA of *E. coli* with the primers.

To express *sll1797*, *slr1099*, and *sll0936* as N-terminal GST fusion proteins in *E. coli* BL21AI, the respective coding sequences were amplified with the primers *sll1797* F, 5′-CAC CAT GAA GCT TTC TCC GCC CTG TTC C-3′; *sll1797* R, 5′-TTA CTC AAA TTT TGG CCT AGG CAA TTT C-3′; *slr1099* forward, 5′-CAC CAT GAA GCT TTC TCC GCC CTG TTC C-3′; *slr1099* reverse, 5′-CTA GAT CTT TAC ACG TCA TAG CCA AAC AAG TTG-3′; and *ubiD* reverse, 5′-CTA GAT CTT TAC ACG TCA TAG CCA AAC AAG TTG-3′ and *ubiD* was amplified from genomic DNA of *E. coli* with the primers.

For the determination of the ubiquinone-8 content in *E. coli* strains, the respective wild type *E. coli* strains were cultivated in 200 ml of LB medium supplemented with 30 mM succinate and 50 μg/ml carbenicillin in a final concentration of 1 μM, 2-solanesylphenol; 5, 2-solanesyl-1,4-benzoquinol; 6, 3-methyl-5-solanesyl-1,4-benzoquinol; 7, plastoquinol; R, solanesyl.

For the determination of the ubiquinone-8 content in *E. coli* cells, overnight cultures were diluted to D_{600} 0.1 in 100 ml of medium. At a D_{600} 0.5, gene expression was induced with iso-
determined. Ubiquinone-8 was extracted and analyzed by HPLC essentially as described (8).

Expression Studies in E. coli BL21AI—Heterologous gene expression in E. coli cells harboring pDEST15 expression constructs with the Synechocystis sp. genes sl1797, slr1099, and sll0936 was induced in liquid cultures at a D600 0.6 by addition of L-arabinose (final concentration, 0.2%) to the medium. The cultures were incubated for 2 h at 37 °C, and the cells were then harvested, washed, and disrupted by sonication to prepare crude cell extracts. Subcellular fractions were obtained by differential centrifugation. To this end, the crude cell extract was centrifuged at 5,000 × g, and the total protein extract was separated by 100,000 × g centrifugation in a soluble and a membrane protein fraction. For immunoblot analyses, the protein samples were separated by discontinuous SDS-polyacrylamide gel electrophoresis and then transferred onto PVDF membranes by semidry blotting. The tagged proteins were immunolabeled with horseradish peroxidase-conjugated GST antibodies (Roche Applied Science). After incubation with LumiLight (Roche Applied Science) substrate, chemiluminescence signals were visualized with a LAS1000 system (Fuji).

Plate Growth Assay and Growth Rates in Liquid Cultures of Synechocystis sp. Mutants—The sl1797::aph, slr1099::aph, and sll0936::aph mutant strains and the wild type were cultivated for 1 week in BG11 liquid medium. Mutant strains were grown in medium supplemented with 50 μg/ml kanamycin. The growth of the mutant strain sl1797::aph was dependent on the supply with 4-hydroxybenzoate that was added to the BG11 medium to a final concentration of 100 μM. For plate growth assays, the cells were sedimented by centrifugation, washed twice with BG11 medium, and resuspended in BG11 to a D730 of 0.1, followed by three 10-fold serial dilutions. Two microliters of each dilution were spotted onto nonselective BG11 agar plates and were incubated at 30 °C for 2 weeks.

The growth rates of Synechocystis sp. wild type and mutant strains were determined in BG11 liquid medium. The cultures were inoculated in nonselective BG11 medium to a D730 of 0.05, and progress of cell growth was determined by measuring the increase in the D730.

RNA Isolation and Real Time Quantitative PCR—Cells from 10 ml of liquid culture with exponentially growing Synechocystis sp. were pelleted by centrifugation at 4 °C and resuspended in 5 ml of TRIzol (17). The cell suspensions were incubated with 2-hydroxybenzoate as an internal standard. The ethyl acetate extracts were compared with those of calibration curves. The values for enzymic 4-hydroxybenzoate formation were corrected for nonenzymic breakdown of chorismate during assay incubation.

Chorismate Pyruvate-Lyase Assays—Protein extracts were assayed for chorismate pyruvate-lyase activity in 200-μl reaction mixtures containing 50 mM Bis-Tris propane/HCl, pH 8.0, and 450 μM chorismate. After 5 min of incubation at 30 °C, the assays were stopped and extracted with 300 μl of 3 M acetate buffer, pH 4.0, and 500 μl of ethyl acetate containing 2-hydroxybenzoate as an internal standard. The ethyl acetate extracts were subsequently analyzed by HPLC using an Agilent 1100 system with a C18 column (Macherey Nagel) and acetonitrile/water (1:1; v/v) with 0.1% trifluoroacetic acid (v/v) as solvent system at a flow rate of 0.5 ml/min at a wavelength of 255 nm. 4-Hydroxybenzoate and 2-hydroxybenzoate were synthesized enzymatically with recombinant Slr0926 as described previously (8). Briefly, E. coli membranes containing Slr0926 (20 μg total protein) were incubated in 1-ml reaction mixtures with 50 mM Bis-Tris propane/HCl, pH 7.0, 5 mM magnesium acetate, 50 μM [14C]-4-hydroxybenzoate (850 dpm/pmol), and 50 μM prenyl diphosphate at 30 °C for 30 min. The assays were stopped and extracted with 3 ml of chloroform/methanol (2:1, v/v) and 1 ml of 0.9% NaCl solution. Labeled 4-hydroxy-3-octaprenylenzoate was obtained by incubating E. coli ΔubiX mutant cultures with 14C-labeled 4-hydroxybenzoate because ΔubiX accumu-
lating the respective intermediate (18). The cells were harvested, resuspended in 1 ml of water, and disrupted with ceramic beads, and the lipophilic compounds were extracted two times with 10 ml of acetone/petroleum ether (3:2, v/v). The organic phases containing radiolabeled 4-hydroxy-3-prenylbenzoate were collected and dried under a stream of nitrogen gas, and the residues were dissolved in chloroform. The radiochemical purity of the 4-hydroxy-3-prenylbenzoate was analyzed via TLC using a silica gel-coated TLC plate and acetone/petroleum ether (3:7, v/v) as mobile phase and a FLA3000 bioimaging system for detection. The radiochemical concentration of the 14C-labeled 4-hydroxy-3-prenylbenzoate was determined by scintillation counting.

**Decarboxylase Assays**—The crude cell extracts from *E. coli* containing recombinant *Synechocystis* sp. protein were typically incubated in 400-μl reaction mixtures containing 50 mM Tris-HCl, pH 8.0, and ~1 μM 4-hydroxy-3-farnesyl-[U-14C]benzoate (850 dpm/pmol). The assays were started by the addition of 1.25 mg of protein. After 45 min at 30 °C, prenyllipids were extracted with 800 μl of chloroform/methanol (2:1, v/v) and analyzed via TLC using acetone/petroleum ether (3:7, v/v) as the solvent system. Labeled products were visualized with a FLA3000 bioimaging system and quantified by scintillation counting.

**Synechocystis sp. Mutagenesis**—To generate disruption cassettes, the *Synechocystis* sp. open reading frames in pQE60 constructs were partially deleted by restriction with AarI (pQE60-sll1797), Nael and SphI (pQE60-slr1099), and ClaI and NotI (pQE60-sll0936), and a kanamycin resistance cassette was amplified from pENTR/SD/D-TOPO (Invitrogen) with respective extensions and was inserted into the linearized plasmids. The constructs were used to generate *Synechocystis* sp. gene disruption mutants by homologous recombination (19). Transformants were grown for several plating cycles on BG11 medium containing increasing concentrations of kanamycin to obtain complete segregation. The highest kanamycin concentration was 75 μg/ml. Cultivation media for the sll1797::aph mutant were supplemented with 100 μM 4-hydroxybenzoiate.

**Decarboxylase Assays**—The crude cell extracts from *E. coli* containing recombinant *Synechocystis* sp. protein were typically incubated in 400-μl reaction mixtures containing 50 mM Tris-HCl, pH 8.0, and ~1 μM 4-hydroxy-3-farnesyl-[U-14C]benzoate (850 dpm/pmol). The assays were started by the addition of 1.25 mg of protein. After 45 min at 30 °C, prenyllipids were extracted with 800 μl of chloroform/methanol (2:1, v/v) and analyzed via TLC using acetone/petroleum ether (3:7, v/v) as the solvent system. Labeled products were visualized with a FLA3000 bioimaging system and quantified by scintillation counting.

**Synechocystis sp. Mutagenesis**—To generate disruption cassettes, the *Synechocystis* sp. open reading frames in pQE60 constructs were partially deleted by restriction with AarI (pQE60-sll1797), Nael and SphI (pQE60-slr1099), and ClaI and NotI (pQE60-sll0936), and a kanamycin resistance cassette was amplified from pENTR/SD/D-TOPO (Invitrogen) with respective extensions and was inserted into the linearized plasmids. The constructs were used to generate *Synechocystis* sp. gene disruption mutants by homologous recombination (19). Transformants were grown for several plating cycles on BG11 medium containing increasing concentrations of kanamycin to obtain complete segregation. The highest kanamycin concentration was 75 μg/ml. Cultivation media for the sll1797::aph mutant were supplemented with 100 μM 4-hydroxybenzoiate.

**Oxygen Measurement**—*Synechocystis* sp. cells were sedimented from cultures with a D730 of 2.0 and resuspended in BG11 medium to a final chlorophyll concentration of 2 μg/ml. The cell suspensions were then transferred into a liquid phase oxygen electrode chamber (Hansatech) and exposed to 50 μmol·m⁻²·s⁻¹ white light. The oxygen evolution rates were measured at 30 °C.

**Determination of Protein and Chlorophyll Contents**—Protein concentrations of total cell extracts were assayed by the Bradford method (20). The chlorophyll contents were determined in 80% acetone according to Lichtenthaler (21). The knowledge on ubiquinone biosynthesis in bacteria is mainly derived from *E. coli* mutant analyses coupled with genetic analyses. It is widely accepted that in *E. coli*, two unrelated proteins, UbiX and UbiD, catalyze the decarboxylation reaction that follows the prenylation of 4-hydroxybenzoate. Like other ubiquinone-deficient mutants, ubiX and ubiD insertional mutants are impaired in aerobic growth especially in media containing nonfermentable carbon sources (12, 18). Moreover, inactivation of either ubiX or ubiD alone resulted in the accumulation of the decarboxylase substrate 4-hydroxy-3-
octaprenylbenzoate, suggesting that both proteins have decarboxylase activity (12, 18). The *Synechocystis* sp. genome encodes two proteins, *Slr1099* (22.2 kDa) and *Sll0936* (55.2 kDa), which show 40 and 43% sequence identity to *E. coli* UbiX and UbiD, respectively. They represented, therefore, good candidates for the third reaction step in the PQ biosynthetic pathway (Fig. 1). Similarly to the *E. coli* proteins, the sequence identity between *Slr1099* and *Sll0936* is only 12%. Fig. 3 depicts the phylogenetic relationship of *Slr1099* and *Sll0936* with *UbiX*- and *UbiD*-like proteins from bacteria. The maximum likelihood tree was then generated, and bootstrap values were performed with 1000 replications (values shown next to branches). The scale bar indicates 0.5 amino acid substitutions per site. With the exception of the *Synechocystis* sp. proteins and the *E. coli* proteins *UbiX* and *UbiD*, all other proteins were shown to contribute to 4-hydroxybenzoate decarboxylation in vitro (30–34).

The reversible multisubunit hydroxyarylic decarboxylases/phenolic carboxylases of *Sedimentibacter hydroxybenzoicus*, *Bacillus subtilis*, *Streptomyces* sp. D7, and *E. coli* O157:H7 are encoded by the three clustered genes: B, C, and D. The products of the genes B and C are *UbiX*- and *UbiD*-like proteins, respectively. The *Synechocystis* sp. genes *slr1099* and *sll0936* are not clustered: *S. hydroxybenzoicus* ShdB (AA67850), ShdC (AAD50377); B. *subtilis*, BsdB (CA12157), BsdC (CA12158); *E. cloacae*, UbiX (CA19476), UbiD (CA19477); *Streptomyces* sp., VdcB (AAD28781), VdcD (AAD28782); *E. coli*, UbiX (NP_288885), UbiD (NP_418285); *Synechocystis* sp., *Slr1099* (NP_440078), *Sll0936* (NP_440197); C. *pneumoniae*, UbiD (NP_300232); *E. coli*, EcdB (NP_311620), and EcdC (NP_299287).

*E. coli* leads to reduced growth rates on media with succinate as the sole carbon source because of a severe decrease in ubiquinone content. As given in Fig. 4A, expression of the *Synechocystis* sp. gene *slr11797* in the *E. coli* mutant complemented the *ΔubiC* mutant phenotype despite the very low sequence identities between *Slr11797* and *UbiC* (Fig. 2). The complemented mutant showed growth rates even higher than the wild type control (Fig. 4A).

In addition, we tested *slr1099* and *sll0936* for their ability to complement an *E. coli* *ΔubiX* mutant to gain a first insight into the catalytic activity of these *Synechocystis* sp. proteins. Fig. 4B depicts the results from the complementation experiments with the *ΔubiX* mutant. Although expression of *slr1099* rescued the growth defect of the *ΔubiX* mutant, no complementation was obtained by expressing the *Synechocystis* sp. *slr1099* gene (Fig. 4B) or the *E. coli* *ubiD* gene (results not shown). The results were further confirmed by the analyses of the ubiquinone content in the *E. coli* mutants (Fig. 4, C and D). In these experiments, the *E. coli* mutants *ΔubiC* and *ΔubiX* expressing *slr11797* and *slr1099*, respectively, accumulated ~80% of the wild type ubiquinone levels.

Because no *ΔubiD* mutant was available from the Keio collection (11), further complementation experiments were performed in the *E. coli* strain AN66 that carries a point mutation in *ubiD* (2). To our surprise, neither gene expression of *ubiD* nor of *sll0936* or *slr1099* improved the growth rate and the ubiquinone content of AN66 in comparison to mutant cells with an empty vector (results not shown).
Intensive efforts were therefore made to develop an *E. coli* ubiD knock-out mutant. However, in contrast to Liu and Liu (35), who constructed an ubiD::amp mutant strain, we were not able to generate a viable ubiD knock-out mutant via insertional mutagenesis. Our results are in line with those of Baba et al. (11), who likewise failed to knock out the ubiD gene in *E. coli*.

These conflicting data suggest that the ubiD gene is essential for *E. coli* under certain conditions.

**Localization of the Synechocystis sp. Proteins in E. coli**—To determine the subcellular localization of the *Synechocystis* sp. proteins in *E. coli*, the recombinant proteins were expressed as GST fusion proteins. Total protein extracts were prepared, separated by high speed centrifugation in a soluble and a membrane protein fraction, and analyzed by SDS-PAGE and Western blotting using an antibody against the GST tag. Fig. 5 depicts results of Western blot analyses. A strong signal was detected for each fusion protein in the 100,000 × g membrane fraction, and a very weak signal was detected in the soluble protein fraction (100,000 × g supernatant). The data were surprising because none of the *Synechocystis* sp. proteins was predicted to possess typical transmembrane domains (36). Experiments with fusion proteins in which the N-terminal GST sequence was substituted by an N-terminal His epitope gave results similar to those shown in Fig. 5 (results not shown).

Attempts to support the subcellular localization of the *Synechocystis* sp. proteins by *in vitro* enzyme assays were successful with regard to the chorismate pyruvate-lyase fusion protein (GST-Sll1797), which showed severalfold higher specific activities in membrane than in soluble protein fractions (Fig. 5B).
by divalent cations or, in contrast to the *E. coli* chorismate pyruvate-lyase (37), by increasing the ion strength with NaCl or Bis-Tris propane buffer.

To optimize the conditions for decarboxylase assays, radio-labeled 4-hydroxy-3-prenylbenzoate substrates were synthesized enzymatically as described previously (8) and used as substrates for determining decarboxylase activity. Based on the results depicted in Fig. 5B, crude cell extracts from *E. coli* ΔubiX mutant cells expressing slr1099 were used for these experiments. After assay incubation, chloroform extracts of the reaction mixtures were subjected to TLC analyses as described under “Experimental Procedures.” Fig. 7 depicts some properties of the recombinant Slr1099. The *Synechocystis* sp. enzyme catalyzed the decarboxylation of 4-hydroxy-3-prenylbenzoate to 2-prenylphenol in vitro and accepted 4-hydroxy-3-prenylbenzoates with different prenyl side chain lengths up to 40 carbon atoms as substrate (Fig. 7C). It was slightly more active with 4-hydroxy-3-farnesylbenzoate than with 4-hydroxy-3-geranylgeranylbenzoate (Fig. 7D). We did not determine the dependence of Slr1099 on 4-hydroxy-3-octaprenylbenzoate, because this substrate was obtained by in vivo labeling with a too low specific activity. The highest Slr1099 activities were obtained at pH 8.5 with 4-hydroxy-3-farnesylbenzoate, an incubation temperature of 30 °C, and an incubation time of 45 min. The addition of divalent cations did not stimulate the Slr1099 activity. Under the optimized assay conditions, no native decarboxylase activity was detectable in protein extracts from *E. coli* ΔubiX cells containing the empty vector. In accordance with the results from complementation experiments (Fig. 4), we were unable to obtain detectable levels of 2-prenylphenols from assays with crude cell extracts from *E. coli* ΔubiX mutant cells expressing sll0936.

**Characterization of Synechocystis sp. Mutants Deficient in PQ Biosynthesis**—To demonstrate that the open reading frames *sll1797, slr1099*, and *sll0936* code for proteins involved in cyanobacterial PQ biosynthesis, we inactivated the wild type genes by inserting a kanamycin resistance gene (*aph*) into the open reading frames. The complete segregation of the wild type alleles was confirmed by PCR amplification (results not shown). In contrast to the PCR products of *sll1797* and *slr1099*, those of *sll0936* were restricted with *Pvu*I prior to electrophoretic analyses to allow univocal identification of wild type and mutated alleles. For each of the three *Synechocystis* sp. mutants DNA fragments of the corresponding mutated alleles were amplified, whereas the shorter wild type fragments were undetectable, suggesting that the mutants were homozygous for the *sll1797::aph, slr1099::aph*, and *sll0936::aph* gene disruptions. These results were further validated by analyzing the transcript levels of the three genes in the mutants in comparison to the respective wild type levels by real time quantitative PCR (Fig. 8). The data provided clear evidence that we succeeded in the development of *Synechocystis* sp. single-gene knock-out mutants lacking the chorismate pyruvate-lyase Sll1797, the decarboxylase Slr1099, or the putative decarboxylase Sll0936. Moreover, these results were supported by analyzing the growth rates of the *Synechocystis* sp. mutants. As depicted in Fig. 9A, cultivation of the mutated *Synechocystis* sp. strains

---

**Figure 5. Subcellular localization and enzyme activity of *Synechocystis* sp. proteins expressed in *E. coli*.** A, GST-Sll1797, GST-Slr1099, and GST-Sll0936 were expressed in E. coli BL21AI. Proteins of the 5,000 × g supernatant (cell extract, ce), 100,000 × g supernatant (s), and 100,000 × g sediment fraction (membranes, m) were separated by SDS-PAGE and analyzed by immunoblotting with GST antibodies. B, the enzyme activities of recombinant Sll1797 and Slr1099 in subcellular fractions of *E. coli* mutant cells ΔubiC and ΔubiX, respectively, are shown. 100% corresponds to relative specific activities of 4.5 μmol·min⁻¹·(μg protein)⁻¹ and 1.2 μmol·min⁻¹·(mg protein)⁻¹ for Sll1797- and Slr1099-containing fractions, respectively. The error bars represent the standard deviations of six independent sets of experiments. nd, not detectable; ce, 5,000 × g supernatant; s, 100,000 × g supernatant; m, 100,000 × g sediment.
under standard growth conditions in BG11 liquid medium resulted in drastically decreased growth rates compared with the parental strain. Under these conditions, the \textit{sll1797}::\textit{aph} mutant showed hardly any growth, but the addition of 4-hydroxybenzoate to the medium rescued the growth defect of the mutant. It showed growth rates even higher than that of the wild type control (Fig. 9A). Unlike the \textit{sll1797}::\textit{aph} mutant, both the \textit{slr1099}::\textit{aph} and \textit{sll0936}::\textit{aph} mutant strains were able to grow in BG11 medium but with growth rates distinctly lower than that of the wild type control. Moreover, cultivation of the different strains on BG11 agar plates illustrated the differences in growth rates of \textit{Synechocystis} sp. mutants and wild type strain even more impressively (Fig. 9B).

To determine whether the growth rates correlated with the PQ contents in the \textit{Synechocystis} sp. mutant strains, lipid extracts of the cultures were analyzed by HPLC. In line with the lowest growth rate, the \textit{sll1797}::\textit{aph} mutant strain had the lowest PQ content (Table 1). It was nearly 30-fold lower than that of the parental strain. The \textit{slr1099}::\textit{aph} and \textit{sll0936}::\textit{aph} mutants showed growth rates higher than \textit{sll1797}::\textit{aph} but lower than those of wild type cultures consistent with the determined PQ contents (Table 1). These low PQ levels, however, appeared to...
Initial Steps in Cyanobacterial Plastoquinone Biosynthesis

Phyloquinone was not affected in the mutants (Table 1). Disruption of one of the three *Synechocystis* sp. genes appeared to influence cell size/structure as reflected by the higher cell numbers/s/D₆₇₃₀ ratio, especially in cultures of *sll1797*∷*aph* and *sll0936*∷*aph* in comparison to the wild type control.

**DISCUSSION**

Previous studies indicated that cyanobacteria possess an alternative PQ biosynthetic pathway compared with the one in higher plants (6, 8). Here, we continued our studies on *Synechocystis* sp. PCC6803 as a model organism to gain further insight into cyanobacterial PQ biosynthesis. The enzymatic activity and properties of the hypothetical *Synechocystis* sp. protein Sll1797 were investigated. This small protein shares low sequence identities with the *E. coli* UbiC protein, which catalyzes the conversion of chorismate to 4-hydroxybenzoate and pyruvate, the initial step in ubiquinone biosynthesis. Sequence alignment with the sequences of UbiC and different putative chorismate pyruvate-lyases allowed us to predict two conserved amino acid residues (Arg-30 and Glu-120) likely forming part of the active site of Sll1797. The chorismate pyruvate-lyase activity of the *Synechocystis* sp. protein was demonstrated by complementation experiments in an *E. coli* ∆ubiC mutant lacking the respective enzymatic activity (Fig. 3) and by *in vitro* enzyme assays with crude and purified enzyme fractions from *E. coli* mutant cells expressing *sll1797* (Figs. 5 and 6). The data were supported and extended by the development and analysis of the *sll1797*∷*aph* knock-out mutant of *Synechocystis* sp. The mutant hardly produced any PQ, its photosynthetic electron transport rates were severely impeded (Table 1), and it required 4-hydroxybenzoate, the reaction product of the chorismate pyruvate-lyase, for growth (Fig. 9). Hence, these results provide compelling evidence that the chorismate pyruvate-lyase encoded by *sll1797* catalyzes the initial and essential step in PQ biosynthesis in *Synechocystis* sp.

Although the *Synechocystis* sp. protein Sll1797 possesses no typical transmembrane domains (results not shown), it was largely detected as membrane-associated protein in *E. coli* (Fig. 5). However, membrane association of Sll1797 in *E. coli* appeared to be rather weak, and purification of the recombinant *Synechocystis* sp. protein resulted in a soluble chorismate pyruvate-lyase protein (results not shown). Provided that membrane association occurs also in the native cellular environment, such an interaction can facilitate the transfer of the Sll1797 reaction product to the membrane bound 4-hydroxybenzoate solanesyltransferase, Slr0926, for the second step in PQ biosynthesis.

**FIGURE 8.** Expression analyses of the developed *Synechocystis* sp. mutant strains. Partial cDNA sequences were amplified by real time quantitative PCR with gene specific primers from total RNA of *Synechocystis* sp. WT and mutant strains. After 40 PCR cycles, the DNA products were separated by agarose gel electrophoresis. The expression levels were compared with the housekeeping genes *lysC* and *trpA*.

**FIGURE 9.** *Synechocystis* sp. gene disruption mutants have severe defects in growth. *A*, the mutant strains and the WT were cultivated in BG11 liquid medium under standard growth conditions. The growth defect of *sll1797*∷*aph* was rescued by the addition of 4-hydroxybenzoate (4-HB) to the liquid medium. The final concentration was 100 μM 4-hydroxybenzoate. Mean values of two independent sets of experiments with S.D. < 0.01 are given. *B*, serial dilutions of cultures from the wild type and the *Synechocystis* sp. mutant strains *sll1797*∷*aph*, *sll1099*∷*aph*, and *sll0936*∷*aph* were cultivated on a BG11 agar plate under standard conditions (10⁶ corresponds to A₆₇₃₀ 0.1).

be sufficiently high to sustain photosynthesis rates similar to those of wild type cultures. On the other hand, the very low PQ content of the *sll1797*∷*aph* mutant strain was reflected in an extremely weak photosynthesis rate, which comprised ~4% of the wild type only. In contrast to the PQ content, the level of
4-hydroxy-3-prenylbenzoate decarboxylase. The enzyme was functionally expressed and compensated the defect in growth rate and in ubiquinone synthesis of the E. coli ΔubiX mutant (Fig. 4). Like Sll0936, Slr1099 was predominantly found in the membrane fractions of E. coli (Fig. 5), whereas decarboxylase activity was detectable only in crude protein fractions harboring the recombinant Slr1099 protein. Perhaps separation of the soluble proteins from the membrane bound ones by high speed centrifugation (100,000 \( \times g \)) removed a compound from the membranes and/or disturbed a membrane-associated complex required for the catalytic active conformation of the decarboxylase. Biochemical studies on E. coli described that protein complexes are involved in ubiquinone biosynthesis (38, 39), and it was suggested that 2-octaprenylphenol, the decarboxylase product, may play a critical role in stabilizing or activating the O-methyltransferase UbiG, a downstream enzyme in ubiquinone biosynthesis (40). Similarly, cyanobacteria may require protein complexes for PQ biosynthesis, and intermediates like 4-hydroxy-3-solanesylbenzoate and 2-solanesylphenol might be important for stabilizing proteins such as the decarboxylase (Fig. 1).

Despite intensive efforts, conclusive evidence for the catalytic activity of Sll0936 and UbiD could not be provided. The sequence homologies to UbiD like aromatic decarboxylases (Fig. 3), however, suggest that both proteins have decarboxylase activity. Our finding that UbiD expression did not restore the functional head group (Fig. 10). For ubiquinone production, three steps involve presumably enzymes with altered substrate specificities in comparison to the E. coli proteins for ubiquinone biosynthesis. Plants, on the other hand, synthesize PQ via a pathway that is, apart from the isoprenoid precursor, identical to the first reaction steps during vitamin E biosynthesis. The vitamin E biosynthetic pathway is conserved in cyanobacteria

### Table 1

|          | WT       | sll1797:aph | slr1099:aph | sll0936:aph |
|----------|----------|-------------|-------------|-------------|
| Cells \( \times 10^9 \) \( \mu g^{-1} \) | 0.84 ± 0 | 2.52 ± 0.01 | 1.18 ± 0.07 | 1.91 ± 0.02 |
| Chlorophyll \( \mu g \) \( A_{730}^{-1} \) | 1.8 ± 0.51 | 1.3 ± 0.08 | 2.0 ± 0.63 | 2.4 ± 0.10 |
| PQ \( \mu g \) \( A_{730}^{-1} \) | 226 ± 15 | 8 ± 3 | 42 ± 16 | 67 ± 7 |
| Phylloquinone \( \mu g \) \( A_{730}^{-1} \) | 120 ± 16 | 117 ± 18 | 126 ± 17 | 119 ± 2 |
| Photosynthesis rates \( \text{nmol} \text{O}_2 \text{s}^{-1} (\mu g \text{Chl})^{-1} \) | 17.9 ± 0.45 | 0.7 ± 0.57 | 16.5 ± 0.59 | 14.9 ± 0.29 |

The data represent mean values and standard deviations of at least four independent sets of experiments.
FIGURE 10. The first steps of the PQ biosynthetic pathway in the cyanobacterium Synechocystis sp. in comparison to ubiquinone biosynthesis in the proteobacterium E. coli and PQ biosynthesis in the higher plant A. thaliana. The dashed arrows indicate that further hydroxylation and methylation reaction steps are required for ubiquinone and PQ biosynthesis.

and plants. A 4-hydroxyphenylpyruvate dioxygenase converts 4-hydroxyphenylpyruvate to homogentisate, the aromatic precursor for PQ (Fig. 10). In the next step, a bifunctional prenyltransferase catalyzes the decarboxylation as well as the prenylation of homogentisate so that 2-methyl-6-solanesyl-benzoquinol is formed. This intermediate is finally converted to PQ by the activity of a methyltransferase (Fig. 10).

Acknowledgment—We gratefully acknowledge help from Stephan Buchkremer in the characterization of Slr1099.

REFERENCES

1. Schultz, M., Foxberich, B., Ruxreth, S., Dyczmons, N. G., Roegner M., and Appel, J. (2009) Localization of cytochrome b6f complexes implies an incomplete respiratory chain in cyttoplasmic membranes of the cyanobacterium Synechocystis sp. PCC6803. Biochim. Biophys. Acta 1787, 1479–1485

2. Collins, M. D., and Jones D. (1981) Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. Microbiol. Rev. 45, 316–354

3. Scherer, S., Alpes, I., Sadowski, H., and Böger, P. (1988) Ferredoxin-NADP* oxidoreductase is the respiratory NADPH dehydrogenase of the cyanobacterium Anabaena variabilis. Arch. Biochem. Biophys. 267, 228–235

4. Sadre, R., Frentzen, M., Saeed, M., and Hawkes, T. (2010) Catalytic reactions of the homogentisate prenyl transferase involved in plastoquinone-9 biosynthesis. J. Biol. Chem. 285, 18191–18198

5. Cheng, Z., Sattler, S., Maeda, H., Sakuragi, Y., Bryant, D. A., and DellaPenna, D. (2003) Highly divergent methyltransferases catalyze a conserved reaction in tocopherol and plastoquinone synthesis in cyanobacteria and photosynthetic eukaryotes. Plant Cell 15, 2943–2956

6. Dähnhardt, D., Falk, J., Appel, J., van der Kooij, T. A., Schulz-Friedrich, R., and Krupinska, K. (2002) The hydroxyphenylpyruvate dioxygenase from Synechocystis sp. PCC6803 is not required for plastoquinone biosynthesis. FEMS Lett. 523, 177–181

7. Sakuragi, Y., and Bryant, D. A. (2006) In photosystem I. The Light-Driven Plastocyanin. Ferredoxin Oxidoreductase (Golbeck, J. H., ed) pp. 205–222, Springer, Dordrecht, The Netherlands

8. Sadre, R., Pfaff, C., and Buchkremer, S. (2012) Plastoquinone-9 biosynthesis in cyanobacteria differs from that in plants and involves a novel 4-hydroxybenzoate solanesyltransferase. Biochem. J. 442, 621–629

9. Ashby, M. N., and Edwards, P. A. (1990) Elucidation of the deficiency in two yeast coenzyme Q mutants. Characterization of the structural gene encoding hexaprenyl pyrophosphate synthetase. J. Biol. Chem. 265, 13157–13164

10. Ripplk, R. (1988) Isolation and purification of cyanobacteria. Methods Enzymol. 167, 3–27

11. Baba, T., Ara, T., Hasegawa, M., Takai, Y., Okumura, Y., Baba, M., Datsenko, K. A., Tomita, M., Wanner, B. L., and Mori, H. (2006) Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants. The Keco collection. Mol. Syst. Biol. 2006.0008

12. Cox, G. B., Young, I. G., McCann, L. M., and Gibson, F. (1969) Biosynthesis of ubiquinone in Escherichia coli K-12 location of genes affecting the metabolism of 3-octaprenyl-4-hydroxybenzoic acid and 2-octaprenylenol. J. Bacteriol 99, 450–458

13. Monod, J., Cohen-Bazire, S., and Cohn, M. (1951) Sur la biosynthèse de la p-galactosidase (lactase) chez Escherichia coli. La spécificité de l’induction. Biochim. Biophys. Acta 7, 585–599

14. Datsenko, K. A., and Wanner, B. L. (2000) One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR cloning programs. Proc. Natl. Acad. Sci. U.S.A. 97, 6640–6645

15. Link, A. J., Phillips, D., and Church, G. M. (1997) Methods for generating precise deletions and insertions in the genome of wild-type Escherichia coli. Application to open reading frame characterization. J. Bacteriol 179, 6228–6237

16. Nakao, M., Okamoto, S., Kohara, M., Fujishiro, T., Fujisawa, T., Sato, S., Tabata, S., Kaneko, T., and Nakamura, Y. (2010) Cyanobase. The cyanobacteria genome database update 2010. Nucleic Acids Res. 38, D379–D381

17. Chomczynski, P., and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162, 156–159

18. Gulmezian, M., Hyman, K. R., Marbois, B. N., Clarke, C. F., and Javor, G. T. (2007) The role of UbiX in Escherichia coli coenzyme Q biosynthesis. Arch. Biochem. Biophys. 467, 144–153

19. Williams, J. G. (1988) Construction of specific mutations in photosystem II photosynthetic reaction center by genetic engineering methods in Synechocystis 6803. Methods Enzymol. 167, 766–778

20. Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254

21. Lichtenthaler, H. K. (1987) Chlorophyll and carotenoids pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382

22. Aravind, L., and Anantharaman, V. (2003) Hct/FarR-like bacterial transcription factors of the GntR family contain a small molecule-binding domain of the chorismate lyase fold. FEMS Microbiol. Lett. 222, 17–23

23. Altshul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (1997) Gapped BLAST and PSI-BLAST. A new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402

24. Quevillon, E., Silventoinen, V., Pillai, S., Harte, N., Mulder, N., Apweiler, R., and Lopez, R. (2005) InterProScan. Protein domains identifier. Nucleic Acids Res. 33, W116–W120

25. Marchler-Bauer, A., Lu, S., Anderson, J. B., Chitsaz, F., Derbyshire, M. K., DeWeese-Scott, C., Fong, J. H., Heer, L. Y., Heer, R. C., Gonzales, N. R., Gwadz, M., Hurwitz, D. I., Jackson, J. D., Ke, Z., Lanczycki, C. J., Lu, F., Lipscomb, W., and Lipman, D. J. (1999) GenBank, Nucleic Acids Res. 27, 209–212

26. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P., McWilliam, H., Valentin, F., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J., and Higgins, D. G. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23, 2947–2948

27. Tabata, S., Kaneko, T., and Nakamura, Y. (2010) CyanoBase. The cyanobacteria genome database update 2010. Nucleic Acids Res. 38, D379–D381

28. Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., and Kumar, S. (2011) MEGA5. Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28, 2731–2739

29. Smith, N., Roitberg, A. E., Rivera, E., Howard, A., Holden, M. J., Mayhew, H. K., and Cohn, M. (1951) Sur la biosynthèse de l'induction. Biochim. Biophys. Acta 7, 585–599
Initial Steps in Cyanobacterial Plastoquinone Biosynthesis

M., Kaistha, S., and Gallagher, D. (2006) Structural analysis of ligand binding and catalysis in chorismate lyase. *Arch. Biochem. Biophys.* **445**, 72–80

30. Rangarajan, E. S., Li, Y., Iannuzzi, P., Tocilj, A., Hung, L. W., Matte, A., and Cygler, M. (2004) Crystal structure of a dodecameric FMN-dependent UbiX-like decarboxylase (Pad1) from *Escherichia coli* O157:H7. *Protein Sci.* **13**, 3006–3016

31. Liu, J., Zhang, X., Zhou, S., and Tao, P. (2007) Purification and Characterization of a 4-Hydroxybenzoate Decarboxylase from *Chlamyphila pneumoniae* AR39. *Curr. Microbiol.* **54**, 102–107

32. Matsui, T., Yoshida, T., Hayashi, T., and Nagasawa, T. (2006) Purification, characterization, and gene cloning of 4-hydroxybenzoate decarboxylase of *Enterobacter cloacae* P240. *Arch. Microbiol.* **186**, 21–29

33. Lupa, B., Lyon, D., Shaw, L. N., Sieprawska-Lupa, M., and Wiegel, J. (2008) Properties of the reversible nonoxidative vanillate/4-hydroxybenzoate decarboxylase from *Bacillus subtilis*. *Can. J. Microbiol.* **54**, 75–81

34. Liu, J., and Liu, J. H. (2006) Ubiquinone (Coenzyme Q) biosynthesis in *Chlamyphila pneumoniae* AR39. Identification of the ubiD gene. *Acta Biochim. Biophys. Sin.* **38**, 725–730

35. Krogh, A., Larsson, B., von Heijne, G., and Sonnhammer, E. L. (2001) Predicting transmembrane protein topology with a hidden Markov model. Application to complete genomes. *J. Mol. Biol.* **305**, 567–580

36. Siebert, M., Severin, K., and Heide, L. (1994) Formation of 4-hydroxybenzoate in *Escherichia coli*. Characterization of the *ubiC* gene and its encoded enzyme chorismate pyruvate-lyase. *Microbiology.* **140**, 897–904

37. Leppik, R. A., Stroobant, P., Shineberg, B., Young, I. G., and Gibson, F. (1976) Membrane-associated reactions in ubiquinone biosynthesis. *Biochim. Biophys. Acta* **428**, 146–156

38. Knöll, H. E. (1979) Isolation of a soluble enzyme complex comprising the ubiquinone-8 synthesis apparatus from the cytoplasmic membrane of *Escherichia coli*. *Biochim. Biophys. Res. Commun.* **91**, 919–925

39. Gulmezian, M., Zhang, H., Javor, T., and Clarke, C. F. (2006) Genetic evidence for an interaction of the UbiG O-methyltransferase with UbiX in *Escherichia coli* coenzyme Q biosynthesis. *J. Bacteriol.* **188**, 6435–6439

40. Tian, L., DellaPenna, D., and Dixon, R. A. (2007) The pds2 mutation is a lesion in the *Arabidopsis* homogentisate solanesyltransferase gene involved in plastoquinone biosynthesis. *Planta* **226**, 1067–1073

41. Johnson, T. W., Shen, G., Zybalov, B., Kolling, D., Reategui, R., Beuparlant, S., Vassiliev, I. R., Bryant, D. A., Jones, A. D., Golbeck, J. H., and Chitnis, P. R. (2000) Recruitment of a foreign quinone into the A1 site of photosystem I. Genetic and physiological characterization of phylloquinone biosynthetic pathway mutants in *Synechocystis* sp. PCC 6803. *J. Biol. Chem.* **275**, 8523–8530

42. Shimada, H., Ohno, R., Shibata, M., Ikegami, I., Onai, K., Ohno, M. A., and Takamiya, K. (2005) Inactivation and deficiency of core proteins of photosystems I and II caused by genetical phylloquinone and plastoquinone deficiency but retained lamellar structure in a T-DNA mutant of *Arabidopsis*. *Plant Cell* **17**, 1627–1637

43. Lefebvre-Legendre, L., Rappaport, F., Finazzi, G., Ceol, M., Grivet, C., Hopfgartner, G., and Rochaix J. D. (2007) Loss of phylloquinone in *Chlamydomonas* affects plastoquinone pool size and photosystem II synthesis. *J. Biol. Chem.* **282**, 13250–13263

44. Kruk, J., Jemiola-Rzeminska, M., and Strzalka, K. (1997) Plastoquinol and α-tocopherol quinol are more active than ubiquinol and α-tocopherol in inhibition of lipid peroxidation. *Chem. Phys. Lipids* **87**, 73–80