A Homolog of Albino3/OxaI Is Essential for Thylakoid Biogenesis in the Cyanobacterium *Synechocystis* sp. PCC6803*

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YidC/OxaI play essential roles in the insertion of a wide range of membrane proteins in *Escherichia coli* and mitochondria, respectively. In contrast, the chloroplast thylakoid homolog Albino3 (Alb3) facilitates the insertion of only a specialized subset of proteins, and the vast majority insert into thylakoids by a pathway that is so far unique to chloroplasts. In this study, we have analyzed the role of Alb3 in the cyanobacterium *Synechocystis* sp. PCC6803, which contains internal thylakoids that are similar in some respects to those of chloroplasts. The single *alb3* gene (slr1471) was disrupted by the introduction of an antibiotic cassette, and phototrophotrophic growth resulted in the generation of a merodiploid species (but not full segregation), indicating an essential role for Alb3 in maintaining the photosynthetic apparatus. Thylakoid organization is lost under these conditions, and the levels of photosynthetic pigments fall to ~40% of wild-type levels. Photosynthetic electron transport and oxygen evolution are reduced by a similar extent. Growth on glucose relieves the selective pressure to maintain photosynthetic competence, and under these conditions, the cells become completely bleached, again indicating that Alb3 is essential for thylakoid biogenesis. Full segregation could not be achieved under any growth regime, strongly suggesting that the slr1471 open reading frame is essential for cell viability.

The insertion of newly synthesized membrane proteins is a complex process that has been studied in a variety of experimental systems. In cases where the proteins insert post-translationally, such as with chloroplast and mitochondrial membrane proteins, hydrophobic proteins must avoid aggregation while exposed to aqueous environments and then insert into the correct membrane bilayer with the correct topology. During this process, hydrophilic loop regions need to be transferred across the entire bilayer. Many aspects of the overall insertion process have been studied, and popular model systems have included the bacterium *Escherichia coli*, as well as mitochondria and chloroplasts, both of which evolved from prokaryotes. In *E. coli*, it is known that most plasma membrane proteins initially interact with soluble translocation apparatus in the form of a signal recognition particle (SRP) and FtsY. These factors bind nascent or newly synthesized membrane proteins and transfer them to membrane-bound translocation apparatus that typically include the SecYEG translocon and an associated factor, YidC. Transfer into the membrane involves GTP hydrolysis by SRP and FtsY. A subset of inner membrane proteins does not require the SRP/Sec apparatus but, nevertheless, rely on YidC for efficient insertion, prompting suggestions that this protein may act as a “translocon” in its own right. With either pathway, the “positive-inside” rule exerts a major influence in determining the topology adopted by the protein during insertion.

Although descended from prokaryotes, mitochondria lack SRP, FtsY, and Sec apparatus (at least in most cases, including yeasts and mammals) and, instead, insert inner membrane proteins by other means. Carrier proteins are inserted during import by a pathway that appears to be unique to mitochondria (5, 6), but other proteins insert from the matrix side. These proteins include mitochondrial translation products together with cytosolically synthesized inner membrane proteins that are first imported into the matrix before insertion. Although the insertion mechanisms have not been fully elucidated, this pathway requires the action of Oxa1, a homolog of *E. coli* YidC (7).

In chloroplasts, the biogenesis of thylakoid membrane proteins occurs by at least two distinct pathways. Most are imported from the cytosol, after which a subset follows an SRP/FtsY-dependent pathway that resembles that of *E. coli* in many respects (8, 9) (reviewed in Ref. 10). The Oxa1 homolog Albino3 (Alb3) plays a vital role in the insertion of SRP substrates into the thylakoid membrane (11, 12). One possible difference, when compared with the *E. coli* pathway, is that the Sec apparatus may not be used for the insertion of membrane proteins. Thylakoids do contain a Sec system (13–15), but the available evidence suggests that it is used solely for the transport of luminal proteins (16). Although it has not been fully established that this Sec system is not involved in membrane protein insertion, current models suggest that Alb3 acts independently, as appears to be the case with its mitochondrial counterpart. Whether acting independently or in conjunction with other apparatus, it has been proposed that Oxa1 family proteins act as membrane-bound chaperones that stabilize transmembrane segments during the insertion process (17).

Although YidC and Oxa1 play vital roles in the insertion of a wide range of membrane proteins in *E. coli* and mitochondria, the same does not apply to Alb3 in chloroplasts. The SRP/Alb3 pathway is used by a surprisingly small set of thylakoid membrane proteins (reviewed in Ref. 10). In *vitro* insertion assays have shown that Alb3 plays a critical role in the insertion of the major light-harvesting chlorophyll-binding protein LcbH1 and a few other light-harvesting chlorophyll-binding proteins, but...
the vast majority of other proteins do not require SRP, FtsY, Sec apparatus, Alb3, or any known form of chloroplastic translocation apparatus for efficient insertion into thylakoids (12, 18, 19). In keeping with this finding, studies on a Chlamydomonas reinhardtii mutant lacking functional Alb3 have shown that only this subset of proteins are depleted, with other thylakoid membrane proteins present at wild-type levels (20). Because these proteins do not require either nucleoside triphosphates or a proton motive force for insertion, it has been suggested that they may insert spontaneously into the thylakoid, although this has not been formally proven.

In this context, cyanobacteria represent interesting model systems, because they are prokaryotes but contain internal thylakoid membranes that resemble those of chloroplasts in several respects. This raises the question as to whether the Alb3 pathway is important for the majority of proteins, as in E. coli, or whether the novel “spontaneous” pathway predominates in the thylakoids of this organism. One possibility raised by previous studies is that Alb3 is important for the insertion of plasma membrane proteins, but not for thylakoid membrane biogenesis. In this study, we have addressed these questions by disrupting the gene encoding the single Alb3 homolog in Synechocystis sp. PCC6803. We show that the Alb3 homolog plays a vital role in thylakoid biogenesis and/or maintenance; depletion of the proteins leads to a severe loss of thylakoid content and organization, and further growth on glucose in the dark leads to a total loss of photosynthetic competence and subsequent cell death.

EXPERIMENTAL PROCEDURES

Growth of Synechocystis sp. PCC6803-G (Glucose-tolerant)—The glucose-tolerant laboratory strain of Synechocystis sp. PCC6803 (21), which exhibits phototrophic growth in the presence of 5 mM glucose, was used in this study. The cultures were maintained in 100-ml aliquots of BG11 minimal media (22) and grown at 30 μE/m²/s with continuous shaking.

Transformation of Synechocystis sp. PCC6803—Transformation was essentially as described in Ref. 21. An exponentially growing culture was resuspended to give a final cell concentration of $1 \times 10^9$ cells ml$^{-1}$.
Ten microliters of plasmid DNA were added to 150 µl of cells and incubated under normal growth illumination (30 µE m⁻²s⁻¹) at 30 °C for 60 min. The cells were then plated onto BG11 plates without selection and incubated for 16–18 h in the light. Antibiotic was introduced underneath the plate to the desired concentration (50 µg ml⁻¹ spectinomycin). Transformants appeared in 7–10 days and were streaked at least three times onto selective plates to ensure segregation. Final cultures of transformed Synechocystis were grown at 30 µE m⁻²s⁻¹ at 30 °C in 100-ml cultures of sterile BG11 supplemented with 50 µg ml⁻¹ of spectinomycin with constant shaking at 150 revolutions/min. Integration of the antibiotic cassette was confirmed by Southern blotting.

Generation of an slr1471 Disruption Mutant in Synechocystis sp. PCC6803—The forward Fslr1471 ACCGTTCTCGGCATAGCCTG (5′–3′) and reverse Rsrlr1471 GCTCGAAGAACTTCTCACTC (5′–3′) primers were used to generate a 1511 bp PCR product including the slr1471 open reading frame (ORF) and flanking regions. The PCR product was TA-cloned into the pCR2.1 vector according to the manufacturer’s instructions (TA Cloning® Kit, Invitrogen). The unique restriction site HincII within the cloned slr1471 ORF was used to linearize the vector, and the ω fragment was ligated in using a standard ligation protocol provided by the manufacturer (T4 DNA ligase, Fermentas Life Sciences), resulting in the disruption of the slr1471 gene. The ω fragment was restricted from the pH4511 plasmid (23) using flanking Smal restriction sites. Both HincII and Smal generate blunt-ended fragments allowing for a blunt-ended ligation. The resulting plasmid p1471TA was transformed into Synechocystis cells, with selection based on spectinomycin resistance from the presence of the ω cassette. Restriction digestion with BamHI was used to confirm the presence of the ω cassette, giving a restriction pattern of 2056 bp (ω fragment), 4478 bp, and 954 bp.

Chlorophyll Fluorescence and Oxygen Evolution Measurements—Chlorophyll fluorescence was measured at 30 °C using a pulse amplitude-modulated fluorometer (Hansatech, King’s Lynn, UK). Maximum photochemical efficiency was calculated as Fm(∅dark) − F/Fm(∅dark) and the quantum yield of photosystem II photochemistry during illumination was calculated as Fm − F/Fm.

Oxygen evolution experiments were carried out essentially according to Mannan and Pakrasi (24). Cells in liquid culture were harvested by centrifugation at 4000 revolutions/min for 5 min at room temperature. Following harvest, the cells were resuspended into a chlorophyll concentration of 5 µg in 3 ml of fresh BG11. Whole chain electron transport was measured using a Clark-type electrode in the presence of 10 mM NaHCO₃ at a constant temperature of 30 °C. Pigmentation measurements were taken according to Myers et al. (25).

Confocal Microscopy—Bleaching experiments were performed using a Leica DM RE microscope equipped with a Leica confocal scanner TCS SP2. In the absence of Chl autofluorescence of the wild-type Synechocystis, the alb3 mutant was monitored between 600 and 700 nm. The physiological application pack included in the Leica Confocal Software 2.5 was used for bleaching. Twenty-five scans at 400 Hz in 75 s were carried out and autofluorescence monitored per scan number.

Transmission Electron Microscopy—The cells from wild-type and mutant strains were collected and fixed in an equal volume of 6% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2, for 2 h. After fixation, the cells were washed in distilled water three times for 10 min. The fixed cells were then incubated in 1% osmium tetroxide, 0.1 M sodium cacodylate buffer, pH 7.2, containing 0.8% potassium ferricyanide for 60 min followed by washing in distilled water three times for 10 min. The cells were then embedded in 2% agar and incubated in 70% ethanol for 30 min at room temperature followed by a further overnight incubation in 100% ethanol. Following dehydration, the cells were embedded in Spurr’s resin (26), and 60–90 nm sections were cut using a Reichert ultracut 4 ultramicrotome. Ultrathin sections were then contrasted with 2% aqueous uranyl acetate followed by Reynolds lead citrate and viewed using a JEOL 1220 transmission electron microscope (JEOL UK Ltd., Images were recorded on either Kodak SO-163 electron microscopy film or captured with an SIS Megaview III digital camera (Soft Imaging Systems, Gmbh).

RESULTS

Depletion of Alb3 in Synechocystis—Data base searches of the Synechocystis sp. PCC6803 genome reveal the presence of a single open reading frame, slr1471, encoding a protein homologous to the Oxl/YidC/Alb3 family of proteins (27). The 42.6 kDa slr1471 gene product is 38.5% identical to the Arabidopsis thaliana Alb3 protein and is predicted to contain several transmembrane spans (not shown). The slr1471 gene product is hereafter termed Alb3 in view of the fact that it is more homologous to chloroplastic Alb3 than to other members of the Oxl family of proteins.

The presence of a single alb3 gene in Synechocystis is interesting, because it implies that this protein may facilitate the insertion of proteins into both the plasma and thylakoid membranes. However, it is also possible that thylakoids do not require this protein, because all of the chloroplastic Alb3 substrates (major three-membrane-span light-harvesting chlorophyll-binding proteins) are absent in Synechocystis. To address this issue, we set out to delete the Alb3 protein in Synechocystis using standard techniques in which the slr1471 ORF is disrupted with a spectinomycin cassette by homologous recombination. Fig. 1A shows the location of the slr1471 ORF within the Synechocystis genome. The adjacent ORFs, slr1470 and slr1472, encode hypothetical proteins of unknown function, and the available evidence from data base tools, such as the genome atlas program (www.cbs.dtu.dk/services/GenomeAtlas) indicates that each has its own promoter. Of the other ORFs, slr1384 encodes a protein homologous to DnaJ, and the slr1383 gene product is an apparent myo-inositol-1-(or 4)-monophosphatase. None are involved in protein insertion processes or
photosynthesis, and each appears to be transcribed independently; hence, insertional inactivation of slr1471 should not lead to pleiotropic effects through polar effects on adjacent genes. The construct used, p1471TA/H9024, is shown in detail in Fig. 1B.

Glucose-tolerant Synechocystis sp. PCC6803 cells were transformed with p1471TA/H9024 and grown on plates in the presence of spectinomycin as detailed under “Experimental Procedures.” This wild-type strain differs from obligatorily photoautotrophic strains in containing a single point mutation in a two-component signaling system that confers the ability to grow heterotrophically on glucose in the dark (in practice, low intensity blue light is required for cell viability (28), but this
level of light is too low to support photosynthesis, and these conditions are hereafter referred to as dark-grown. Initial studies were carried out under phototrophic conditions, i.e., in the absence of glucose, where the cells rely totally on photosynthesis as an energy source. Resistant colonies were analyzed by Southern blotting using the slr1471 coding sequence as probe to test for the integration of the Ω cassette. Fig. 2 shows a Southern blot of HincII-digested DNA from wild-type cells and Δslr1471Ω cells grown photoautotrophically in the light or in the dark on glucose. Fig. 2, lanes 1–3 show the results from the two different forms of wild-type cells, namely glucose-intolerant cells grown in the light (lane 1) and the glucose-tolerant strain used in this study, grown in the light (lane 2) or with glucose in the dark (lane 3). The blot shows the presence of the expected 2.1- and 2.8-kbp hybridizing bands (see Fig. 1 for restriction sites in this region). The remaining lanes contain HincII-digested DNA from the Δslr1471Ω transformant grown photoautotrophically in the light (Fig. 2, lanes 4 and 5, two independent cultures) or grown in the dark on glucose (lane 6). In each case, the 2.1- and 2.8-kbp bands are evident, together with a 4.1-kbp band that represents the 2.1-kbp HincII fragment with an inserted ω cassette. These data show that the cells are merodiploid, containing a mixture of wild-type and disrupted slr1471 ORFs, and identical results were obtained with every Δslr1471Ω culture studied, whether grown in the light or dark. Full segregation could not be achieved, and further increases in antibiotic concentration led to cell death, indicating that Alb3 is essential for viability in general.

Depletion of Alb3 Leads to Major Changes in Thylakoid Morphology under Photoautotrophic Growth Conditions—Merodiploid Δslr1471Ω cells were generated by growth in the light in the presence of spectinomycin and initially analyzed by electron microscopy; Fig. 3 shows a group of four wild-type cells (grown under identical conditions apart from the absence of antibiotic) and two panels of Δslr1471Ω cells. The wild-type cells exhibit the usual morphology in which the thylakoids are found in the periphery of the cells, organized in several discrete groups that are each apparently tethered to the plasma membrane. In contrast, the Δslr1471Ω cells fall into two groups. In a minority of cells (3% of those analyzed in this study), distinct thylakoid membranes can be visualized, although at levels lower than in wild-type cells; four examples are shown in panel Δslr1471Ω (1). The remainder, and vast majority of the cells, are as shown in panel Δslr1471Ω (2). The periphery of the cell contains electron-dense material that is pigmented and photosynthetically competent (see Fig. 6); this region thus does contain thylakoids, but the morphology of these membranes is very different, and they are not organized to any detectable extent. It is notable that the thylakoids do not exhibit the level of separation that is evident with wild-type thylakoids. The Δslr1471Ω cells are also slightly smaller than wild-type cells, with the diameter reduced by an average of 10%.

Δslr1471Ω Cells Contain Reduced Levels of Pigment but Are Photosynthetically Competent—Whole cell absorption spectra for Δslr1471Ω cells under these conditions show an overall decrease in pigmentation in the mutant compared with wild-type cells (Fig. 4A). Decreases are seen in the absorption maxima of Δslr1471Ω cells at wavelengths of 625 and 686 nm, corresponding to phycocyanin and chlorophyll, respectively. In addition, absorption in the Soret region is lower in the mutant compared with the wild type. Direct measurement of pigment content/unit cell confirms the decreases in both phycocyanobilin and chlorophyll in the Δslr1471Ω mutant, as shown in the tabular data below the absorption spectra. In both cases, the pigment level is approximately one-third of the wild-type levels.

Membranes were prepared from both wild-type and Δslr1471Ω cells and the Coomassie-staining polypeptide profile is shown in Fig. 4B. Many of the bands present in the wild-type membranes are also present in the Δslr1471Ω membranes, but there are notable differences, and the asterisks denote polypeptides that are enriched in one membrane or the other. These polypeptides have yet to be identified, and it is presently unclear whether they are located in the thylakoid or plasma membrane.

Light saturation curves for oxygen evolution were measured for both wild-type and Δslr1471Ω cells (Fig. 5). Maximum rates of photosynthesis are lower for Δslr1471Ω cells when compared with the wild type, with values of 580 μmol O₂/mg chl/hr and 1600 μmol O₂/mg chl/hr, respectively. However, the values are expressed on a unit chlorophyll basis and mirror the decreases in chlorophyll content/cell shown in Fig. 4, which in turn reflects the variable loss of thylakoids observed in the electron micrograph images. As with the chlorophyll measurements, the lower values of photosynthetic rate observed in Δslr1471Ω samples represent an average decrease across the cell sample.

Room temperature chlorophyll a fluorescence was used to
monitor the maximum photochemical efficiency of photosystem II (PSII) in the dark \( (F_m^{\text{dark}} - F_s/F_m^{\text{dark}}) \) and the quantum yield of PSII during constant illumination \( (F_m^0/F_m^-) \). Relative decreases in the maximum photochemical efficiency of PSII in the dark measure photoinhibition (29). Fig. 6A shows measurements of \( F_m^{\text{dark}} - F_s/F_m^{\text{dark}} \) for wild-type and mutant cells following exposure to moderately high saturating irradiance \((800 \text{ mol quanta m}^{-2} \text{s}^{-1})\). Both wild-type and \( \Delta \text{slr}1471\Omega \) cells showed a slight decrease in \( F_m^{\text{dark}} - F_s/F_m^{\text{dark}} \) during 2 h of exposure to the saturating irradiance. The similarity in response to saturating light treatment suggested that the mutant was equally resistant to photoinhibition when compared with wild-type Synechocystis.

The chlorophyll fluorescence parameter \( F_m^0 - F_s/F_m^- \) measures the quantum yield of PSII during constant illumination and reflects the efficiency of PSI electron transport. When measured across a range of actinic irradiance, the quantum efficiency of PSII is slightly lower than the wild type (Fig. 6B). However, the shape of the irradiance dependence curve for \( F_m^0/F_m^- \) is similar in both the mutant and the wild type, suggesting that, despite slightly lower overall efficiencies, the \( \Delta \text{slr}1471\Omega \) mutant is capable of appreciable rates of photosynthetic electron transport across a range of light intensities. Taken together, these data and the resistance to photoinhibition indicate that the thylakoid membranes remaining in the \( \Delta \text{slr}1471\Omega \) mutant are reasonably functional.

Although the disorganized thylakoids are depleted but relatively functional in photosynthetic terms, other studies show that the thylakoids in the \( \Delta \text{slr}1471\Omega \) cells bleach more rapidly than do those of wild-type cells under some circumstances. The lower panels of Fig. 7 show confocal microscopy images of wild-type and \( \Delta \text{slr}1471\Omega \) cells before and after scanning with the 543-nm confocal laser. The images confirm that the \( \Delta \text{slr}1471\Omega \) cells are slightly smaller than wild-type cells, as found in the electron micrographs, and that red autofluorescence from pigments is lower than that of wild-type cells (reflecting reduced pigmentation). The graph in Fig. 7 shows a quantitative measure of the autofluorescence from the entire field of cells after repeated exposure to illumination from the laser. Very little bleaching is observed with the wild-type cells, whereas that of wild-type cells was reduced by 40%, whereas that of wild-type cells was reduced by only 4.5%. These data, in conjunction with the electron microscopy and photosynthetic assays, indicate that the \( \Delta \text{slr}1471\Omega \) cells are affected in terms of thylakoid organization and both the capacity and integrity of the photosynthetic apparatus as a whole.

Growth on Glucose Results in a Complete Loss of Pigment and Photosynthetic Competence—Complete segregation of the disrupted \( \text{alb}3 \) gene cannot be achieved when the cells are grown photoautotrophically, which points to an essential role of the \( \text{alb}3 \) protein in thylakoid and/or plasma membrane biogenesis. The former is likely because thylakoid function is essential under these circumstances, and even partial segregation results in a >50% loss of pigment, an equivalent loss of photosynthetic competence, and a major change in thylakoid morphology. Presumably, this merodiploid state represents a compromise between the expression of the \( \omega \) cassette growing on spectinomycin and the expression of functional \( \text{alb}3 \) protein in maintaining photosynthetic competence. To further analyze...
the role of Alb3, we grew the Δslr1471Ω cells in the dark in the presence of glucose, as detailed under “Experimental Procedures.” Under these conditions, the selective pressure to maintain photosynthesis is removed, and we observed a major difference with the Δslr1471Ω cells. These cells rapidly became bleached, and the confocal images in Fig. 8 show that, at this point, they contain essentially no photosynthetic pigments. Fig. 8, upper panels, show the pigment autofluorescence from wild-type cells that exhibit the characteristics shown in Fig. 7. Fig. 8, middle panels, show Δslr1471Ω cells grown under photoautotrophic conditions with reduced fluorescence and slightly smaller dimensions, as shown in the upper panels. Fig. 8, lower panels, show cells from the same culture after 4 days of growth in the dark in the presence of 5 mM glucose. In parallel cultures,
wild-type glucose-tolerant cells maintained full photosynthetic competence, and the thylakoids are essentially identical after this period on glucose, as found in previous studies (21, 28, 29) (not shown). However, the lower panel of Fig. 8A shows that the Δslr1471Δ cells lose all photosynthetic pigments and become completely bleached. This is confirmed by the absorbance spectrum shown in Fig. 8B, which shows a complete absence of absorbance maxima for chlorophyll and phycocyanin.

Southern blotting studies (see Fig. 1) show that the cells still remain merodiploid at this stage, and cell death is inevitably observed after a further period of culture. During this period, the cells hardly grow at all. Even wild-type cells grow very slowly under these conditions when compared with photoautotrophic growth rates (21, 28), and the mutant cells may therefore be weakened to the extent that even expression of the ω cassette fails to support growth. Overall, these data strongly suggest that the Alb3 protein is required both for thylakoid biogenesis and cell viability.

DISCUSSION

OxaI family proteins play crucial roles in the insertion of membrane proteins in E. coli and mitochondria, but Alb3 appears to have a much more specialized function in chloroplast thylakoids. The initial alb3 null mutant in A. thaliana exhibited dramatic defects in chloroplast development, suggestive of a similarly important role in thylakoid biogenesis (30). However, more recent studies have shown that, although Alb3 is important for the insertion of a small (but important) group of light-harvesting chlorophyll-binding proteins (11, 12), the vast majority of chloroplast thylakoid membrane proteins insert by Alb3-independent mechanisms (12, 19, 20). Because these proteins insert by mechanisms that require none of the known translocation apparatus or energy sources, it has been suggested that they may insert spontaneously into the thylakoid membrane (12, 19). This raises the question of how the proteins avoid insertion into the wrong membrane after synthesis, and one possible explanation is that the highly unusual lipid composition may enable membrane proteins to insert into thylakoids but not other membranes. Thylakoid membranes are primarily composed of galactolipids (>80%), whereas other membranes in plant cells are phospholipid-based (31). Moreover, the level of desaturation in thylakoid lipids is significantly higher than in most other membrane types, suggesting that the overall fluidity may be correspondingly higher.

Cyanobacteria, such as Synechocystis, represent an interesting system in this context, because they too contain internal thylakoid membranes that are composed largely of galactolipid (32). However, these cells do not contain the three-span light-harvesting chlorophyll-binding proteins that are the exclusive substrates of chloroplast thylakoid Alb3; instead, light-harvesting is carried out by phycobilisomes that differ radically in both organization, composition, and pigment content. Prior to this study, we considered it possible that all thylakoid proteins insert by Alb3-independent mechanisms in Synechocystis and that it may be involved exclusively in plasma membrane biogenesis. In this study, we have addressed this issue by disruption of the Synechocystis alb3 gene, and several points have emerged.

First, there are clear indications that Alb3 plays an essential role in thylakoid biogenesis and morphology in Synechocystis. Photoautotrophic growth of the Δslr1471Δ transformant leads to partial segregation of the disrupted gene and even this partial loss of Alb3 activity leads to a consistent loss of thylakoid contents in the form of chlorophyll, phycocyanins, and photosynthetic electron transport capacity in general. Although we do not know the precise effects involved, it seems highly likely that these effects stem from defects in thylakoid protein biogenesis. However, it is notable that the thylakoid membranes are disorganized under these conditions and the Alb3 protein may therefore influence, directly or indirectly, the organization of thylakoid membranes, as well as the insertion of individual membrane proteins. In general, the data point to a fundamentally important role for Alb3 in this cyanobacterium, contrasting with the relatively specialized role that Alb3 plays in chloroplasts.

Growth on glucose in the dark results in an even more severe phenotype, emphasizing the vital nature of the Alb3 activity, and it is notable that the thylakoids again appear to be most badly affected under these conditions in the period prior to cell death. The complete loss of thylakoid electron transport indicates that Alb3 is essential for thylakoid maintenance, at a point where the plasma membrane is still intact and the cells are otherwise viable. Notably, complete segregation could not be achieved at any point, and the cells stop dividing and ultimately die. Once again, it is interesting that the reduced levels of Alb3 activity are unable to maintain viability under this growth regime, strongly suggesting that Alb3 is also essential for the biogenesis of the plasma membrane, as in E. coli.

Our data differ from those obtained in a previous study involving the disruption of the slr1471 ORF in Synechocystis (33). Here, the authors stated that full segregation could be achieved, and at this point, the cells were photosynthetically competent and viable. The major phenotype was an inhibition of cell division, reminiscent of the effects on chloroplast division when the homologous Artemis gene is disrupted in Arabidopsis (33). These authors did not use the glucose-tolerant strain of Synechocystis, but this strain differs from the photoautotrophic wild-type strain in only a single point mutation in a two-component signaling system and it is unlikely, in our opinion, that this difference would render the cells more dependent on Alb3 activity. Moreover, we did not observe any inhibition of cell division in the transformants used in this study. Further studies are required to reconcile these data, but in our studies, it should be emphasized that full segregation was never observed, and photoautotrophic growth inevitably led to a reproducible loss of thylakoid functionality.

Further work is required to unravel with more precision the role of Alb3 in this and other cyanobacteria, and it is especially interesting that a single form of Alb3 appears to be required for the biogenesis of the two different membrane systems. The mechanisms governing subcellular compartmentation in cyanobacteria are poorly understood at present, and it is not known whether membrane proteins are initially directed to the correct membrane or indiscriminately to either membrane. The latter scenario would appear likely if a single form of Alb3 is involved in the insertion of both thylakoid and plasma membrane proteins, but this would require subsequent “sorting” of the proteins by mechanisms that are very vague at present. Further studies on the location and role of Alb3 may contribute significantly to this area.

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