11
Structure of the Phycobilisome Antennae in Cyanobacteria and Red Algae

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11.1
Introduction

11.1.1
Photosynthetic Antennas

All photosynthetic organisms contain a two-component photochemical apparatus comprised of an antenna complex and a reaction center [1, 2]. One can thus assume that useful biological conversion of light-energy requires the presence of a high enough input of energy by the antennas to sustain efficient electron transfer by the reaction centers. In the most primary sense, antenna complex proteins have been designed by evolution to perform three tasks: bind high densities of pigments (chlorophylls, bilins and carotenoids); create a functional funnel that efficiently transfers the absorbed energy into the reaction center; and self-assemble into units that allow the performance of the former tasks. These antenna structures must function correctly while preventing potentially deleterious side effects that could occur due to the proximity of excited molecules with other proteins. A variety of very different geometric solutions has evolved to attain these very same goals, o a much greater extent than the differences between reaction centers [3–5]. The four major antenna forms are: the circular transmembrane light-harvesting complexes (LH1 and LH2) of purple non-sulfur bacteria [6]; the compact transmembrane light-harvesting complexes (LHC-I, LHC-II) of prochlorophytes, green algae, and plants [7, 8]; the membrane associated phycobilisome of cyanobacteria and red algae [9, 10]; and, the organelle-like chlorosome of green sulfur and green filamentous bacteria (*Chlorobi* and *Chloroflexi*) [11]. As a result of the exceptionally different morphological nature of these antenna systems, each one is described in a separate chapter. This chapter describes in depth the knowledge obtained from the determination of near-atomic resolution crystal structures of isolated components of the phycobillisome. Models of the entire complex based on these structures will be presented along with a description of how the phycobillisome performs its tasks.

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11.1.2
Photosynthetic Organisms that Utilize the Phycobilisome

The phycobilisome serves as the major light-harvesting antenna complex of cyanobacteria, rhodophyta (eukaryotic red algae), and cryptophyta. These organisms collectively make up earth's largest producers of oxygen and absorbers of CO_2. They can be found in environments ranging from oceans to hot-springs (up to ~70 °C) to dry deserts and to the Arctic [12]. In all environments they can use light throughout the visible spectrum due to the coverage provided by the phycobilisome and reaction center pigments. The phycobilisome pigments absorb strongly in the region between 550–660 nm, which complements the spectral region covered by chlorophyll *a* in the blue (soret band) and red regions. Red algae can grow in much deeper water due to the addition of a larger compliment of phycoerythrin (PE), the blue absorbing phycobiliprotein (PBP).

The study of the phycobilisome and its component protein subunits began almost 150 years ago (for a detailed description of the early period of phycobilisome research see [13]). However, the real first structural studies that led to insights on phycobilisome function began in the 1960s and 1970s in the research groups of E. Gantt, A. Glazer, D. Bryant, H. Zuber, and their coworkers [14–20]. These original structural studies coupled the visualization by electron microscopy (EM) of the phycobilisome along the thylakoid membranes, with biochemical and genetic studies that associated the phycobilisome with both the bilin-bearing PBPs and the colorless linker proteins. This was followed by additional EM studies on isolated phycobilisomes using negatively stained samples from different organisms. The intricate structure revealed in these micrographs showed that the phycobilisome is assembled by a large number of seemingly similarly sized disks. The fragility of the complex during sample fixation had a notable affect on its shape and integrity, as can be seen by the lack of uniformity between complexes. However, it became quite clear that the phycobilisome contains two main substructures – a set of rod like stacks of disks surrounding a core of two to five close-packed disks [21]. The number of disks in the core was found to be species dependent [22]; but, in all cases, the rods appeared to radiate out from the core. The fashion and architecture of rod attachment to the core is of major functional importance and will be the focus of the final section of this review.

11.2
Basic Structural Characteristics of the Phycobilisome Component Proteins

All modern PBPs (those that exist in present day organisms), can be traced back to a common founder gene product – an ancestral PBP [23]. This ancestral gene can be associated even further back to the globin family of proteins [24]; thus, one can chart the development of the phycobilisome through comparisons of protein sequences and cofactor changes. The primary unit of all PBPs is a heterodimer

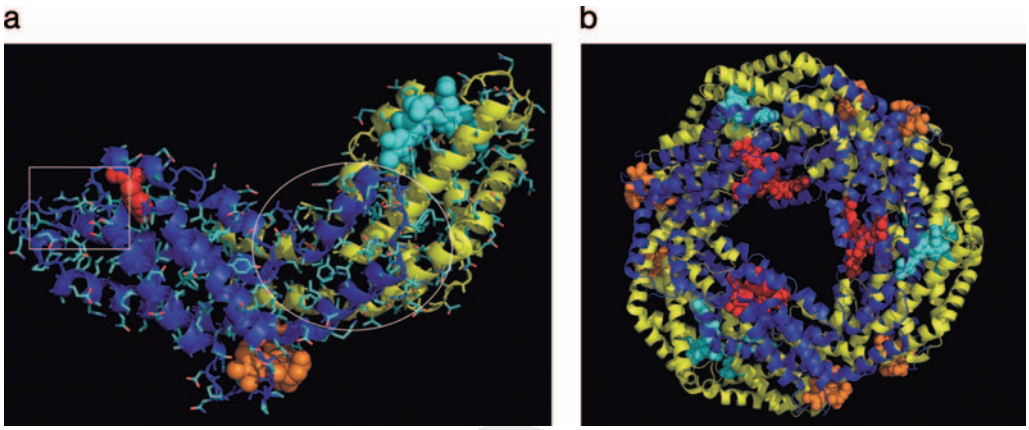

Figure 11.1 **(a)** (αβ) phycocyanin monomer. The monomer formation surface formed by the X-Y helices of both subunits is identified by the white oval. The white square identifies the position of two conserved phycobiliprotein modifications: the βThr^{77} residue (with non standard peptide dehydral angles) and βAsn^{72} which is methylated. Amino acid residues are depicted in stick representation and colored according to the usual CPK color code. **(b)** $(\alpha\beta)_6$ hexameric form of phycocyanin. In both panels the α and β subunits are depicted in yellow and blue cartoon helices, respectively. The PCB chromophores are shown in sphere representation: αPCB^{84} in cyan, βPCB^{84} in red and βPCB^{155} in orange. Figures 11.1, 11.2 and 11.4 were prepared using Pymol (pymol.sourceforge.net).

composed of two homologous subunits, α and β, known as the (αβ) monomer, shown in Figure 11.1a. There are four major forms of PBPs, classified by the number and type of bilin cofactor to which they are associated. These are allophycocyanin (APC, λ_{max} = 652 nm), phycocyanin (PC, λ_{max} = 620 nm), phycoerythrin (PE, λ_{max} = 560 nm), and the infrequently found phycoerythrocyanin (PEC, λ_{max} = 575 nm). The molecular weights of the α and β subunits are 16–20 kDa, depending on the type of PBP. The major structural facet of all PBP subunits is a compact globular structure made up of six α-helices (A, B, E, F, F′, and H) that are denoted according to their similarity to other members of the globin family (Figure 11.1a). All cofactors are covalently bound to these core helices or to additional loops that have arisen by insertion into the gene sequence. Two additional α-helices (X and Y) extend out from the core and serve as the assembly interface of the monomer and of additional levels of assembly (Figure 11.1a, white circle).

All PBPs, except for a special class of soluble PE found only in cryptomonads (see Section 11.2.3.5), further assemble into $(\alpha\beta)_3$ trimers. Further levels of assembly of PC, PEC, and PE occur rapidly: $(\alpha\beta)_3$ trimers can associate into $(\alpha\beta)_6$ hexamers and these then associate further into extended structures called **rods**. Figure 11.1a and b show a representative PC monomer and hexamer (respectively) from the *Thermosynechococcus vulcanus* crystal structure at the highest resolution determined to date (1.4 Å, deposition into the PDB in progress). The APC trimers associate in a somewhat different manner, forming cylinders containing four

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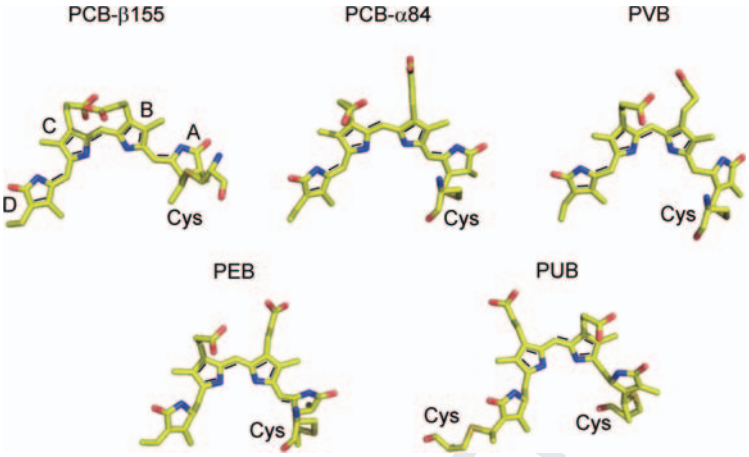

Figure 11.2 Three-dimensional structures of bilin chroophores thio-ether linked to cysteine residues. The coordinates of each bilin structure were carved out of the crystal structures: phycocyanoblins (PCB) from *T. vulcanus* PC–[1KTP], phycoviolobilin (PVB) from *M. laminosus* PEC–[1C7L], phycoerythrobilin (PEB) and doubly linked phycourobilin (PUB) from *G. monilis* PE–[1B8D]. The positions of the conjugated double bonds systems are denoted by black lines. Note that identical bilins can have different conformations (see two PCBs) and that the orientations of the propionic acid groups is highly variable.

trimers. Two to five of these cylinders pack into what is called the **core** of the phycobilisome.

All of the cofactors of the phycobilisome belong to the bilin family (Figure 11.2). These molecules are linear tetrapyrroles, which differ by the number and position of the conjugated double bonds that serve to tune the general region of light absorption. Both APC and cyanobacterial PCs contain only phycocyanobilin (PCB) cofactors, which have eight conjugated double bonds, while rhodophyte PC and all PECs and PEs can contain phycoviolobilins (PVB, seven conjugated double bonds), phycoerythrobilins (PEB, six conjugated double bonds), and phycoeurobilins (PUB, five conjugated double bonds). Some PVBs, PEBs, and PUBs can be covalently linked to two cysteine residues via a thio-ether linkage, while all PCBs are singly linked. The molecular structures shown in Figure 11.2 have been carved out of the PDP coordinates, as determined by X-ray crystallography of the isolated PBPs (two-dimensional representations of the bilins can be seen in (Grossman and coworkers, 1993)). In this way, the bent and twisted nature of the cofactors can be appreciated. It can be clearly seen that the degree of linearity, the positions of the propionic acids, and the relative orientation of the bilins with respect to the cysteines (to which each bilin is covalently bound) differ. These structural modifications are of course caused by the polypeptide environment surrounding each bilin. It is apparent that, by use of the open chain structure, the proteins can apply a second level of tuning by bending and twisting the molecules. Such tuning results in a broader range of absorption spectra and, potentially, serves to induce

directed energy transfer from the outside of the phycobilisome down into the core and finally into the reaction center (see Section 11.4.2).

11.2.1
Isolation of Phycobilisomes and Phycobiliproteins

One of the major questions that must be addressed when describing information obtained by structural methods is the state of the molecule to be analyzed. Unfortunately, the state of the molecule in vivo is rarely exactly the same as it is in its isolated form prior to the manipulations required for the performance of measurements (such as crystallization, concentration, or flash-freezing). Upon performance of such manipulations, the state may change again. The resulting structures are very often spectacular in the amount of information obtained; thus, in many cases, the question of the relevance of the structure to the true state of the molecule is ignored. The relevance of crystal structures of membrane bound LHCs has been debated [3], and indeed the results obtained at high-resolution by X-ray crystallography do not always agree with lower-resolution electron microscopy (performed on less traumatized samples). In the case of phycobilisome, this has certainly been a major problem throughout the history of its structural investigation. To date, crystallography has provided structural information on isolated PBPs [9] that may, or may not be entirely useful in the building of larger phycobilisome structures. In many of the published reports of PBP crystallization, the level of organization (see above) obtained during isolation is not noted. While this does not negate the importance of the structural results on the level of the monomeric protein, it does limit the applicability of the structure to the understanding of the function of the entire phycobilisome.

PBPs are readily isolated from a variety of organisms. The amount of protein in each cell is quite high, and the covalent nature of the bilin binding to the protein makes monitoring the purification of the protein quite simple. Upon cell disruption, large amounts of PBP, which can be easily purified by standard biochemical methods, are typically released. Experience shows that in low ionic strength buffers, much of the isolated PBPs remain in $(\alpha\beta)_3$ trimeric form. In almost all reported crystal forms, these trimers, or perhaps in some cases monomers, reform during the crystallization process into $(\alpha\beta)_6$ hexameric form, or even further into infinitely long rods.

Intact phycobilisomes are rarely found in the supernatant of disrupted cells. This may indicate that the phycobilisome are rather loosely held together. Most of the published EM studies of thin sections of cyanobacteria show series of similarly sized complexes lining the thylakoid membrane [25]. Each phycobilisome may actually be surrounded by densely packed cytosolic proteins, perhaps preserving complex integrity. Upon disruption and dilution, these proteins (bound to the phycobilisome in a specific manner or not) may dissociate from the phycobilisome, allowing the complex to fragment. In order to obtain intact phycobilisome, cell disruption must be performed in the presence of high concentrations of phosphate buffer (0.5–0.9 M); this buffer appears to mimic, in some fashion, the

conditions that preserve the complex [19]. To date, isolated phycobilisomes have only served for EM studies (negatively-stained and flash-frozen cryoprotected samples [26]). Crystallization of such an immense complex is certainly a difficult prospect, but the availability of even a low-resolution structure would be extremely helpful, as it could serve as a template for the building of a true structure based model.

11.2.2
Crystallization of Phycobilisomes

Following their isolation, PBPs are typically readily crystallized. Crystals have been obtained from both polymer (e.g. polyethylene glycols) and salt (e.g. ammonium sulphate) precipitating reagents [9]. Many of the crystals obtained belong to trigonal or hexagonal space groups, including rhombohedral space groups in hexagonal habit, as a result of the threefold symmetry of the basic $(\alpha\beta)_3$ trimer. However, a number of additional space groups have been obtained including monoclininc and orthorhombic. In these cases, the asymmetric unit contains multiple trimers or hexamers which are, in some cases, in non-physiologically related packing arrangements [27].

The ease with which PBPs, especially PC, are crystallized is most likely due to their propensity to self-assemble in vivo. While the presence of chaperones in the self assembly process has been suggested [28], it has not been experimentally verified. If the same chemical properties come into play during both self assembly and crystallization, this would indicate that the extended crystal structures, beyond that of the asymmetric unit, are informative as far as understanding the actual in vivo substructures that make up the phycobilisome. It is interesting to note that the interaction surfaces of the different hierarchal levels of phycobilisome assembly (α and β subunits to monomer, monomers to trimers, trimers to hexamers, etc.) are quite flat and without prominent invaginations. The details of the interactions between PBPs at different association levels will be discussed in Section 11.3.

11.2.3
X-Ray Structures of PBPs

To date, a total of 23 PBP crystal structures exist in the Protein Data Bank (www.rcsb.org). They include three APC structures (PDB codes [1ALL], [1B33] and [1KN1]), seven unique (from different organisms) PC structures ([1CPC], [1F99], [1GH0], [1HA7], [1I7Y], [1JBO], [1KTP], [1ON7], [1PHN], and [2BV8]), one unique PEC structure ([2C7J], [2C7K], and [2C7L]), four PE structures from the phycobilisome containing organisms ([1B8D], [1EYX], [1LIA], and [2G9M]), and one unique cryptophyte PE structure ([1QGW], [1XF6] and [1XG0]). Only a single linker protein has been visualized by crystallographic methods within the [1B33] APC structure [27]. In the following sections the details of each PBP class are described. The structures are of varying diffraction quality, with the resolution of diffraction ranging between 1.45 Å to 3 Å. The crytophyte, PE_{545}, structures are unique in their

superior quality. The PE_{545} crystals diffracted to better than 1 Å, enabling true atomic resolution and determination of anisotropic B-factors. Most PBP structures contain the entire polypeptide chain (due to the fact that the entire chain participates in the formation of tertiary/quaternary interactions), all cofactors, and various (but almost always significant) numbers of solvent molecules [9]. Refinement of the structures has typically converged at good R_{crys}/R_{free} values with relatively low average B-factors. On the basis of these crystallographic parameters, it is safe to assume that these structures represent very close approximations of the actual protein structures found in vivo. However, the general lack of linker proteins within these structures may indicate that significant conformational changes may occur within the trimeric/hexameric internal cavities.

In the following sections, each of the various components of the phycobilisome are described in detail. The amino acid residues mentioned in the text will be denoted according to the sequence numbering of the C-phycocyanin structures, as proposed by Huber and coworkers [29, 30]. For an extensive study of PBP sequence alignment see [23].

11.2.3.1 **Allophycocyanin**

APC is the only type of PBP found in phycobilisome cores. While only about 25–30% sequence identity exists between APC, PC, and PE from the same species [23], the three-dimensional structures are highly similar; typically there is pair-wise r.m.s.d. of ~1.3 Å between equivalent α-carbons. Each APC monomer covalently binds two phycobillisome cofactors to Cys^{84} of each subunit. The molecular structures and protein environment of the βPCB^{84} are almost identical to those found in PC, while the environment of the αPCB^{84} cofactors is quite different. It has been suggested that the significant shift in the λ_{max} of APC versus PC (652 nm and 620 nm respectively) in the total $(\alpha\beta)_3$ APC absorption spectra is a result of this change in the αPEB^{84} environment [31]. However no direct proof has yet been obtained that this is indeed the case. It has previously been noted that the conserved βTyr^{62} residue strongly interacts with the αPCB^{84} during trimer assembly [32]. The phenolic half penetrates in between the two PCB propionic acids, potentially modifying its absorption properties. Interestingly, the small linker protein visualized in the [1B33] structure [27] also contains a tyrosine residue at (Tyr^{33}) which upon assembly penetrates in a similar fashion into one of the βPCB^{84} cofactors. Thus four out of six APC cofactors interact in a similar fashion with Tyr residues. This could explain the increase in the 652 absorption peak and concomitant decrease in the 620 nm shoulder in the APC trimer + linker spectrum in comparison with linker-less APC trimer [27]. No such Tyr-PCB interaction exists in PC, PEC or PE subunits.

The phycobilisome core can contain two to five cylinders (each cylinder containing two to four trimers) depending on the species. Two cylinders associate with the outer surface of the thylakoid membrane (basal cylinders); the remaining cylinders are stacked as a second layer in closed packed fashion (Figure 11.3). Second level cylinders typically contain only APC monomers (the gene products of the *apcA* and *abcB* genes) and a small core linker. The two basal cylinders contain

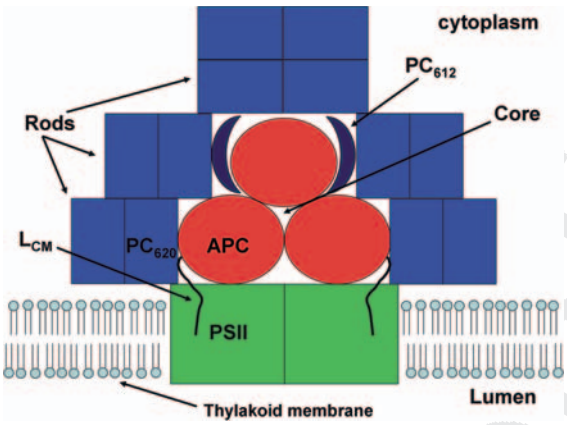

Figure 11.3 Model of a tricylindrical core phycobilisome consistent with crystallographic and biochemical studies. A Photosystem II (PSII) dimer (green) is situated in the thylakoid membrane. The phycobilisome core containing allophycocyanin (APC, red circles) is attached to PSII via the L_{CM} subunit on the cytoplasmic side of the membrane. Six rods are attached as three doublets surrounding the core. The rods always contain phycocyanin (PC) hexamers (shown as blue rectangles, but actually round disks shown side-on). In some species, additional hexamers of phycoerythrocyanin (PEC) or phycoerythrin attach to the rod distal end. Linker proteins found in the internal spaces of the core cylinders and rods are not depicted.

both regular $(\alpha\beta)_3$ APC and trimers that contain modified APC monomers: either a modified α subunit, called α^B or a modified β subunit, called β^{18} (encoded by the *apcD* and *apcF* genes respectively). The β^{18} is paired with the L_{CM} core-membrane linker protein, encoded by the *apcE* gene [22]. These minor constituents all have a single PCB cofactor that absorbs further to the red than the bulk APC PCBs, probably to complete the overlap with the PSII chlorophyll *a* pigments and assist in efficient energy transfer. Of the subunits that physically link the entire phycobilisome to the reaction center of Photosystem II (PSII) (see Chapter 4) within the membrane, L_{CM} is the largest – between 70–128 kDa, depending on the number of core cylinders. It has been suggested that the C-termini of the L_{CM} subunits penetrate into PSII by way of a cavity formed between the D1/D2 subunits and the peripheral small PSII subunits (psbJ, psbK, and cytochrome b_{559}; [33, 34]). The recently determined PSII crystal structures reveal a dimeric structure with the longest dimension, ~205 Å, closely matching the combined diameters of two APC cylinders, ~220 Å. Figure 11.3 shows schematically the position of the L_{CM} anchor penetrating into PSII. APC crystal structures and to date have been obtained only for regular $(\alpha\beta)_3$ APC trimers and not for any of the minor APC forms.

One of the major differences in the APC subunit is the formation in crystal of a more loosely associated hexamer, arising from slight sequence variations in the APC versus PC or PE [31, 32]. It is not clear what the source of this looseness might be; however, the somewhat lower rigidity could facilitate the perpendicular association between the APC core and the rods. Evidence that the native form of

APC, within the context of the complete phycobilisome, is a trimer has been suggested [35]; whether or not a hexameric nature is actually necessary for APC function is unclear. Concentrated APC can, however, form long rod-like crystalline needles [18], showing that APC also has the ability to form long stacks. In the [1B33] APC structure [27], APC is found in trimeric form. Two trimers make up the asymmetric unit; they are associated in a perpendicular orientation that is clearly non-physiological. This mode of association is not similar to the fashion of rod-core assembly as visualized in EM studies, and may be due to the presence of the internal linker protein which may disturb the association of the trimers into hexamers in crystal. In the other two APC structures, the linker is absent and the association into hexamers occurs, although the hexamers are not equivalent. In the [1ALL] *S. platensis* structure, the hexamer is formed via interactions between β subunits [31]; while, in the [1KN1] *P. yezoensis* structure, the association is via the α subunits [32] and is similar to that found for PC hexamers. Since each core cylinder is made up of four APC trimers, it is possible that these two crystal structures mimic two different trimer-trimer association modes. Clearly, crystallization of a larger complex (either a core subcomplex or the entire phycobilisome) will help in solving this problem.

Isolated APC, in either monomeric or trimer form, has served as a model system to study the effects of absorption and energy transfer [36–39]. This system is especially attractive because of the large red-shift that occurs in the absorption spectra following trimerization. A detailed review of the photophysical experiments performed on APC was recently published by MacColl [40]. Further details on possible modes of energy transfer in the phycobilisome are detailed in Section 11.4.2.

11.2.3.2 **Phycocyanin**

Phycocyanin (PC) is found in all phycobilisome and is the major rod component. Typically isolated in trimeric form, this PBP has an absorption maxima at 620 nm, as a result of the overlap of its three cofactors, α^{84}, β^{84}, and β^{155} (Figure 11.1a), which absorb between 600–630 nm [41]. In the presence of bound linkers, the λ_{max} is red-shifted by up to 20 nm [42]. The overall molecular dimensions of the trimeric discs are the same as found for APC; however, an additional loop made up of nine residues (residues 146–154) is inserted into the β subunit sequence between helices G and H. Such loops protrude from the outer circumference of the trimeric/hexameric disk (Figure 11.1b) and partially shield ring A of the βPCB^{155}, which is covalently linked to βCys^{155}, from the solvent. It has been proposed that cofactors on the outside circumference of the disks enable efficient cross-rod energy transfer. The extra loop may prevent movement of ring A (Figure 11.2), and perhaps ring B, but allow movement of rings C and D upon further complex assembly. Both α and β subunits in the monomer have thio-linked PCB chromophores at symmetry related positions, Cys^{84}, located between helices E, F′, and G. The chemical surroundings of these two PCBs is, however, very different upon formation of the higher-order $(\alpha\beta)_3$ trimer. The α PCB^{84} is almost totally buried and hidden from the surrounding solvent (Figure 11.1b), unlike the βPCB^{84} which extends out into the trimeric/hexameric disc interior.

PC crystal structures have been determined from seven species of cyanobacteria, (four mesophiles and three thermophiles) and three rhodophytes, (two mesophiles and one thermophile). Two of the early PC structures have not been deposited in the PDB; however, the published reports are quite detailed and informative [29, 30, 43]. The structures obtained are at high resolution and provide an excellent molecular description of the structural characteristics required for efficient energy transfer, preserved during evolution. These structures also provide insights into the fine-tuning required by species that have evolved in different surroundings, and clues on the process of phycobilisome assembly. The crystal structures, [1I7Y], [1KTP], and [1JBO], of two thermophilic cyanobacterial species show a significant change in the relative position of pyrolle ring D of the βPCB^{155}. The altered conformation of this ring was first identified in the [1I7Y] *T. vulcanus* structure determined at room temperature [44], and then further confirmed at cryogenic temperature [45]. This alteration in ring orientation was proposed to occur at a critical site with respect to the PC assembly process. This position may stabilize the $(\alpha\beta)$ monomer interaction interface as well as the $(\alpha\beta)_6$ hexamer formation interface. In both structures, solvent molecules are located in almost identical positions, about equidistant from αAsp^{28}, βAsn^{35}, and αArg^{33} from the lower $(\alpha\beta)_3$ trimer. The presence of bound water in these positions at the typical growth temperatures of 55–60 °C is probably transient, but could add a significant amount of stabilization energy to both the $(\alpha\beta)$ monomer and the $(\alpha\beta)_6$ hexamer. Additional solvent molecules were located in close contact, 2.6–3.4 Å, with each of the three cofactors. Those solvent molecules associated with βPCB^{84} and βPCB^{155} interact with the molecular edge adjacent to the bulk solvent, while those associated with $PCB\alpha^{84}$ are positioned in the trimer association interface. These waters may have a role similar to that of the surrounding protein residues in stabilizing the PCB configuration to obtain optimal functionality.

The two internal pyrolle rings, B and C, of all bilin chromophores contain propionic acids whose functions are not clear (Figure 11.2). One possibility is that they may be required to help keep the bilins in a less folded configuration, thereby modifying their absorption spectra. Interactions between the propionic acids and polar residues have been previously identified for the PCBs in a number of crystal structures; these residues are highly conserved. In the low temperature *T. vulcanus* structure ([1KTP]), all three chromophores bind solvent molecules in the gap between the two propionic carboxyl groups [45]. When a superposition of the three cofactors is performed, these solvent molecules spatially coincide. It can be thus suggested that these solvent molecules are important for the positioning of the propionic side chains in specific conformations. Indeed, molecular dynamics simulations of the propionic acid positions in the absence, or presence, of the intervening solvent molecules show that in their absence, the propionic acids both repel each other and are attracted to adjacent positively charged residues. This induces a greater degree of separation and strain on the PCB structure. Examination of other PC structures, [1CPC] from *F. diplosiphon,* [1JBO] from *T. elongatus,* and so on, show solvent molecules in similar positions, indicating that this requirement is not a characteristic trait limited to PC from thermophilic species. By

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obtaining their proper position, the propionic acid groups may maintain the two central pyrolle rings on almost the same plane, and thus may be important for efficient energy transfer. The propionic acids may also interact with linker proteins, as was visualized in the *M. laminosus* [1B33] APC structure [27].

As indicated above, isolated PC maximal absorption is at 620 nm in its $(\alpha\beta)_3$ trimeric form (PC_{620}). PC monomer absorption is blue-shifted to 614 nm [41] while the absorption of trimers isolated in the presence of linkers is red-shifted (629–638 nm). These observations reaffirm the complexity of clarifying the mechanism of energy transfer in the phycobilisome using isolated components. However, spectroscopic measurements made on isolated components, in conjunction with high-resolution studies, continue to serve as the major source of information on phycobilisome function. In the course of isolation of the phycobilisome and APC from *T. vulcanus,* a minor fraction of PC was isolated that absorbed maximally at 612 nm while in $(\alpha\beta)_3$ trimeric form [46]. This form of PC, PC_{612}, was characterized crystallographically ([1ON7]) and appeared to have a number of unique structural traits. The difference in absorption was proposed to be the result of the lack of methylation of βAsn^{72} (see below). This residue has been found to be methylated in all PBPs [47, 48], and to affect the absorption characteristics of the conserved βPCB^{84} (see Section 11.2.4.2). Although crystallized under conditions similar to that of *T. vulcanus* PC_{620}, the PC_{612} did not form $(\alpha\beta)_6$ hexamers. This flexibility could be advantageous in the formation of the interaction surface between the end of the rod and the circumference of the APC core cylinders. The presence of a trimer at the end of two of the rods could serve as a "filler" ring to make up for the differential in position between the lower base core cylinders and the upper cylinder (Figure 11.3). This hypothesis is strengthened by the fact that the PC_{612} was only isolated in conjunction with APC cores, and thus it is probably not a small population of unmethylated PC. The lack of methylation could allow the rod linker protein, present at the end of the rod closest to the core, to interact strongly with the β^{84} cofactor, affording a red-shift towards 630–640 nm. This, in turn, would serve as a functional bridge between the bulk PC_{620} in the rods and the APC in the cores.

Along with the detailed three-dimensional description of the PC structures, one of the most important goals of all of the crystallographic studies of PC was to describe the possible pathways of energy transfer between adjacent cofactors. Since, in many cases, the crystallized protein was either in the $(\alpha\beta)_3$ or $(\alpha\beta)_6$ forms, the clarification of more extensive energy transfer pathways requires the assumption that the further assembly of the trimeric rings into hexamers and rods during the crystallization process resembles the organization of the phycobilisome in vivo. This assumption has been a mainstay of all structural studies, despite the fact that all PC structures lack linker components. The relevance of the crystal structures to the phycobilisome structure will be detailed in Sections 11.3 and 11.4.

11.2.3.3 **Phycoerythrocyanin**

PEC is a relatively minor PBP component, found in only some cyanobacterial species [41, 49, 50]. It is similar to PC, with a PVB chromophore bound to the

α-subunit instead of PCB. This results in an absorption shift to the blue, positioning this PBP between PE and PC in the energy transfer funnel. While first crystallographically determined in 1990 [51], the PEC structure from *M. laminosus* was not deposited in the PDB. However, this same protein was recently studied crystallographically under different conditions of data collection – room-temperature versus cryogenic temperature, and monochromatic versus polychromatic Laue irradiation, yielding a molecular view of this protein [49]. The major motivation for this study is that the PVB chromophore goes through a reversible, photochemically induced *Z/E* isomerization, involving a rotation of pyrolle ring D, with respect to the rest of the chromophore. The crystals obtained in this study allowed a more precise determination of the chromophore structures within the protein environment, an important aspect for the understanding of the PEC functional absorption and transmission properties. The authors also report the first successful use of the Laue method, which can potentially be used for time-resolved crystallographic measurements. Since, to date, all structures are static, it would be interesting to see if any protein dynamics occur on the time-scale of energy transfer within the complex.

The *M. laminosus* PEC described in this study crystallized in the same space group as the [1ON7] structure of unmethylated PC from *T. vulcanus* (Section 11.2.3.2). In both cases, the $(\alpha\beta)_3$ trimers used for crystallization did not associate further into $(\alpha\beta)_6$ hexmers. Both structures show elevated B-factors for the helix-turn-helix motif adjacent and interacting with the α-subunit chromophore, PCB in PC and PVB in PEC. The elevated B-factors reveal a high degree of flexibility in the motif, which may afford the small proportion of the PVB found to undergo isomerization in the trimeric form. Isomerization could modify the functional properties of the PEC by loosening the trimer-trimer interactions in the hexamer.

11.2.3.4 **Phycoerythrin**

PE exists as an additional PBP only in some cyanobacterial species, but exists in all red algae. The basic PE structure is a $(\alpha\beta)_6$ hexamer in cyanobacteria and a $(\alpha\beta)_6\gamma$ in red algae: the unique chromophore-bearing 30 kDa γ subunit is found inserted within the hexameric disk of red-algal PE. The first PE structures to be determined were those of *Porphyridium sordidum* bPE [52] and *Porphyridium cruentum* bPE – organisms that differ in the number of PE/PUB chromophors bound to the (αβ) monomer and to the γ subunit. Unfortunately, the coordinates of these structures were not deposited in the PDB. A number of years later, four additional PE structures were determined and their coordinates deposited in the PDB. These structures are all red algal PE that contain both singly and doubly linked cofactors, which has a noticeable effect on their conformation and absorption. All proteins prior to crystallization had the internal γ subunit, except for the bPE structure; the presence of the protein in crystal was identified by SDS-PAGE of solubilized crystals. However, except for a very small number of residues, the γ subunit structure could not be visualized in the electron density. In the [1B8D] structure, three residues of the γ subunit were built into electron density within the $(\alpha\beta)_6$ disk [53],

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thus providing evidence for the general position of this subunit. The identified residues were located in the vicinity of the PE chromophores. It was proposed that the reason for the absence of the γ subunit in the electron density was due to the threefold crystallographic averaging applied during structure determination [52, 53], although heterogeneity in the position of this subunit cannot be discounted. Another possibility is that crystal packing forces on the PE disks change the affinity toward the γ-subunit to a degree that allows it to bind in a less specific manner, thereby lowering its apparent occupancy in the crystal structure.

The absorption of PE is blue-shifted compared to PC; some organisms take advantage of this difference to change their absorption cross-section by a mechanism known as complimentary chromatic adaptation (CCA) [54]. In this process, cells that contain phycobilisome rods with PC grown under red light modify the expression of PBP encoding genes, as a result of a shift to green light. The newly expressed genes encode for variants of PC, PE, and for the appropriate linker proteins. PE now becomes the terminal subunit on the newly assembled phycobilisome. A number of excellent reviews describe the mechanism of CCA in detail [41, 54, 55]. In normal sunlight, the phycobilisome contains intermediate levels of PC and PE, indicating that cells can respond correctly to the actual light quality, affording maximal energy transfer. Changes in light intensity, with or without additional changes in the light quality, have also been known to have an effect on both the number of phycobilisome and the length of the phycobilisome rods. These observations show that the phycobilisome is a dynamic complex, as far as composition, and requires the presence of additional accessory proteins in order to obtain its functional structure.

11.2.3.5 **Crytophyte Phycoerythrin**

Cryptophytes are fresh-water, or marine unicellular, organisms with a unique photosystem arrangement, different from cyanobacteria, red algae, green algae, and so on. The crytophytes contain plastid organelles with a red algae origin that have lost the ability to form phycobilisomes. Instead, proteins similar to PE β-subunits, in complex with a unique non-PBP α-subunit, form a ααββ tetramer that binds chromophores and is translocated into the membrane lumen. Within this space, they are apparently soluble, perhaps interacting with the reaction center via an additional protein. Since these proteins are not phycobilisome forming, they are outside the scope of this review. However, it is important to note that this protein class has afforded crystals that diffract to the highest resolution (0.97 Å) of any photosynthetic antenna protein, thus allowing a true atomic resolution structure of an energy-harvesting protein [56, 57].

11.2.3.6 **Linker Proteins**

First identified biochemically in the 1970s [58, 59], the linker proteins (LPs) that lack bound chromophores have remained an enigma, both in functional and structural terms. Using electrophoretic methods on purified phycobilisomes, the number and molecular weight of these proteins could be estimated. More precise analysis of electron micrographs showed a protein component within the core

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cylinders and rod disks, promoting the idea that the role of these proteins is to serve as physical and stabilizing links within the phycobilisome. With the advantage of genomics, the ability to predict the entire linker complement of a number of phycobilisome containing organisms became possible, and the genes identified could be associated with the proteins identified (by molecular weight) biochemically. In accordance with the putative linking role, four different classes of LPs were suggested: (i) core linkers (L_C); (ii) rod linkers (L_R) that can be either large, ~25–30 kDa, for linking hexamers to hexamers, or small, ~8 kDa, that cap or terminate the rods; (iii) linkers involved in rod-core attachment (L_{RC}); and, (iv) the special APC subunit, which links the entire assembly to the membrane, called L_{CM}. This last LP is the product of gene fusion between a gene encoding for an APC α-subunit and a number of repetitions of genes encoding for LPs. The size of the L_{CM} is proportional to the size of the core, between two to five cylinders. In red algae, the PE hexamers contain a γ subunit that serves as both linker and additional chromophore-binding subunit. The γ subunits are dissimilar in sequence from both other linkers and the PE α and β subunits, and are a relatively new addition to the phycobilisome. These γ proteins are encoded by nuclear genes, unlike the other phycobilisome genes which are found in the plastid genome.

With the crystallographic visualizations of the PC and PEC trimeric/hexameric structures, it became clear that if the linkers are present in the disk cavity, they will come into contact with the chromophores that jut out into this solvent accessible space (for instance, the βPCB[84] in PC, PEC, and APC). Since the chromophore environment has a very strong effect on its spectral characteristics, the presence of different LPs could potentially modify the functionality of these similar, or even identical, chromophores. By specifically altering the environment of certain chromophores, the LPs could induce directed energy flow down the rods and into the core, and from there into the reaction center. Thus, a second role for LPs was proposed. Indeed, biophysical measurements made on isolated PBPs show a significant shift in their absorption spectra in the presence or absence of LPs [41]. No phycobilisome has yet been isolated that does not contain LPs; but, mutagenesis that causes the lack of the LPs structural presence results in the loss of the phycobilisome.

Over 20 crystal structures of PBPs have now been determined, yet only a single crystal structure has an identifiable linker protein – the [1B33] APC structure [27]. In this structure, in which the APC trimers associate in a perpendicular fashion, the linker is seen completely embedded within the trimer ring. The linker contains a three strand β-sheet with two short α-helices, and makes contact with only two of the three APC monomers. Most of the contact surface is with residues and rings II and IV of the bilin on the APC β-subunits, with concomitant structural changes to both. The presence of the linker has a significant effect on the entire trimer, inducing a significant conformational change that restrains the natural curvature of the trimer, making it somewhat more flat. The asymmetric unit of this APC-linker form included two trimers; the positions of the linkers within each trimer were not identical, suggesting that the linker binding may be somewhat flexible. This flexibility may be compounded by the additional heterogeneity in the APC

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cylinders due to the presence of the minor forms of APC, as described above. It should be noted, however, that the ability to visualize the linker in the crystals negates to a certain extent the reasoning behind that lack of visualization of the γ subunits of PE (see above). It was proposed that this subunit, which serves as both linker and additional chromophore binding protein, does not appear in the electron density maps because of threefold averaging around the hexamer ring. However, if the linkers bind asymmetrically within the cavity, it would be expected that the unit cell would be larger, and the crystals would belong to a space group that would allow for the presence of this protein. While it is certainly possible that binding of all linkers is not identical, rather being bound in symmetrical fashion aligned along the threefold axis, it is also possible that in the 1B33 structure, the linker was stabilized in a non-physiological position. Only the successful crystallization of other PBP-LP complexes will resolve this question.

11.2.4 Phycobiliprotein Post-translational Modifications

Following translation, the PBPs assemble into the subunits that form the phycobilisome. During this process, they undergo a number of structural and chemical modifications required for proper functionality. One of the non-covalent changes that occurs is the stabilization of the almost invariant Thrβ^{77} residue (Figure 11.1a, white square), in a configuration of dihedral angles that belong to a typically non-permitted region of the Ramachandran plot [51]. This is due to constraints that occur during the trimerization process that brings this residue into close contact with the α84 bilin, thereby shielding it from the solvent and shifting its absorption to the red. This configuration could also, potentially, shield the α84 bilin from the effects of linker binding, unlike the β84 bilin which is accessible to the linker in the trimer cavity.

11.2.4.1 Bilin Lyases

The phycobilisome is unique in that all chromophores are covalently linked to the polypeptide components. Thus, the most important of all post-translational modifications to the PBPs is the creation of thio-ether linkages between conserved Cys residues and the appropriate bilins. In the cases of PEC and PE, this requires the linkage of different bilin types to the same protein, indicating that the enzyme that catalyzes this bond formation has specificity for both substrates. Lyases have been studied for a number of systems [60–64]. Attachment of bilins to PC and PE α-subunits requires a two-protein complex consisting of the products of the genes *cpcE/F*. Very recently, a second set of genes (particularly *cpcS* and *cpcT o,r cpeS and cpeT)* that encode lyases that catalyze the attachment of bilins to the β subunits were identified [63, 64]. The lyases bind the chromophore and substrate apoprotein and may modulate the chromophore configuration prior to transfer to the PBP [61]. While there are cases where the PBP contains its own lyase activity [65] and autocatalytically creates the thio-ether linkage, other PBPs are totally dependent on the presence of lyase components. The recent expression of functional

α-PC and α-PE subunits in *E. coli* was totally dependent on the coexpression of the *cpcE/F* and *pecE/F* lyase genes, respectively [66, 67].

11.2.4.2 Methylation of βAsn^{72}

7 Glazer and coworkers first identified this post-translational modification of βAsn^{72} in *Anabaena variabilis* APC, today properly named *Nostoc* PCC7937. The asparagine residue was found to be modified by the addition of a methyl group to the γ-nitrogen of the side chain [47] to form N-methyl asparagine (NMA). The enzyme responsible for this modification was isolated, [48] and was shown to perform the methylation the βAsn^{72} residue in vitro, indicating that this modification may occur after trimer assembly in vivo. NMA residues were identified in PC, PE, and PEC and were also identified crystallographically by reinterpretation of the electron density maps of early PC structures [52, 68]. Utilization of the three-dimensional structures visualized the NMA position, showing that it is in van der Waals contact with ring II of the β155bilin chromophore, close to the propionic acid, thereby modifying the polar environment of this chromophore which, in turn, could alter its energy absorption and transfer properties (Figure 11.1a, white square). When compared to PC isolated from methylase-lacking mutant strains, methylated PC is more efficient in energy transfer from PC to APC. Consistent with this idea, the linker visualized with APC [27] does interact with the NMA residue.

The existence of Asn residues at this position is highly conserved, but there are species in which not all of the PBPs contain this residue [23]. This indicates that, for certain cases, perhaps due to changes in the linker protein, methylation is not required, or is deleterious. It is possible to envision that isolation of the β84 bilin from the solvent could be achieved by a combination of natural amino-acid substitutions coupled with the linker protein. Indeed, one could envision that the evolutionary development of a methylation system might only be required if it served to control some aspect of function, thus suggesting that under other conditions, non-methylated βAsn^{72} might be required. As mentioned in Section 11.2.3.2, a non-methylated βAsn^{72} PC might serve as a functional bridge between some of the bulk PC rods and the APC in the tricylindrical core (Figure 11.3). In this case, the absence of NMA and the presence of the proper linker protein, could modify the absorption of the PC further to the red, efficiently transferring energy from the rods to the core. It is possible that this fraction is unique to this thermophilic fresh-water species – such a blue-shifted fraction of PC was isolated from the eukaryotic thermophilic cyanobacteria *S. lividus* [69].

11.2.4.3 Phosphorylation and Glycosylation of Linker Proteins

The presence of phosphorylation of cyanobacterial thylakoid proteins has been experimentally investigated on a number of systems, with somewhat contradictory results [70, 71]. The latter study indicated that dephosphorylation was required
8 prior to complex disassembly (see below) and that phosphorylation of the linker proteins could have a stabilizing influence on the entire structure. However, the experimental evidence for this modification lacks further corroboration. A similar
Q1 state of uncertainty exists for the glycosylation of linker proteins, with one positive

report [72] and one negative [73]. Obviously, further studies of the linker proteins, including crystal structures, will help clarify these matters.

11.3
Self Assembly and Disassembly of the Phycobilisome

An intriguing aspect of phycobilisome function is its ability to assemble into its extremely large, multi subunit structure. With the structural information obtained by X-ray crystallography of the isolated subunits described in Section 11.2, this propensity becomes even more remarkable as a consequence of the high degree of homology between the different subunits on all structural levels.

11.3.1
Phycobilisome Assembly

Assembly of protein subunits is typically considered to be an ordered sequence of events. In the case of PBPs, each newly synthesized α and β apo-subunit must be rapidly converted into (αβ) heterodimers, the basic monomeric PBP unit, perhaps, with attached chromophores. As shown by Anderson and coworkers, lack of either bilin binding or conversion to the heterodimer results in rapid protein degradation [28, 74–76]. This indicates that these non assembled proteins are very quickly recognized as incorrectly folded by the cellular proteolytic machinery, requiring their immediate removal. One of the potential results of lack of removal of misfolded protein subunits may be the formation of associations between subunits of different components, such as APC with PC, and so on. This could occur as a result of the similarity in the conserved residues identified as important for assembly.

Using the basic PC monomer as an example and using the standard PC sequence numbering, it is seen that all of the interactions between the two subunits are between α-helices X, Y (Figure 11.1a, white circle), and A (residues 1–42) and the E-F′ helix-turn-helix structure (residues 92–110) between both subunits in a symmetrical fashion. The surface electrostatic potential has the ability to attract, or repulse, at a greater distance than other chemical forces, and probably plays a role in the initial positioning of the subunits. The entire surfaces of both α and β subunits on the side that forms the dimer is almost completely flat, thus potential residues that will position the two subunits in the correct orientation must be found. Helices on the surface that do not interact have both positive and negative patches; however, a major positive patch can be identified on the E helices of both subunits. These patches could potentially repulse further overlap of the two subunits during association. In the αβ interaction surface, a few prominent electrostatic interactions form: between αMet^{1}-βAsp^{3}-αArg^{30}, αArg^{93}-βAsp^{13}, and βArg^{93}-αAsp^{13}-βArg^{110}. These electrostatic locks are invariable and can be found in APC, PEC, and PE structures as well. There must be additional locking molecules that impart specificity and prevent the formation of mixed PBPs. In the case of PC, such a specificity element is αArg^{42}-βAsp^{25}; while in APC, using the *M.*

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laminosus [1B33] structure as the structural example, the specificity element is formed by αLys^{26}-βGlu^{35}. Both of these positive residues, αArg^{42} in PC and αLys^{26} in APC, jut out significantly from the main body of the protein. An additional specificity element occurs in PC from cyanobacterial thermophiles, between αAsp^{28} and the N atom of ring D of βPCB^{155}-βAsn^{35}.

These electrostatic interactions are, however, not sufficient to explain the stability of the PBP monomers, whose separation into subunits requires more than just the disruption of electrostatic interactions. Isolation of the subunits from higher aggregates typically requires the use of chaotropic agents (i.e. urea or KSCN) to induce polypeptide denaturation and separation [77, 78]. The ability of urea to disrupt the structures of polypeptides has been described as a result of direct interaction with backbone contacts (including non-polar interactions) which lead to secondary and tertiary structure destabilization [79]. Thus, along with polar interactions, it is clear that there are important hydrophobic interactions which stabilize the monomer formation interface. The major hydrophobic locks are due to Phe and Tyr residues, whose side chains extend out from one subunit and become buried in the second. Positions 18 and 97 are typically either Tyr or Phe residues and serve as additional common locks on all PBPs (both subunits). Additional aromatic residues further strengthen the monomer interaction in a more PBP specific manner, such as: βPhe^{5}, conserved in PC, PEC, and PE, absent in APC; αPhe^{31}, conserved in PC and PEC, but not in APC or PE; or, $\beta Tyr^{30}/\beta Phe^{31}$, conserved in APC and PE, but not in PC or PEC. The proximity of such a large number of aromatic residues in each subunit prior to monomer formation may exert forces which keep the subunits in a partially unfolded conformation that is then recognized by either the other subunit, followed by rapid monomer formation, or by the proteolytic system, in cases of the presence of one excess subunit. Figure 11.4 shows the interaction surface of the monomer, with the unique locking residues (in *T. vulcanus* PC) encircled.

Further evidence of the importance of the hydrophobicity in the stabilization of the monomer interaction surface was obtained by the isolation of α-subunits of PEC from intact phycobilisomes [80]. Isolation required the application of rather harsh conditions, 0.3% formic acid which induces destabilization and denaturation, followed by isocratic hydrophobic interaction chromatography. The isolated subunit undergoes substantial aggregation, which could be alleviated by the proteolytic removal of the N-terminal helices X and Y. The initiation of aggregation was found to be caused by the formation of α_2 homodimers, reinforcing the need for the subunit specificity elements to prevent such non-physiological association. Removal of the helices prevented aggregation, but was found to have a destabilizing effect on the tertiary structure of the remaining helices.

Following monomer assembly, the process of trimer formation follows a similar pattern, including both polar and hydrophobic interactions. The buried monomer-monomer interaction surface that occurs during trimer formation is only about 30% of the α and β subunit interaction surface in the monomer. However, this interaction is quite strong, resisting decomposition at even very low protein con-

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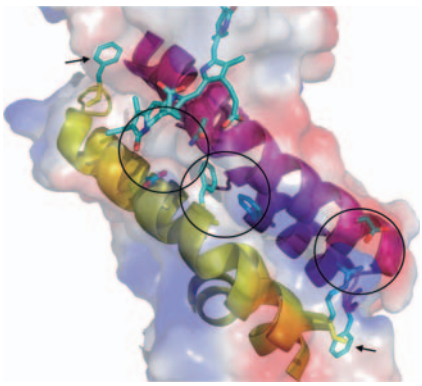

Figure 11.4 Specific residue locks in the PC αβ interaction interface. The subunits are in cartoon representation (α–yellow, β–purple). Sequence specific interactions (not conserved) mentioned in the text are encircled. Arrows show Phe^{18} conserved residues (on both subunits) that demarcate the interface.

centrations. In thermophilic organisms, additional polar locking residues can be found at both the monomer and trimer interfaces. These additional stabilizing contacts can be considered to be of intermediate strength, with distances of 4–8 Å. Thus protein structure thermostability requires only small sequence modifications, which do not change the other characteristics of the PBPs, such as their absorption spectra.

The next steps in phycobilisome assembly are hexamer, rod, and core formation, again utilizing similar principles. Most of the PC, PEC, and PE crystal structures contain such extended structures indicating that assembly can occur in the absence of LPs in vitro, at least in the presence of high PBP concentrations and dehydrating crystallization precipitants. In vitro reconstitution experiments performed on a number of systems indicated the importance of LPs in nucleating, extending, and terminating the rod structures. It thus appears that the major structural facets needed for assembly exist within the PBPs themselves. The role of the LPs may be to modify, control, and stabilize these contacts in the phycobilisome.

Figure 11.3 shows a model of the phycobilisome that is consistent with many of the structural details outlined in this and other, reviews. It should be pointed out that other models exist, with the aesthetically pleasing form showing the rods radiating out at equivalent angles from the core. Recent cryo-electron microscopy results [26] show doublets of rods at right-angles surrounding the core, in a fashion similar to that shown in Figure 11.3. These measurements show that all of the rods surround the core on the same plane, excluding the possibility that the rods are in a staggered formation. A description of the phycobilisome architecture that accounts for the actual sizes of the rods and cores can be found in [10, 46].

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11.3.2 Phycobilisome Disassembly

Cyanobacteria can be exposed to changes in their typical growth conditions that modify both the number and characteristics of the phycobilisome. As already noted, the expression of the genes encoding for phycobilisome components can be altered by external environmental changes within a short period of time. However, more long-term changes that require changes in the phycobilisome makeup may occur. One such change is the response of cyanobacteria to the lack of specific nutrients, especially nitrogen and sulfur. Under these conditions, the cyanobacterial cells change color from blue-green to yellow-green in a process known as chlorosis, or bleaching [81]. Using spectroscopy, and other techniques, it was found that the bleaching occurs within a few hours of the onset of nutrient starvation and was a result of the loss of the phycobilisome in an ordered fashion [55]. It has been proposed that the disassembly of the phycobilisome can have two beneficial roles. During starvation the cellular metabolism decreases, while the rate of photosynthesis may be unchanged. This imbalance could lead to excessive absorption of excitation energy, which then leads to the production of harmful radical species [82]. Under extreme conditions this may lead to cell death and must be avoided. Since the rate of photosynthesis is directly coupled to the rate of energy absorption, uncoupling and degradation of the major antenna will be highly beneficial in avoiding overexcitation. A second role for phycobilisome degradation is to serve as an internal nutrient reservoir. The phycobilisome can supply the cell with amino acid residues for the synthesis of protein systems required for high affinity nutrient uptake. Additionally, by further degradation, it can supply building blocks for the synthesis of other metabolites or as a source of energy. Collier and Grossman used a genetic screen to identify mutants unable to degrade phycobilisomes during nutrient starvation in order to identify the molecular pathway of phycobilisome degradation [83]. The non-bleaching A, *nblA,* gene was thus identified and has been found to be present in all cyanobacteria. Nitrogen is the major nutrient whose lack can lead to a 50-fold increase in the amount of NblA protein [83]. In some cyanobacteria, such as *Synechococcus* sp. PCC 7942, sulfur and phosphorus limitation can also induce the *nblA* response [83]; while, in other species, sulfur limitation does not influence the expression of *nblA* [84]. During starvation, wild-type cells, but not *nblA* mutants, degrade two rod-linker proteins, L_R 33 and L_R 34.5, indicating a possible functional connection between the linkers and the NblA protein [84]. This observation supports previous data showing that wild-type cells degrade the phycobilisome by first decreasing the length of the rods [28, 85]. It was shown that in the cyanobacterium *Tolypothrix* PCC 7601, the NblA protein has affinity for both PC and PE subunits, but not for APC or for PBPs from other cyanobacterial species [86].

Beside the *nblA,* there are four other genes that have been identified as being related to phycobilisome degradation: *nblR, nblS, nbl,B,* and *nblC*. The NblR protein controls *nblA* expression and is critical for survival under stress conditions [87]. It has been suggested that *nblR* is controlled by *nblS*, a sensor histidine kinase that

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has a PAS domain [88]. The NblB protein is necessary for phycobilisome degradation during starvation; but, unlike the NblA protein, the NblB levels are similar prior to, or during, nutrient starvation [89]. This protein is partially homologous with PCB lyases, the enzyme involved in chromophore attachment to the PC apoprotein. The most recent addition to the arsenal of proteins involved in the disassembly of the phycobilisome is the nblC protein, which is also required for *nblA* expression [90].

The size of proteins belonging to the NblA family is quite small, ranging from 54 to 65 residues with molecular masses of about 7–7.5 kDa. The sequence identity between NblA proteins is relatively poor, ~30%, which is somewhat surprising since the phycobilisome components on which the NblA interacts are highly homologous, >70%. The first crystal structure ([1OJH] of a member of the NblA protein family (from *Anabena* sp. PCC 7120) was recently determined by X-ray crystallography [91]; this has been followed up with two additional NblA members (from *T. vulcanus* ([2Q8V] and [2QDO])and *S. elongatus* sp. 7942;[92]). All structures are extremely similar, exhibiting a helix-turn-helix motif that dimerizes into a flat four-helical bundle. Based on direct measurements between PC and the NblA (both wild-type and mutant forms), Bienert and coworkers suggested a mode of activity whereby the NblA dimer interacts with PC monomers via its amino and carboxyl termini [91]; however no proposal as to the method by which the NblA mechanically disassembles the phycobilisome is presented by the authors. Alternative interaction modes are certainly possible, especially between similar helix-turn-helix motifs found in all PBPs, but await further investigation to prove their validity.

11.4
Phycobilisome Function

11.4.1
Phycobilisome Binding to PSII and PSI

The phycobilisome is primarily bound to the thylakoid membrane via the L_{CM} subunit that penetrates into the reaction centers of PSII. A typical phycobilisome thus provides the absorptive power of more than 600 chromophores to a single PSII dimer [21]. Taking into account the number of chlorophyll *a* molecules attached to PSII, this brings the total antenna potential for energy absorption to more than 350 chromophores per reaction center. This can be compared to about 100 chlorophylls per cyanobacterial Photosystem I (PSI) monomer [93]. In plant and green algae systems, the number of chromophores is also large [94], but still smaller than that provided by the phycobilisome. The result of this enlarged antenna allows many cyanobacterial species to increase the ratio of PSI/PSII to between three and six [95], as opposed to the green algae and plant situation, where the ratio is close to one [94]. Lowering the amount of PSII may be physiologically beneficial due to the well known requirement of PSII to replace

the D1 protein under normal illumination conditions. Under extreme conditions, high light stress or a combination of light and temperature stress, the cells can be photoinhibited, leading to cell death [82, 96]. One method of prevention of photoinhibition can be achieved by rapid replacement of the PSII D1 subunit, something that can be achieved more efficiently by lowering the total amount of PSII.

An additional protective route would be via disconnection of the phycobilisome from PSII, leading to increased fluorescence. Indeed, the phycobilisome has been shown to have the potential to become disconnected from PSII and to functionally associate with Photosystem I (PSI) (see Chapter 2). A brief period of heat treatment also has the effect of disconnecting the phycobilisome from PSII, without direct damage to either complex [97]. Direct measurements using confocal microscopy have indicated that the phycobilisome is quite mobile in vivo, much more than the photosystems [98, 99], indicating that the association between antenna and photosystems in cyanobacteria my be much looser than in other systems. Recent experiments performed on cyanobacterial cells in vivo show that the mobility of the phycobilisome and its disconnection from PSII is light dependent [100]. Disconnection occurs rapidly and thus this phenomenon has been equated to state 1–state 2 transition in plants that occurs via the phosphorylation-induced disconnection of LHCII from PSII. In this study, the product of the gene *rpaC* was found to be required for the occurrence of the state transition at very low light intensities. *rpaC*$^-$ deletion mutants exhibited no state transitions, but were unaffected in comparison to wild-type cells, at high light. Thus, multiple mechanisms for protection of PSII may exist.

Many reports exist showing the ability of the phycobilisome to transfer energy to PSI in vitro [101] and in vivo [102]; however, whether binding to PSI is mediated by specific interactions or by transient association is unclear [103]. Heat treatment appears not to affect the phycobilisome-PSI functional interaction in the same manner as it does the phycobilisome-PSII interaction [97]. The report by Rakhimberdieva and coworkers [102] on *Spirulina* cells indicates that most of the phycobilisome is functionally connected to PSI, both trimers and monomers, and not to PSII. This could correlate with the fact that, while there is a PSI : PSII ratio of ~6, electron micrographs of thylakoid membranes appear to show almost complete coverage of the stromal side of the thylakoid membrane with phycobilisome complexes [15, 25]. Since L_{CM} has been indicated as the source of phycobilisome-PSII binding, it would be unlikely that phycobilisome-PSI binding would occur via the same mechanism. Indeed, there are indications that the phycobilisome binds to PSI via interactions of rod PBPs, mostly PC, with the ferredoxin-$NADP^+$ oxidoreductase (FNR) subunit of PSI [104]. It is certainly possible that these auxiliary-like phycobilisome units not connected to PSII loosely interact with PSI, both structurally and functionally, with the potential to increase its potential for both linear and cyclic electron transfer.

This auxiliary population of phycobilisomes could have a second physiological role, in addition to light absorption. The phycobilisome has been identified as an emergency source of nutrients to be used in the case of nitrogen, sulfur, or carbon

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starvation [83, 84, 105]. Use of the phycobilisome as a nutrient source requires its ordered disassembly, as described in the previous section.

11.4.2 Energy Transfer within the Phycobilisome

The existence of crystal structures has revealed many of the structural requirements for the assembly of an efficient energy transfer antenna complex. Relatively high quality images of entire antenna complexes have been obtained from non-oxygenic bacteria [106–109], PSI [93, 110] and PSII [33, 111]. This has enabled the formulation of more complete models of light harvesting [2] that propose the positions of chromophores are determined by universal physical constraints such as photon flux, time scales of photon absorption, energy transfer, and competing processes. The design of antennas has been optimized through evolution to provide maximum energy transfer.

The mechanism of energy transfer in the phycobilisome has been extensively studied by a variety of spectroscopic techniques on the entire complex, but more often on isolated subunits [112]. However, since the crystal structures that exist contain, at most, one $(\alpha\beta)_6$ hexameric unit, the actual method of directed energy transfer down to the reaction center can only be conjectured. Species-specific differences not-withstanding, energy transfer rates are extremely fast [113] and overall quantum yields are high – about 95% [21, 41, 114]. The phycobilisome differs from the chlorophyll-based antennas by having rather large distances between cofactors. In the chlorophyll-based antennas, distances between adjacent cofactors are typically close enough to envision closely interacting absorption rings or aggregates. In the bacterial LH2 complex, the two rings of bacteriochlorophylls have spacing of ~9 Å and ~20 Å, center to center, respectively; the distance between rings is also ~20 Å. The addition of carotenoid molecules makes this antenna quite chromophore dense. In the antenna beds of both PSI and PSII, there are many chlorophyll molecules, positioned with very complex arrangements, which can roughly be separated into two groups, on either face of the membrane – however typical distances are on the order of 10 Å. The densest of all chlorophyll-based systems is the chlorosome found in green sulfur (*Chlorobiaceae*) and green filamentous bacteria (*Chloroflexaceae*). In these, an entire organelle encloses thousands of pigment molecules stacked in close proximity, without intervening protein subunits [115–117].

Within each minimal PC unit, the distances between the α^{84} and both the β^{84} and β155 bilins is ~50 Å, while the β^{84} and β^{155} bilins are separated by ~40 Å (Figure 11.1a). Association of the PC monomers into trimers and hexamers (Figure 11.1b) results in a slightly shorter distance between certain bilins, but none are closer than ~20 Å [29, 118]. In assembled PE hexamers, which contain five bilins per monomer, the density is slightly higher, with the nearest approach between two bilins on the order of 15 Å [52]. Thus, it can be assumed that efficient energy transfer does not particularly require the dense packing of cofactors, rather, that dense packing maximizes the potential for light absorption with the lowest expenditure on protein synthesis. Since this is not the case for the phycobilisome, it

could be assumed that the secondary role of the phycobilisome, serving as a reservoir of nutrients for starved cells, must be of great enough importance to have preserved this form of antenna system.

The central mechanism for energy transfer proposed by Förster [119] requires the existence of weak coupling between electronic energy levels of nearby chromophores. Due to the relatively large distances between cofactors in the phycobilisome, this coupling is weak; structure-based theoretical energy transfer rate calculations utilizing the Förster mechanism match experimentally obtained values well. Debreczeny and coworkers measured energy transfer rates in isolated PC and obtained values between 50–500 psec for different pathways within the $(\alpha\beta)$ monomer [120]. Formation of $(\alpha\beta)_3$ trimers allowed for much faster energy transfer in the 0.5–1 psec range [36], showing the importance of the level of complex formation when dealing with the functional characteristics of the phycobilisome. Ultra fast, two-color pump-probe spectroscopic measurements on different organizational states of APC have revealed energy-transfer at less than 100 fsec, which has been suggested to occur due the formation of dimer-exciton states [37, 38]. These states can only exist between the PCBs on adjacent monomers; the very fast decay component is probably outside of the time-frame accessible to Förster resonance energy transfer. It is still unclear what the effect of further aggregation to full cylinders, cores, or phycobilisomes will have on the energy transfer rates, especially with the presence of linker proteins. However, use of isolated subunit also allows for extremely high quality descriptions of the cofactor/protein/solvent environments during absorption and energy transfer [39, 121]. Detailed discussions on energy transfer within isolated PBP can be found in a number of excellent reviews [21, 41, 113, 122].

11.5 Final Remarks

The details of phycobilisome structure and function described here cannot be deemed complete until a true, experimentally derived structure of the cores, rods, and phycobilisome is determined. This may require the bridging of high resolution crystallography with lower resolution microscopic techniques. It is clear that visualization of the phycobilisome will not only expand our understanding of biological energy harvesting and transfer, but it will also serve as an extraordinary example of nanometric self-assembly and disassembly. Clarification of the chemical rules that lead to the specific association of molecules will be used in the future development and production of true nano-devices.

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Abstract

In cyanobacteria and red alga, the major light-harvesting pigment–protein complex is called the phycobilisome. The crystal structures of isolated components of different phycobilisomes have been determined at intermediate to high resolutions, allowing a detailed picture of the functional surroundings of the pigment molecules. These structures also suggest the mechanisms of assembly of the entire complex, as well as its manner of association with the membrane bound photochemical reaction centers. In this chapter, the structural information obtained on isolated components of the phycobilisome will be described and correlated with a model of the entire complex and with the proposed mechanism of energy absorption and transfer.

Keywords

phycobilisome antennae, cyanobacteria, red algae, phycobilisome component proteins, phycobilisome self assembly and disassembly, phycobilisome function

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