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Review

Light-harvesting complexes of vascular plants

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Abstract. Light-harvesting complexes (LHCs) located in the thylakoid membrane of plant chloroplasts are the collectors of solar radiation that fuel photosynthesis, and thus enable life on our planet. They consist of pigments that are non-covalently bound to light-harvesting proteins (Lhc proteins), which form a family whose members share a significant sequence identity. Due to their central role in photosynthesis, LHCs belong in several respects to the best-analysed membrane proteins. In the past decade, tremendous progress has been made in identifying new members of the Lhc family, in localising the LHCs within the photosystems, and in elucidating the structure and function of LHCs, which is summarised in this review. By contrast, gaining insight into the assembly process and the degradation of the LHCs could not keep pace. Therefore, topics for the next decade will be the elucidation of the location(s) and the operating mode of steps in the assembly and degradation process.

Keywords. Photosynthesis, photosystem, light-harvesting complexes, protein structure, protein folding, protein-protein interactions, protein oligomerisation, proteolysis.

Introduction

To increase light absorption, photosynthetic organisms invented antenna systems that consist of pigments or complexes formed by pigments and proteins. Depending on the developmental level and the endosymbiotic event phycobilisomes, fucoxanthin-binding proteins, LI818 (-like) proteins, peridinin-binding proteins, and finally the light-harvesting proteins (Lhc proteins) of green algae and higher plants emerged [1–5]. Light-harvesting complexes (LHC) consist of pigments, which are non-covalently attached to the Lhc proteins and are located in the periphery of photosystem (PS) I and II. Since Lhc proteins of higher plants bind chlorophyll (Chl) *a* and *b* besides several carotenoid (Car) species, they are also called chlorophyll *a*+*b*-binding (cab) proteins. Lhc proteins form a multigene family together with one-helix proteins, stress-enhanced two-helix proteins, early light-inducible three-helix proteins, and the PsbS protein with four helices [6–9]. Although Lhc proteins possess a considerable amino acid identity/similarity in some regions, especially in the transmembrane regions, there are prominent differences in the overall amino acid sequences, which may be of importance with regard to functions exceeding the common functions of LHCs [6, 10]. According to current knowledge, LHCs possess the following functions: (1) they increase the amount of absorbed quanta per photosystem to fuel photosynthetic activity under normal light conditions; (2) under high light, LHCs can dissipate energy absorbed in excess by non-photochemical quenching (NPQ) and thus regulate the excitation energy transmission to the reaction centres [11–16]; (3) unbalanced excitation of both PSI and II can be evened out by state transition achieved by a mobile pool of LHCII that can switch back and forth between PSII and PSI [17–19]; and (4) some

LHCs of PSII are important for the structure of the thylakoid membrane, as they are involved in stacking of grana thylakoids [20–22]. The differences in the amino acid sequences of Lhc proteins may indicate additional, up to now unknown, functions specific for the individual Lhc proteins and the LHCs they form, respectively. Other consequences of the sequence differences of Lhc proteins are their different molecular weights and the properties of the formed LHCs as summarised in Table 1.

The present article gives an overview about the presently identified Lhc proteins and LHCs against the background of the history of their detection. In the following chapter the current knowledge regarding the binding sites and position of LHCs in their PS is described, a topic that immensely benefited from the advent of the x-ray structure of PSI and various mutant plants depleted in one of the Lhcb proteins. Recently, high-resolution x-ray structures obtained for the major LHC of PSII provided detailed insight into the structure and cofactor binding of LHCs, which form a focus of this review. These structures provided the basis for a better understanding of some mechanisms underlying NPQ. Finally, the various steps in the life cycle of a light-harvesting complex are delineated.

History and variety of LHCs

In the 1940 s ultracentrifugation of solubilised chloroplasts provided first evidence that photosynthetic pigments are attached to protein. It took another 20 years until the existence of two protein fractions with associated pigments (called complex I and II) was demonstrated by polyacrylamide gel electrophoresis [23, 24]. It was assumed that the so-called complex I and II correspond to PSI and PSII. Detailed analyses of a chlorophyll *b*-less barley mutant revealed the presence of PSII activity in the absence of complex II. Therefore, it was concluded that complex II solely functions in light absorption and energy transmission to the reaction centre. Due to this proposed function the name light-harvesting complex was introduced [25] and complex II was called the major LHCII due its abundance, which amounts to about half of the chlorophyll and one third of the protein present in thylakoids and makes it to the most frequent membrane protein on our planet [26]. However, it should be noted that the designation LHCII is somewhat misleading since a fraction of this LHC can also be associated with PSI when an imbalance in excitation of PSII and PSI occurs (see next chapter for more details). LHCII is made up by three apoproteins, called Lhcb1–3 (c.f. Table 1) according to the nomenclature introduced by Jansson et al. [27], which form homotrimers (Lhcb1, Lhcb2) or heterotrimers (Lhcb1, Lhcb2, Lhcb3) [28–30]. Subsequent refinement of membrane solubilisation and electrophoretic conditions demonstrated that on one hand all Chl is bound to protein [31] and on the other hand the presence of additional LHCs. Around 1980 another pigment protein with an apparent molecular mass of 29 kDa could be resolved, which is made up by the Lhcb4 protein and was called chlorophyll protein (CP) 29 [32, 33]. The existence of an LHC in PSI was first postulated due to loss of Chl *b* from PSI upon detergent treatment and ultracentrifugation [34]. Subsequently an LHCI holocomplex was first described [35], which can be split into two populations [36], called LHCI-680 and LHCI-730 because of their 77K fluorescence emission maximum [37]. Both consist of two apoproteins: Lhca1 and Lhca4 form LHCI-730, and Lhca2 and Lhca3 are components of LHCI-680 (Table 1) [38, 39]. The latter authors concluded from electrophoretic mobilities that LHCI-730 represents a dimer and that the LHCI-680 band contains monomers. Later-on evidence was obtained that LHCI-730 is a heterodimer formed by Lhca1 and Lhca4 [40, 41]. Use of more gentle separation techniques demonstrated that Lhca2 and Lhca3 also form dimers [42], and the x-ray structure recently obtained from PSI [43] revealed the presence of two slightly different Lhc proteins at the location of LHCI-680, confirming the presence of an Lhca2/3 heterodimer. In 1996, the smallest LHC belonging to PSII was detected and called CP24 on the basis of the molecular mass of its apoprotein Lhcb6 [44]. Finally, CP26, the fourth LHC attached to PSII, was isolated [45]. It is formed by the apoprotein Lhcb5.

Concomitant to these biochemical analyses, genetic techniques were applied to learn more about the corresponding genes. Starting with the first cloning of a gene encoding the Lhcb1 protein [49], it became obvious that the individual Lhc proteins are encoded in the nucleus, some by several genes each [6, 10, 26, 50]. In the completely sequenced *Arabidopsis thaliana* genome, *e.g.*, five, four, and three genes coding for Lhcb1, Lhcb2, and Lhcb4, respectively, were found (Table 1). In the case of the major LHCII, the number of genes coding for Lhcb1, Lhcb2, and Lhcb3 roughly corresponds to the amount of these proteins in the LHC. Interestingly, only three out of the five genes coding for Lhcb1 give rise to identical proteins (Lhcb1.1 to Lhcb1.3), whereas the remaining two yield very similar (Lhcb1.4 and 1.5) proteins. Recently, proteomic studies of various plants demonstrated the parallel expression of Lhc isoforms [47, 51–54]. However, the reason for this gene diversity is still unclear.

Table 1. Overview about the various light-harvesting proteins (Lhc proteins) biochemically identified in higher plants. Shown is the number of genes coding for the individual Lhc protein in *Arabidopsis thaliana*, their molecular mass, the light-harvesting complexes (LHCs) they form, the photosystem they are (preferentially) attached with, and their oligomerisation state. Molecular masses of apoproteins are given on basis of the nucleotide sequences for the *A. thaliana* proteins assuming N termini given in [6]. Additionally the chlorophyll amount of individual LHCs is shown in percentage of total chlorophyll in thylakoids.

Protein	Number of genes in *Arabidopsis* [6]	Molecular mass of mature apoprotein (kDa)	Formed LHC	Attached to photosystem	Oligomerisation state	% of total chlorophyll in thylakoids [48]
Lhcb1	5	24.7–24.9		PSII (+PSI)		
Lhcb2	4	24.9	LHCII [26]	PSII (+PSI)	Trimer [46]	40
Lhcb3	1	24.3		PSII		
Lhcb4	3	27.3–28.2	CP29 [32, 33]	PSII	Monomer [33]	4
Lhcb5	1	26.1	CP26 [45]	PSII	Monomer [45]	4
Lhcb6	1	23.2	CP24 [44]	PSII	Monomer [44]	4
Lhca1	1	21.5	LHCI-730 [36]	PSI	Dimer [40, 41]	
Lhca2	1	23.2	LHCI-680 [36]	PSI	Dimer [42, 43]	18
Lhca3	1	24.9	LHCI-680 [36]	PSI	Dimer [42, 43]	
Lhca4	1	22.3	LHCI-730 [36]	PSI	Dimer [40, 41]	
Lhca5	1	23.5	?	PSI	Dimer [47]	?

Complete sequencing of the genomes of *Arabidopsis* and poplar and the availability of databases with expressed sequence tags of these and other plants revealed the presence of additional, hitherto unknown *lhc* genes [6, 55, 56] whose expression is differently regulated as compared to the abundantly expressed *lhca*1–4 and *lhcb*1–6 genes [56]. Meanwhile expression of Lhca5 and its attachment to PSI could be demonstrated [47, 53, 55, 57]. For another Lhc protein, which presumably is part of PSII and therefore called Lhcb7, several expressed sequence tags are present indicating its expression [56]. Additionally, genes with only very limited deviation in the sequence as compared to Lhca2 and Lhcb4 have been recognised and it is not clear yet whether they represent isoforms of these proteins or novel proteins as was suggested by Jansson [6] and Klimmek et al. [56], who named them Lhca6 and Lhcb8. Since these four additional *lhc* genes are expressed in other tissues and under different environmental conditions than the other ten abundantly expressed Lhc proteins, their function(s) remains to be elucidated [56].

Arrangement of LHCs in PSI and PSII

PSII. Due to the lack of an x-ray structure of the eukaryotic PSII with its attached LHCs, the current structure models are based on (i) the high resolution x-ray structures of an LHC-free cyanobacterial PSII [58] and trimeric LHCII [21, 59], (ii) the biochemical identification of associations of LHCs of PSII [60, 61], and (iii) the single-particle analyses of PSII [62, 63] of plants with repressed expression of Lhcb1 and Lhcb2 [64], Lhcb4 and Lhcb5 [65], and Lhcb6 [22]. From these analyses the current view about PSII structure emerged, which is summarised in Figure 1A. In this, a complex of trimeric LHCII, CP29, and CP24 (which is only present in some preparations of dimeric PSII) abut on the inner antenna CP47, while an association of trimeric LHCII and CP26 is connected with the other PSII core antenna CP43 [67, 68]. Repression of the minor LHCs CP24, CP26, and CP29 revealed that these proteins have specific binding sites at the core and that their positions cannot be adopted by other Lhc proteins. Interestingly, suppression of Lhcb1 and Lhcb2 resulted in an increased synthesis of Lhcb5 [64]. This forms, partly together with Lhcb3, trimers in this special situation and takes the position of the trimers usually composed of Lhcb1 and Lhcb2 [69]. Thus, Lhcb5 appears to be the only Lhcb protein, which has some flexibility in its oligomerisation behaviour and binding specificity to PSII under special conditions.

Single-particle analyses of mildly solubilised PSII membranes resulted in the identification of three

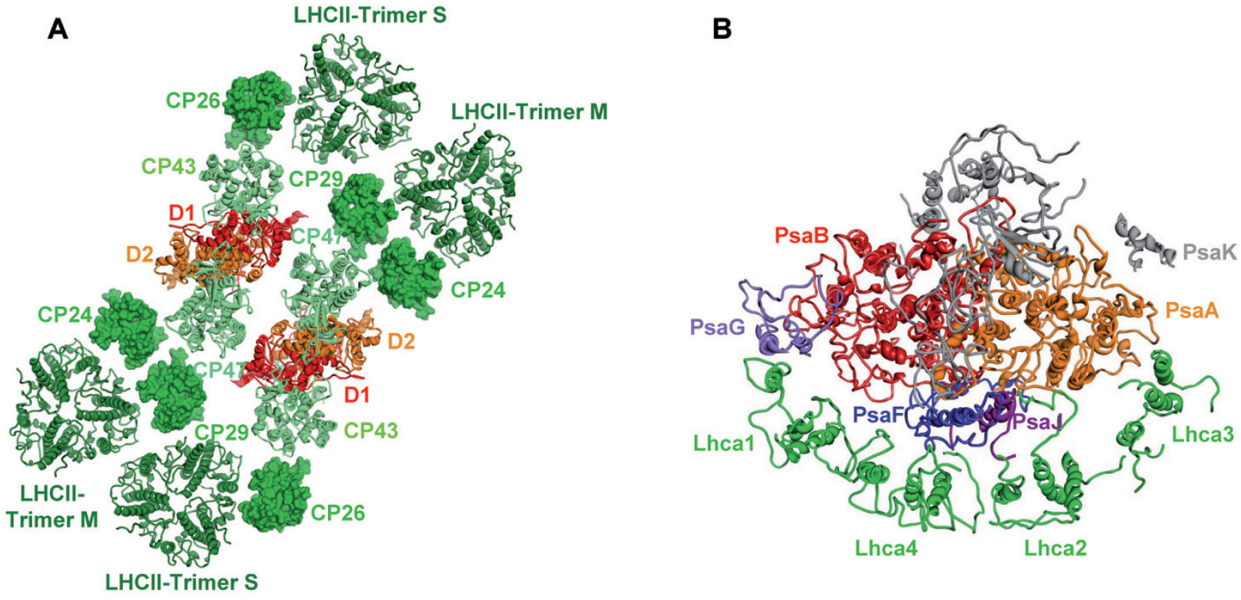

Figure 1. Arrangement of light-harvesting complexes (LHCs) in photosystem (PS) II (*A*) and PSI (*B*). PSII was assembled with chimera [66] using the D1, D2, CP43, and CP47 protein from the structure of the dimeric cyanobacterial PSII (pdb entry 2AXT; [58]) and the trimeric LHCII of higher plants (pdb entry 2BHW; [21]). The transmembrane regions of one monomer of such a trimer were used to create a crude surface structure for the minor CP29, CP26, and CP24, which should indicate that no high-resolution structure is available yet for them. These proteins were positioned with regard to the core antenna proteins CP47 and CP43 according to recent PSII models [67]. The decrease of chlorophyll *b* concentration towards the centre of the photosystem is visualised by the transition of dark to light green. In (*B*) the PSI (pdb entry 2O01; [83]) is depicted and proteins involved in Lhca protein binding are designated in colour.

populations of PSII particles with different sizes due to their different contents of LHCII trimers [62]. The trimer associated with CP26 is always present in PSII and therefore it is called strongly bound (S-trimer). Furthermore, many PSII particles were detected with an additional LHCII trimer, which is associated with CP29 and CP24. Since this is apparently more easily released from PSII, it was named moderately bound (M-trimer). Very rarely, larger PSII complexes were observed and interpreted to bind a third, very loosely bound trimer (L-trimer), which is not considered in Figure 1A. Although these differently sized PSII complexes occur apparently *in vivo* [70], it is not clear yet, whether they fulfil particular physiologically functions [67].

However, the number of LHCII molecules per PSII as determined by single-particle analyses apparently does not agree with the LHCII content found in thylakoid membranes. Biochemical analyses of thylakoid membranes demonstrated a 1:1:1:4 ratio of CP29:CP26:CP24:trimeric LHCII per monomeric PSII core [50, 60]. Although there is some variability in the LHC size of PSII in dependence of the growth light intensity [71–76], which affects especially Lhcb1 and Lhcb2, obviously there is a discrepancy between the LHCII number per PSII found in thylakoids and in PSII preparations. This conflicting data can be explained by the finding of 'free' LHCII in grana membranes, which possibly is either only in functional contact with PSII cores located in the adjacent grana membrane or forms larger aggregates [63], or by the attachment of some of the LHCII to PSI [77].

PSI. Early single-particle analyses suggested the presence of eight [78] or six [79] Lhca proteins in PSI of higher plants. However, the first high-resolution x-ray structure of PSI revealed the presence of only four Lhca proteins (Fig. 1B) [43]. Upon biochemical re-examination, the existence of only four Lhca proteins per PSI could be confirmed [80, 81]. By comparison of the PSI structure of pea with the high-resolution structure of *Synechococcus* PSI [82], which corresponds to the core complex of the higher plant PSI, assignment and location of four additional Lhca proteins was possible due to detection of their transmembrane helices (TMH). As is shown in Figure 1B, the Lhca proteins are arranged like a belt on the 'southern hemisphere' of the PSI core complex [43]. The four Lhca proteins were assigned to the abundantly expressed Lhca1, Lhca4, Lhca2, and Lhca3 protein from the left to the right (Fig. 1B). According to a refined x-ray structure, Lhca1 is bound to PsaG and Lhca4 to PsaF [83, 84], pointed out in Figure 1B by colouring. Lhca2 is associated with PsaA and in addition to PsaJ *via* gap chlorophylls. Lhca3 is in contact with PsaA and in close proximity to PsaK. The

location of Lhca5, which is not present in the crystal structure, is equivocal. Cross-linking studies demonstrated its association with Lhca2 in *Arabidopsis* wild-type plants, whereas in an Lhca4 knockout mutant Lhca5 appears to occupy the binding site usually reserved for Lhca1 and/or Lhca4 [85], thus indicating some flexibility in the binding of Lhca5. From its substoichiometric expression in comparison to Lhca1–4 and the presence of two isoforms of Lhca4 in tomato PSI, the existence of differently composed PSI can be inferred [47]. The same conclusion can be drawn from the analyses of barley and *Arabidopsis* mutants showing altered Lhc compositions [55, 86, 87]. Whether Lhca6 actually accumulates and associates with PSI is questionable, as it could not be detected by mass spectrometry in PSI of ten different plants [53].

Additionally, light conditions may also have an effect on the Lhca composition of PSI. Two studies demonstrated a decrease especially of Lhca4 in high-light treated *Arabidopsis* plants [74, 75]. However, in another study, using more strongly irradiated *Arabidopsis*, no changes in the content of individual Lhca proteins were observed [76]. Therefore, this issue requires further clarification. Interestingly, immunoblot analyses of subfractionated thylakoid membranes indicated the presence of PSI with a reduced Lhca protein content in grana cores in comparison to the PSI in the grana margins and stroma thylakoids [88]. Additionally, PSI with probably one LHCII trimer was found in the stroma thylakoids, designated PSIβ, whereas PSI with more than one LHCII trimer was found in the grana margins, which represents PSIα.

Thus, an extra level is added to PSI heterogeneity by the presence of LHCII in PSI, which was observed by biochemical and single-particle analyses [77, 88, 89]. The docking site of an LHCII trimer is in the 'north-eastern' part of a portion of PSI [89], which is formed by PsaA and the small subunits PsaH, PsaL and PsaK [77, 90, 91]. The reason for the LHCII presence in PSI can be a predominant excitation of PSII that leads to the detachment of part of the LHCII, the mobile LHCII, which possibly represents an M-trimer [89], from PSII. This detachment is triggered by phosphorylation of LHCII by a kinase, which is regulated by the reduction state of the plastoquinone pool [17]. Recently, a kinase has been identified that is, either alone or in collaboration with other kinases, responsible for the phosphorylation of LHCII [18]. Following detachment from PSII, possibly by charge repulsion or a phosphorylation-induced conformational change, phosphorylated LHCII docks to PSI and serves as a light-harvesting antenna there. Mass spectrometry of PSI-enriched stroma thylakoids obtained after illumination of spinach thylakoids with different light intensities demonstrated the diverse migration of the isoforms of Lhcb1 and of Lhcb2 [54]. This indicates the existence of different LHCII populations in the grana membranes (PSII bound and/or free ones), whose migration appears to be differently regulated. The other way round, this implies the presence of PSI-LHCII with diverse composition of the LHCII moiety. Further mass spectrometric analyses should provide better insight into the presence of isoforms of single Lhcb proteins in PSI under special conditions, and may contribute to unravel the enigmatic presence of several genes coding for one Lhc protein.

Interestingly, LHCII attached to PSI also contains unphosphorylated Lhcb1 and Lhcb2 proteins [54, 77]. Up to now, it is not clear whether Lhcb1 and Lhcb2 migrate as monomers or trimers. Since it binds in a trimeric form to PSI, one may speculate that they migrate in this form and that only one or two Lhcb subunits of an LHCII trimer have to be phosphorylated to become disconnected from PSII. Otherwise, it may indicate that a fraction of PSI always has some LHCII attached. This interpretation is in line with the finding that LHCII is associated with PSI not only under light conditions selectively exciting the PSII antenna (state 2), but also when PSI is preferentially excited (state 1) [77], indicating that binding of LHCII to PSI is not strictly dependent on light conditions favouring state 2, which is in accordance with former biochemical analyses [92, 93].

Pigment content of the various LHCs

Native LHCs. According to more recent biochemical, biophysical, and structural analyses, PSI of higher plants binds ~170 chlorophyll molecules [43, 81, 83, 94], although, especially in earlier years, slightly higher values were also reported [80, 93]. The crystal structure of PSI at 3.4 Å revealed 168 Chl molecules [83], of which 102 are ligated to the core complex and the rest remains for the external antenna [83, 84]. The latter 66 chlorophylls can be subdivided into three classes according to their locations either within a folded Lhca protein, between adjacent Lhca proteins (linker Chls), or at the interface between Lhca proteins and the neighbouring core proteins (gap Chls) [43, 95]. Depending on the assignment of the latter Chls, lower (13, 13, 12, and 13) [95] or higher (14, 15, 15, and 14) [84] Chl numbers have been published for Lhca1–4. As shown in Table 2, biochemical analyses revealed only 10 Chls per Lhca protein for a total LHCI preparation [42]. Studies with further dissected LHCI antenna revealed about 11 and 9 Chls

Table 2. Pigment contents of native LHCs and reconstituted (r-) Lhc proteins.

Pigment → LHC ↓	Chl *a*+*b*	Chl *a*	Chl *b*	Σ Carotenoids	Neo	Vio	Lut	β-Car	References
LHCI	10	7.9	2.1	2.15	–	0.55	1.2	0.4	[98]
LHCI-730	11.4	8.6	2.9	1.94	–	0.54	0.99	0.42	[96]
LHCI-680	9.0	6.4	2.6	1.76	–	0.43	0.92	0.40	[96]
LHCII	14	8	6	4	1	1	2	–	[59]
CP29	8	6	2	2	0.47	0.64	0.89	–	[102]
CP26	9	6.2	2.8	2.01	0.61	0.38	1.02	–	[103]
CP24	10	5	5	2.03	0.003	0.95	1.08	–	[104]
r-Lhca1	12	9.3	2.7	2.41	0.34	0.3	1.71	0.05	[112]
r-Lhca2	12	8.3	3.7	2.06	0.12	0.22	1.67	0.05	[112]
r-Lhca3	12	10.3	1.7	3.1	0.36	0.31	2.23	0.2	[112]
r-Lhca4	12	8.7	3.3	1.96	0.14	0.2	1.56	0.05	[112]
r-Lhca5	12	8.7	3.3	2.69	0.34	0.28	1.89	0.18	[57]
r-Lhca6	12	8.9	3.1	4.08	0.57	0.42	2.79	0.3	Storf and Schmid
r-Lhcb1	14.9	7.8	7.1	3	0.9	0.1	2	–	[30]
r-Lhcb2	14.9	8.	6.9	2.9	0.8	0.1	2	–	[30]
r-Lhcb3	14.5	8.3	6.1	2.9	0.7	0.1	2	–	[30]

per apoprotein of LHCI-730 and LHCI-680, respectively [94, 96], thus also yielding 10 Chls per apoprotein on the average. The discrepancy between these values and those obtained from crystallised PSI can be explained by the release of gap and linker Chls in the course of LHCI isolation, which yield a significant amount of unbound pigment, even if mildly solubilised PSI is subfractionated very cautiously by density gradient ultracentrifugation [80, 93, 94, 96]. Investigation of the density gradient bands with free pigments, LHCI, and PSI core supported the assumption that they represent gap and linker Chls [80]. One drawback of the recent PSI structure is that its resolution is not sufficient for detecting the carotenoids (and lipids) in LHCI. Biochemical analyses demonstrated the coordination of lutein (Lut), violaxanthin (Vio), and β-carotene (β-car) [94, 96–98], of which two to three molecules are bound per Lhca apoprotein (Table 2). Conversely, the LHCs belonging to PSII contain no β-car, but neoxanthin (Neo) in addition to Lut and Vio, which is absent in LHCIs *vice versa.* With regard to the Chl load, Lhca proteins are similar to the major LHCII, which binds 14 Chls (8 Chl *a* and 6 Chl *b*), 2 Lut, 1 Neo, and 1 violaxanthin cycle pigment (VCP; *i.e.*, violaxanthin, antheraxanthin, or zeaxanthin (Zea); Fig. 2A) [21, 59, 99]. The existence of a Vio binding site was questioned for a long time, because of its substoichiometric presence in earlier analyses [60, 100]. By very mild solubilisation and gentle purification, it was possible to retain Vio at LHCII [99] and meanwhile there is ample evidence for the presence of one VCP in LHCII [21, 59, 101]. The minor LHCIIs exhibit a lower Car and Chl content than the major LHCII (Table 2). For CP29 and CP26, the presence of two [102, 103] or three [99] carotenoids was suggested in addition to eight or nine Chls, respectively. Lhcb6 binds two carotenoids and ten Chls to form CP24 [104]. Unlike CP24, CP29 and CP26 have a strong preference for binding Chl *a* in comparison to Chl *b* (Table 2). In this feature and in the number of biochemically identified Chls, the minor LHCIIs resemble the LHCIs. Possibly, a future crystal structure of a PSII-LHCII supercomplex will show that also these minor Lhcb proteins possess peripherally ligated Chls, which are released during the isolation, pretending too low a Chl content.

Reconstituted LHCs. Pigment analyses of the native LHCIs and the major LHCII only provide insight into the pigment composition and content of dimeric and trimeric complexes with heterogeneous protein composition, which does not allow studying the pigment-binding properties of the individual Lhcb1–3 and Lhca1–4 proteins. This problem can be overcome by the reconstitution technique initially described by Plumley and Schmidt 1987 [105]. By this method, individual Lhc proteins can be folded in the presence of pigments to LHCs, which are comparable in pigment composition and spectral properties to their native counterparts [106]. This makes them useful objects for investigations aiming to clarify specific properties and/or functions of the individual Lhc proteins. At first, pigments and proteins isolated from thylakoids were used. Later, the availability of cDNAs

and expressed sequence tags of *lhc* genes allowed the use of proteins, expressed in *E. coli*, for reconstitutions [107, 108]. Using this approach, the pigment-binding properties of all abundantly expressed as well as of the rarely expressed Lhca5 and Lhca6 proteins could be determined (Table 2) [41, 57, 96, 98, 104, 107–110]. Analyses of monomeric LHCs obtained with Lhca1–4 demonstrated similarities in Chl contents and dissimilarities in Car binding. One subunit of both LHCI-730 (Lhca1) and LHCI-680 (Lhca3) binds about three carotenoid molecules, whereas the two other ones (Lhca4 and Lhca2) apparently ligate only two such molecules [96, 98, 111, 112]. The pigment composition of reconstituted Lhca5 closely resembled that of Lhca1 [57], whereas Lhca6 had a strongly increased Car:Chl ratio due to a high Lut content (Storf and Schmid, unpublished). This way, it was possible to demonstrate that Lhca6 actually binds pigments and, thus, is principally able to fulfil a light-harvesting function in plants. Contrary to the subunits of LHCI-730 and LHCI-680, examination of reconstituted Lhcb1–3 revealed similar Chl and Car contents for these three proteins (Table 2) [29, 30]. While the Chl content was comparable to that of the trimeric LHCII, the Car content was not, since the VCP is strongly reduced indicating a weak binding at a peripheral binding site, which is in concordance with the crystal structure [21, 59].

Taken together, the Lhc proteins show differences in the pigment content and composition despite of the considerable conservation of the amino acid sequences in their transmembrane regions, where these pigments are almost exclusively attached. On the basis of the pigment content of native LHCs given in Table 2 and under the assumptions that (i) thylakoids on average possess a PSII/PSI stoichiometry of 1.4 [113, 114], (ii) there are eight LHCII trimers per dimeric PSII [50], and (iii) the gap and linker Chls in PSI belong to the Lhca proteins; 69 % of the Chl present in thylakoids is associated with Lhc proteins.

Molecular architecture of the LHCs

Due to its abundance in thylakoid membranes and the easy purification procedure, LHCII has been the main subject for structure analyses. Hydropathy plots and protease digestion patterns suggested that the most prevalent apoprotein of the major LHCII, Lhcb1 or LHCP, possesses three TMH [115, 116].

Protein backbone. The first higher resolution (6 Å) structure obtained by electron microscopy of two-dimensional crystals of pea LHCII confirmed the presence of three TMH, called B, C, and A (or alternatively as in Fig. 2A, TMH I, II, and III) from the N to the C terminus [117]. Improved electron microscopic techniques paved the way for the first high resolution structure at 3.4 Å, which allowed the location of most cofactors (12 chlorophylls, 2 carotenoids) and the identification of a small amphipathic helix in the C-terminal region (V in Fig. 2A) [118]. Finally, x-ray analyses of three-dimensional crystals of spinach LHCII and further refined analyses of two-dimensional crystals of pea LHCII allowed resolutions of 2.72 Å [59] and 2.5 Å [21], respectively. All these studies have in common that they employed native heterotrimeric LHCII, which is composed of Lhcb1 and Lhcb2 (and probably Lhcb3 as a minor constituent). The structures below 3-Å resolution provided detailed insight into the location of all cofactors (14 chlorophylls, 4 carotenoids, 2 lipids) and the arrangement of the extrinsic protein regions, which revealed the presence of another short amphipathic helix located in the luminal loop between TMH I and II, which is named helix IV in Figure 2A. The 2.5-Å resolution allowed tracing of the entire polypeptide chain except for the nine N-terminal amino acids. One explanation for their absence in the resolved structure is the heterogeneous protein composition of LHCII, since Lhcb1–3 vary mainly in their N-terminal sequence. Another explanation is a possible conformational heterogeneity in this domain even in the crystal. The most prominent feature of the protein backbone are the 32-amino acids-long TMH I and III, which intertwine and form a super helix (Fig. 2A) that protrudes from the membrane. TMH I and III are inclined by about 30° relative to the membrane normal. By contrast, the shorter TMH II (20 or 21 amino acids in pea and spinach, respectively) is only slightly tilted to the membrane plane [59, 117]. The two amphipathic helices at the luminal surface of the thylakoid membrane consist of nine (helix IV) and ten (helix V) amino acids and have some inclination with regard to the membrane plane [59].

LHC structure details are also available for the Lhca1–4 proteins from the 3.4-Å structure of PSI [83]. With this it became obvious that the abundantly expressed Lhca proteins share the same three-dimensional folding pattern with the LHCII proteins in the membrane region except for Lhca3, whose TMH I and III are almost parallel and much closer to each other. However, this alteration may be the consequence of the tight packing of the individual Lhca proteins in the LHCI belt, since analyses of the individual pigmented protein did not indicate stronger differences in comparison to the other Lhca proteins [119]. According to the Protein Data Bank (pdb) file 2O01, the resolution of the extrinsic protein segments decreases from Lhca1 on the PsaG side to Lhca3 on the PsaK

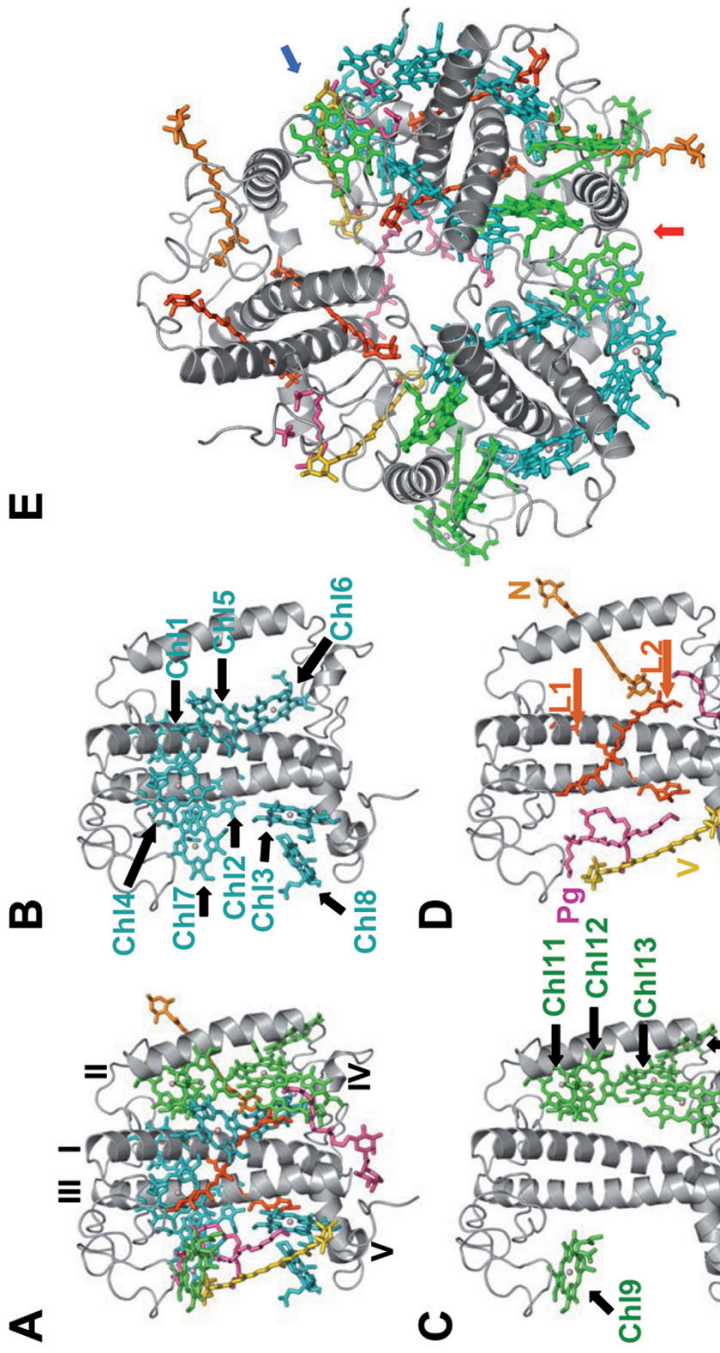

Figure 2. Structure of the major light-harvesting complex of photosystem II at a resolution of 2.5 Å. Side views of a monomer with the full set of cofactors (*A*) or with individual cofactor classes (*B–D*) are shown. Chlorophyll (Chl) numbering is according to Standfuss et al. [21] (c.f. Table 3). Phytyl chains were omitted for clarity. In (*E*) the top view of a trimer from the stromal side is depicted. For each monomer a different set of cofactors is shown for optimal clarity. The colouring is the same in *A–E*: Chl *a*, blue; Chl *b* (green); lutein (L), neoxanthin (N), and the violaxanthin cycle pigment (V), orange and yellow; digalactosyl diacyl glycerol (DGDG) and phosphatidyl glycerol (PG), magenta. See the text for further explanations.

side of PSI and still needs improvement to further elucidate the potential involvement of these regions in interaction of Lhca proteins with each other and with components of the PSI core. Since the amino acid sequence identity of all Lhc proteins is especially high in the membrane-embedded regions [10], it is assumed that the other Lhcb proteins, for which no crystal structure is available yet, possess a comparable structure in the transmembrane region like LHCII and the Lhca proteins.

Chlorophyll binding. The presumed common structure of the protein backbone in the transmembrane region of Lhc proteins provides the scaffold for the highly ordered binding of the pigments, allowing very efficient energy transfer between them and the Chls located closer to the centres of the PS. As is shown for a monomeric LHCII in Figure 2A–C, eight Chl *a* and six Chl *b* molecules are attached to the polypeptide backbone in two layers, one facing the luminal side and one the stromal side of the thylakoid membrane, resulting in a Chl concentration as high as 300 mM [21]. The numbering of the chlorophylls in this figure refers to the nomenclature introduced by Standfuss et al. [21], which is opposed to other currently used nomenclatures in Table 3. Chls 1, 2, 4, 5, 7, 9, 11 and 12 comprise the layer facing the stroma, whereas Chls 3, 6, 8, 10, 13 and 14 are closer to the lumen. Interestingly, Chl *a* molecules are arranged around TMH I and III (Fig. 2B), and Chl *b* molecules are in the vicinity of TMH II (Fig. 2C) close to the interface with the neighbouring monomer, which might be of importance for energy equilibration within a functional trimer (see red arrow in Fig. 2E). Chlorophylls are attached to Lhc proteins *via* their central Mg^{2+} and this binding can occur in different ways (Table 3). Seven chlorophylls are bound to amino acid side chains of histidine, asparagine, glutamic acid, and glutamine (Chls 1, 2, 3, 4, 5, 8 and 12). Two are bound to the oxygens of carbonyl groups (Chls 9 and 14), one to phosphatidyl glycerol (Chl 7) and another four *via* water molecules (Chls 6, 10, 11 and 13) [21, 59]. Besides the ligation *via* the magnesium, all Chl *b* and some Chl *a* are additionally ligated to the protein by hydrogen bonds formed with the formyl group of Chl *b* and the keto group at C13 of Chl *a*, respectively. Detection of phytyl residues in structures below 3 Å revealed their very variable structure.

For determining the spectral properties of Chls within one LHC, the aforementioned reconstitution technique is of great use when apoproteins are employed that are mutated in a single potential Chl-coordinating amino acid. Investigations of Lhca1 [120], Lhca3 [119], Lhca4 [121], Lhcb1 [122–124], and Lhcb4 [102] revealed that mutations of single putative Chl-binding amino acids frequently resulted in the loss of not only one Chl, but of two or even more Chls (highlighted in bold face in Table 3). Nevertheless, in many instances absorption, fluorescence and dichroism measurements of such samples facilitated assignment of absorption properties to individual Chls and provided detailed insight into the excitation energy transfer pathway [125]. By this approach, it could be demonstrated that especially in Lhca proteins spectral properties often arise from strong excitonic coupling of nearby Chls. This is of special importance with regard to long wavelength properties of LHCI, which originate from Chls ligated to the binding sites A5 and B5 or Chl 12 and Chl 5, respectively [119, 120, 126, 127].

Carotenoids. Each LHCII monomer has in its centre two Lut molecules at the so-called L1 and L2 site (Fig. 2D). They are located in grooves formed by TMH I and III and stabilise the LHC by the resulting cross-brace construction [118]. The β-cyclohexane rings of both Lut molecules are located towards the lumen. Conversely, the ε-cyclohexane rings are positioned close to the stromal membrane surface [21, 59]. The cyclohexane rings are in van der Waals and hydrogen bonding distance to consensus sequences of the Lhcb1 protein and other Lhc proteins (Fig. 3) [10]. For the Lut at the L1 site there is a contact to amino acids in the range of Ser160 and Leu164 on the stromal side and another one to Gln197 at the luminal side [118]. Binding partners for the Lut 2 are Asp47, Thr48, or Ala49 on the stromal side, and residues between Trp97 and Ala100 on the luminal side. Additionally, one binding site for both Neo and a VCP exists in LHCII, which are labelled N and V in Figure 2D. Neo is the only xanthophyll that protrudes into the lipid bilayer (Fig. 2D, E). The other end of Neo with its epoxidated cyclohexane ring is in close proximity to the pyrrole ring I of Chl 13 and is hydrogen bonded by Tyr112 [59]. Several amino acids of TMH II together with Chls form a hydrophobic pocket that harbours the polyene chain [59]. Reconstitution experiments with mutated Lhcb1 protein indicated that the Neo is kept in place by the Chls attached to the binding sites of Chl 12 and 13 [122, 128]. The remaining VCP is embedded into a hydrophobic environment at the interface of two monomers that is formed by hydrophobic amino acids and Chls (blue arrow in Fig. 2E) [59]. One cyclohexane ring is positioned by a hydrogen bond between its hydroxyl group and the carbonyl group of the propionic acid carrying the phytyl group of Chl 10, and the other one by a polar interaction with the glycerol of a phosphatidyl glycerol (PG; Fig. 2E) [21]. For the minor LHCIIs and the LHCIs no information about Car binding sites is available from

crystal structures. However, detailed reconstitution analyses were performed with varying pigment mixtures and mutated proteins that provided insight into the number and specificity of these sites (Table 4). Given that each CP29, CP26, and CP24 bind only two carotenoids (Table 2), it was assumed that Lut is specifically bound to the L1 site and that the L2 site accommodates Vio or Neo [102, 103, 128]. However, other biochemical data [99] and recent reconstitution results obtained with Lhcb5 and Lhcb6 mutated in the tyrosine involved in the binding of Neo in LHCII indicate that there is a specific N site in Lhcb5 and Lhcb6 (Table 4). Due to the substoichiometric presence of Neo in these proteins (c.f. Table 2), this result implies that this pigment (as well as Vio, which is also present in substoichiometric amounts) is easily released during solubilisation and purification of these LHCs and thus is lost in a fraction of the isolated LHCs. In the Lhca1–4 proteins, Lut is probably always bound to the L1 site [96, 98, 111]. The L2 site is supposedly occupied by Lut (Lhca1; [98]) or alternatively by Lut or Vio or β-Car (Lhca2–4; [96, 98, 111]). Additionally, there seems to be an N site filled by Vio in Lhca1 and a V site in Lhca3 that is occupied by Vio [111, 112].

Lipids. One of the two lipid molecules bound in LHCII, PG, is essential for the trimeric state [134]. The high-resolution structure shows that PG contains one molecule of trans-Δ^3-hexadecenoic acid, which occurs exclusively in LHCII among LHCs of PSII [135]. This fatty acid runs parallel to the Vio and may promote the connection of monomers [21]. Besides, one molecule digalactosyl diacyl glycerol (DGDG) is present at the luminal side of LHCII, possibly possessing a space-filling function. Biochemical analyses of the minor LHCs of PSII revealed an exceptionally high content of lipids, which are somewhat enriched in highly unsaturated fatty acids [136]. Due to the inherent problem that one does not know whether lipids attached to the LHCs are true constituents or contaminating appendages, as long as no high-resolution crystal structure is available, it is not clear whether they fulfil a special function or are merely intramolecular space fillers. Alternatively, they could establish a specific link of these proteins with their neighbours in PSII. For LHCI-730, it was shown that PG is required for heterodimerisation and that increasingly unsaturated fatty acids improved the interaction capability of the two subunits, indicating that the fatty acid moiety may contribute to the interface between the two monomers [137].

Table 3. Nomenclature of chlorophyll-binding sites and their occupation in various Lhc proteins according to reconstitution experiments with mutated apoproteins. An exception from this is Lhcb5 where the assignment was based on LHC reconstitutions with varying Chl *a*/*b* ratios and on homologies with Lhcb1 and Lhcb4. n.s., reconstitution yielded no stable LHC. -, not analysed. Mutations leading to a loss of more than one chlorophyll in the reconstituted LHC are in bold letters.

Protein → Binding site ↓	Lhcb1			Lhcb1 [124]	Lhcb4 [102]	Lhcb5 [103]	Lhca1 [120]	Lhca3 [119]	Lhca4 [121]
Standfuss et al. [21]	Liu et al. [59]	Kühlbrandt et al. [118]	Mg^{2+} coordinated by						
Chl 1	Chl *a* 610	Chl a1	Glu 180	**Chl** *a*	n.s.	Chl *a*	n.s.	n.s.	–
Chl 2	Chl *a* 612	Chl a2	Asn 183	**Chl** *a* + *b*	Chl *a*	Chl *a*	**Chl** *a* +*b*	**Chl** *a*	–
Chl 3	Chl *a* 613	Chl a3	Gln 197	Chl a or b	Chl *a* or *b*	Chl *a* or *b*	**Chl** *b* +*a*	n.s.	n.s.
Chl 4	Chl *a* 602	Chl a4	Glu 65	**Chl** *a* + *b*	n.s.	Chl *a*	**Chl** *a* +*b*	n.s.	Chl *a*
Chl 5	Chl *a* 603	Chl a5	His 68	Chl a	Chl *a*	Chl *a*	**Chl** *a* +*b*	**Chl** *a* + *b*	**Chl** *a* + *b*
Chl 6	Chl *a* 604	Chl a6	Water 309	–	–	–	–	–	–
Chl 10	Chl *b* 607	Chl a7	Water 308	–	–	–	–	–	–
Chl 11	Chl *b* 608	Chl b1	Water 302	–	–	–	–	–	–
Chl 7	Chl *a* 611	Chl b2	Phosphatidyl-glycerol	–	–	Chl *b*	–	–	–
Chl 8	Chl *a* 614	Chl b3	His 212	Chl *a* or *b*	Chl *b* or *a*	Chl *b* or *a*	Chl *a* or *b*	Chl *a*	Chl *a*
Chl 12	Chl *b* 609	Chl b5	Glu 139	**Chl** *b* + *a*	Chl *b* or *a*	Chl *b* or *a*	**Chl** *b* + *a*	**Chl** *a* + *b*	**Chl** *a* + *b*
Chl 13	Chl *b* 606	Chl b6	Water 310	**Chl** *b* + *a*	**Chl** *a* + *b*	Chl *b* or *a*	Chl *b* or *a*	Chl *a*	Chl *b*
Chl 9	Chl *b* 601	–	Tyr 24*	–	–	–	–	–	–
Chl 14	Chl *b* 605	–	Val 119*	–	–	–	–	–	–

* Coordination of the Mg^{2+} occurs via the oxygen of the backbone carbonyl group.

Table 4. Occupancy of the carotenoid binding sites in light-harvesting complexes of photosystem I and II. Lut, lutein, Neo, neoxanthin; Vio, violaxanthin; VCP, violaxanthin cycle pigment (violaxanthin, antheraxanthin or zeaxanthin); β-Car, β-carotene.

Carotenoid binding site → LHC ↓	L1	L2	N	V	Reference
Lhcb1–3	Lut	Lut (Vio)	Neo	VCP	[59, 99, 130, 131]
Lhcb4	Lut	Vio	Neo	–	[99, 102, 129]
Lhcb5	Lut	Vio	Neo	–	[103, 129, 132]
Lhcb6	Lut	Vio	–	–	[132, 133]
Lhca1	Lut	Lut or Vio or β-Car	Lut or Vio	–	[96, 98, 112]
Lhca2	Lut	Lut or Vio or β-Car	–	–	[96, 111]
Lhca3	Lut	Lut or Vio or β-Car	–	Lut or Vio	[96, 111, 112]
Lhca4	Lut	Lut or Vio or β-Car	–	–	[96, 98]

Structure flexibility. The above description of the LHCII structure may be suggestive of being a rigid structure. However, this clearly is an oversimplification.

The second explanation for the failure to trace the nine N-terminal amino acids in the LHCII structure proposes different conformations of the N terminus. Such a conformational change was proposed as a consequence of phosphorylation in the context of state transitions, which results in detachment of LHCII from PSII [138]. EPR measurements of LHCII tagged with spin labels at different positions of the protein actually indicated two conformational states of the N terminus [139]. However, a more detailed knowledge of the location of the N terminus is required, especially towards a better understanding of the detachment/attachment process underlying the state transitions.

Changes in the structure of Lhc proteins are also important for NPQ [11, 15]. This phenomenon is triggered by protonation of the PsbS protein [140], which subsequently interacts with LHCs of PSII and probably induces their conformational alteration [15, 141]. This elicits a reversible rearrangement of pigments, which switches the LHC from a light harvesting to an energy dissipating conformation, thus regulating the amount of excitation energy transmitted to the reaction centre. In LHCII, twisting of Neo apparently induces a closer location of the Lut 1 towards the nearby Chls 7+2 and Chl 1. This relocation should change the energy levels of these pigments in a way that allows retransfer of excitation energy from Lut 1 to these Chls with a low energy level, which function as a sink and dissipate the energy [14, 142]. In CP29, the alteration in protein conformation supposedly changes the distance and/or orientation of a Zea molecule at the L2 site relative to a Chl pair (Chl 5 and 12), thereby forming a strongly coupled pigment trimer [16]. This creates the site of charge transfer quenching, which includes charge separation (leading to a Chl^{-}* and Zea^{+}*) and subsequent charge recombination that leads to de-excitation of the excited state [143]. Thus, at least two different quenching mechanisms seem to contribute to the rapidly reversible non-photochemical quenching in the light-harvesting antenna of PSII that dissipates energy absorbed in excess [14–16, 144]. Despite this very recent progress in understanding the interrelation between pigment orientation/location and energy dissipation, no information about the underlying conformational changes of the protein are available. Due to the outstanding significance of efficient light-harvesting and energy quenching, respectively, and of state transitions for optimal regulation of photosynthetic performance under fluctuating light conditions, detailed insight into the accompanying changes in the protein structure is urgently awaited.

Life cycle of LHCs

Expression, transport and membrane insertion of LHCs. Expression of the nuclear-encoded Lhc proteins is under the control of the phytochrome system [145, 146] and follows a circadian rhythm being highest around noon [147]. They are synthesised in the cytosol with an N-terminal transit sequence, which marks them for chloroplast import. Two translocons traversing the outer (Toc) and inner (Tic) envelope membrane achieve protein import into the chloroplast stroma [148, 149]. The existence of several genes for individual components of the Toc [150, 151] and one component of the Tic [152] in *Arabidopsis* implicates diversity in the composition of Toc and Tic complexes with potentially different specificities. Lhc proteins and other proteins of the photosynthetic apparatus apparently utilise a light-induced pathway employing a special Toc. It remains to be clarified whether a specifically composed Tic complex is also involved in their import [152–154]. Regardless of the precise

envelope passage, the transit peptide is cleaved off upon arrival in the stroma. Afterwards two different routes apparently exist by which Lhc proteins can reach their destination in the thylakoid membrane.

The first pathway depends on recognition of Lhc proteins, possibly with the exception of Lhcb4, by a plastidic signal recognition particle (cpSRP). CpSRP consists of a homologue of the 54-kDa protein of cytosolic SRP, cpSRP54 [155], and a second protein, cpSRP43, which substitutes for the RNA present in cytosolic SRP [156]. Analyses with LHCP, which is a synonym for Lhcb1, revealed binding of cpSRP43 to the so-called L18 domain of mature Lhcb1, located between the second and third TMH of Lhc proteins (Fig. 3) [157, 158]. Subsequently, cpSRP54 binds to cpSRP43, probably by electrostatic attraction, and to hydrophobic TMH regions of Lhcb1 [159, 160]. Assembly of this complex allows binding of the FtsY protein, which is a homologue of the bacterial SRP receptor. It has been proposed that FtsY attaches to the SRP-LHCP transit complex and, possibly together with other up to now unidentified factors, directs this complex to thylakoids and initiates its GTP-dependent membrane integration [160–162]. This apparently occurs in both stacked and unstacked thylakoids [163]. For the insertion of LHCP, the membrane-embedded protein Alb3 is required [164], which forms a protein family with the mitochondrial Oxa1 and bacterial YidC [165]. These are insertases or/and foldases and may be additionally involved in establishing protein-protein interactions accomplishing the formation of multi protein complexes like PSI and PSII [166]. Alb3 presumably has five TMH and a large C-terminal segment located in the stroma, which may provide a docking point for FtsY with the transit complex [167], thereby effecting membrane insertion accompanied by pigment binding. Recently, a homologue of Alb3 was detected in the chloroplast, designated Alb4, which fulfils apparently a distinct role in chloroplast and thylakoid biogenesis as compared to Alb3 [168].

The second pathway is mainly based on findings with *Chlamydomonas* (mutants) [169, 170] and recent chloroplast import studies with Lhcb1 and Lhcb4 protein of *Arabidopsis* showing the requirement of the functional, Tic-associated, chlorophyllide *a* oxygenase (CAO), and thus chlorophyll(ide) *b*, for the import of these proteins [171]. According to this hypothesis, CAO in the inner envelope membrane is associated with the Tic complex and participates in Chl delivery to Lhcb1 and Lhcb4 during its concomitant insertion into the inner envelope membrane. Following assembly, the LHCs become incorporated into budding vesicles and move this way to the developing thylakoid membrane. Vesicle transport in chloroplasts, especially developing ones, is widespread among land plants [172, 173], thus supporting this hypothesis.

Up to now, the conditions under which one of the two pathways prevails are not clear. Increased vesicle formation and thus potentially increased transport of assembled LHCII from the envelope membrane to the thylakoids during formation of the thylakoid membrane system were interpreted as giving a preference of this pathway during earlier developmental stages [169]. Results obtained with cpSRP54/43 double-knockout mutants demonstrated that the plants are viable, although thylakoid membrane accumulation of some Lhc proteins is strongly affected [159, 174, 175]. While Lhca1, Lhca3, and Lhcb3 were not detectable in thylakoids, the concentration of Lhca2, Lhca4, Lhcb1, and Lhcb6 was reduced by more than the half of their normal content. By contrast, Lhcb4 even appeared to be enriched. Interestingly, almost complete lack of Lhcb1 and Lhcb4 import into chloroplasts deficient in functional CAO implies that this pathway may be of especial importance for the latter protein. This may indicate that some Lhc proteins have a clear preference for one of the two pathways. As the pathways split up at the Tic complex, one may speculate that differently composed Tics exist, *e.g.*, with CAO associated or not, which decide about recognition

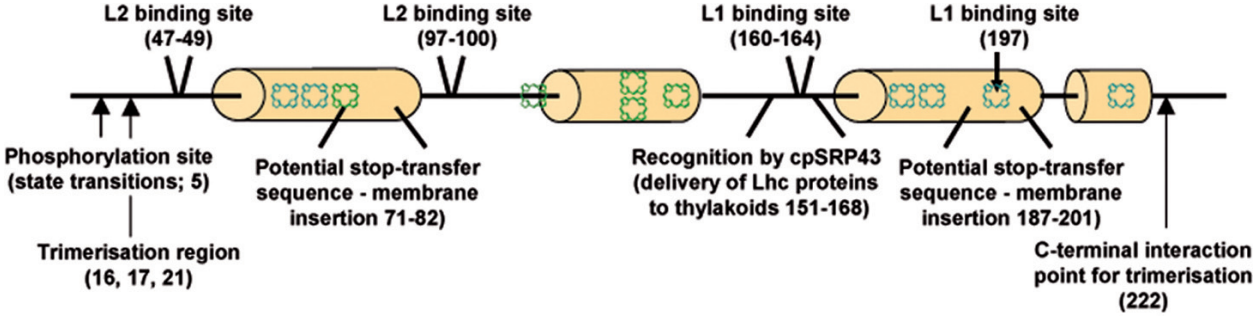

Figure 3. Overview of the functions in different regions of the Lhcb1 (or LHCP) protein. Numbers given in parentheses represent the precise position(s) in the pea protein. Starting from the N terminus these are: T5 phosphorylation site [138], trimerisation motif spanning W16 and/or Y17 and R21 [213], lutein and chlorophyll (Chl *a* in cyan, Chl *b* in green) binding sites except for those of Chl 6, 7 and 9 [10, 21, 59, 118], supposed stop sequences for membrane integration in the transmembrane helices I and III [170], L18 binding region of the chloroplast signal recognition particle [157], and the C-terminal trimerisation point W222 [214].

and processing of individual Lhc proteins, and thereby, determine the further route towards the destination. Generally, the dual location of CAO, the key enzyme for Chl *b* synthesis, in the inner envelope and thylakoid membrane in pea and *Arabidopsis* [171, 176, 177] supports the idea that two different locations for LHC assembly exist. Regardless of the location, the question arises how coordination of protein insertion and pigment binding proceeds.

Assembly of LHCs. Hoober and coworkers [169, 170] proposed a model about the LHCII assembly process in the inner envelope membrane during chloroplast import, which can be subdivided in four steps: (1) the first step envisages that membrane insertion of LHCP stops as soon as a non-polar stop-transfer region in the second half of the TMH I of the protein (Fig. 3) enters the membrane; (2) for a successful insertion, attachment of Chl *b* and *a, e.g.*, Chl 9 and Chl 5 (see Fig. 2; Table 3), are required allowing the protein to be retained in the membrane and to adopt a stable insertion conformation, possibly by additional binding of Chl 14 by Val at position 119; (3) subsequently, accompanied by the binding of other pigments, the rest of the protein is transported over/into the membrane, which permits the protein segments corresponding to TMH II and III to capture their transmembrane positions and completes the membrane insertion step according to [178]; (4) further folding of the protein follows and comprises binding of additional pigments and formation of the ion bridges between the glutamates and arginines present in TMH I and III, resulting in the formation of the completely assembled LHC. The overall model and its initial steps are in accordance with findings obtained for the D1 reaction centre protein and the Lhc proteins of *Chlamydomonas*. The co-translationally assembled D1 apparently halts at specific intervals allowing for concomitant Chl *a* ligation before synthesis continues until the entire molecule is produced [179, 180]. An interrelationship between pigment biosynthesis and Lhc protein accumulation in thylakoids was also observed in *Chlamydomonas* and interpreted to mean that pigment synthesis is directly linked to the presence of the Lhc protein [181]. Additionally, stable insertion of LHCP requires the availability of Chl *b* in higher plants since no persistent insertion occurs into inner etioplast membranes or thylakoids as long as no Chl *b* or a substitute for it is present [182–184].

In view of the reproducible pigment composition of individual LHCs (Table 2), the question arises how the specificity of pigment binding into individual sites comes about to yield LHCs of at least ten distinct Lhc proteins that are assembled in close proximity. One suggestion is that free Chls and Cars, possibly in close contact allowing the carotenoids to quench the Chls if required [169], are present at the assembly sites and that binding of them depends on the affinity of the individual Chl and Car binding sites for the individual pigment species. In fact, detailed *in vitro* reconstitution analyses revealed different affinities of Chl [103, 185, 186] and Car binding sites [103, 130, 131]. However, occupation of individual Chl and Car binding sites is not always strict *in vivo*. Mutants deficient in specific xanthophylls show similar Chl:Car ratios as the wild types, indicating that individual Cars are, at least partially, replaceable against each other [187, 188]. Likewise, Chl *b* free mutants or plants grown under intermittent light conditions, when only few Chl *b* is synthesised, are still able to assemble some of the various Chl *b* containing LHCs [184, 189–191]. Interestingly, enhanced Chl *b* synthesis achieved by overexpression of CAO resulted in an enrichment of LHCs relative to the Chl *a* binding core components [192] and in a replacement of Chl *a* by Chl *b* in the LHCs [193]. In addition, *in vitro* reconstitutions with pigment mixtures of diverse composition or in the absence of individual pigments showed some flexibility in pigment binding of the different Lhcb [104, 105, 108–110, 130, 131, 186, 194–197] and Lhca proteins [57, 96, 112, 137, 195, 197]. For instance, reconstitution experiments with pigment mixtures corresponding to the pigment composition of thylakoids revealed that Lhca proteins are able to bind Neo instead of β-Car, which is not bound to Lhca proteins *in vivo* [96]. This clearly demonstrates a considerable flexibility of at least some pigment-binding sites listed in Tables 3 and 4 under *in vivo* and *in vitro* conditions and implies that different affinities of the Chl- and Car-binding sites cannot solely be sufficient to attain the exact pigment composition. Therefore, other elements may be needed that participate in the controlled pigment supply to individual binding sites. Such a control could be exerted either directly by the last enzymes of Chl or Car biosynthesis, which are possibly organised in Chl- [177, 198, 199] or Car-forming centres [200], or indirectly by pigment carrier proteins. A prerequisite for the direct mode is a close neighbourhood between the newly inserted Lhc proteins and several pigment-forming centres, whose number would depend on their composition. However, such a scenario would require a proper alignment of pigment forming centres around a folding protein, which represents a steric challenge. Whether the concept of an indirect mode with pigment carrier proteins actually plays a role in adjusted pigment delivery has to be questioned because of limitations of the proposed candidates, which are early light-inducible proteins [201] and the PsbS protein [202]. As

discussed earlier, these proteins would have to fulfil several prerequisites like a close spatial proximity, a lower affinity to Chls and Cars as compared to Lhc proteins, and prevalence under conditions of strong LHC assembly [201, 203]. Recent analyses with native and recombinant PsbS, however, raised scepticism whether it actually binds Chls or pigments at all [141, 204, 205]. In contrast, early light-inducible proteins only bind a subset of the pigment species ligated to a single Lhc protein [201]. Hence, other unidentified proteins would have to assist if one assumes that Lhc proteins receive all pigments by carrier proteins. Alternatively, LHCs with a uniform pigment composition could be obtained from a mixture of LHCs with diverse pigment content, which are subject to exchange of individual pigments by early light-inducible proteins. However, this would only allow exchange of pigments located at the surface or which are at least accessible from the surface. Taken together, no concept without pitfalls is currently at hand about the pigment-binding mechanism, perhaps because important actors are still unknown.

Regarding the later steps of the LHC assembly process, it depends on whether assembly occurs in the thylakoid membrane or in the inner envelope membrane. In the latter case, only the stromal loop has to be translocated across the membrane. By contrast, assembly in the thylakoid membrane would require translocation of the luminal loop and the C terminus across the membrane, which could be experimentally confirmed [206]. Knowledge about the folding process is very scarce. *In vitro* reconstitutions demonstrated the interdependence of protein folding and pigment binding [207] and that there are two phases during LHCII formation. During the initial fast phase Chl *a* and xanthophylls are bound, while in the second phase Chl *b* is attached [208, 209]. The final assembly step might be to establish the tertiary structure by formation of the aforementioned ion bridges between TMH I and III and the concomitant binding of the lacking pigments. This may 'lock' the structure and enables the LHC to interact with its prospective partner(s).

Oligomerisation and PS integration. Monitoring biogenesis of Lhc proteins in the thylakoid membrane demonstrated that formation of monomeric LHCs precedes assembly of oligomeric LHCs [210–212]. The trimerisation of Lhcb1,2,3 to form LHCII and the dimerisation of LHCI-730 requires amino acid (groups) at the N and C termini of Lhcb1 [213, 214] and Lhca1 [215] as well as amino acids in the TMH I of Lhcb1 [123] and TMH II of Lhca4 [216]. The interaction mode of Lhca1 and Lhca4 may also apply for Lhca2 and Lhca3 forming the other dimer of PSI [83]. For LHCI-730 it was found that dimerisation coincides with the ligation of additional Chl [41], and Chls as linkers appear to be of general importance for Lhca interaction in the LHCI belt [43]. Furthermore, PG plays a decisive role in the formation of dimeric LHCI-730 [137] and trimeric LHCII [134]. Upon oligomerisation, LHCs in developing plastids are subsequently attached to the core complexes of both PS, which are centrifugally assembled [217, 218].

Degradation of LHCs. Degradation of LHCs is very slow and is scarcely detectable under normal growth conditions [219, 220]. For *Lemna*, a half-life of about 10 h was determined for Lhc proteins [220], whereas no LHCII degradation at all was observable within 24 hours in bean [221]. However, upon light to dark transition [219], dark-induced senescence [222] and low light to high light transfer [223–225] degradation of LHCII becomes accelerated. Analyses of LHCII degradation under these conditions revealed the involvement of different proteases. In spinach, a serine- or cysteine-type protease is involved in high-light acclimation [223]. It reversibly binds to stroma thylakoids and degrades non-phosphorylated LHCII monomers, but no trimers [224, 225]. In bean, a serine-type protease was identified [221] that is, possibly *via* cations, attached to stroma exposed thylakoids. In barley and *Arabidopsis* a zinc-dependent metalloprotease was detected that degrades Lhcb3 in the dark-induced senescent state [222, 226]. Identification of the corresponding gene (FtsH6) and use of knockout plants revealed that this protease causes the high-light acclimation-induced degradation of Lhcb1 in addition to the aforementioned Lhcb3 degradation and that it is constitutively expressed [222]. Thus, several proteases seem to be involved in the degradation of the major LHCII, but it is not clear whether there is a redundancy. As the proteases were found in association with stroma thylakoids and nonappressed grana thylakoids, it was inferred that degradation occurs in these thylakoid regions. In senescent leaves, degradation of LHCs means not only digestion of the Lhc protein, but also of the Chls. Since Chls that are not attached to proteins along with Cars generate toxic oxygen species upon illumination, LHCII degradation clearly needs a concerted, well-balanced action of both degrading pathways [227]. The inhibition of degradation of pigments present in the major LHCII (Chl *a*+*b*, Lut, Neo) in a stay-green mutant and the concomitant constant amount of the major LHCII indicates that LHCII disintegration starts with protein cleavage and not with pigment degradation [228]. Control of protease activity is apparently not subject to a common regulation mechanism since the activity of various different proteases has to be independently adapted to different developmental/environmental

conditions [229]. For the major LHCII the signal responsible for triggering a molecule for degradation may be the transition from the trimeric state into the monomeric one as monomeric LHCII is the main substrate [222, 225]. Protease action apparently starts by recognition of amino acids in the N-terminal segment of LHCII apoproteins [225, 230]. The luminal loop region was suggested as another place, where LHCII disintegration could start [231]. Recently, a protein was identified in stay-green plants that might be involved in the initial destabilisation process [232]. The failure to detect degradation products *in vivo* implies that degradation proceeds very rapidly and that the rate-limiting step is the initiation of the process.

Observation of degradation of other LHCs demonstrated that the minor LHCIIs and the LHCIs are degraded much faster than the major LHCII [228]. However, no proteases degrading these proteins have yet been identified [233]. In summary, degradation of LHCs requires the cooperation of several distinct proteases for the different Lhc proteins and currently only few tesserae of the whole story are known.

Summary and perspectives

During the last few years, probably the last Lhc proteins of vascular plants have been identified and considerable progress has been achieved with respect to elucidation of the structure and function of LHCs, especially the major LHCII. By contrast, gain of knowledge about biogenesis of the LHCs and their further fate could not keep pace with the progress in these fields. Because there is still rather limited insight into the assembly process of LHCs, important future questions could be aimed at locating this process under various developmental and environmental situations and at identifying further proteins possibly involved in it. In addition, the elucidation of the pigment-binding mechanism responsible for the reproducible pigment binding by the individual Lhc proteins deserves further attention. Likewise, the reason for the specific binding of LHCs to only one PS awaits its clarification. Further insight into this topic may also shed light on the formation of differently composed PS and their relevance under varying environmental conditions. Finally, identification and characterisation of further components involved in LHC degradation will be required to obtain a better understanding of the life cycle of Lhc proteins. Availability of cDNAs of components of both degradation pathways allows their heterologous expression and the design of *in vitro* assays suitable to elucidate the interrelationship between protein and pigment degradation.

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