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Photosynthetic Pigments: Perplexing Persistent Prevalence of 'Superfluous' Pigment Production

Phycobilins function as light-harvesting pigments in most cyanobacteria and red algae. Although green cyanobacteria of the genus *Prochlorococcus* express genes encoding enzymes that direct the synthesis of phycobilins, these pigments do not appear to play a role in light harvesting in *Prochlorococcus*. Now, it is shown that cyanophages infecting *Prochlorococcus* also contain genes for phycobilin-synthesizing enzymes, and these are expressed in *Prochlorococcus*, raising further questions as to the role of phycobilins in the host and the virus.

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Phycobilins are accessory photosynthetic pigments that are found in cyanobacteria and red algae [1]. In their functional state, these pigments are covalently bound to proteins called phycobiliproteins. The latter, along with other, nonpigmented proteins, are organized into semispherical arrays called phycobilisomes that are attached to the surface of the photosynthetic membranes — the thylakoids — which contain the photosynthetic reaction centers [2]. The energy of photons of light absorbed by the phycobilins is transferred to chlorophyll *a* molecules in the reaction centers by the Förster excitation transfer mechanism. The indirectly photoexcited chlorophylls in the reaction centers then undergo the photosynthetic 'light reaction' — essentially a photoionization. The phycobilisomes act as a light-absorbing antenna for photosynthesis, and are able to efficiently absorb light in the green and orange spectral region where chlorophyll absorbs poorly. Phycobilisomes therefore confer an advantage to autotrophic organisms in aquatic environments, where light in the red wavelength range, which chlorophyll can absorb efficiently, is attenuated by water. Two major phycobilins are phycocyanobilin, which absorbs light in the 620 nm region, and phycoerythrobilin, which absorbs in the 560 nm region. Spectroscopic and biochemical studies have shown that energy from light absorbed by phycoerythrobilin must first be transferred to phycocyanobilin before it can reach chlorophyll and contribute to photosynthesis. Thus, the presence of phycocyanobilin is necessary for phycoerythrobilin to have a light-harvesting function [3].

Land plants and green algae do not have phycobilin-based antennae. Instead, these organisms harvest light with pigment–protein complexes that contain chlorophylls along with certain carotenoids. Unlike the phycobilisomes, which are located on the surface of thylakoid membranes, the chlorophyll-containing light-harvesting complexes are integrally embedded in the thylakoid membranes. The predominant chlorophylls of the light-harvesting complexes are chlorophyll *a* and a modified form, chlorophyll *b*. The latter is generally absent from organisms that use phycobilins as light-harvesting pigments.

Several years ago an exceptional group of cyanobacteria was discovered that does not contain phycobilisomes but, like land plants and green algae, uses chlorophylls *a* and *b* as its light-harvesting pigments (actually, these pigments are minor variants of the plant and green-algal chlorophylls, but they function identically to them). Aside from their use of chlorophylls instead of phycobilins as photosynthetic light-harvesting pigments, these organisms, named prochlorophytes, are typical cyanobacteria [4]. One such prochlorophyte group, the free-living marine *Prochlorococcus*, is most closely related to the *Synechococcus* group of cyanobacteria. It has recently become evident that *Prochlorococcus* is responsible for a large portion of total carbon fixation in marine environments [5].

Careful biochemical and genomic analysis of several *Prochlorococcus* strains has revealed that, even though they do not use phycobilins as photosynthetic pigments, they retain the capability to synthesize phycobilins as well as some phycobiliproteins [6–9]. In these strains, intact genes encoding enzymes needed for synthesis of phycoerythrobilin and phycocyanobilin from heme are present in the genome, and expression of these genes in *Escherichia coli* yields active enzymes. Depending on the strain and the light environment, *Prochlorococcus* cells also express some or all of these genes, and can even covalently attach phycoerythrobilin to apoproteins to form phycoerythrin. However, the cells appear to lack genes encoding phycocyanobilin-binding proteins. The absence of these genes indicates that energy from light absorbed by phycoerythrin cannot be transferred to the photosynthetic apparatus and thus phycoerythrin cannot contribute to photosynthesis. Spectroscopic analysis has confirmed that phycoerythrin contributes very little if anything to photosynthetic light harvesting in *Prochlorococcus* [10,11].

The above observations might suggest that the ability of *Prochlorococcus* to form phycobilins and phycoerythrin are vestiges carried over from an ancestral phycobilisome-containing cyanobacterium and that phycobilins have no current physiological role in *Prochlorococcus*. However, the fact that the genes are expressed and the encoded biosynthetic enzymes are functional argues against this interpretation. An alternative role for phycoerythrin as a storage protein seems to have been ruled out by observations that phycoerythrin protein level does not decrease in cells cultured under nitrogen deprivation [12]. In other cyanobacteria, phycobiliproteins are degraded to serve as a source of nitrogen for growth under nitrogen-deficient conditions [13]. A remaining possibility is that the

bilins and/or phycoerythrin might function as photosensors. Although a photosensory role has not been excluded, there is no evidence to support it. Determining the function, if any, of phycobilins in *Prochlorococcus* will ultimately require studies of the consequences of their absence. Unfortunately, neither targeted gene disruption nor antisense suppression has been reported in *Prochlorococcus*.

The role of phycobilins in *Prochlorococcus* has now become more perplexing. In a recent issue of *Current Biology*, Dammeyer *et al.* [14] found that certain cyanophages that infect *Prochlorococcus* carry their own genes for phycobilin biosynthesis. This finding is surprising because phages are thought to be under strong selective pressure to keep chromosome size and gene content to a minimum. Nevertheless, the cyanophages described by Dammeyer *et al.* [14] contain genes for fully functional phycobilin biosynthetic enzymes and these genes are expressed in *Prochlorococcus*. Moreover, there is a significant difference between cyanophages and cyanobacteria in the enzymology of phycoerythrobilin synthesis: whereas cyanobacteria, including *Prochlorococcus*, convert biliverdin to phycoerythrobilin in two separate, two-electron reduction steps catalyzed by two different enzymes, PebA and PebB, a cyanophage gene codes for a single enzyme, PebS, that catalyzes the entire four-electron reduction. Because this gene is absent from all cyanobacterial genomes described so far, it appears that the genetic basis for phycobilin biosynthesis in the cyanophages is not the result of a genetic exchange with the host, but, rather, has evolved independently and has not undergone frequent genetic exchange with the host cells. The apparent strong positive selection for the retention of phycobilin-biosynthetic genes in the cyanophages suggests that the ability to augment phycobilin synthesis in their hosts confers an important advantage to the cyanophages and possibly also to the *Prochlorococcus* cells.

An alternative to the above interpretation is that the cyanophages have two (or more) hosts, at least one of which is a phycobilisome-containing cyanobacterium. As a precedent, other cyanophages have been reported to infect both *Prochlorococcus* and *Synechococcus* [15,16]. If that were the case for the cyanophages described by Dammeyer *et al.* [14], and if the genes for phycobilin and phycoerythrin synthesis confer an advantage when expressed in an alternative host, then it is possible that these genes are simply carried over when the host is *Prochlorococcus*. Clarification of this interpretation will require determination of the host range of the cyanophages and the phenotypic effects of their presence in alternative hosts.

Some lessons can be learned and some questions are raised following the thought-provoking report by Dammeyer *et al.* [14]. Firstly, we clearly don't yet fully understand all of the roles of phycobilins in cyanobacteria and, in particular, how possession of these pigments by *Prochlorococcus* confers competitive or survival advantages if they do not function in photosynthetic light harvesting. Secondly, given that *Prochlorococcus* has the ability to synthesize phycobilins, we don't know what might be gained from the introduction of additional phycobilin-biosynthetic machinery into cells by the infecting cyanophages. Finally, considering the ability of a single cyanophage-encoded enzyme, PebS, to convert biliverdin to phycoerythrobilin, while two enzymes, PebA and PebB, are required for the same conversion in the host cells, phycoerythrobilin biosynthesis could provide an enlightening case study on enzyme evolution that might reveal the evolutionary direction from one enzyme to two (or vice versa) and the advantages conferred by the change.

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