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Review Article

LHC-like proteins involved in stress responses and biogenesis/repair of the photosynthetic apparatus

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LHC (light-harvesting complex) proteins of plants and algae are known to be involved both in collecting light energy for driving the primary photochemical reactions of photosynthesis and in photoprotection when the absorbed light energy exceeds the capacity of the photosynthetic apparatus. These proteins usually contain three transmembrane (TM) helices which span the thylakoid membranes and bind several chlorophyll, carotenoid and lipid molecules. In addition, the LHC protein family includes LHC-like proteins containing one, two, three or even four TM domains. One-helix proteins are not only present in eukaryotic photosynthetic organisms but also in cyanobacteria where they have been named high light-inducible proteins. These small proteins are probably the ancestors of the members of the extant LHC protein family which arouse through gene duplications, deletions and fusions. During evolution, some of these proteins have diverged and acquired novel functions. In most cases, LHC-like proteins are induced in response to various stress conditions including high light, high salinity, elevated temperature and nutrient limitation. Many of these proteins play key roles in photoprotection, notably in non-photochemical quenching of absorbed light energy. Moreover, some of these proteins appear to be involved in the regulation of chlorophyll synthesis and in the assembly and repair of Photosystem II and also of Photosystem I possibly by mediating the insertion of newly synthesized pigments into the photosynthetic reaction centers.

Introduction

Chlorophyll-binding proteins play a key role in the growth and development of eukaryotic photosynthetic organisms. These proteins are localized in the thylakoid membranes of plants and algae and form light-harvesting chlorophyll–protein complexes called light-harvesting complex (LHC) that funnel the absorbed light excitation energy to the reaction centers of photosystem II (PSII) and photosystem I (PSI) to create charge separations across the thylakoid membrane. These events lead to the photo-oxidation of water by PSII and electron flow along the photosynthetic electron transport chain from PSII to the plastoquinone pool, the cytochrome b6f complex, plastocyanin and PSI where a second light-triggered charge separation occurs followed by a reduction of ferredoxin and of $NADP^+$ to NADPH with concomitant proton translocation into the thylakoid lumen. The resulting proton gradient is used for ATP synthesis by the ATP synthase.

LHC proteins generally consist of three α-helices that bind chlorophyll *a* and other chlorophylls (*b* or *c*), carotenoids and lipids [1,2]. While most of these proteins are mainly involved in harvesting light energy and accumulate in large amounts, they have distant relatives, LHC-like proteins, in land plants, algae and cyanobacteria which contain between one and four transmembrane (TM) domains with putative chlorophyll-binding sites referred to as LHC motifs. They include the one-helix proteins (OHPs) also called HLIPs (high light-induced proteins) in cyanobacteria, two-helix stress-enhanced proteins (SEPs), three-helix proteins involved in various light responses and including LHCSRs and ELIPs (early light-induced proteins) and the four-helix PSBS protein that is unable to bind pigments (see Table 1). The HLIPs and SEPs are the likely ancestors of extant three-helix LHC proteins which

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Table 1 LHC-like proteins involved in stress responses and assembly of photosynthetic complexes

Protein	Organism	Number of helices	Function
PSBS	Land plants, mosses, algae	4	qE-NPQ (land plants, mosses) acclimation to UV-B
LHCSR1	Green and brown algae, non-vascular plants, diatoms	3	qE-NPQ
LHCSR3	Green and brown algae, non-vascular plants, diatoms	3	qE-NPQ
CrLHCBM1	*C. reinhardtii*	3	qE-NPQ
CrLHCBM9	*C. reinhardtii*	3	Acclimation to high light
ELIP	Land plants, mosses, algae	3	Response to high light
MSF1	*C. reinhardtii*	3	Assembly of photosynthetic complexes
SEP/LIL	Land plants, algae	2	Acclimation to stress, chlorophyll biosynthesis
OHP1, HLIP	Land plants, algae, cyanobacteria	1	Acclimation to stress, assembly of photosynthetic complexes
OHP2	Land plants, algae, cyanobacteria	1	Acclimation to stress, assembly of photosynthetic complexes

could have been derived from the former through gene duplication, fusion and deletion events [3,4]. Many of these proteins are induced under specific stress conditions such as high light, UV-B light, nutrient limitation and elevated temperatures. However, their precise role and mode of action in most cases has not yet been elucidated although they are clearly important for acclimation, optimal photosynthetic function and plant survival under stress. In this review, some of these proteins are presented and discussed. Several excellent reviews on this topic have been published in recent years [5,6].

LHC-like proteins, induced by stress, involved in non-photochemical quenching

Because photosynthetic organisms are subjected to frequent and rapid changes in light irradiance, they have evolved efficient protection mechanisms against photo-oxidation. One of these is energy-dependent non-photochemical quenching (qE-NPQ) in which the excess absorbed light energy is dissipated as heat [7]. This process is rapidly induced and relaxed within seconds to minutes. After transfer of the illuminated plants to the dark, the quenching relaxation kinetics reveals different components: besides the fast qE component, slower components appear, including qT/qZ due to state transitions and/or zeaxanthin binding to the LHC proteins [8], qH related to abiotic stress [9] and qI caused by photoinhibition [10,11]. When the absorbed light energy exceeds the capacity of the photosynthetic apparatus, acidification of the lumen occurs and leads to changes in pigment composition through the xanthophyll cycle and the LHC proteins switch from an energy absorption mode to an energy dissipation mode [12,13]. The process involves pH-sensing proteins such as PSBS [14] and/or members of the LHC-like family including LHCSRs [15] and HLIPs (see below).

In the absence of PSBS, the quenching relaxation kinetics reveals another slower component of fluorescence decay named qM, which is triggered by blue light through the Phot2 photoreceptor, and distinct from qE, qT/qZ and qH, which depend on the formation of a TM proton gradient. In contrast with qE, qM is independent of xanthophyll or LHC composition [16].

PSBS is an LHC-like protein containing four TM domains that originated most likely from a two-helix progenitor [4]. qE-NPQ is triggered by the acidification of the thylakoid lumen that occurs following high light treatment. Under these conditions, the pH of the lumen is between 5.5 and 5.8, two pH units below that of the stroma [17]. This leads to the protonation of two Glu residues of PsbS exposed to the thylakoid lumen. It was proposed that upon protonation of these residues, the conformational state of the protein changes thereby promoting dissipation of the absorbed light energy as heat [18]. Determination of the structure of PSBS has

revealed that two long intertwined TM helices, TM1 and TM3 form a central supercoil and are surrounded by two short helices TM2 and TM4. These helices are linked by two short lumenal loops and an elongated stromal loop [19]. Besides the additional helix TM4, there are significant structural differences with the common LHC proteins. Notably, PSBS forms a compact structure which does not allow for the formation of pigment-binding sites. Thus, PSBS is not a canonical pigment-binding protein [19]. However, its crystal structure has not provided clear insights into how it acts. PsbS forms homodimers in the thylakoid membrane and it was suggested that activation of PSBS by low pH may be mediated through a conformational change involving altered lumenal intermolecular interactions of the PSBS dimer [19] in line with the idea that PSBS acts as a pH-sensitive trigger for qE perhaps by promoting a reorganization of the photosynthetic membranes which in turn induces the formation of quenching sites in the PSII antenna system [20–23]. Alternatively, the activated PSBS dimer could interact directly with neighboring LHCs and induce quenching within these LHCs. Pull-down assays [24] and *in vitro* reconstitution of the quenching process with proteoliposomes containing PSBS, LHCII and zeaxanthin [25] support such a direct interaction between PSBS and LHCs during qE-NPQ. The specificity of these interactions is also supported by a genetic analysis of LHC proteins showing that quenching occurs within specific antenna proteins, namely the monomeric antenna CP29 [26] and the Lhcbm1 component of trimeric LHCII [27,28]. These interactions do lead to rearrangement of PSII supercomplexes in the grana [22,29].

The need of PSBS for inducing NPQ when the pH of the lumen is sufficiently low was questioned based on two observations. First, quenching of fluorescence was still observed in the PSBS-deficient *npq4* mutant of *Arabidopsis* after long-term illumination although at a reduced rate. Second, treatment of the *npq4* mutant with the quinone analog diaminodurene that mediates cyclic electron flow around PSI and thereby enhances the pH gradient, induced a rapidly reversible qE-type NPQ in isolated intact chloroplasts of the mutant [30] suggesting and that the role of PSBS is to rapidly turn on NPQ at physiological lumen pH values [30]. However, it was later shown that the fluorescence quenching observed in the *npq4* mutant upon illumination which accounts for nearly 50% of the qM amplitude in wild type, is due to the chloroplast light avoidance response which can be induced only with white or blue light but not with red light [16,31]. This process is mediated by the blue light receptors phototropins. Thus, this fluorescence decrease is mostly caused by a decreased light absorption because of the light-induced relocation of chloroplasts rather than by a change in quenching activity. Moreover, the dark reversibility of the quenching process observed in the *npq4* mutant treated with diaminodurene could not be reproduced by one of us (R. Bassi and S. Dall'Osto, unpublished results) and thus remains a controversial issue. The current evidence supports a direct and essential role of PSBS for triggering the quenching reactions of qE.

PSBS genes have been detected in plants and green algae but not in other oxygenic photosynthetic organisms which are not part of the green lineage [32]. Although two PSBS genes are present in the nuclear genome of the green alga *Chlamydomonas reinhardtii*, initial attempts to detect the protein under several different growth conditions were unsuccessful [23]. However, transcriptomic studies reveled that these genes are expressed under specific conditions such as nitrogen deprivation [33] or during a dark to light shift [34]. Further studies revealed that PSBS accumulates rapidly and transiently upon light stress [35]. Moreover, constitutive overexpression of PSBS in the chloroplast of *Chlamydomonas* increased qE-NPQ [36].

Interestingly, PSBS is strongly induced by UV-B light together with LHCSR1 (see below) both of which contribute to qE under these conditions. Upon UV irradiation, the cytoplasmic dimeric UVR8 receptor monomerizes and interacts with the E3 ubiquitin ligase COP1 (Constitutively Photomorphogenic 1), moves to the nucleus and induces changes in gene expression [37–40]. PSBS and LHCSR1 are prominent among the proteins up-regulated in this response [41]. They provide a direct mechanistic link between UVR8 receptor signaling and acclimation and photoprotection of the photosynthetic machinery of *Chlamydomonas* [42].

A genetic screen for NPQ-deficient mutants of *Chlamydomonas* identified *npq4*, deficient in LHCSR3, a new component involved in qE [15]. There are three LHCSR genes in this alga called LHCSR1, LHCSR3.1 and LHCSR3.2. The latter two encode identical proteins that are both missing in the *npq4* mutant. LHCSR was first identified as a light-induced mRNA called LI818 [43] with a different expression pattern from most other LHCs involved in light harvesting [44]. The LHCSR transcripts accumulate in cells subjected to photooxidative stress such as high light [45] and deprivation of carbon dioxide [46], iron [47] or sulfur [48]. The mRNAs of LHCSR1 and LHCSR3 appear to be regulated by different signals because they respond differently to low carbon dioxide [49]. In comparison with PSBS, LHCSR3 is much more abundant during high light stress and plays a major role in photoprotection.

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With its three TM domains, LHCSR3 is structurally very similar to LHC proteins and it is also able to bind chlorophylls and carotenoids [50]. Moreover, it responds to low pH. Possibly, LHCSR3 could be the energy quencher because of its ability to bind pigments and of its capacity to undergo a transition to a very short fluorescence lifetime state [50–52]. LHCSR proteins are present in many photosynthetic organisms including green and brown algae, diatoms, non-vascular plants but they are absent from most vascular plants and in Rhodophyte algae [32] which dissipate excess light excitation energy from phycobilisomes in a different way than qE [53]. In the moss *Physcomitrella patens*, qE-NPQ operates both in an LHCSR- and PSBS-dependent way [54]. In this organism, LHCSR is localized both in grana margins and stroma lamellae [55] whereas in *Chlamydomonas*, LHCSR3 is associated with both PSI and PSII depending on the light conditions [56,57]. LHCSR1 from *Physcomitrella* has been overexpressed in tobacco and shown to be active in NPQ [55]. The purified recombinant protein bound Chl *a* plus lutein and violaxanthin when purified from dark-adapted leaves while zeaxanthin replaced violaxanthin upon light adaptation.

Single molecule spectroscopy of LHCSR1 revealed that this protein undergoes rapid conformational changes and that the protein conformational dynamics control switching between two dissipative states one of which is activated by pH and the other by binding of zeaxanthin [58] through direct energy transfer from the chlorophyll *a* Qy state to the zeaxanthin S1 state and to lutein through the transient formation of a carotenoid radical cation [59,60]. These two states allow the organism to adapt to either step or gradual changes in solar irradiance. The quenched and unquenched conformations of LHCSR1 have life times of 80 ps and 3.7 ns, respectively, implying that the unquenched protein does act as a common light-harvesting antenna, feeding excitation energy into the reaction centers but competes with their exciton trapping when in its quenched state [61].

Besides reducing qE, loss of LHCSR3 leads to an increase in the chlorophyll *a*/*b* ratio, suggesting some minor remodeling of the antenna [15]. However, the other components of the antenna systems accumulate normally when LHCSR3 is absent, including LHCBM1, which is known to also play a role in qE [62].

Chlamydomonas cells need to be exposed to high light stress for several hours for inducing the accumulation of LHCSR3 and qE-NPQ [15]. One might expect that photodamage occurs during this period. However, this is prevented because state transitions occur within a few minutes of light stress and promote the transfer of a large part of the PSII antenna from PSII to PSI which acts as an efficient quencher of the absorbed light energy [56]. Comparison of fluorescence quenching, photosynthetic activity and ROS production in wild type, *npq4*, *stt7*, a mutant deficient in state transitions [63] and in the double mutant *npq4stt7* revealed that photodamage is significantly more pronounced in the double mutant than in both single mutants indicating that qE and state transitions contribute both to NPQ in this alga under high light [56]. Under steady state conditions, qE plays a major role whereas state transitions appear to be important during the early phase of NPQ induction in this alga.

Until recently, it was thought that photoperception by specific photoreceptors and photoprotection mediated by qE-NPQ are distinct independent processes. However, new molecular links have been uncovered between these two processes in *Chlamydomonas*. Recent work indicates that energy dissipation in green algae is controlled by the blue light UV-B receptor UVR8 and the blue light receptor phototropin. Activation of UVR8 by UV-B light mediates the induction of PSBS and LHCSR1 and to a lesser extent LHCSR3 [42], while activation of phototropin by blue light together with a chloroplast signal controlled by photosynthetic activity induces expression of LHCSR3 [64]. The nature of this signal is still unknown but it is not linked to the redox state of the plastoquinone pool. It possibly relies on the accumulation of ROS species [65]. These two signaling pathways appear to be independent, but complementary because induction of qE-related proteins is not dependent on electron flow.

Induction of the 10 ELIP genes in *Chlamydomonas* is also mediated by two independent pathways. Expression of ELIP1,5,6 is preferentially induced by UV-B [41] whereas that of ELIP2,3,4,9,10 is induced by white light [34].

Although the genome of *Chlamydomonas* does not contain phytochrome genes, this alga uses bilins, the chromophores of phytochromes in land plants, to regulate photosynthesis-associated nuclear gene expression. Bilins are derived from heme by heme oxygenase (HMOX1) and ferredoxin-dependent bilin reductase both of which are localized in chloroplasts. High light-induced expression of genes such as LHCSR1 and PSBS is suppressed by biliverdin supplementation in *Chlamydomonas* suggesting that these bilins act as negative signals under these conditions to regulate a gene network for mitigating light-induced oxidative stress [66]. However, the underlying molecular mechanisms of this retrograde signaling chain are still unknown.

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LHC-like proteins involved in assembly/repair of photosynthetic complexes and induced under stress conditions

LHC genes form a large family in plants and algae. In *C. reinhardtii*, there are at least 20 different LHC isoforms that are expressed [67,68]. In spite of their similar sequences, several observations indicate that specific members are involved in specific processes. Thus, mutant strains deficient in LHCBM1 are affected in qE-NPQ [62] whereas LHCBM2, LHCBM5 and LHCBM9 are involved in state transitions [69,70]. In the case of PSI, LHCA3 has been proposed to be required for maintaining LHCI [71].

While most three-helix LHC proteins are abundant, some LHC-like proteins accumulate only in small amounts or are undetectable under normal conditions but are strongly and transiently induced under specific stress conditions. This is particularly true for ELIPs (early light-induced proteins) which form a large family in pro-and eukaryotic organisms. These proteins are inserted in thylakoids through the SRP pathway responsible for targeting the LHC proteins from the chloroplast envelope to the thylakoids [72] and accumulate transiently mostly under light stress and have photo-protective functions [73]. They also accumulate during induction of photomorphogenesis of greening etiolated seedlings [74,75]. ELIPS has three TM domains of which the first and third helices display high sequence identity with the corresponding helices of LHC proteins and they contain four putative chlorophyll-binding residues [76]. As compared with other LHC proteins, ELIPs have an unusual pigment composition consisting mostly of chlorophyll *a* and large amounts of lutein and they have a low excitonic coupling between chlorophyll *a* molecules [74]. Expression of these proteins is induced under high light [77], high salinity [78], UV-B irradiance [79] and desiccation [80]. The genes of ELIP1 and ELIP2 are induced by blue light through the photoreceptor CRY1 [81].

ELIPs have been localized in the stroma-exposed thylakoids where PSII repair takes place. In this repair process, damaged PSII moves from the grana to the stroma thylakoids where newly synthesized reaction center protein D1 is synthesized and inserted together with chlorophyll into the PSII complex. Also, constitutive expression of ELIPs was shown to rescue the photosensitivity of *chaos*, a mutant deficient in the SRP pathway unable to rapidly accumulate ELIPs under high light and chilling and therefore highly photo-sensitive [82]. Taken together, these data strongly suggest that ELIPS have a photo-protective role.

Constitutive expression of ELIP2 in *Arabidopsis* led to a 50% decrease in leaf chlorophyll content and photosystems which were fully assembled and functional [83]. Analysis of the chlorophyll biosynthetic pathway in this mutant revealed that two important regulatory steps are affected with a decrease in glutamyl tRNA reductase and Mg chelatase subunits CHLH and CHLI. These observations suggest that ELIPs may act as chlorophyll sensors that modulate chlorophyll synthesis to prevent accumulation of free chlorophyll and hence prevent photooxidative stress [83]. Surprisingly, however, loss of the two unique ELIPS in an *Arabidopsis elip1/elip2* mutant did not change its sensitivity to photoinhibition and photodamage or its ability to recover from light stress compared with wild type and led only to a modest decrease in chlorophyll [84]. Also, in this mutant, there was no increase in uncoupled pigments which appear when chlorophyll–protein complexes are incompletely assembled or damaged. Moreover, upon high light stress, there was no compensatory increased expression of other ELIP-like proteins such as OHP and SEP. These results raise questions about the proposed role of ELIPs in photoprotection. The observation that the level of zeaxanthin was reduced in *elip1/elip2* under high light and cold raises the possibility that ELIPs may modulate the xanthophyll cycle [84].

Among ELIP-like proteins, the MSF1 protein was identified through a screen for mutants of *Chlamydomonas* impaired in photosynthetic activity [85]. This protein is structurally related to ELIPS and accumulates only under certain stress conditions such as elevated temperatures, dark-light transitions, nutrient stress with deprivation of iron and copper, and also in aging cells (Table 2). Although the level of MSF1 rises transiently upon high light stress, there is no change in its mRNA abundance, in striking contrast with ELIP mRNAs [73]. MSF1 is required for stability and maintenance of protein–chlorophyll complexes, mainly PSI because the loss of MSF1 leads to a 4-fold decrease in PSI with only minor effects on the levels of other photosynthetic complexes. Although clear evidence for chlorophyll binding could not be demonstrated, MSF1 appears to be linked to the chlorophyll biosynthetic pathway based on the observation that it interacts with CTH1, a subunit of the aerobic chlorophyll cyclase and co-fractionates in a 150 kDa complex with CTH1 and CGL178 [85]. CTH1 catalyzes the synthesis of protochlorophyllide, one of the last steps of chlorophyll synthesis and CGL178 is involved in the regulation of the chlorophyll cyclase. Expression of both CTH1 and CGL178 is

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Table 2 Conserved LHC motif in LHC-like proteins

The LHC motif is indicated in some of the transmembrane domains of the listed proteins. Conserved amino acids are highlighted. The position of the consensus motif is indicated for each protein in brackets. In common LHC proteins, E_1 and R_6 from this motif in helix 1 form two ion pairs with oppositely charged residues from helix 3 and are involved both in locking these two transmembrane helices together, and with $(N/H)_4$ they act as ligands for the central Mg^{++} of chlorophyll molecules (1, 2). Note that $(N/H)_4$ is not conserved in PSBS consistent with the fact that this protein does not bind chlorophyll (18, 19). Cr, *Chlamydomonas reinhardtii*; *At, Arabidopsis thaliana*.

CrPSBS2 (Cre01.g016750)	ELFVGRLAMVGFSAS	(71–85)
	ELFVGRAAQLGFAFS	(159–173)
LHCSR1 (Cre08.g365900)	EITHGRVAMLAALGF	(81–95)
	ELNNGRLAMIAIAAF	(191–205)
LHCSR3 (Cre08.g367400)	EITHGRVAMLAALGF	(87–101)
	ELNNGRLAMIAIAAF	(197–211)
LHCBM1 (Cre23.g766250)	ELIHARWAMLGALGC	(90–105)
	EIKNGRLAMFSMFGF	(205–219)
LHCBM9 (Cre06.g284200)	ELIHARWAMLGALGC	(87–101)
	ELKNGRLAMFSMFGF	(202–216)
ELIP (Cre13.g576760)	EINNGRIAMVSVVTA	(67–81)
	EKINGRAAMMGLTSL	(346–360)
ELIP (Cre09.g394325)	EIVNGRLAMLGFVSA	(103–117)
	ELLNGRAAMIGFAAM	(171–185)
Msf1 (Cre14.g626750)	ETINGRAAMLGFVAA	(87–101)
	EKVHGRLAMLGLTTL	(166–170)
CrLIL3	EKLNGRAAMMGYVLA	(162–176)
AtLIL3	ELLNGRAAMIGFFMA	(174–188)
AtOHP1	EIWNSRACMIGLIGT	(69–83)
AtOHP2	EISNGRWAMFGFAVG	(130–144)
SynHliA	EKLNGRLAMIGFVAL	(36–40)
Consensus	E**NGR*AM*G HA A	

decreased in the absence of MSF1. These observations raise the possibility that MSF1 could represent a potential candidate for linking chlorophyll biosynthesis to the maintenance of photosynthetic chlorophyll–protein complexes under specific stress conditions. Assuming that it is capable of transiently binding chlorophyll, it could mediate the transfer of newly synthesized chlorophyll to the nascent photosynthetic complexes thereby linking chlorophyll biosynthesis to the assembly and/or repair of PSI and to a lesser extent of other complexes [85]. This would prevent the release of free chlorophyll which would cause photooxidative damage. It is noteworthy that among photosynthetic complexes, PSI contains the largest number of chlorophylls. These molecules need to be inserted in a co-ordinated way during the assembly of the reaction center. Specific factors such as MSF1 may have evolved for this role which is especially important under stress conditions. While MSF1 is clearly derived from ELIPs, its function appears to have been tailored for assembly and/or maintenance of PSI and chlorophyll–protein complexes during evolution.

Functional links with chlorophyll synthesis have been observed for other LHC-like proteins, in particular, for LIL3, a protein with two TM domains, one of which resembles the LHC chlorophyll-binding motif (Table 2). In the absence of both isoforms of LIL3 of *Arabidopsis*, geranylgeranyl reductase (CHLP) and protochlorophyllide oxidoreductase (POR), are destabilized thus compromising the supply of the two metabolites, chlorophyllide and phytyl pyrophosphate, needed for the final steps of chlorophyll synthesis [86,87]. In barley seedlings, LIL3 specifically accumulates during the de-etiolation stage and assembles as chlorophyll-binding protein complex [88]. LIL3 could thus play an important role in efficiently channeling tetrapyrrole intermediates and their products to their final destination within the photosynthetic complexes thereby preventing photooxidative damage.

Another atypical LHC protein is LHCBM9 from *C. reinhardtii*. While most LHC genes are expressed to high levels under normal growth conditions, LHCBM9 is only weakly expressed. However, its expression is strikingly enhanced at the mRNA and protein level under various stresses including exposure of cells to high light, nitrogen deprivation, sulfur-deprived anaerobic conditions which induce hydrogen production in this alga [89–92]. The latter process is thought to act as safety system whereby excess protons and electrons produced by the photosynthetic reactions are scavenged [93,94]. In addition, expression of LHCBM9 is also increased upon inhibition of chloroplast transcription and translation indicating that LHCBM9 is a general stress protein [95].

Similar to other LHCII proteins, LHCBM9 binds chlorophyll *a* and *b*. The presence of an amino acid motif essential for LHC trimer formation in LHCBM9 is consistent with the observation that it is enriched in trimeric complexes and in PSII supercomplexes [92]. Under nutrient-deficient conditions, LHCBM9 appears to replace other LHCII proteins in the LHCII antenna and thereby enhances light energy dissipation and prevents the formation of ROS. This is particularly important because under these conditions, PSII is partly degraded and the resulting free LHCII trimers can no longer dissipate the absorbed energy by photochemical quenching.

Reduction in LHCBM9 by 50–70% in knockdown lines led to decreased photosynthetic activity upon illumination and reduced levels of PSII supercomplexes. Moreover, hydrogen production was severely affected in *Chlamydomonas* [92]. Further functional analysis revealed that in the presence of LHCBM9, chlorophyll fluorescence decay is faster and less singlet oxygen is produced indicating that this protein is a better quencher than the other LHCBM proteins or is a preferential docking site for LHCSR proteins. Hence, it is important for photoprotection during stress conditions by facilitating light energy dissipation and by stabilizing PSII supercomplexes. LHCBM9 expression appears to be regulated through some hypothetical retrograde signaling pathways which remain to be elucidated. The observation that LHCBM9 mRNA and protein accumulation is induced within the timeframe of hours suggests that the protective function of LHCBM9 becomes important when the stress conditions prevail for hours or more. Taken together, these studies suggest that LHCBM9 participates in an acclimation process to general stress conditions that involves the remodeling of PSII–LHCII supercomplexes and results in reduced ROS formation and thus enhanced photoprotection.

SCPs, small cab-like proteins: possible roles

The SCP proteins (small cab-like proteins) also called OHPs or HLIPs (high light-inducible proteins) in cyanobacteria, are small CAB (chlorophyll *a*/*b* binding)-like proteins containing one trans-membrane domain that is related to the first and third TM helices of LHCII proteins which contain the chlorophyll-binding sites (Table 2). It was proposed that these proteins act as carrier proteins of chlorophyll and it was indeed shown that they bind chlorophyll *in vitro* [96]. SCPs/OHPs have been implicated in the assembly of the PSII reaction center [97]. During this process, insertion of cofactors such as chlorophyll and carotenoids constitutes an

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essential step but is prone to photo-oxidative damage. These proteins have also been suggested to function in the PSII repair cycle by serving as a temporary pigment reservoir when PSII components are being replaced [98,99]. This could explain their low affinity for pigments. SCPs appear to prevent degradation of chlorophylls associated with PSII and could mediate chlorophyll reutilization during repair of photodamaged PSII [100]. In cyanobacteria, the OHP proteins HLIC and HLID together with the short chain dehydrogenase/reductase YCF39, the ortholog of chloroplast HCF244, form a complex binding chlorophyll *a* and β-carotene. This complex binds to the PSII D1/D2 reaction center complex but it is not essential for its assembly [101].

Chlorophyll synthase CHLG can be co-immunoprecipitated with HLID and YCF39 [102]. HLID appears to be the key factor for assembling this CHLG protein–pigment complex important for proper chlorophyll biosynthesis. Whereas HLID binds chlorophyll *a* and β-carotene in a 3:1 ratio, more pigments are bound to the CHLG complex. These include chlorophyllide *a*, chlorophyll *a* and three different carotenoids: myxoxanthophyll, zeaxanthin and β-carotene. These results suggest that the HLID–YCF39 complex may mediate the delivery of chlorophyll to the newly synthesized D1 and D2 proteins during assembly of the PSII reaction center complex and protect it from photodamage. Besides being associated with PSII, these proteins have also been proposed to interact with trimers of PSI in cyanobacteria [103]. It is possible that some of the functions ascribed to the SCPs are related to those of MSF1 mentioned above.

Similarly, in land plants, OHP1 appears to deliver chlorophyll to the PSII reaction center and can be co-purified with D1/D2, HCF136, HCF244 and HCF173 [97]. Loss of OHP1 or OHP2 leads to a pale-green phenotype and accumulation of the photosystems is reduced [97,104]. Thylakoid organization is altered in mutants lacking OHP1 with a nearly complete absence of stromal lamellae and swelling of the marginal thylakoids [105]. Recently, it was shown that OHP1 and OHP2 form a heterodimer which could mimic the crossed arrangement of the first and third helix critical for chlorophyll binding of LHC proteins (Table 2). Moreover, the stromal part of OHP2 interacts with HCF244. OHP2 is required for the stability of OHP1 and HCF244 and specifically involved in the assembly of the PSII complex [106]. Formation of the PSII reaction center complex is specifically inhibited in the absence of OHP1 and OHP2 in *Arabidopsis* [105]. Moreover, changes in the critical residues of the chlorophyll-binding sites of these proteins interfere with their function further suggesting that they are chlorophyll-binding proteins. Further studies revealed that OHP1, OHP2 and HCF244 together with D1, D2, PSBI and Cytb559 form a PSII–RC-like complex for a limited period at an early stage of PSII assembly and also during PSII repair [105]. OHP1, OHP2 and HCF244 are subsequently released from the PSII–RC-like complex and replaced by other PSII subunits. This complex is distinct from the RC center sub-complex in the intact PSII complex and appears to be the counterpart of the cyanobacterial YCF39–HLID complex in which HLID binds chlorophyll *a* and β-carotene and dissipates absorbed energy [101,102]. Ultrafast absorption spectroscopy reveals that this process occurs through a direct energy transfer from the chlorophyll *a* Qy state to the β-carotene S1 state [107]. Taken together, these studies indicate that the mechanisms governing PSII-RC assembly are highly conserved both in prokaryotic and eukaryotic photosynthetic organisms and that there is a tight coordination between chlorophyll synthesis and assembly of the photosystem complexes.

Conclusions

The picture which is emerging from numerous studies of the large LHC protein family is that even though the members of this family are very similar, some of these proteins perform specific functions mostly related to dissipation of excess absorbed light excitation energy when the capacity of the photosynthetic machinery is saturated. They appear to play a key role in photoprotection as seen by the fact that in many cases plants lacking any of these proteins are significantly more vulnerable to photodamage under various stress conditions. However, many of these stress-induced LHC proteins differ from the common LHC proteins of the light-harvesting systems in three major aspects. First, whereas common LHC proteins stably accumulate during most of the plant/algal life cycle, this is not the case for the stress-induced LHC proteins. These proteins are often barely detectable under normal growth conditions, but are strongly induced under stress conditions such as high light, elevated temperature, desiccation and nutrient deprivation. Second, while common LHC proteins contain invariably three TM domains, some stress-induced proteins contain one, two, three or four TM helices with characteristic chlorophyll-binding motifs (Table 2). Third, besides their role in photoprotection, some of these proteins participate actively in the assembly and/or repair of the reaction centers of PSII and PSI. This is particularly true for the OHP proteins OHP1 and OHP2 which together with ORF244 form a transient

complex, called RC-like complex with the PSII reaction center proteins D1, D2 and CYTB559 [105]. Interestingly, a similar complex has been reported in cyanobacteria with YCF39, the ORF244 ortholog, and the PSII reaction center proteins [108] indicating that this assembly pathway has been conserved in photosynthetic organisms. Although the action of these OHPs is not yet fully understood at the molecular level, a possible model is that they bind newly synthesized chlorophyll molecules transiently and insert them into the reaction center in a co-ordinated way during assembly. In this way, no free chlorophyll is released and thereby photo-damage of the cells is minimized. A similar scheme may apply to MSF1 in *Chlamydomonas*, a three-helix LHC-like protein which appears to be preferentially involved in the assembly of the PSI core complex [85].

From an evolutionary point of view, these small LHC-like proteins are of special interest because they suggest possible evolutionary pathways in which three-helix LHC proteins evolved presumably from the prokaryotic one-helix HLIPs through a series of duplications and fusions [73,109].

Among the stress-induced proteins involved in photo-protection, several play key roles in qE-NPQ. This is particularly the case or the four-helix PSBS protein in land plants although it is still not clear how this protein act in the quenching process in spite of the determination of its crystal structure [19]. Because this protein does not bind any pigment, it is unlikely to contain the quenching site and probably acts as a trigger by interacting with nearby LHC proteins of the antenna once it is activated by the low pH of the thylakoid lumen. Although PSBS has also been conserved in green algae and is induced under high light, it does not appear to be involved in qE-NPQ. In these organisms, this process is mediated by the LHCSR proteins containing three TM helices. In contrast with PSBS, the LHCSR proteins bind pigments and could harbor the quenching site. Whereas PSBS and LHCSR require a low lumenal pH to be activated for dissipation of the light excitation energy by qE-NPQ, this is not the case for LHCBM9 of *Chlamydomonas*, a three-helix LHC protein that accumulates under stress conditions even if the pH is neutral. Interestingly, LHCBM9 substitutes common LHCII proteins in the antenna trimers and stabilizes PSII supercomplexes in an energy-dissipative mode. How LHCBM9 is induced and how it acts in the antenna complexes remains to be explored.

Taken together, these studies reveal that stress-induced LHC proteins play key roles not only in the dissipation of excess light excitation energy and prevention of photo-oxidative damage but that at least some of these proteins actively participate in the assembly pathway of photosynthetic complexes possibly by mediating the transfer of newly synthesized chlorophylls into the nascent complex. A challenging task will be to understand how these proteins promote these processes at the molecular level.

Abbreviations

LHC, light-harvesting complex; OHPs, one-helix proteins; PSI, photosystem I; PSII, photosystem II; TM, transmembrane.

Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

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