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The chloroplast signal recognition particle (CpSRP) pathway as a tool to minimize chlorophyll antenna size and maximize photosynthetic productivity

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ABSTRACT

The concept of the Truncated Light-harvesting chlorophyll Antenna (TLA) size, as a tool by which to maximize sunlight utilization and photosynthetic productivity in microalgal mass cultures or high-density plant canopies, is discussed. TLA technology is known to improve sunlight-to-product energy conversion efficiencies and is hereby exemplified by photosynthetic productivity estimates of wild type and a TLA strain under simulated mass culture conditions. Recent advances in the generation of TLA-type mutants by targeting genes of the chloroplast signal-recognition particle (CpSRP) pathway, affecting the thylakoid membrane assembly of light-harvesting proteins, are also summarized. Two distinct CpSRP assembly pathways are recognized, one entailing post-translational, the other a co-translational mechanism. Differences between the post-translational and co-translational integration mechanisms are outlined, as these pertain to the CpSRP-mediated assembly of thylakoid membrane protein complexes in higher plants and green microalgae. The applicability of the CpSRP pathway genes in efforts to generate TLA-type strains with enhanced solar energy conversion efficiency in photosynthesis is evaluated.

Contents

1. Introduction

Photosynthesis relies on the absorption and utilization of sunlight by the photosystems, embedded in the chloroplast thylakoids. Higher plants and green microalgae have developed an extensive light-harvesting system, composed of highly coordinated chlorophylls and carotenoids that absorb and funnel the energy of sunlight towards the photochemical reaction centers. Green algae and plants have developed rather large arrays of such light-harvesting complexes associated with the reaction centers of photosystem-I (PSI) and photosystem-II (PSII). These light-harvesting holocomplexes are assembled and become associated with PSI and PSII in the developing thylakoid membrane of chloroplasts. The *Lhca* gene subfamily encodes for proteins of the light-harvesting complex I (LHCI) associated with PSI, while the *Lhcb* gene subfamily encodes for proteins of the light-harvesting complex II (LHCII), associated with PSII (Jansson et al., 1992). The large light-harvesting antenna size found in wild type plants and algae is thought to confer survival advantages for the cell in the wild: it enables chloroplasts in these organisms to operate the photochemical reaction centers at maximum capacity even under low sunlight intensities. Thus, cells can grow under a wide range of light conditions, which are encountered in the natural environment, from the early morning hours right after sunrise, when the light intensity is low, over midday where the light intensity reaches a maximum, to late evening before sunset. Limiting light conditions for microalgae could arise from growing further down in the water column, where the light intensity is low even at midday or when plants grow in

Abbreviations: CpSRP, chloroplast signal recognition particle; TLA, truncated light-harvesting antenna; D1, the psbA-encoded 32 kD PSII reaction center protein; GTP, guanosine triphosphate; LHC, light-harvesting complex; NPQ, non-photochemical quenching; Chl, chlorophyll; PS, photosystem.

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the lower canopy of trees and forests. In both cases, the intensity of sunlight is attenuated often to below saturation for the process of photosynthesis. Independently, attenuation of sunlight intensity occurs due to cloud cover in the atmosphere (Kirk, 1994). It has been hypothesized that possessing a large light-harvesting chlorophyll antenna size in its chloroplasts affords the organism a competitive growth advantage under these light-limiting conditions. The other advantage of a large light-harvesting antenna size is indirect; it can be plausibly argued that by virtue of shading competing organisms in the same ecotype, enables an organism to grow more efficiently, while slowing down growth of competitors.

Having a large light-harvesting Chl antenna size results in over-absorption of direct sunlight, not all of which can be used for photosynthesis, as this is limited by the rate of the carbon reactions and the associated biochemical steps. Excess absorbed irradiance from direct sunlight is then dissipated by the process of non-photochemical quenching (NPQ), a mechanism designed to prevent photodamage of the photosystems in the chloroplast (reviewed by Müller et al., 2001). In summary, evolution has applied selective pressure for the assembly and function of large light-harvesting Chl antenna complexes in all photosynthetic systems and, at the same time, efficient non-photochemical quenching processes, that are both characteristics of the photosynthetic apparatus in wild type plants and algae.

When plants and algae are grown under direct sunlight in high-density unicultures for product generation, e.g. fuel and chemicals, efficient conversion of sunlight energy to product by the culture as a whole is highly desirable. This requirement is compromised by the over absorption and wasteful dissipation of sunlight by cells at the surface of an alga culture, or upper canopy of plants, thereby compromising productivity (Melis, 2009). In such production systems, it is desirable to prevent the over-absorption of sunlight, to alleviate unnecessary shading, and to enable better sunlight penetration and more efficient utilization, thus enabling cells deeper in a culture or canopy to perform photosynthetically. Engineering strains that do not over-absorb and wastefully dissipate sunlight, but photosynthetically utilize all of it, could theoretically afford substantial productivity benefits to mass cultures. This was recognized long ago (Kok, 1953, 1960; Myers, 1957), but a solution could not be found. The concept of the truncated light-harvesting antenna (TLA) in photosynthetic organisms has recently attracted attention, as a way by which to optimize growth and productivity in mass cultures under bright sunlight conditions. This review discusses the conditions upon which a strain with a truncated light-harvesting antenna (TLA) would be more productive in mass-culture compared to a corresponding wild type. The work also summarizes recent progress in the elucidation of the CpSRP assembly pathway for the integration of the light-harvesting proteins into developing thylakoid membranes and examines how this process can be exploited in biotechnology to generate strains with a truncated light-harvesting chlorophyll antenna size.

2. Minimizing the light-harvesting chlorophyll antennae to maximize photosynthetic productivity

There is current interest and on-going efforts to renewably generate fuel and chemicals for human industrial and domestic consumption, through the process of microalgal and plant photosynthesis. Such bioproducts include H_2 and other suitable hydrocarbon and alcohol fuel molecules (Greenwell et al., 2010; Hankamer et al., 2007; Hu et al., 2008; Mata et al., 2010; Melis, 2007, 2012), and antigens (Dauvillée et al., 2010; Mayfield et al., 2007; Michelet et al., 2011). To aid the economic aspects of such effort, sunlight energy conversion efficiency in photosynthesis must occur with the maximum possible, as this would help to make renewable fuel and chemical processes economically feasible. In plants and algae, the solar energy conversion efficiency of photosynthesis is thus a most critical factor for the economic viability of renewable biomass, fuel and chemical production (Melis, 2009).

At low sunlight intensities, i.e., below those required for the saturation of photosynthesis, all absorbed photons are utilized efficiently to drive electrons in the electron-transport chain. Under these conditions, photosynthetic productivity increases linearly with the level or irradiance (Fig. 1, 0–500 μmol photons m^{-2} s^{-1} range). As the level of irradiance increases further, photosynthesis becomes saturated and reaches a plateau due to the fact that the carbon reactions cannot keep up with the linear increase in light absorption (Fig. 1, linear). Strains with a wild type light-harvesting antenna system (Fig. 1, WT) reach this light intensity for saturation, I_s, at lower levels of irradiance than their TLA counterparts (Fig. 1, *tla3*). The light-saturation of photosynthesis signifies that the sunlight harvested by the chlorophyll antenna exceeds the maximal operational capacity of the electron-transport chain and of the carbon reactions of photosynthesis, rendering the excess absorbed photons useless. Under bright sunlight conditions (2500 μmol photons m^{-2} s^{-1}) wild type strains with their fully developed light-harvesting antenna utilize photons inefficiently; only about 20% of the incoming sunlight energy is converted into useful photosynthesis (Fig. 1), excess absorbed energy is dissipated by the NPQ process. This inefficiency can theoretically be alleviated upon minimizing the light-harvesting chlorophyll antenna size (Kok, 1953, 1960; Myers, 1957) to limit sunlight absorption. It has been shown that cultures populated with TLA strains are more productive, when grown under high cell densities and sunlight intensities (Formighieri et al., 2012; Melis, 2009; Melis et al., 1999; Nakajima and Ueda, 1997, 1999; Polle et al., 2003). Greater culture productivity results by diminishing over-absorption of sunlight at the surface of the culture and thus minimizing wasteful dissipation of energy, while at the same time allowing for a far greater transmittance of sunlight deeper into the culture by eliminating unwanted shading. Ideally, no saturation of photosynthesis should occur within the range of the natural daylight intensities (0–2500 μmol photons m^{-2} s^{-1} range, Fig. 1), such that every single absorbed photon is utilized in photosynthesis. Such corrective action could improve productivity of mass cultures by over 500% under bright sunlight conditions, as evident by comparing the measured productivity of a wild type strain at 2500 μmol photons m^{-2} s^{-1}, which is about 100 mmol O_2 per mol Chl per s for the wild type (Fig. 1, WT) to over 500 mmol O_2 per mol Chl per s, as the case would be when photosynthesis could increase linearly with light intensity in the entire spectral range (Fig. 1, linear). Practically, the theoretical max efficiency is not achieved under ambient sunlight, as photosynthetic productivity is

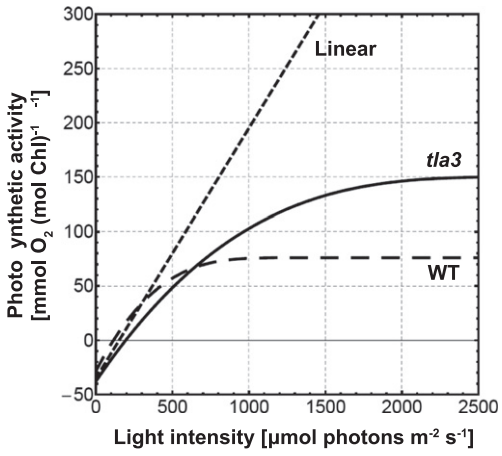

Fig. 1. The light saturation curves of photosynthesis. Activity was measured in O_2-evolution as a function of light intensity. Dotted line: theoretical maximal photosynthetic efficiency, which is linear with light intensity; dashed line: WT, wild type strains showed saturation of photosynthesis at about 500 μmol photons m^{-2} s^{-1}; solid line: *tla3* strain (Kirst et al., 2012b) shown here as an example for a TLA-type strain, showed saturation of photosynthesis at about 1000 μmol photons m^{-2} s^{-1}. A light intensity of 2500 μmol photons m^{-2} s^{-1} corresponds to that of bright sunlight.

attenuated due to over-absorption and wasteful dissipation of excess photons. It is thus desirable to generate strains that possess only the PSII-core and PSI-core complexes, and which possess a "core" light-harvesting antenna, that cannot be further reduced in size without disrupting the functionality of the reaction centers (Glick and Melis, 1988). The minimal number of chlorophyll molecules associated with a functional reaction center was measured by the spectrophotometric–kinetic method (Melis, 1989) to be 37 chlorophyll *a* (Chl *a*) molecules for PSII, and 95 Chl *a* molecules for PSI (Glick and Melis, 1988). These measurements were independently confirmed from the crystal structural analysis of the PSII-core complex, which showed 35 Chl molecules at a resolution of 1.9 Å molecules (Umena et al., 2011). Similarly, 96 Chl molecules where shown in the crystal structure of isolated PSI-core complexes (Jordan et al., 2001). A fully assembled light-harvesting antenna complex entails the assembly of peripheral Chl-protein complexes, encompassing both Chl *a* and Chl *b*, and thereby increasing the number of associated Chl molecules per reaction center up to 300 Chl molecules per PSII (Harrison and Melis, 1992; Ley and Mauzerall, 1982; Smith et al., 1990) and 250 per PSI (Melis and Anderson, 1983; Smith et al., 1990).

To explore the potential of TLA strains for improved photosynthetic productivity, when in mass culture under bright sunlight conditions, we used the light saturation curve of the *tla3* mutant (Kirst et al., 2012b) and calculated the anticipated productivity of a high-density culture over the course of a day, compared to that of a wild type culture under the same growth conditions and at the same chlorophyll concentration. The *tla3* mutant has a PSII Chl antenna size of 93 Chl molecules and a PSI Chl antenna size of 120 Chl, which is about half the size of the wild type. It is thus expected to perform better than the wild type but not as well as a strain with the absolute minimal antenna of the core PSII and PSI complexes. The light-saturation curves of photosynthesis provide information on the quantum yield of the process and offer a measure of the capacity of photosynthesis for the particular sample [Powles and Critchley, 1980]. The light saturation curves of photosynthesis are shown in Fig. 1 for wild type and the *tla3* mutant. Wild type cells showed a light-saturated rate (P_{max}) of ~80 mmol O_2 (mol Chl)$^{-1}$ s^{-1} with $I_S \cong 500$ μmol photons m^{-2} s^{-1}. The *tla3* mutant reached a P_{max} of ~150 μmol O_2 (mol Chl)$^{-1}$ s^{-1}, i.e., about 2-times greater than that of the control. This difference is attributed to the smaller Chl antenna size for the *tla3* cells, translating into higher per Chl productivity for the latter.

To calculate the productivity of the *tla3* culture compared to a wild type we used the light intensity profile over the course of a day, as measured during a sunny day with no cloud cover in Berkeley, California (Melis et al., 1999). Cultures of the two strains, having enough density to absorb all incoming irradiance would show a similar per Chl productivity at sub-saturating light intensities, i.e. intensities below the $I_S =$ 500 μmol photons m^{-2} s^{-1} level. A culture of the *tla3* strain was estimated to out-perform the wild type culture whenever the light-intensity exceeded 500 μmol photons m^{-2} s^{-1}, which is the case for about 8 h during the course of a cloudless day (Fig. 2). At incident intensities less than 500 μmol photons m^{-2} s^{-1} the wild type and the *tla3* strain would perform about the same and a productivity increase of the *tla3* strain could only be demonstrated if the incident irradiance exceeds the $I_S =$ 500 μmol photons m^{-2} s^{-1} level.

Attenuation of light intensity occurs as a function of culture depth, and this parameter would depend strongly on the pigment content of wild type or TLA cells. On the basis of the light-saturation curves shown in Fig. 1, and experimental results of the light intensity gradient with the depth of a liquid culture with cells suspended at a chlorophyll concentration of 13 μM Chl (Melis et al., 1999), we were able to integrate the photosynthetic productivity of wild type and the *tla3* strain across the depth of the culture, taking into consideration the attenuation of intensity with distance from the surface (Fig. 3). The culture with a *tla3* strain outperformed the wild type at the surface but both strains showed a very similar productivity once the light dropped

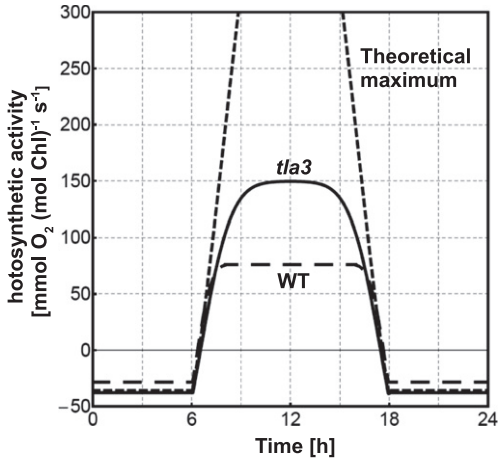

Fig. 2. Photosynthetic activity as a function of time over the course of a day without cloud cover (Melis et al., 1999). The integrated area underneath each curve is proportional to the daily productivity of the strains. Dotted line: theoretical maximal achievable photosynthetic productivity; dashed line: WT; solid line: *tla3* strain (Kirst et al., 2012b). The *tla3* strain outperforms the WT when the light intensity is greater than the saturation intensity of the wild type (500 μmol photons m^{-2} s^{-1}), reached early in the morning, i.e. at about 8 AM of a sunny day. Photosynthesis in the *tla3* mutant saturates later in the day, and reaches a higher level of productivity, as it requires a higher light intensity for the light reactions to reach the rate-limiting step imposed by the carbon reactions.

below 500 μmol photons m^{-2} s^{-1} (culture depth > 5 cm). To calculate how the productivity of the wild type and the *tla3* culture compared over the course of the day, we combined both graphs (Figs. 2 and 3) to form an area of productivity as a function of daytime and across the depth of the culture, shown in Fig. 4. In this analysis, the *tla3* strain outperformed the wild type at the surface of the culture for several hours in the course of the day, but there was no substantial difference between wild type and *tla3* strain deeper in the culture, where the light intensity was lower than 500 μmol photons m^{-2} s^{-1}, or in the early morning and late evening hours, when again the incident solar intensity was lower than 500 μmol photons m^{-2} s^{-1}. In terms of biomass productivity, a higher per Chl respiration rate of the *tla3* strain would

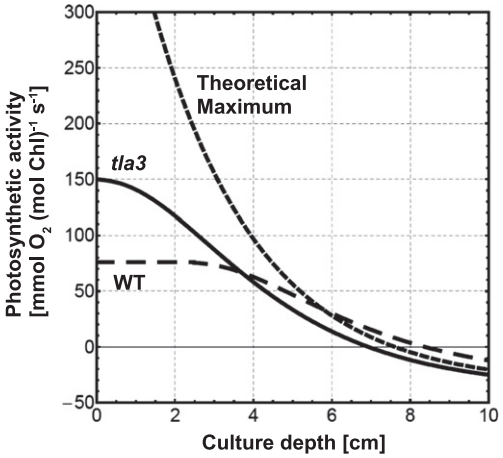

Fig. 3. Photosynthetic activity on a per Chl basis as a function of liquid culture depth (Melis et al., 1999). Dotted line: theoretical maximal achievable photosynthetic efficiency; dashed line: WT; solid line: *tla3* strain (Kirst et al., 2012b). The productivity of chlorophyll in the *tla3* strain is greater than that of the wild type at the surface of the culture, where the light-intensity is high. Deeper in the culture, where light-intensity is low, *tla3* mutant and wild type show the same productivity on a per Chl basis. In this analysis, 10^6 cells per ml for the wild type and 5×10^6 cells for the TLA strain were assumed, translating to equal Chl per ml in the two cultures.

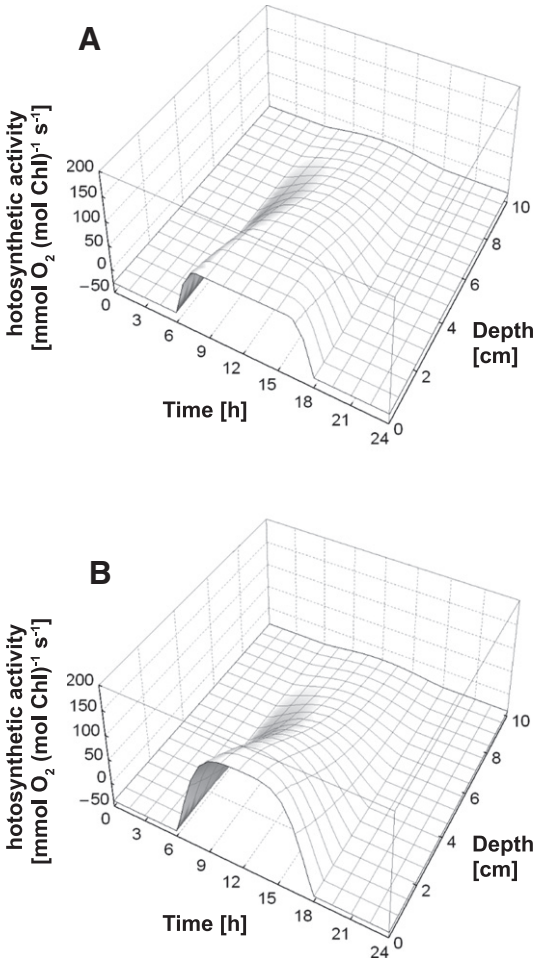

Fig. 4. Photosynthetic activity as a function of time over the course of a day and across culture depth. (A) Wild type; (B) *tla3* strain. The *tla3* strain shows a higher per Chl productivity at the surface of the culture during the period of high light intensity in the course of the daytime but is slightly compromised at night due to a higher respiration rate per Chl.

tend to catabolize more biomass at night compared to the wild type, lowering the overall production advantage of the former in the mass-culture.

The integrated volume underneath the photosynthetic activity profile area of Fig. 4 affords a measure of photosynthetic production in the course of the day and across culture depth. We calculated the maximal achievable photosynthetic production using both strains at the same Chl content while keeping the culture depth as a variable. The *tla3* strain reached an overall productivity during the course of the day that was about 20% greater compared to the maximal achievable production using a wild type strain. However, the pigmentation properties and Chl antenna size of the *tla3* mutant is not minimal yet, as evident by the higher than minimal number of Chl molecules associated with each reaction center (Table 1) (Kirst et al., 2012b). An even greater productivity could be achieved in mass cultures with a strain possessing the absolute minimum light-harvesting antenna size. Thus, there is room for improvement. The above calculation did not include adverse effects caused by photoinhibition of photosynthesis, which are far greater at bright sunlight for wild type than for TLA strains. Wild type is more susceptible to photoinhibition, because of the larger light-harvesting antenna size, forcing the non-photochemical dissipation of a far greater number of photons than would be the case for TLA strains (Nakajima et al., 1998).

Table 1
Summary of the antenna size of the photosystems and production properties calculated of the wild type and *tla3* mutant (Kirst et al., 2012b).

Parameter	WT	*tla3*
Average PSII Chl antenna size	245 ± 30	94 ± 5
Functional PSI Chl antenna size	230 ± 26	120 ± 10
Quantum yield, relative units	100 ± 25	104 ± 20
Half-saturation intensity, [μmol photons m^{-2} s^{-1}]	210	>600
Maximal photosynthetic productivity in mass culture, relative units	100	120

3. The CpSRP pathway, a potential target to generate TLA type strains

In higher plants, the Chl *a*–*b* light-harvesting proteins and some PSII-core and PSI-core proteins are assembled and integrated into the thylakoid membrane via a pathway similar to the signal recognition partial (SRP) pathway in bacteria (Pool, 2005). The chloroplast equivalent of the signal recognition particle pathway (CpSRP) catalyzes the post-translational and co-translational protein insertion into the lipid bilayer of the developing thylakoid membrane (Fig. 5A). The co-translational function is essential for the insertion of proteins encoded by the chloroplast genome, like the PSII reaction center proteins (Dewez et al., 2009; Nilsson and van Wijk, 2002; Pilgrim et al., 1998; Zhang and Aro, 2002). It involves the chloroplast signal recognition particle protein CpSRP54, potentially the chloroplast SRP receptor CpFTSY, the chloroplast SRP insertase (ALB3) and the SEC translocase CpSECY (Klostermann et al., 2002; Pasch et al., 2005; Zhang et al., 2001). Mechanistically, CpSRP54 binds to newly synthesized proteins and then potentially to CpFTSY, which guides the protein-CpSRP54 complex to ALB3 and CpSECY for co-translational integration into the developing thylakoid membrane.

The post-translational integration of thylakoid membrane proteins requires two proteins forming the signal recognition particle (CpSRP), namely CpSRP54 and CpSRP43, the signal recognition receptor CpFTSY, and the insertase ALB3. CpSRP43 is a molecular chaperon that prevents and also reverses aggregated light-harvesting proteins following import into the chloroplast (Falk and Sinning, 2010). It recognizes a specific motif between the transmembrane helices 2 and 3 of light-harvesting proteins termed L18 motif (DeLille et al., 2000; Tu et al., 2000). CpSRP54 binds to the sequence of the third transmembrane helix of light-harvesting proteins (High et al., 1997), but also recognizes a chromo protein domain on CpSRP43, a protein domain often found in protein–protein interactions (Goforth et al., 2004; Jonas-Straube et al., 2001). The chloroplast signal recognition receptor, CpFTSY, recognizes this complex, presumable by interaction with CpSRP54 (Moore et al., 2003), thus forming a membrane bound complex (Moore et al., 2003). This large complex is thought to glide along the developing thylakoid membrane until it reaches ALB3. Upon hydrolysis of guanosine triphosphate (GTP) catalyzed by the GTPase domains in CpSRP54 and CpFTSY, the target protein becomes integrated into the thylakoid membrane (Tu et al., 1999). The molecular details of some fundamental interactions between these proteins and with their substrate, e.g. the structure of CpSRP43 in complex with the L18 (Stengel et al., 2008) and the interaction of the CpSRP43 chromodomain with cpSRP54 (Holdermann et al., 2012) have been discussed. A recent review (Richter et al., 2010) summarizes current aspects of the CpSRP pathway.

Recently, the CpSRP pathway for the integration of thylakoid-membrane proteins has also been shown to operate in green microalgae (Bellafiore et al., 2002; Göhre et al., 2006; Kirst et al., 2012a, 2012b). While the function of the CpSRP pathway in green microalgae is overall very similar to the one in higher plants, there are some unique differences (Fig. 5B). The co-translational and post-translational functions for the insertion of proteins into the lipid bilayer of developing thylakoids appear to be separate from each other. The green microalgae CpSRP system has two ALB3 homolog proteins, namely ALB3.1 and ALB3.2. The

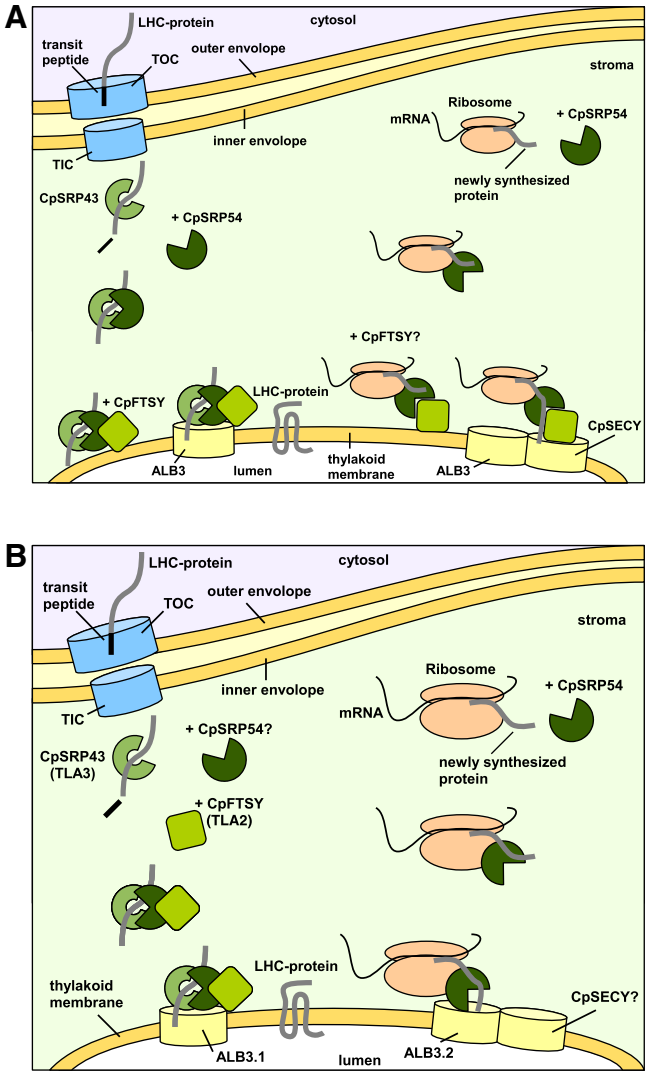

Fig. 5. Model of the CpSRP pathway for protein integration in the developing thylakoid membrane. Post-translational (A and B left side) and co-translational (A and B right side) pathways are illustrated. For simplicity no stoichiometric ratios of the proteins involved are shown. (A) Schematic of the CpSRP pathway as it is known to operate in higher plants. (B) Schematic of the CpSRP pathway as it is known to operate in green microalgae. In the post-translational pathway, precursor light-harvesting proteins are targeted to the chloroplast via a transit peptide and are imported into the chloroplast via the TOC and TIC envelope-localized complexes. To prevent misfolding and aggregation, the molecular chaperon CpSRP43 binds to the imported light-harvesting proteins. In higher plants, CpSRP54 binds to the CpSRP43–LHC protein complex but it is not known if this also occurs in green microalgae. In plants this complex binds to a membrane-bound CpFTSY, while in green algae the CpFTSY is soluble (Kirst et al., 2012a). This LHC–CpSRP43–CpSRP54–CpFTSY complex is then guided to the ALB3.1 insertase in green microalgae or the ALB3 protein in higher plants. Upon GTP hydrolysis, the light-harvesting protein is integrated into the developing thylakoid membrane and the CpSRP complex disassembles to become available for another carry-and-integration cycle. In the co-translational pathway, a chloroplast-encoded protein is newly synthesized by a ribosome. A transmembrane region of the newly synthesized proteins is recognized by the CpSRP54 protein, which also binds to the large subunit of the ribosome. In higher plants, CpFTSY presumably guides this complex to the ALB3 insertase that works in tandem with the CpSECY translocase for co-translational insertion of the chloroplast-encoded protein. The CpFTSY protein is probably not needed for this process to function in green algae.

ALB3.2 has been reported to be essential for cell function and for the assembly of the photosystems, while integration of the light-harvesting proteins is not affected by the presence or absence of the ALB3.2 (Göhre et al., 2006). The ALB3.1 protein, on the other hand, functions exclusively in the post-translational insertion of light-harvesting proteins into the developing thylakoid membrane (Bellafiore et al., 2002). Further evidence of the differential function of the post-translational and co-translational mechanisms in green microalgae for the insertion of thylakoid membrane proteins is provided by the phenotype of the CpFTSY deletion mutant in *Chlamydomonas reinhardtii* (Kirst et al., 2012a). This mutant showed depletion in light-harvesting proteins in the thylakoid membrane and a slight reduction in the number of reaction centers in chloroplasts, but survival of the organism was not compromised by the mutation. Δ*cpftsy* mutants were fully functional and able of photoautotrophic growth with doubling times similar to those of the wild type (Kirst et al., 2012a). Like in green microalgae, deletion of CpFTSY in higher plants diminished the abundance of the light-harvesting proteins in the thylakoid membrane, but additionally affected the fitness of the plant in terms of light sensitivity (Kugelmann et al., 2013) and growth (Asakura et al., 2004, 2008; Kugelmann et al., 2013). Kugelmann et al. (2013) reported an *Arabidopsis thaliana* T-DNA insertion line in the CpFTSY gene to have a dwarf phenotype, while two different T-DNA insertion lines investigated by Asakura et al. (2004, 2008) showed inability of the organism to develop beyond the cotyledon or first true-leaf stage, when grown photoautotrophically. These phenotypes indicated that the function of the CpFTSY protein in higher plants is not exclusively limited to the integration of the light-harvesting proteins into the developing thylakoid membrane, but may play a significant role in the proper integration of other thylakoid membrane proteins, which are essential for photosynthesis and growth.

To the best of our knowledge, no Δ*cpsrp54* mutants have been identified or described in green microalgae. It would be of interest to know if the CpSRP54 protein function in green microalgae is limited to co-translational integration of thylakoid membrane proteins, or whether it might be involved in both post-translational and co-translational integration processes, as the case appears to be in plants.

The CpSRP43 protein has been reported to be a molecular chaperon specific to light-harvesting proteins (Falk and Sinning, 2010) and the phenotypes of the Δ*cpsrp43* mutants in *Arabidopsis thaliana* (Amin et al., 1999; Klimyuk et al., 1999) and *C. reinhardtii* (Kirst et al., 2012b) support this contention. Δ*cpsrp43* mutants showed a similar and specific reduction of light-harvesting proteins in the developed chloroplasts, indicating that the CpSRP43 protein plays a very conserved role in green microalgae and higher plants. A summary of the various CpSRP mutants and their properties, as described in the literature, is given in Table 2.

Genes of the CpSRP pathway make an ideal target in efforts to generate TLA type mutants by which to increase the photosynthetic productivity of microalgae and crop plants under high density mass-cultures or canopy conditions. Certainly, the above analyses suggest the CpSRP pathway genes as suitable targets for Chl antenna size modification. However, not all CpSRP pathway genes are equally suitable. In higher-plants, knockout of the CpSRP43 gene would confer a truncated Chl antenna size without exerting an adverse effect on organism photoautotrophic growth. There are more obvious options in green microalgae because the CpSRP pathway functions slightly differently from that of higher plants, as described above. Suitable targets to generate TLA type mutants would include the *ALB3.1*, *CpFTSY*, *CpSRP43* genes because mutants of all three are capable of photoautotrophic growth. A caveat in this respect is that single-gene knockout mutants of the *ALB3.1*, *CpFTSY*, *CpSRP43* genes are leaky, i.e., show some limited assembly of light-harvesting proteins, suggesting either a redundant/overlapping function among the CpSRP proteins, permitting a limited assembly of light-harvesting proteins in the total absence of one of the CpSRP proteins. Alternatively, it cannot be excluded that another pathway for thylakoid membrane protein integration is partially able to compensate for the loss of the CpSRP function. The two alternatives can be addressed and delineated upon deletion of two or all of the *ALB3.1*, *CpFTSY*, *CpSRP43* genes, seeking to test whether the absence of more than one of the CpSRP genes might entirely eliminate the leaky phenotype and the partial assembly of light-harvesting proteins, leading to a mutant with the smallest possible Chl antenna size.

Table 2
Knockout or RNAi down regulation mutants of CpSRP proteins. Chlorophyll content is given relative to the wild type.

Mutated protein	Mutant synonym	Strain	Chl content	Affected chloroplast proteins	Viability	References
CpSRP54 (knockout)	*ffc*	*A. thaliana*	25% in first true leaves	Reduction of most LHC's, PSI and PSII	Viable	(Amin et al., 1999; Kugelmann et al., 2013; Pilgrim et al., 1998)
CpSRP43 (knockout)	*cao*, *chaos*	*A. thaliana*	50%	Reduction of most LCHs, Normal PSI and PSII	Viable	(Amin et al., 1999; Klimyuk et al., 1999)
TLA3-CpSRP43 (deletion)	*tla3-cpsrp43*	*C. reinhardtii*	15%	Strong reduction of LCH's, slight reduction of PSI and PSII	Viable	(Kirst et al., 2012b)
CpSRP54/CpSRP43 (knockout)	*ffc*/*cao*	*A. thaliana*	15%	Strong reduction of LHC's, PSI and PSII	Viable	(Amin et al., 1999)
CpFTSY (knockout)	*cpftsy*	*A. thaliana*	6–7%	Strong reduction of LHC's, PSI and PSII	Lethal/retarded growth	(Asakura et al., 2008; Kugelmann et al., 2013)
CpFTSY (knockout)	*csr1*	*Zea mays*	12%	Strong reduction of LHC's, PSI and PSII	Seedling lethal	(Asakura et al., 2004)
TLA2-CpFTSY (deletion)	*tla2-cpftsy*	*C. reinhardtii*	20%	Reduction of LHC's, slight reduction of PSI and PSII	Viable	(Kirst et al., 2012a)
ALB3 (knockout)	*alb3*	*A. thaliana*	5%	Strong reduction or absence of LHC's, PSI and PSII	Seedling lethal	(Asakura et al., 2008; Kugelmann et al., 2013 Sundberg et al., 1997)
Alb3.1 (knockout)	*ac29*	*C. reinhardtii*	30%	Strong reduction of LHC's, PSI and PSII affected to a lesser extend.	Retarded growth	(Bellafiore et al., 2002; Ossenbühl et al., 2004)
Alb3.2 (RNAi)	*alb3.2*	*C. reinhardtii*	25%–50%	Strong reduction of PSI and PSII, LHC's affected to a lesser extend	Lethal	(Göhre et al., 2006)

Plastids in certain higher-plants and green algae encode a structural RNA, which is thought to play a role in the SRP process, probably originating from the prokaryotic SRP system preceding the endosymbiosis event (Packer and Howe, 1998; Richter et al., 2008; Rosenblad and Samuelsson, 2004). A recent study revealed that the plastid SRP RNA in *Physcomitrella patens* enhances the GTPase activity of the CpSRP54 protein (Träger et al., 2013). Thus, if the plastid SRP RNA is deleted, it could also bring about a TLA phenotype.

Along these lines, the *Arabidopsis thaliana* LTD (Light-harvesting chlorophyll-binding protein Translocation Defect) protein acts to facilitate import of light-harvesting proteins into the chloroplast (Ouyang et al., 2011). LTD knockout mutants of *A. thaliana* were viable but showed a severe reduction of light-harvesting proteins and a higher than wild type Chl *a*/*b* ratio, both suggesting a truncated light-harvesting antenna size in the LTD photosystems. This protein is also predicted to be present in green algae and thus could serve as a target for the generation of a TLA phenotype.

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