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Chapter 14

Improving Photosynthetic Solar Energy Conversion Efficiency: the Truncated Light-harvesting Antenna (TLA) Concept

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14.1. Introduction

Cyanobacteria and green microalgae are organisms of oxygenic photosynthesis. The process of oxygenic photosynthesis in these microorganisms, as well as multicellular algae and plants, can be divided into two distinct stages, namely the light-reactions and the carbon reactions (see Chapter 1). The light-reactions absorb and convert the energy of sunlight into chemical energy, stored in the form of the energy-rich molecules such as reduced ferredoxin (FD_{red}), nicotinamide adenine dinucleotide phosphate (NADPH), and adenosine triphosphate (ATP). The energy stored in FD_{red}, NADPH, and ATP is used mainly by the carbon reactions of photosynthesis to reduce CO_2 and to form sugar molecules. The high-potential energy electrons stored in FD_{red} can also be used for the production of molecular hydrogen (H_2) (see Chapters 2, 7, 8, 9, and 10). The light-reactions are highly conserved in all organisms of oxygenic photosynthesis, as are the two photosystems that perform it. There are, however, substantial variations in the light-harvesting pigments' content, composition, and organization between taxonomically different organisms, and also in the organization of the two photosystems in photosynthetic thylakoids. However, the core and reaction center complexes of the photosystems are similar in green microalgae and cyanobacteria. The latter taxonomic groups are of interest as, in addition to the basic functions of photosynthesis described above, they are endowed with the property of molecular hydrogen (H_2) production. Thus, their photochemical apparatus organization and solar-to-product energy conversion efficiency has attracted a lot of attention.

The photosystem II (PSII) core complex comprises a light-driven water-to-plastoquinone oxidoreductase, encompassing a heterodimeric protein-cofactor complex with a molecular weight of about 600 kDa. It includes at least 22 protein subunits, containing 60–65 cofactors per monomer. Among these, there are 37 chlorophyll *a* and 12 β-carotene molecules, as well as pheophytin, Fe^{++}, tyrosine, Mn^{++}/Mn^{+++}, Ca^{++} and plastoquinone-9.[1–4]

The photosystem I (PSI) core is a light-driven plastocyanin-to-ferredoxin oxidoreductase. It comprises at least 12 protein subunits, and more than 110 cofactors, including the PSI-core light-harvesting antenna pigments of 95 chlorophyll *a* molecules,[1] most of which are bound to the heterodimeric core complex of PSI consisting of the PsaA and PsaB subunits.[5]

14.1.1. Green Microalgal Chlorophyll-protein Light-harvesting Antenna Complexes

Proteins of the peripheral light-harvesting complex in plants and green algae belong to the same large family, which presumably arose by multiple gene duplication and fusion events.[6] They share a general structure of three transmembrane helices with the N-terminus being exposed to the soluble phase on the stromal side of the thylakoid membrane, while the

C-terminus is in the lumen. Each of these pigment-proteins contain 10–15 Chl *a* and Chl *b* molecules and 3–4 xanthophylls.[7–10] Light-harvesting holocomplexes for PSII and PSI, each encoded by a different set of genes, assemble as peripheral to the PS-core complexes and can be divided into inner and outer components. The *LHCA* gene subfamily encodes proteins of the light-harvesting complex I (LHCI) associated with PSI, while the *LHCB* gene subfamily encodes proteins of the light-harvesting complex II (LHCII) associated with PSII.[11]

The auxiliary light-harvesting complex of photosystem II in higher plants consists of six distinct pigment-containing proteins, namely LHCB1-6. Multiple copies of genes typically encode these proteins. In *Arabidopsis thaliana*, for example, the LHCB1 protein is encoded by five copies of the LHCB1 gene.[12] Within the LHCB1-6 proteins there are two groups, the minor (inner) and major (outer) light-harvesting proteins, distinguished by their relative abundance in the thylakoid membrane.[13] The minor proteins, namely LHCB4-6, are found in close proximity to the PSII-core. They do not form higher-order complexes with each other and are thus believed to be monomeric. Typically, one of each minor light-harvesting proteins is found per PSII reaction center. The major light-harvesting proteins, LHCB1-3, form trimers with each other. Compared to the minor antenna, they are more loosely associated with the inner LHC and the PSII-core, and their abundance can vary substantially depending on environmental conditions. Biochemical quantification of the LHCB proteins, in tandem with a precise measurement of the Chl *a* and Chl *b* molecules contained in a PSII holocomplex (the PSII functional antenna size), helped define a stoichiometry of $(LHCB1)_9$ $(LHCB2)_3$ $(LHCB3)_2$ $(LHCB4)_2$ $(LHCB5)_1$ $(LHC6)_1$ per PSII complex in barley chloroplasts.[13]

The light-harvesting complex of PSII in the model green alga, *Chlamydomonas reinhardtii*, is also composed of minor and major light-harvesting Chl-proteins. Two genes encode for the minor light-harvesting antenna proteins, *LHCB4* and *LHCB5*.[12] The major light-harvesting proteins are encoded by nine genes, *LHCBM1-LHCBM6*, *LHCBM8*, *LHCBM9* and *LHCBM11*.[12]

LHCII assembly takes place in a stepwise fashion. A newly assembled PSII-core first acquires a partial complement of peripheral light-harvesting antenna, increasing its Chl content from 37 to about 120–130 chlorophyll molecules per reaction center. This structural configuration constitutes the so-called $PSII_\beta$ form of PSII.[14] It is normally found in stroma-exposed thylakoid membranes (Melis 1991). Addition of trimeric peripheral light-harvesting protein complexes to $PSII_\beta$ converts the latter to the fully pigmented $PSII_\alpha$ supercomplex, possessing a fully developed LHCII with up to 250 Chl *a* and Chl *b* molecules. The stacked membranes of grana thylakoids are the locus of $PSII_\alpha$.[15] This stepwise process of LHCII assembly is reversible and helps facilitate the PSIIα disassembly that occurs during phosphorylation for the repair of PSII from frequently occurring photodamage.[16,17]

The light-harvesting complex associated with photosystem I (LHCI) in the green alga *Chlamydomonas reinhardtii* is encoded by nine genes,

LHCA1-LHCA9. These light-harvesting proteins occur in the periphery of the PSI-core complex.[18] Structural analysis of a PSI supercomplex in *C. reinhardtii* revealed 9 LHCI proteins forming the peripheral light-harvesting antenna complex of PSI,[18] which is in good agreement with the spectroscopically determined number.[19]

14.1.2. Cyanobacterial Bilin-protein Light-harvesting Antenna Complexes

Cyanobacteria have evolved a different auxiliary light-harvesting antenna system, the phycobilisome (PBS) that allows absorption of sunlight, primarily in the 575–675 nm region. The PBS enables unidirectional excitation energy transfer toward the chlorophyll-pigment bed of PSII reaction centers. Each phycobilisome consists of two main structural parts, the core-cylinders and the peripheral rods. Core cylinders are made of allophycocyanin $(\alpha\beta)_3$ discs stacked next to each other, with the cylinder axis oriented in parallel to the plane of the thylakoid membrane, with at least two of three cylinders resting with their long axes on the stromal side of the thylakoid membrane. These provide a structural and excitation energy transfer link between the peripheral rods and the chlorophyll-pigment bed of the PSII reaction centers.[20–24] In *Synechocystis* sp. PCC 6803 (*Synechocystis*), there are three allophycocyanin core cylinders, two of which rest directly on the thylakoid membrane. A third cylinder is resting on the stromal side of the furrow formed by the other two core cylinders.[24] Core cylinders contain the pigment-proteins allophycocyanin-α and allophycocyanin-β, encoded by the *APCA* and *APCB* genes, and a small linker polypeptide LC, encoded by the *APCC* gene.[22,25,26] They are linked to the thylakoid membrane and the PSII dimer chlorophyll-pigments by a PBS terminal excitation-acceptor allophycocyanin pigment containing the linker polypeptide L_{CM}, encoded by the *APCE* gene.[27,28] The latter functions together with the products of the *APCD* and *APCF* genes to facilitate efficient excitation energy transfer from the phycobilisome toward the PSII reaction center.[29–32]

Peripheral to the allophycocyanin core cylinders are phycocyanin-containing rods, also in cylinder form, physically extending outward from the allophycocyanin core cylinders.[21,22,24] Similar to the allophycocyanin, the phycocyanin rods are composed of stacked discs, each one consisting of six hetero-dimers of the pigment containing CPC-α and CPC-β proteins, encoded by the *CPCA* and *CPCB* genes, respectively.[22,25,26] The CPC-α and CPC-β dimers are connected by linker polypeptides, encoded by *CPCC1, CPCC2,* and *CPCD* genes.[25,33] Genes *CPCA*, *CPCB*, *CPCC1*, *CPCC2* and *CPCD* are clustered in a single operon in *Synechocystis*, which is referred to as the *CPC*-operon.

The phycobilisome substantially increases the sunlight absorption cross-section of PSII,[21,22] thereby countering a potential imbalance in excitation energy distribution due to the high PSI : PSII stoichiometric ratio in cyanobacteria,[34,35] and the fact that most of the chlorophyll is associated with PSI in

these microorganisms.[21,36] Up to 450 phycocyanin (Phc) and allophycocyanin (AP) pigments can be associated with the PBS in S*ynechocystis*.

14.2. Competition for Light-harvesting Among Photosynthetic Organisms

14.2.1. Size of the Light-harvesting Antenna

Given the modular composition and assembly of the photosystems, it is understood that the size of the peripheral light-harvesting complexes is not fixed but could vary substantially depending on the genetic, developmental, and even environmental light conditions. With constraints imposed by the genetics of the system, the Chl antenna size appears to be regulated in photosynthetic organisms inversely with the level of irradiance.[19,37] A smaller PS absorption cross-section under high-light conditions limits the rate of excitation trapping and charge separation in each reaction center, helping to conserve energy resources and leading to less photodamage and photoinhibition of photosynthesis.

Adjustments in the antenna size of PSII are implemented mainly by the removal or addition of the trimeric LHCB subunits.[37,38] While under low-light conditions, these peripheral trimers are abundant in the LHCII, they are depleted under high irradiance growth conditions.[37–39] In green microalgae, experimental evidence has shown that up to 300 chlorophyll molecules can be associated with PSII under low-light growth, whereas as few as only ~60 Chl *a* molecules per PSII reaction center were measured under high-light growth conditions.[13,19,40] The light-harvesting antenna complex of PSI can also vary with the light intensity during growth. A maximum of ~250 Chl molecules per PSI was measured under low-light growth conditions and a minimum of ~100 Chl molecules was reported under high irradiance growth.[19,40]

Such high-light-induced lowering of the absorption cross section of PSI and PSII can be generated in the lab upon growth under continuous high-intensity illumination of the cultures, when shading is avoided. This high-light-induced "truncated light-harvesting antenna" of the photosystems cannot be achieved under ambient (natural and diurnal) sunlight in nature. The intensity of sunlight varies substantially over the course of a day, even without cloud cover, from low light intensities in the morning to very bright light conditions at noon to, again, very low light later in the afternoon. More importantly, in a multi-species ecotype, competition for sunlight forces photosynthetic organisms to attain a large light-harvesting antenna size. Such large light-harvesting antenna sizes ensure that the organism can grow at maximum photosynthetic capacity even in the early morning and late afternoon hours, when the light-intensities are generally low. Furthermore, if the organism is shaded by competitors or if the light-intensity drops by cloud cover, a large antenna size ensures the organism will make the best use of the

limited number of transmitted photons. Thus, photosynthetic organisms compete with one another for light capture in the wild, under conditions that require capturing more light for the individual, even if it is wasted, and preventing light capture by competing neighbors.[41] Obviously, this property is detrimental to the yield and photosynthetic productivity in a dense monoculture designed for product or biomass generation.

There are other indications that nature selects in favor of photosynthetic systems with a large light-harvesting antenna size. Photosynthetic organisms evolved a large number of light-harvesting proteins and, depending on the organism, there are 10–13 different LH proteins in green algae and higher plants.[12] In addition, some of these proteins are encoded by multiple genes; *e.g.*, LHCB1 is encoded by 5 different genes, *LHCB1.1-LHCB1.5,* in *Arabidopsis thaliana*.[12] Such complexity and redundancy is a good indicator for a high selective pressure toward providing the proteins needed to increase the photon-absorption cross-section of the photosystems.

Overall, maximizing light-absorption increases the chances of survival of individual plants, algae, or cyanobacteria, as it affords the opportunity for the organism to operate photosynthesis at the highest possible capacity, alleviating as much as possible irradiance as the growth-limiting factor. At the same time, a large absorption cross-section helps to shade competitors, inhibiting their growth. Thus, an organism with the largest light-harvesting antenna size has a competitive advantage over other photosynthetic organisms in the wild, a survival feature that explains the high selective pressure to increase the Chl light-harvesting antenna size.

14.2.2. Sunlight-to-biomass Energy Conversion Efficiency in Photosynthesis

A consequence of a large light-harvesting antenna size is over-absorption of photons at high sunlight intensities and wasteful dissipation of most of them, as these far exceed the capacity of the thylakoid membrane for electron-transport and the biochemical rate of the carbon reactions of photosynthesis. Photosynthesis saturates at about 500 μmol photons m^{-2} s^{-1}, which is less than a fourth of full sunlight intensity. At incident sunlight intensities greater than 500 μmol photons m^{-2} s^{-1}, photons absorbed by the light-harvesting antenna of the photosystems cannot be used for photosynthesis. This means that at bright sunlight (about 2200 μmol photons m^{-2} s^{-1}) three quarters of the energy that could drive photosynthesis is instead wasted, substantially lowering the sunlight-to-biomass energy conversion efficiency.

Table 14.1 shows the theoretical maximum sunlight to biomass energy conversion efficiency of photosynthesis (8–10%), the anticipated productivity (75 g dw m^{-2} d^{-1}), and the actual achieved under optimal ambient growth conditions in the field (rows 2–5). Given the absorption properties of photosynthetic pigments, green algae and terrestrial plants are theoretically able to convert about 8–10% of the energy of sunlight into biomass.[40–42] However,

Table 14.1. Estimated and actual solar energy conversion efficiencies and biomass accumulation based on an annual cycle. For meaningful comparison purposes, photosynthetic productivities were normalized on the basis of biomass accumulation in g dry weight per m^{-2} per d. (Adapted from Melis 2009[41]).

Photosynthetic system considered	Solar energy conversion efficiency (sunlight-to-biomass)	Photosynthetic biomass accumulation productivities, g dw m^{-2} d^{-1}
Theoretical max based on the inherent efficiency of photosynthesis	8–10%	75
Chlorella (green microalga) mass culture	2–3%	15–22
Spirulina (cyanobacterium)	0.4–1.1%	3–8
Switchgrass	0.13–0.27%	1–2

under otherwise optimal growth conditions, only a maximum of about 2–3% could be demonstrated with microalgae, although greater efficiencies and yields have been reported. For example, Wang and Seibert[43] recently reported a productivity of 35.6 g dw m^{-2} d^{-1} for continuous growth of the diatom *Chaetoceros* sp. Reported efficiencies and productivities are lowered to 0.4–0.8% in cyanobacteria (Spirulina) and 0.1–0.3% in switchgrass. Similarly, low values are expected from other C3 plants with variations depending on the plant species examined in such field experiments. When molecular hydrogen (H_2) is the final product, emanating from the light-reactions of photosynthesis,[44] theoretical solar-to-hydrogen energy conversion efficiency was estimated to be 12–14%. However, even in this case, over-absorption and wasteful dissipation of sunlight due to the large antenna size of the photosystems would limit yields (Figure 14.1A).

This severe limitation is independent of photobioreactor design and growth conditions and curtails the ability to sustainably generate H_2 (or other bioproducts). As sunlight-energy is the only energy input into a system for H_2 production, it should be used with maximum efficiency to ensure high yields and low costs. Minimizing the over-absorption of sunlight by the photosynthetic apparatus and ensuring a better distribution of photons through the depth of the culture can substantially increase yields.

14.2.3. The Principle of Light-harvesting Antenna Engineering for Mass Cultures

To improve the sunlight-to-biomass energy conversion efficiency, light-absorption by the photosystems needs to be minimized so as to prevent the early saturation of photosynthesis. Ideally, photosynthesis should saturate at higher light-intensities, potentially close to the intensity of bright sunlight. In principle, this can be achieved in two ways, either by (*i*) accelerating the photosynthetic electron-transport and carbon reactions, the rate

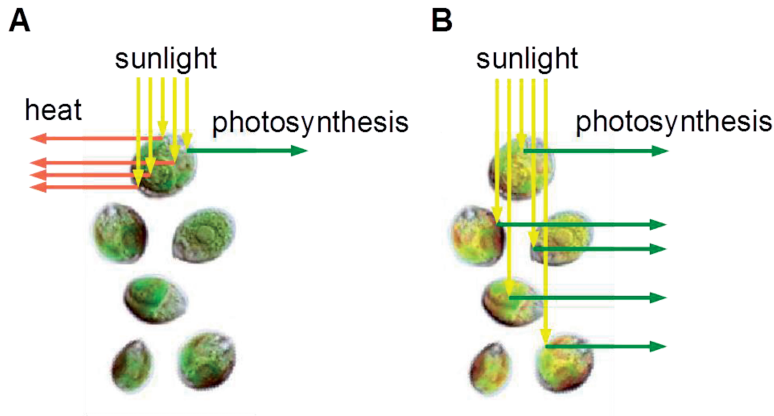

Figure 14.1. Schematic of incident sunlight absorption and processing. A. Fully pigmented (dark green) microalgae in a high-density culture would over-absorb incoming sunlight (more than can be utilized by photosynthesis), and dissipate most of it in the form of heat, thus limiting useful photosynthesis. It is evident that a high probability of absorption by the first layer of cells would cause shading, *i.e.*, prevent cells deeper in the culture from being exposed to sunlight. B. Individual cells with a truncated Chl antenna size have a diminished probability of absorbing sunlight, thereby permitting greater penetration and more uniform distribution of irradiance throughout the culture volume. This alleviates heat losses and enhances photosynthetic productivity by the culture as a whole.

or capacity of which needs to be substantially increased so that they can accommodate much higher rates of photon absorption, or (*ii*) decreasing the photon absorption cross-section of the photosystems so as to prevent the early saturation of photosynthesis and to enable maximum use of incident sunlight. Changing the photosynthetic capacity of the system is not trivial, it would involve changing the kinetics and/or abundancy the enzymes involved in electron-transport and the carbon reactions of photosynthesis. The current rate-limiting steps in photosynthesis are believed to be the diffusion/oxidation of plastohydroquinone at the cytochrome *b-f* complex, and the turnover of RuBisCO (the Ribulose-1,5-bisphosphate carboxylase/oxygenase), the enzyme that performs the primary carboxylation reaction. Little can be done to improve diffusion rates of plastohydroquinone through the thylakoid membrane lipid bilayer, spanning the distance between PSII and the cytochrome *b-f* complex. Moreover, Rubisco is already expressed to high levels in the cell and nature, through evolution, must have improved its catalytic properties to the maximum possible. This makes approaches to further improve its catalytic activity or abundance challenging. Potential approaches to improve RuBisCO's activity have been recently reviewed.[45,46]

Truncating the light-harvesting antenna of the photosystems is a simpler and more feasible approach. This so-called TLA (*T*runcated *L*ight-harvesting

*A*ntenna) concept aims to lower the rate of photon-absorption by minimizing the absorption cross-section of the photosystems, permitting each cell to absorb only the quantity of photons needed to operate photosynthesis, without an over-absorption of sunlight. This property of diminished ability to absorb photons at the cellular level would permit excess sunlight to pass through the top layers of a culture, thus affording the possibility of cells deeper in the culture to perform photosynthesis (Figure 14.1B). It should be noted that improvement of photosynthetic productivity by this approach is achieved not on a single cell (chloroplast) basis but rather on an entire culture volume or canopy level basis.

14.3. Minimizing the Chlorophyll Antennae to Maximize Photosynthetic Efficiency and Productivity

14.3.1. Import, Transit, and Assembly of the Light-harvesting Proteins in Developing Thylakoids

The chloroplast of higher plants and algae contains about 3000 different proteins. About 100 of these proteins, depending on the species, are encoded by the chloroplast genome,[47] whereas the vast majority of the chloroplast proteome is encoded in the nucleus. The protein products of these nuclear encoded genes carry a transit peptide comprising 30–130 extra amino acids on the amino-terminal side of the protein. This extra amino acid sequence serves to target the nuclear-encoded protein to the chloroplast envelope and directs import of the newly synthesized protein into the chloroplast. The post-translational import of the nuclear-encoded and cytosol-synthesized precursor protein through the outer and inner envelope membranes of the chloroplast is mediated by the "*translocon at the outer envelope membrane of chloroplasts*" (TOC) complex and the "*translocon at the inner envelope membrane of chloroplasts*" (TIC) complex.[48,49] Upon protein import in the chloroplast stroma, the transit peptide is cleaved off by an ATP-dependent CLPC protease that resides on the stromal-side of the inner envelope. Depending on the final destination of the imported protein, it either assumes its final conformation as a soluble (stroma targeted) enzyme, or is further targeted to the internal thylakoid membrane compartments of the chloroplast. Proteins that are targeted to the thylakoid lumen are processed by the Twin-Arginine Translocation (TAT) pathway, or by a pathway similar to the secretory (SEC) pathway of bacteria. These pathways require an additional target peptide that is unmasked after the chloroplast transit peptide is cleaved off.[50–53] The algal/plant SEC pathway uses an ATP-driven translocation complex to facilitate the transport through the membrane, similar to the SEC translocase found in bacteria.[54–56] The TAT pathway derives its name from a twin-arginine motif in the translocation signal peptide.[53] In contrast to the SEC translocase, the TAT-pathway is capable of transporting proteins in their folded configuration across the thylakoid membrane.[57,58] The translocation of proteins *via* the

TAT pathway is thought to depend on the pH gradient across the thylakoid membrane as indicated by *in vitro* experiments,[59–61] hence the TAT pathway is sometimes referred to as the ΔpH-dependent pathway. However, *in vivo* studies with *C. reinhardtii* could not demonstrate any ΔpH dependence.[62]

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 (reviewed by Richter *et al.*[63]). CpSRP43 is a molecular chaperon that prevents and also reverses aggregated light-harvesting proteins following import into the chloroplast stroma.[64,65] It recognizes a specific motif between the transmembrane helices 2 and 3 of light-harvesting proteins, termed the L18 motif.[66–68] CpSRP54 binds to the sequence of the third transmembrane helix of light-harvesting proteins,[69,70] but also recognizes a chromo-protein domain on CpSRP43, a protein domain often found in protein–protein interactions.[71–74] This multiprotein complex binding is thought to increase specificity, which is supported by the finding that the complex has a higher affinity to the L18 motif compared to each protein by itself.[75,76] The chloroplast signal recognition receptor, CpFTSY, recognizes this complex, presumable by interaction with CpSRP54, thus forming a membrane-bound complex.[77–83] 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 as a transmembrane protein into the developing thylakoid membrane.[66,72,73,84,85] The function of the CpSRP pathway in green microalgae is overall similar to the one in higher plants (Figure 14.2). The green microalgal CpSRP system has two ALB3 homolog proteins, namely ALB3.1 and ALB3.2. The 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.[86] The ALB3.1 protein, on the other hand, functions exclusively in the post-translational insertion of light-harvesting proteins into the developing thylakoid membrane.[87] In addition, the CpFTSY protein was found to operate in the post-translational insertion of the light-harvesting proteins after the latter are imported into the chloroplast, but is not essential for the co-translational insertion of thylakoid membrane proteins encoded by the chloroplast genome.[88]

The Δ*cpftsy* mutant in *C. reinhardtii* showed depletion of light-harvesting proteins in the thylakoid membrane, a smaller (truncated) light-harvesting antenna of the photosystems, and a slightly lower number of reaction centers in chloroplasts, but survival of the organism was not compromised by the mutation. Δ*cpftsy* mutants were fully functional and capable of photoautotrophic growth with doubling times similar to those of the wild type.[88] 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[89] and growth[89–91] Kugelmann *et al.*[89] reported an *Arabidopsis thaliana*

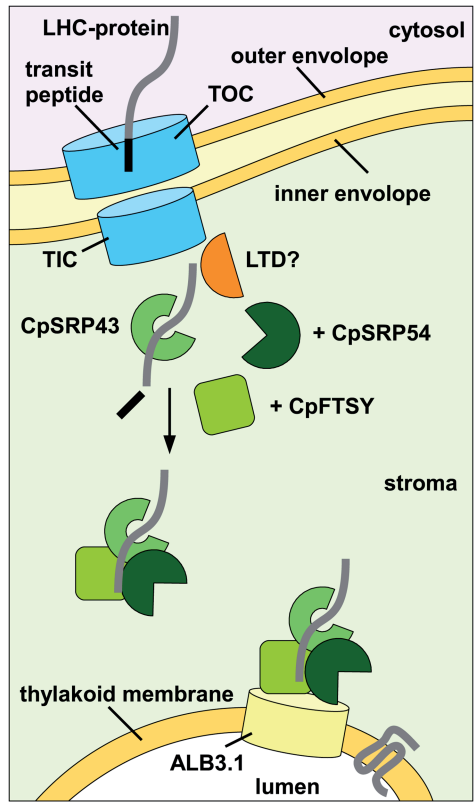

Figure 14.2. Model of the CpSRP pathway for the post-translational integration of proteins into the developing thylakoid membrane in green microalgae. For simplicity, stoichiometric ratios of the proteins involved are not shown. Precursor light-harvesting proteins are targeted to the chloroplast *via* a transit peptide and are imported into the chloroplast stroma *via* the TOC and TIC envelope-localized complexes. To prevent misfolding and aggregation, the molecular chaperon CpSRP43 binds to the imported light-harvesting proteins. The LTD protein, also known to bind to the incoming light-harvesting proteins in higher plants might also be involved in a first recognition process of incoming light-harvesting proteins in green microalgae. Subsequently, CpFTSY binds to the CpSRP43-LHC protein complex, which in turn is postulated to be recognized by CpSRP54. This LHC-CpSRP43-CpFTSY-CpSRP54 complex is then guided to the ALB3.1 insertase. 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.

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.*[90,91] 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.[88]

Likewise, the CpSRP54 protein, appears to function differently in green algae compared to higher plants. Disruption of the *CpSRP54* gene in *C. reinhardtii* resulted in partial failure to assemble the light-harvesting, Chl-binding proteins, phenotypically similar to a Δ*cpftsy* mutant,[92] with the cells retaining the ability to assemble PS reaction centers and autotrophic growth. This is in contrast to the *CpSRP54* gene in higher plants, where deletion of it has a severe effect on chloroplast development, leading to retarded growth.[93] This adverse effect experienced by plants can be explained by the functioning of the CpSRP54 protein both in the assembly of the LHC proteins and in the co-translational integration of chloroplast encoded genes like the PSII reaction center proteins.[54, 94–96] Furthermore, it is unclear how the CpSRP54 protein functions in green algae, because it is not involved in LHC protein recognition, nor does it form a stable complex with CpSRP43.[97]

The CpSRP43 protein has been reported to function as a molecular chaperon specific to light-harvesting proteins,[64] and the phenotypes of the Δcpsrp43 mutants in *A. thaliana*[98,99] and *C. reinhardtii*[100] support this contention. Δ*cpsrp43* mutants showed a similar and specific lowering in the amount of light-harvesting proteins in developed chloroplasts, indicating that the CpSRP43 protein plays a highly conserved role in green microalgae and higher plants.

In higher plants, the chloroplast-localized protein known as LTD plays a role in the light-harvesting apoprotein import into the chloroplast.[101] Consequently, a deletion of the *LTD* gene caused an apparently truncated light-harvesting antenna phenotype. It is not clear whether absence of the LTD protein may also cause defects in reaction center protein assembly, stability, and function,[100] or how this protein may function in green microalgae.

Truncation of the light-harvesting antenna in cyanobacteria can be achieved by replacing the *cpc* operon, encoding the majority of the peripheral phycocyanin rod proteins, with a selectable marker (Δ*cpc*). This approach substantially diminishes the size of the phycobilisome and lowers the absorption cross-section of PSII considerably to about 10% of the wild type. Δ*cpc* strains have been generated and studied in terms of biomass accumulation independently in three different labs.[102–105] However, there is disagreement in the literature as to the functional outcome of the phycocyanin-deletion, which could be either due the use of different selectable markers expressed in the transformants, and/or due to the choice of experimental conditions in assessing the performance of the photosynthetic apparatus and productivity of the cells in wild type and phycocyanin-less mutants.

14.4. Conclusions

Evidence in the literature supports the notion that a TLA property in green microalgae and cyanobacteria can increase the productivity of a mass culture under bright sunlight conditions. As a concept, this may appear to be

counterintuitive, as individual TLA-cells will be handicapped in terms of their ability to absorb sunlight and, therefore, will not be able to successfully compete in the wild. However, a high-density culture with such TLA-cells under bright sunlight conditions collectively will do better than an equivalent high-density culture with WT-cells under the same conditions. Culture productivity has been tested under simulated mass culture conditions. A 50% enhancement in culture productivity was reported by a TLA-strain of the green alga *C. reinhardtii*,[106] and a 57% enhancement in photosynthetic productivity was reported by a TLA-strain of the cyanobacterium *Synechocystis*.[105] However, such improvement in productivity, attained with TLA strains in small-scale cultivations, needs to be more carefully assessed under mass-culture ambient-growth conditions.

In microalgae, but not in cyanobacteria, targeting the genes of the CpSRP pathway can be used as a tool to generate TLA type mutants by which to increase the photosynthetic productivity of the respective cultures under high cell density and bright sunlight conditions. A knockout or knock-down of the *CpSRP43* gene is promising as an approach by which to confer a truncated light-harvesting antenna size in any green system without exerting an adverse effect on the core photosystem proteins, and without affecting the organism's photoautotrophic growth. Because of differences in the function of the CpSRP pathway in green microalgae *versus* higher plants, TLA green algae can also be generated by targeting the *ALB3.1*, *CpFTSY*, *CpSRP43*, and *CpSRP54* genes without exerting additional major adverse effects on the organism fitness and survival. Thus, interference with or inactivation of the CpSRP-assembly pathway for the integration of the light-harvesting proteins into developing thylakoid membranes offers a promising approach to attaining photosynthetic systems with a truncated light-harvesting antenna size. Such organisms with a genetically engineered smaller Chl antenna size would not be as fit as the wild type to competitively survive in the wild. Further, the TLA technology for the enhancement of culture productivities can be applied to green microalgae and cyanobacteria for the generation of hydrogen (H_2), biomass, and other bio-products.[107–109]

14.5. Future Directions

Single-gene knockout mutants of the *ALB3.1*, *CpFTSY*, *CpSRP43*, and *CPSRP54* show leaky phenotypes, meaning that the assembly of light-harvesting proteins is not completely inhibited and a limited light-harvesting antenna can still assemble. This suggests 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 more of the CpSRP pathway genes, seeking to test whether absence of more than one of the CpSRP genes might entirely eliminate the leaky phenotype

and the partial assembly of light-harvesting proteins. With a genome editing method available for the algae,[110–112] double, triple, or quadruple knockouts of the CpSRP pathway can easily be generated and studied. This could lead to a TLA-strain with minimized light-harvesting antenna, offering the possibility to test whether a better sunlight-to-biomass energy conversion efficiency can be achieved, when such TLA-mutants are grown in mass-culture compared to the wild type or to the single mutants currently at hand. Alternatively, genome editing can be used to completely delete all copies of the light-harvesting genes in the photosynthetic organism. Since there are many targets that need deletion, this approach would be more complex but could ensure the generation of TLA-strains with enhanced light-transmission, improved mass culture optical properties, and enhanced biomass or biofuels/H_2 photoproduction.

Some higher-plants and green algae contain plastid SRP RNA, which is thought to be a remnant from the prokaryotic SRP system after endosymbiosis.[63,113,114] A recent study revealed that the plastid SRP RNA in *P. patens* enhances the GTPase activity of the CpSRP54 protein.[115] Thus, deletion of the plastid SRP RNA could potentially lead to a truncated light-harvesting antenna size phenotype. The function of the plastid SRP RNA needs to be investigated, as it is unclear if that may have unforeseen negative consequences on co-translational insertion of the photosystem reaction center proteins.

The selectable marker used in the generation of microalgal TLA mutants constitutes a metabolic burden on the transformed organism, as carbon needs to be invested for synthesis of the marker protein. This foreign protein is needed only for the initial selection but is useless in a subsequent production system. The current generation of TLA strains, all of which carry such a selectable marker, may therefore be suboptimal, and transformant strains may not be as fit as they could, a property that limits their survivability and productivity. Thus, the presence of the selectable marker could have the potential to unnecessarily slow down growth and productivity of the TLA-strains in a scaled-up production system.[116] In future applications, genome editing technologies should be used to remove these markers, a prospect that will improve the performance and yield of TLA strains in terms of energy-conversion efficiency, biomass accumulation, and societal acceptability.

In summary, the current TLA technology has shown improvement in the sunlight-to-biomass/H_2 energy conversion efficiency significantly, in the range of 1.5–1.6-fold over that measured with the wild type. Nonetheless, there is room to improve yields even further, and to get closer to the theoretical maximum of 8–10%.

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