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Review

Biogenesis of light harvesting proteins☆

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ABSTRACT

The LHC family includes nuclear-encoded, integral thylakoid membrane proteins, most of which coordinate chlorophyll and xanthophyll chromophores. By assembling with the core complexes of both photosystems, LHCs form a flexible peripheral moiety for enhancing light-harvesting cross-section, regulating its efficiency and providing protection against photo-oxidative stress. Upon its first appearance, LHC proteins underwent evolutionary diversification into a large protein family with a complex genetic redundancy. Such differentiation appears as a crucial event in the adaptation of photosynthetic organisms to changing environmental conditions and land colonization. The structure of photosystems, including nuclear- and chloroplast-encoded subunits, presented the cell with a number of challenges for the control of the light harvesting function. Indeed, LHC-encoding messages are translated in the cytosol, and pre-proteins imported into the chloroplast, processed to their mature size and targeted to the thylakoids where are assembled with chromophores. Thus, a tight coordination between nuclear and plastid gene expression, in response to environmental stimuli, is required to adjust LHC composition during photoacclimation. In recent years, remarkable progress has been achieved in elucidating structure, function and regulatory pathways involving LHCs; however, a number of molecular details still await elucidation. In this review, we will provide an overview on the current knowledge on LHC biogenesis, ranging from organization of pigment–protein complexes to the modulation of gene expression, import and targeting to the photosynthetic membranes, and regulation of LHC assembly and turnover. Genes controlling these events are potential candidate for biotechnological applications aimed at optimizing light use efficiency of photosynthetic organisms. This article is part of a Special Issue entitled: Chloroplast biogenesis.

1. Introduction

Light harvesting is a fundamental step of primary productivity, and the light use efficiency has indeed been identified as a critical factor for biomass and biofuel production in photoautotrophs [1–3]. In the past decade, progress has been made in elucidating both structural and functional bases of light harvesting, and investigation into regulation of antenna protein assembly, turnover and relative abundance, has been an area of considerable interest.

In the photosynthetic apparatus, excitation energy is rapidly transferred among chlorophylls (Chls) to a reaction center (RC), where the occurrence of charge separation events fuels electron transport chain and leads to water oxidation and NADP$^+$ reduction, and catalyzes the generation of a trans-thylakoid protonmotive force and the synthesis of ATP [4]. Within the photosynthetic machinery, a remarkably high quantum efficiency is achieved by the protein scaffold of photosystems (PSs), which keep the Chls at the right geometry and distance, thus avoiding concentration quenching while favoring excitonic interactions and fast energy transfer [5]. Photosystems I and II are membrane-integral, multisubunit pigment–protein complexes, main actors in the light energy conversion process. Both PSs are composed by a core-complex containing the RC, and by an array of membrane-embedded light-harvesting complexes (LHCs), a modular antenna system surrounding the core. All these structural elements together form a so-called supercomplex [6]. Within thylakoid membranes, PSII is located in the region of stacked membrane disks called grana, while PSI is mainly found in the stromatic lamellae, unappressed regions which connect grana stacks [7]. See [8] for a somehow different view.

Evolution generated a wide group of photoautotrophs, ranging from cyanobacteria to higher plants, which optimized photosynthesis for the most diverse environmental conditions occurring together with the enlargement of LHC protein super-family [9], in contrast with the high conservation in the subunits of the PSI and PSII core complexes [10]. Members of the LHC superfamily comprise about 40% of the protein content in the thylakoid membrane and, together, make the most abundant membrane protein on earth. They all share structural motifs with membrane-spanning regions hosting closely spaced and conserved Chl binding residues [11]. As a result LHC subunits have a lower protein/pigment mass ratio (~2) with respect to the core complex or

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either photosystems (>3), and far lower than non-LHC antenna complexes such as phycobilisomes, which appeared earlier in evolution and were then outclassed by LHCs [12–14].

Besides extending the absorption capacity of the RC supercomplexes, LHC antenna systems regulate PS photochemical efficiency and provide enhanced level of photoprotection. Indeed, while the efficiency of energy conversion is maximal under constant, moderate irradiances, photosynthesis is hampered when the concentration of Chl singlet excited states (^{1}Chl*) in the photosynthetic machinery exceeds the capacity for photochemical quenching [15]. In these conditions the probability for Chl triplet (^{3}Chl*) formation increases leading to release of singlet oxygen ($^1O_2$) [16]. Molecular safety systems are built in LHC proteins which catalyze detoxification of $^1O_2$ [17,18] or prevent its formation by downregulating ^{1}Chl* lifetime [19]. The evolutionary selection of LHCs more efficient in the activation of photoprotective responses has likely been crucial during transition from aquatic to aerial environment in which a concomitant increase in O_2 concentration leads to a higher risk of $^1O_2$ formation and photoxidative damage [13]. The formation of eukaryotic plastids was followed by the diversification of LHC on multiple isoforms, thus leading to a genetic complexity within a conserved supramolecular assembly, whose evolutionary importance emerges by considering that individual gene products are tuned to a different balance between light harvesting and photoprotection capacity [20].

2. LHC: molecular architecture, localization, function

The first structure of a light-harvesting complex obtained by X-ray crystallography was that of trimeric LHCII, the major light-harvesting complex of plants encoded by *lhcb1–3* genes [21,22]. This complex has three membrane-spanning helices (named A, B and C), connected by both stroma- and lumen-exposed loops, and two amphipathic helices (D and E) exposed to the lumenal surface (Fig. 1A). Each monomer binds 4 xanthophylls, 8 Chl *a* and 6 Chl *b* molecules, and two different lipids, phosphatidyl glycerol and digalactosyl diacyl glycerol. More recently, the 2.8 Å resolution structure of the monomeric antenna CP29 (Lhcb4) from spinach was published [23]. The CP29 structure, based on the three membrane-spanning regions and the two amphipathic helices exposed on the lumen surface, revealed great similarities with a LHCII monomer [24]. This protein contains binding sites for 13 Chls and 3 xanthophylls.

In green algae and plants, PSII and PSI are the supramolecular complexes which coordinate the LHC subunits and catalyze the photosynthetic light reactions. The largest PSII supercomplexes purified from plants [6,25] are composed of a dimeric core (C2), surrounded by the nuclear-encoded, outer antenna system which includes four trimeric LHCII, and two copies each of the monomeric antennae CP29, CP26 (Lhcb5) and CP24 (Lhcb6) [26,27]. CP29 and CP26, located nearby the core, mediate the binding of the so-called LHCII-S (strongly-bound, named by their susceptibility to detachment by detergents) [28], while the monomeric subunit CP24 and a trimeric LHCII-M (moderately-bound) enlarge the light harvesting capacity of the complex (Fig. 1B). In green algae, major LHCII components diversified independently with respect to those of higher plants: in *Chlamydomonas reinhardtii*, subunits of trimeric LHCII are encoded by nine Lhcbm genes, called Lhcbm1–m6, m8, m9 and m11 [29], while CP24 orthologs were not detected. The C2S2M2 is the most abundant PSII supercomplex in thylakoid membranes of *Arabidopsis*, either grown in low or high light conditions [6]. The abundance in trimeric LHCII is higher in low light than in excess light (EL) conditions. In both conditions the stoichiometry of trimeric LHCII per monomeric PSII core is higher than two [30,31], suggesting that additional trimers, loosely-bound (LHCII-L) exist that are lost during purification of supercomplexes.

Core complex of PSI is also endowed with a peripheral antenna system called LHCI (light-harvesting complex of PSI), of four antenna proteins (Lhca1–4), one copy per supercomplex [32] (Fig. 1C). Binding of the antenna moiety to the core is strongly cooperative [33] with the Lhca1/4 and Lhca2/3 heterodimers being the minimal building blocks [34]. The composition of the peripheral antenna was found constant irrespective from light conditions [35]. Lhcas were not interchangeable, indeed missing subunits could not be replaced by others in *Arabidopsis* mutants disrupted in individual *lhca* genes [33,36]. In addition to *lhca1–4*, two additional genes, *lhca5* and *lhca6*, were identified in the genome of *Arabidopsis* [11], which encode subunits highly homologous to Lhca1–4, and yet are found in sub-stoichiometric amounts with respect to PSI RC [37]. Consistently, Lhca5 was found to replace missing Lhca4 in a small fraction of PSI supercomplexes [38] and to mediate interaction between PSI and the NADH dehydrogenase-like complex (NDH), forming the supercomplex which drives PSI cyclic electron transport [39,40]. The study of the PSI–LHCI supercomplex in organisms other than higher plants showed differences in the organization [41]. In *C. reinhardtii*, nine Lhca gene products [29] were found to participate to large PSI supercomplexes [42].

Besides the typical three-helix type members, the LHC super-family includes other proteins which share sequence similarity with the former and yet carry significant differences, namely PsbS, LhcSR and the light-harvesting-like (LIL) proteins.

PSBS is a four-helix protein present in all land plants [43], which is essential for the photoprotective mechanism of Excess Energy Dissipation (EED) [44,45] and the EL-dependent reorganization of LHC antenna system within PSII [46,47]. Interestingly, psbS genes are present in many green algae, including *C. reinhardtii*, but the protein was not accumulated in the chloroplast [48], suggesting that this sequence might have a different function in lower organisms.

LHCSR is also essential for EED [49], but in green algae and mosses, while plants lack orthologs. In *C. reinhardtii*, LHCSR has been first described as a stress-related protein, whose transcripts accumulate in response to EL conditions [49,50] as a component of an early photoprotective type [51,52].

LIL [11,37] proteins which are found in both plants and algae differ in their number of transmembrane segments: the three-helix early light-inducible proteins (ELIPs), the one-helix proteins (OHPs) and the stress-enhanced proteins (SEPs) are likely involved in photoprotection rather than in light harvesting [53,54]. Although these subunits are not constitutive components of PSs, biochemical evidences suggest that they can establish weak interactions with PSs [55]. Their mode of action was proposed to include regulation of pigment biosynthesis [56] and/or scavenging of reactive oxygen species (ROS) [57].

The main function of LHCs is to harvest photon energy, delocalize excitons for significant time lengths (ns) and transfer excitation energy to the RCs to drive electron transport [5,58]. Besides light absorption, remarkable properties of the LHC proteins are the ability (i) to actively regulate PSII quantum efficiency and (ii) to catalyze photoprotective reactions (Fig. 2). Fluctuations of light intensity, temperature, nutrients and water availability on a daily as well as seasonal basis yield into changes of excitation pressure on PSII, by affecting the capacity for photochemical quenching of ^{1}Chl* [15] and to increased $^1O_2$ release [59,60]. Activation of photoprotective safety systems is thus mandatory in order to either scavenge ROS or limit their release [61]. LHC subunits have key roles in these processes. Lhcb proteins are ideal candidates for a role in down-regulation of ^{1}Chl* lifetime through the process of EED, that safely dissipates excitation energy in excess [19]: indeed, the depletion of LHC proteins is obtained in *ch1* mutants, and leads to depletion of EED [62] and to a dramatic increase in photosensitivity [18,63]. Xanthophylls bound to the LHC proteins protect the complex against $^1O_2$ formation, by either quenching ^{3}Chl* or directly scavenging $^1O_2$ [64–66]. Additional LHC-dependent regulation is the lateral migration of phosphorylated LHCII trimers, triggered by PQ over-reduction, to stroma-exposed membranes where they connect to PSI, balancing excitation distribution of PSs via the so called state I–state II transition (ST) [67,68]. In *C. reinhardtii*, the amplitude of ST is far larger than in higher plants, possibly due to phosphorylation of CP26 and CP29 in addition to that of LHCII. This appears to dissociate PSII supercomplexes since CP29, CP26 and LHCII trimers were all found to become associated to the PSI–LHCI

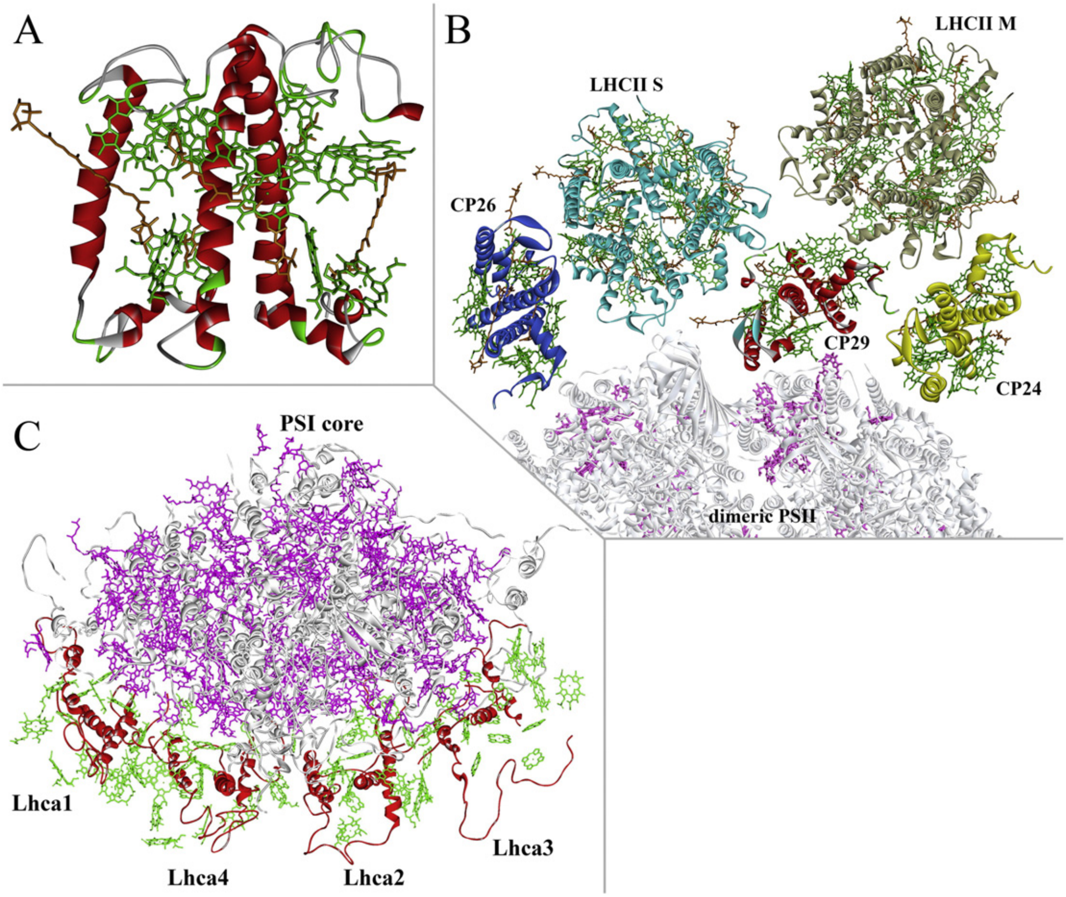

Fig. 1. Model of the structure of (A) monomeric LHCII, side view, (B) photosystem II supercomplex C2S2M2 and (C) photosystem I supercomplex, top view. The C2S2M2 model has been assembled using the crystal structures of monomeric antenna CP29 and trimeric LHCII [21,23], the cyanobacterial PSII core [185], and the plant PSI–LHCI supercomplex [186]. For the monomeric antennas CP26 and CP24, the structure of CP29 has been used. Color legend for the supercomplexes: core complexes, silver; LHCII-M, gray; LHCII-S, cyano; CP29, red; CP24, yellow; CP26, blue; LHCI, red; Chls of core complex, violet; Chls of LHC, green; and xanthophylls, orange. For clarity, the phytol chains of the Chls have been hidden.

under conditions promoting ST [69–71]. The idea of a simple balance between PSs was recently challenged by the finding that the increase in PSI antenna size is much smaller than expected in the assumption that all LHCII disconnected from PSII actually becomes an antenna for PSI [72]. Long-term photoacclimatory responses consist into the stoichiometric reduction of the trimeric LHCII complement, relieving chronic over-excitation on the PSII [73].

3. Biogenesis of LHC: expression, import, membrane insertion and assembly with chromophores

Long-term acclimatory responses in plants and algae allow for a feedback to environmental changes occurring on a time-scale of hours to days [37,74–76]. Such responses include the stoichiometric regulation of PSI/PSII ratio, and the adjustment of antenna size to PS's ability of using excitation energy from harvested photons [35,77]. Regulation of light-harvesting requires fine-tuning in response to environmental cues: indeed, expression of antenna proteins is regulated on multiple levels from mRNA transcription to protein degradation. Acclimation requires the coordinated expression of genetic information located in both nuclear and plastid genomes. In the case of LHC, mRNAs are translated in the cytosol, and precursors are then imported into the chloroplast, addressed to the photosynthetic membrane, folded with chromophores and assembled with the plastid-encoded subunits of the core complex [78] (Fig. 3).

Light strongly regulates transcription of *lhc* genes depending on phytochrome activity. Thus mRNAs encoding antenna subunits undergo circadian as well as shorter term fluctuations [79] with up-regulation in light-limiting conditions and repression in EL. There is evidence of a blue light-dependent mechanism which regulates photoacclimation in algae [80,81] most of which do not have phytochromes, while the importance of photoreceptors in sensing and responding to EL by transcriptional regulation appears negligible in higher plants [82].

Since the different components of PSs are encoded by distinct genomes, matching stoichiometric balance with environmental stimuli requires a concerted gene expression. Coupling light sensing and regulation of *lhc* gene expression involves the so-called retrograde (plastid to nucleus) signals, for which multiple pathways have been proposed [83]. A direct correlation has been reported between level of *lhc* gene transcription and protein accumulation level [37,84–86]. Response to environmental cues involves fast (<1 h) transcriptional regulation, and analysis of expression patterns allowed to identify associations of gene products which participate to the same molecular pathways. However, a number of contrasting results have questioned a major role of *lhc* gene transcriptional regulation in the long term acclimatory response: algal cells grown under limiting CO_2, a condition which severely overexcites PSII, underwent no significant changes in the level of LHCBM mRNAs [87]; the prompt down-regulation of *lhc* gene transcription by EL exposure is relieved within 24 h under EL treatment and does not lead to any decrease of the corresponding gene products [85,88], implying the action of other regulatory mechanisms.

A comparison between transcriptomic and proteomic dataset highlighted a major role of post-transcriptional control of LHC content in barley [89]. In *Arabidopsis*, light intensity strongly affected global translation by cytoplasmic ribosomes, and differentially regulates specific transcripts within the LHC superfamily [90]. A link between *lhc* message translation in the cytosol and photosynthetic

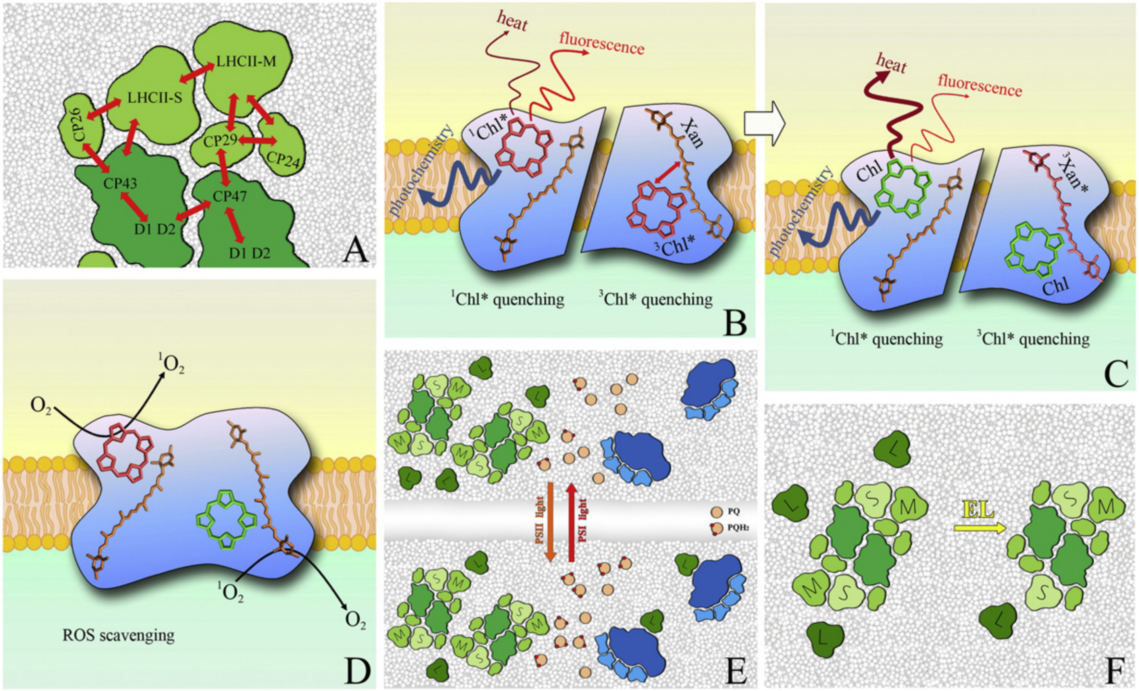

Fig. 2. Functional roles of LHC proteins. (A) Excitation energy transfer: connectivity among antennae and with the core subunits is represented according to [187]. (B, C) Regulation of ^{1}Chl* and ^{3}Chl* de-excitation: conformational changes of LHC to a dissipative state decrease ^{1}Chl* lifetime and promote thermal dissipation of excitation energy in excess; while, zeaxanthin binding to LHC modulates ^{3}Chl* formation in vivo. Both processes lower the yield of potentially dangerous Chl excited states on the complexes, thus preventing ROS formation. (D) ROS scavenging: xanthophylls preserve PSII from photoinactivation and protect membrane lipids from oxidation, being particularly active against singlet oxygen; photoprotection capacity of xanthophylls is enhanced upon binding to LHC. (E, F) Lateral migration of phosphorylated LHCII balances excitation delivery between PSII (green) and PSI (blue) via the so called state I–state II transition (E), while the EL-induced reduction of Lhcb stoichiometry is a long-term photoacclimatory response, aimed at counteracting prolonged over-excitation on the PSII (F). PSII:LHC supercomplexes are depicted according to [30,188].

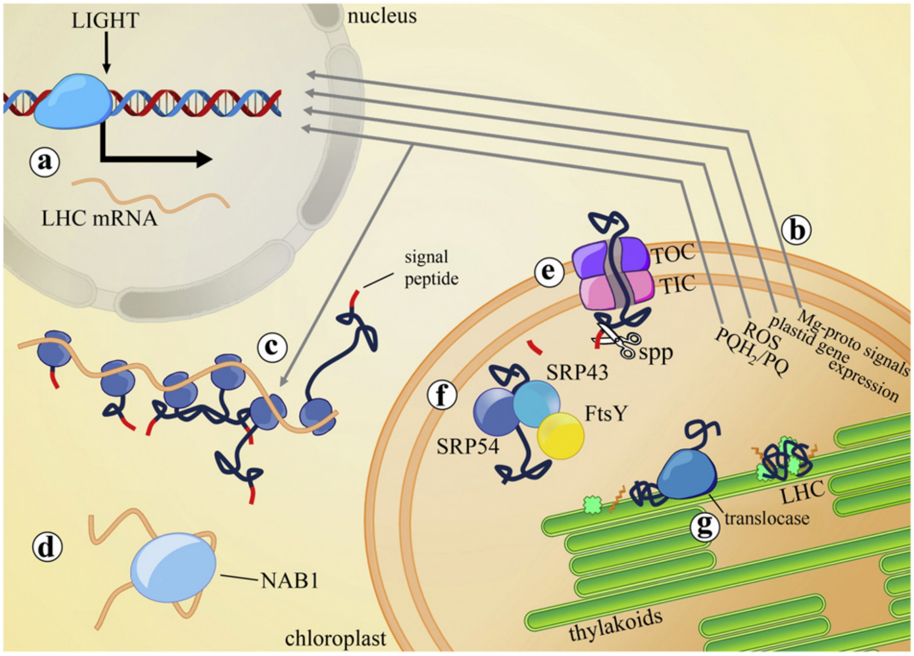

Fig. 3. Life cycle of a LHC. The diagram displays an overview of regulatory events and co-ordination between the nucleus, cytoplasm and chloroplast, which overall adjust LHC expression, import in the organelles and insertion into the photosynthetic membrane. (a) Environmental signals such as circadian rhythm and diurnal fluctuation of lights regulate *lhc* gene transcription. (b) Upon sensing environmental stimuli, chloroplasts communicate their functional status to the nucleus. A number of pathways have been involved in the regulatory signaling of photosynthetic gene expression; PQ redox state and plastid gene expression affect Lhcb transcription. (c) The redox state of PQ pool is linked to post-transcriptional regulation of *lhc* gene expression. (d) In *C. reinhardtii*, the cytosolic RNA-binding protein NAB1 interacts with specific Lhcbm transcripts, leading to translational repression. (e) TOC and TIC, translocons of the envelope membrane, mediate translocation of LHC precursor in the stroma and cleavage of the signal peptide. (f) LHC in the stroma is captured by the dimeric cpSRP; interaction with the SRP receptor cpFtsY brings LHC to the Alb3/Alb4 translocases, which mediate the co-translational targeting of the polypeptide into the thylakoids. (g) In the photosynthetic membrane, pigment binding might proceed by a self-assembly or by means of a folding machinery. Further details are discussed in the text.

activity was observed in seedlings treated with DCMU, an inhibitor of the photosynthetic electron transport: DCMU impaired light-dependent translational induction while did not affect the up-regulation of Lhcb transcripts upon dark to light transition [91]. In *C. reinhardtii*, analysis of polysomal profile upon photoacclimation to EL revealed translational repression of specific LhcbM [76]. NAB1, a cytosolic RNA-binding protein lacking in a *Chlamydomonas* mutant affected on photoacclimation, modulates LhcbM composition by selectively interacting with specific *lhcbm* transcripts [92], which are sequestrated in sub-polysomal mRNA–ribonucleoprotein complexes thus leading to translational repression. Activity of the repressor was regulated through Arg methylation [93] and thiol modification [94], and is thus linked to the redox condition of the cytosol, which in turn is reliant on the photosynthetic electron transport rate. However, many details on the regulation of photosynthetic gene expression still remain elusive, as well as the identity of the retrograde signals which modulate nuclear gene expression to the status of the plastid. Also in *Chlamydomonas*, plastoquinone (PQ) redox state affects expression of several *lhcbm* genes, whose transcription is repressed upon exposure to EL [85]. This is consistent with the redox state of plastoquinol (PQH_2) being the rate-limiting step of photosynthetic electron transport and thus reflects the balance between photon absorption and energy utilization by downstream reactions. In plants, the role of PQ redox state in regulating *lhc* gene transcription is controversial [95,96]. However, in barley [89] redox state of PQ was found to control post-transcriptional regulation of *lhc* genes [89]. Additional pathways have been involved in the regulatory signaling of photosynthetic gene transcription including intermediates of tetrapyrrole biosynthesis, ROS [97], coupling to plastid gene expression [98], secondary metabolites [99] and carotenoid oxidation products [100].

LHC proteins are synthesized on cytosolic ribosomes, in the form of precursors carrying an N-terminal transit peptide for addressing to the protein import machinery of the chloroplast. The latter is a multiprotein complex of the envelope membrane composed by the TOC and TIC translocons [101,102], respectively inserted in the outer and inner envelope membranes, which mediates precursor translocation towards the plastid stroma compartment [103]. Once in the stroma, transit peptide is taken away by the stromal processing peptidase (spp), then the mature LHC polypeptides are targeted and integrated into the thylakoids following a SRP-dependent pathway [104]. TOC34, one of the three subunits forming the core complex of TOC, is encoded by a single gene in *Chlamydomonas* [105], while two paralogs in *Arabidopsis* (AtTOC33, AtTOC34) form functionally different TOC translocons [106]; AtTOC33 depletion led to an impaired assembly of the photosynthetic machinery, and that subunit was proposed as the preferential pathway for import of LHC proteins [107]. The main pathway by which LHC subunits are targeted to the thylakoid membranes involves identification and capture by a plastidic signal recognition particle (cpSRP), a heterodimeric protein complex comprised of cpSRP54 and cpSRP43 [108]. Binding of LHC polypeptide to the cpSRP involves a conserved sequence motif, called L18, which is localized in between helices A and C [109]. Once assembled, this complex interacts with cpFtsY, homologous to the bacterial SRP receptor, which bring LHCs to the Alb3/Alb4 translocases [110]. The latter catalyze the GTP-dependent, co-translational targeting of the polypeptide into the thylakoids, which together with pigment binding yields into a functional light-harvesting complex [108,111].

Mutations affecting components of cpSRP targeting pathway lead to a phenotype of truncated Chl antenna size. *Chlamydomonas tla3* mutant, which exhibited a 90% reduction in the Lhcb complement per PSII RC, was deleted in a gene homolog to the cpSRP43 [112]. *Arabidopsis* mutant plants devoid of both 43- and 54-kDa subunits of the cpSRP had severely impaired accumulation of LHCs in thylakoids although still viable [113]. Interestingly, retention of the different LHCs was uneven: some (Lhca1, Lhcb3) were completely missing, while others (Lhcb1, Lhcb6) were in part retained or even enriched with respect to WT plants (Lhcb4). The maintenance of functional LHC targeting to the thylakoids, even in plants in which cpSRP pathway was missing, suggests that more than one targeting mechanism is active. Indeed, evidence for a second pathway in the import step can be postulated based on evidences in *Chlamydomonas* [114] and in *Arabidopsis* [115]. Association between TIC and chlorophyllide *a* oxygenase (CAO) was suggested to be required for Lhcb1 and Lhcb4 import, with CAO participating to Chl *b* supply to the nascent LHC complexes, before it was delivered to thylakoids via fusion of vesicles budding from the inner envelope membrane [116]. Based on a dual location of CAO, in the inner envelope and thylakoid membranes, the existence of two different import/assembly pathways (cpSRP- and CAO-dependent) for LHCs has been suggested. However, *Arabidopsis* mutant lacking CAO activity [117] showed that Chl *b* depletion did not affect import of LHC precursors, processing to mature form and insertion into thylakoids. Regardless of whether LHC polypeptide accumulation, folding and binding of pigment take place in the inner envelope or in the thylakoid membranes, it is clear that our knowledge on molecular mechanisms for LHC targeting is still poor.

cpSRP is proposed to maintain the antenna complex in an unfolded form devoid of pigments prior to delivery to the membrane [118]. Pigment binding is mandatory for LHC stability into thylakoids; indeed, apoproteins do not accumulate in the absence of chromophores [119]. Little is known on how chlorophylls and xanthophylls are provided to the nascent complexes.

Hoober et al. [114] proposed that LHCII assembly takes place in the inner envelope of the chloroplast and consists of 4 steps: (i) partial LHC insertion until a stop-transfer region within the B helix enters the membrane; (ii) binding of Chl molecules which allows the LHC complex to reach a stable conformation and be retained in the membrane; (iii) insertion of helix A and C domains in the membrane; and (iv) further pigment binding and fixing of the ion bridges between helices A and B, yielding into a fully assembled LHC pigment–protein. Alternatively, it has suggested that assembly of LHCs in the thylakoids requires translocation of both the luminal loop and the hydrophilic C terminus across the membrane. Both these models still await experimental confirmation. It is still possible that LHC insertion and folding with pigments is a spontaneous process following ALB3/4 catalyzed steps. This is suggested by the possibility of obtaining in vitro pigment–protein complexes, indistinguishable from those purified from chloroplasts, from its apoprotein and chromophores [120,121]. The possibility of step-triggering the folding process by sequential addition of individual chromophores has been exploited to track LHC folding dynamics by time-resolved spectroscopy experiments, which have monitored the establishment of excitation energy transfer between Chls *a* and *b* [122] or from Chls to an acceptor dye [123] in the nascent complex. These experiments showed that pigment binding and building of the protein secondary structure are closely coupled events and occur with the same kinetics. In particular, folding of LHCII in vitro occurred into a faster step (10–60 s) followed by a slower process requiring several minutes [124] (Fig. 4). The fast phase was attributed to Chl *a* binding, whereas slow binding events were dependent on Chl *b* binding [123]. Time-resolved circular dichroism spectroscopy showed the formation of α-helices during both phases [125]. Double electron resonance spectroscopy, which measures the distances between two spin label pairs, was recently used to gain insights into LHCII folding [126]. Results showed that achievement of the tertiary structure, through the formation of ion bridges between helices A and B, and binding of the last pigments, were late steps in LHCII folding, which strengthen the complex and enabled molecular interactions with partners in the photosystem.

Although this unique property of LHC proteins is consistent with self-assembly [127], it must be considered that folding in vitro occurs at very high pigment concentration, while it appears unlikely that Chl molecules can freely diffuse in thylakoids without yielding dangerous photochemical reactions [60]. Since individual LHCs showed a highly reproducible pigment composition in vivo [128–132], the question arises how such a specificity is maintained for at least ten distinct antenna proteins that

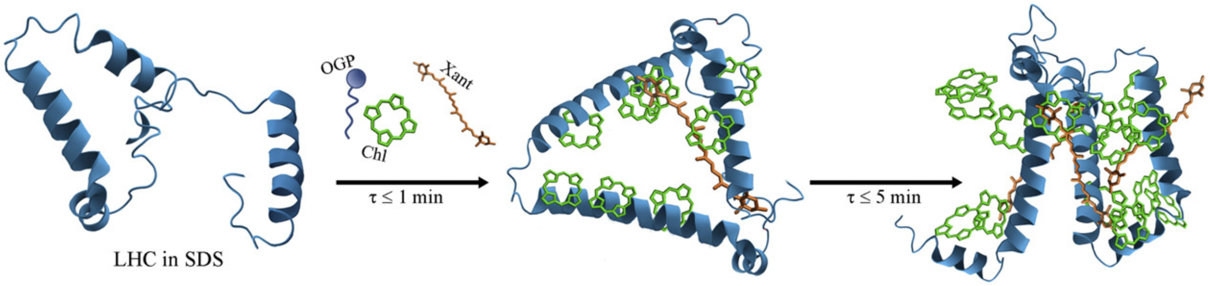

Fig. 4. In vitro folding dynamic of LHCII. The model proposed by [126] is displayed. Upon mixing LHCII apoprotein with pigments, folding was shown to occur in two phases. Both phases are triggered by pigment binding, and correspond to acquisition of complete secondary ($\tau_1 \leq 1$ min) and tertiary ($\tau_2 \leq 5$ min) structures, respectively. Abbreviations: SDS, sodium dodecyl sulfate; OGP, octyl β-D-glucopyranoside; Chl, chlorophylls; and Xant, xanthophylls.

are assembled in close proximity, i.e. with a similar accessibility to chromophores. Whether a pigment delivery step mediated by a specific carrier is involved, or if LHC folding occurs coupled to the final steps of pigment biosynthesis [133,134] is unknown, since no mutants impaired in such a function have been identified so far. The integral membrane complexes Alb3/Alb4 are needed for the accumulation of LHC in the thylakoids [110]. Thus, in the hypothesis that assembly with pigments proceeds under the control of a folding machinery, Alb3/Alb4 appears as potential components of such a still unknown putative assembling supercomplex.

4. Role of chromophores in the biogenesis of LHCs

Chlorophylls and xanthophylls are structural elements of LHCs, and have been shown to be essential for pigment–protein folding in vitro [135]. In vivo, the assembly of LHC apoproteins with their cofactors is a checkpoint in the modulation of LHC abundance, which interacts with transcriptional and translational regulatory networks. Indeed, no LHC proteins accumulate in the absence of pigment synthesis [136,137] and stoichiometric adjustment of the photosynthetic subunits requires a coordinated biosynthesis of apoproteins and chromophores [138].

In particular, Chls *a* and *b* are both required for stabilization of the apoproteins and assembly of the majority of antenna complexes in higher plants [139]; this is consistent with the evidence that lack of Chl *b* in *ch1* mutant of *Arabidopsis* yielded into near-complete depletion in LHCs [18,62]. Instead, lack of Chl *b* does not impair LHC assembly in the green alga *C. reinhardtii* [140]. Marine photoautotrophs evolved a variety of Chl species [141,142] while land plants all have Chls *a* and *b* as the only light harvesting porphyrins, both being needed for LHC biogenesis, thus suggesting a specific function in folding for each. While the universal role of Chl *a* in the photochemistry is well established, it is interesting to assess whether Chl *b* has a regulative role for LHC biogenesis besides the enhancement of light-harvesting cross-section.

Crystal structure of plant LHCs showed distinct and specific Chl *b* binding sites [21], thus suggesting that Chl distribution is regulated by their binding affinity. In vivo changes in the Chl *a*/*b* ratio of antenna complexes by irradiance during growth have never been reported in WT plants. However, the Chl *a*/*b* ratio of thylakoids has been altered by impairing the Chl *b* metabolism through the overexpression of a cyanobacterial CAO gene in *Arabidopsis* [143] leading to an increased Chl *b* abundance and a higher LHCII content with respect to WT. This led to the hypothesis that CAO activity (i) determines the rate of Chl *b* biosynthesis and (ii) regulates LHC biogenesis and light acclimation [144]. Indeed, acclimation of WT plants to either low- or high-light involves modulation of both CAO activity [145] and Chl *b* content, which leads to changes in PSII antenna size. The mechanism responsible to such a Chl *b*-dependent accumulation of LHCs is still unknown, although Tanaka and Tanaka [144] hypothesized that LHCII does not acquire a proper conformation below a minimal Chl *b* threshold, making it a better substrate for proteases. Whether this hypothetical mechanism is actually important in vivo and how it is interfaced with regulation of *lhc* gene transcription/translation regulation in the cytoplasm, is still to be assessed.

LHC chromophores also include the xanthophylls lutein, neoxanthin and violaxanthin. The latter is exchanged with zeaxanthin (Zea) which is only synthesized upon EL exposure. In vitro reconstitution analyses revealed xanthophyll binding sites with high specificity [121]. In vivo, however, xanthophyll binding site appears to be more promiscuous as judged by the observation that xanthophyll biosynthesis mutants showed little modifications of Chl to Car ratio and LHC abundance with respect to WT plants [65,66,146,147].

Exception to this pattern is Zea, the only xanthophyll in the *npq2 lut2 Arabidopsis* mutant which, in consequence, undergoes a decrease of PSII antenna size due to a selective destabilization of LHCs [148]; the effect was also observed in the *C. reinhardtii* mutant *npq2 lor1* [149] with similar xanthophyll composition, suggesting that Zea might down-regulate PSII biochemical antenna size. Since Zea binding to LHCs was shown to trigger a conformational change [150], the Zea-binding LHC might become available for proteolysis; this would allow closing the feed-back loop in which overexcitation leads to thylakoid lumen acidification and activation of VDE (violaxanthin de-epoxidase) yielding into accumulation of Zea, which binds to antenna proteins leading to their degradation and decreased lumen acidification, adjusting LHC complement to the average incident light. Even before LHC degradation, Zea binding increases Chl triplet quenching efficiency and ROS scavenging, thus partially relieving photoxidative damage in both PSs [151,152].

The strongest effect on the biogenesis of LHCs comes from limitation in the relative abundance of the total pool of xanthophyll vs carotenes, rather than from changes in composition within the pools. In *Arabidopsis*, combination of mutations of xanthophyll biosynthesis yielded into an eight-fold decrease in xanthophyll/carotenoid ratio with respect to WT [153]. As a consequence, LHCII to PSII core complex stoichiometry was strongly decreased. This was in striking contrast with the case of PSI, where the LHCI to RC ratio was the same as in WT. Decreasing xanthophylls to carotene ratio had a strong effect into the total amount of PSI, which decreased together with xanthophyll abundance till its complete depletion in the xanthophyll-less mutant [137]. Since PSI core only binds β-carotene, while xanthophylls are components of LHCI, the most obvious conclusion is that PSI requires LHCI for its stability. However, this is not the case since *ch1* mutant lacks Lhca proteins and yet has an efficient PSI activity. The lesion in *ch1* leaves the mutant without Chl *b*, while its content in xanthophylls is nearly WT. We conclude that a xanthophyll-dependent coupled mechanism exists for the co-regulation of PSII antenna size and the PSI core complex, which is consistent with the maintenance of a proper redox state of PQ pool. This mechanism appears to be effective in the regulation of both the antenna size with respect to light intensity and PSI to PSII ratio, in an integrated plot (Fig. 5).

The limitation in PSI accumulation was shown to be due to a decreased translation efficiency of PsaA core complex subunit. The connection with xanthophyll availability is still to be elucidated but it is well possible that xanthophylls might affect the activity of the Alb3/4–cpSRP complex, which acts in both PsaA and LHC insertion into the thylakoid

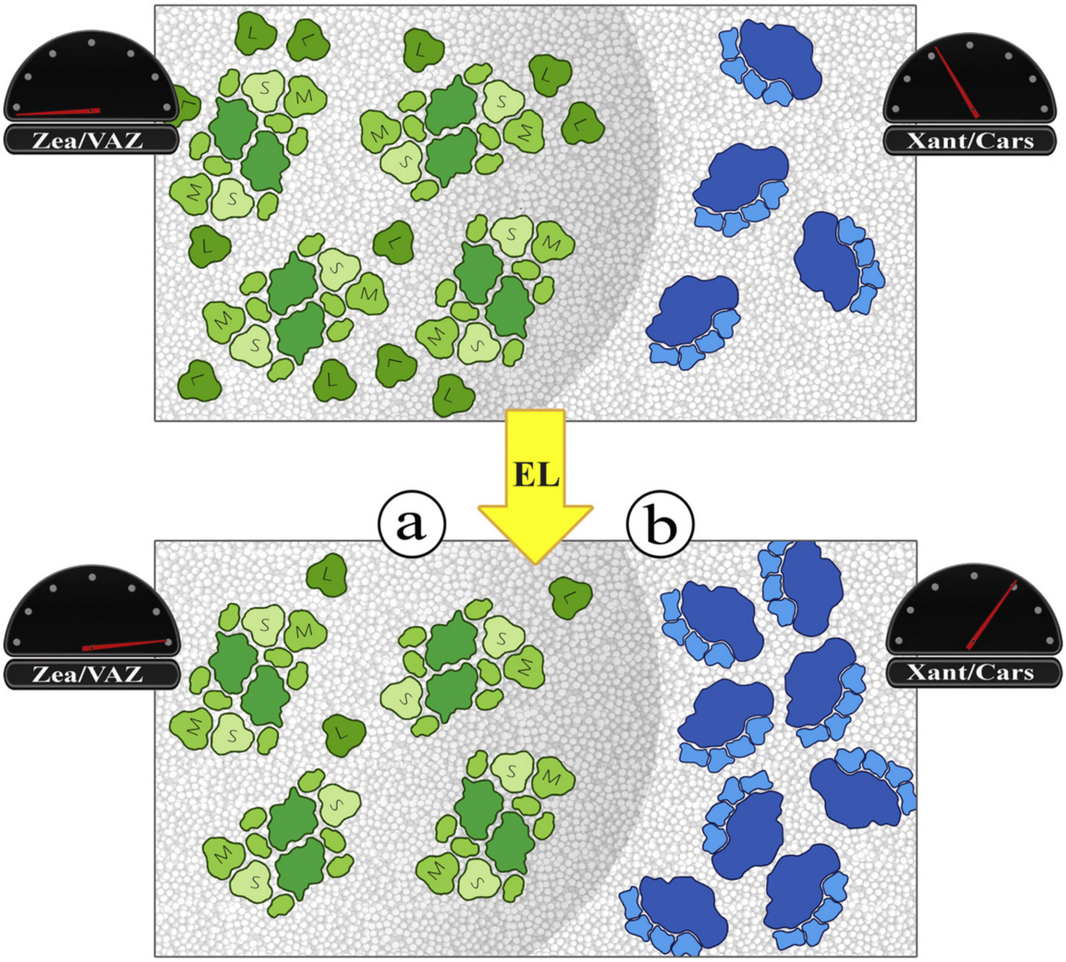

Fig. 5. Scheme of the regulation of PSII antenna size and PSI to PSII ratio as assessed in *Arabidopsis* xanthophyll biosynthesis mutants. (a) Zeaxanthin down-regulates PSII biochemical antenna size: its binding to LHCs triggers a conformational change of the complex, which might become available for proteolysis. (b) The modulation of xanthophyll to carotene ratio in EL would reflect into an adjustment of PSI level. Such a xanthophyll-dependent coupled mechanism, co-regulating PSII antenna size and PSI to PSII ratio, is consistent with the maintenance of a proper redox state of PQ pool. VAZ, violaxanthin + antheraxanthin + zeaxanthin; Xant, xanthophylls.

membrane. Within LHC superfamily, Lhca proteins show a higher affinity for xanthophylls. This effect might be ascribed to the capacity of LHCI of binding small amounts of β-carotene [154], at difference with LHCII.

5. Degradation of LHCs during acclimation and senescence

Despite the paramount importance of intramembrane proteolytic events which take place in the chloroplasts, present knowledge about the identity of proteases involved and the mechanism of their regulation is limited [155]. In particular, even less is known about the proteases involved in the regulation of LHC turnover.

Under low irradiance, turnover of LHCs is very slow, insomuch as being hardly detectable [156]. The half-life of LHCs was determined by short-term labeling in vivo with ^{35}S-methionine, and was found around 10 h in *Lemna minor* [157] and much slower in *Phaseolus vulgaris* [158] since no LHCII degradation was detected within 24 h. However, treatments such as light-to-dark transition, EL conditions or senescence induced by prolonged darkness, speed-up turnover rate of LHCs.

In particular, during photoacclimatory response to EL, a strong decrease in the level of Lhcb polypeptides per PSII RC is detected within a few hours by the application of the stress. These changes are not coupled to any significant change of *lhcb* gene transcription, and were thus attributed to a post-transcriptional regulatory mechanism [90], possibly modulated by the PQ redox state [89]. Another possibility is that regulation of LHC abundance is achieved by tuning turnover rate. Consistent with this idea, EL up-regulates expression of a number of chloroplastic proteases [159]. Up to now, such mechanisms have been mainly investigated in higher plants, and early results suggested that Ser/Cys-type proteolytic enzymes were implicated in LHCII degradation during chloroplast development [160] or participated to the ATP-dependent proteolysis of LHCII upon acclimation to EL [161,162]. The proteolytic enzyme, found associated to the surface of stroma-exposed thylakoid domains, was shown active in degrading de-phosphorylated form of LHCII in vitro. Moreover, a SppA protease was suggested to participate to LHCII degradative regulation [163]. Substrate specificity and mechanism of substrate recognition of EL-induced, thylakoid-bound proteases were studied in vitro by means of wild-type and mutant recombinant LHCII: results showed that the N-terminal domain of the antenna was needed for the protease–substrate recognition, thus suggesting that the degradative event originates at the N-terminal region [164].

More recently, a metalloprotease was identified as responsible for Lhcb1 degradation during chloroplast senescence induced by prolonged darkness [165]; such an enzyme is an integral protein of thylakoids, requires ATP and either Mg^{2+} or Zn^{2+} for activation, and was present even in chloroplasts of illuminated leaves, although in an inactive form. In *Arabidopsis*, a reverse genetic approach allowed to identify FtsH6 as the protease involved in degradation of LHCII, which occurs during photoacclimatory response to EL conditions [166]. In a more recent study [167] a chloroplastic AtFtsH heterocomplex was shown to be involved in stress-dependent degradation of Lhcb1–2–3 apoproteins. In *Arabidopsis* Deg1, a serine-type, thylakoid extrinsic protease, was shown to induce cleavage of the monomeric antennae CP26 and CP29 in response to EL [168,169], while Deg2 triggers degradation of the monomeric antenna CP24 in response to high-temperature and high-light stress [170]. The latter result is consistent with the evidence that CP24 underwent the most rapid decline among the Lhcb complexes during acclimation to EL in *Arabidopsis* [35].

Thus, a number of proteases were shown able to catalyze proteolysis of Lhcb subunits, but the level of redundancy of the system is still unclear. None of the FtsH sequences encoding for chloroplastic proteases were up-regulated in high light [171]. Although degradation of pigment–protein complexes is expected to be a highly regulated process in order to avoid photochemical damage of PSs, a regulatory network for LHC proteolytic activities of the chloroplast has not yet been described.

Monomerization of the major antenna may represent the triggering event of degradative response, indeed the monomeric forms of LHCII are targeted for proteolysis, while the trimeric forms are not [164]. Another possibility is that activation of proteolysis is triggered by the recognition of amino acids on exposed, unfolded region of the target protein, possibly located either at the N-terminal domain [172] or in the luminal loop regions [173]. The chloroplast protein SGR, recently identified by studying a stay-green mutant line in *Oryza sativa* [174], has been associated with the first events of destabilization of the LHC proteins, which are obligatory steps for degradation of Chls and antenna polypeptides [175]. Another possible scenario is that Chl *b* degradation is the first event that precedes and triggers proteolysis [176], this model strengthened by the evidence that FtsH lacks a strong unfoldase activity. However, it is reasonable to assume that initiation of the proteolytic reaction is the rate-limiting step, while the subsequent degradative steps might be very fast, since no degradation products can be detected in vivo once LHC dismantling is triggered by EL or sustained darkness [177].

6. Concluding remarks

During the last decade, considerable progress has been achieved with respect to both molecular and functional characterization of light-harvesting in green algae and plants. Structures of LHCII and CP29 [23] were elucidated at the atomic level, and new insights were gained on the organization and the interactions of PS supercomplexes within the thylakoid membrane [6]. Mutations affecting either the cpSRP-dependent pathway [112] or the cytosolic translation efficiency [94] showed that such mechanisms are target for reducing cellular pigment content and PS antenna size. Therefore, they potentially represent biotechnological strategies for optimizing photosynthetic yield of algal mass culture [3]. A number of studies have led to our present understanding of the photoprotective mechanisms of EED, localized within the LHC moiety [178–181]. Capacity of prompt response to fluctuating light has been predicted to affect canopy photosynthesis by up to 30% [182], thus comprehension of molecular details of thermal dissipation opens the possibility to manipulate light-use efficiency in order to enhance crop productivity [183,184].

By contrast, several aspects of biogenesis of LHC still remain poorly understood. Some of them concern the role of *lhc* expression regulation in the long term photoacclimation. Research on the cross-talk between chloroplast and nucleus in plant cells showed that post-transcriptional control of LHCs is mediated by a signal from the chloroplast, affecting translation in the cytoplasm [90] and whose molecular nature is still unknown. The existence of a retrograde signaling that synchronizes *lhc* gene expression to the status of the chloroplast is largely accepted, but again its molecular identity still remains elusive. Understanding of LHC precursors targeting and insertion in the photosynthetic membranes is still limited with respect to whether assembly of the complete LHC proceeds through a spontaneous folding with recruitment of free chromophores, or involves an assembly supercomplex. Finally, identification and characterization of proteases involved in LHC degradation, and comprehension on how polypeptide and pigment turnover interact with each other, are key steps for understanding the life cycle of antenna proteins.

Evolution has diversified LHCs into a large and diverse group of proteins which, despite only apparent redundancy, were shown to be crucial in adapting to a range of (even extreme) environmental conditions, thus they represent a base of genetic variability which offers perspective for the enhancement of light-to-biomass conversion efficiency, particularly in non-natural environments such as photobioreactors.

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