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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

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