

Minireview

Cryptomonad biliproteins – an evolutionary perspective

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Abstract

Each cryptomonad strain contains only a single spectroscopic type of biliprotein. These biliproteins are isolated as ~50000 kDa $\alpha\alpha'\beta_2$ complexes which carry one bilin on the α and three on the β subunit. Six different bilins are present on the cryptomonad biliproteins, two of which (phycocyanobilin and phycoerythrobilin) also occur in cyanobacterial and rhodophytan biliproteins, while four are known only in the cryptomonads. The β subunit is encoded on the chloroplast genome, whereas the α subunits are encoded by a small nuclear multigene family. The β subunits of all cryptomonad biliproteins, regardless of spectroscopic type, have highly conserved amino acid sequences, which show > 80% identity with those of rhodophytan phycoerythrin β subunits. In contrast, cyanobacteria and red algal chloroplasts each contain several spectroscopically distinct biliproteins organized into macromolecular complexes (phycobilisomes). The data on biliproteins, as well as several other lines of evidence, indicate that the cryptomonad biliprotein antenna system is 'primitive' and antedates that of the cyanobacteria. It is proposed that the gene encoding the cryptomonad biliprotein β subunit is the ancestral gene of the gene family encoding cyanobacterial and rhodophytan biliprotein α and β subunits.

Abbreviations: Chl – chlorophyll; CER – chloroplast endoplasmic reticulum; SSU rRNA – small subunit ribosomal RNA

Introduction

Phycobiliproteins, proteins with linear tetrapyrrole (bilin) prosthetic groups (Beale 1993), are light-harvesting components of the photosynthetic apparatus in cyanobacteria and in two groups of eukaryotic algae, the rhodophytes (red algae) and the cryptomonads (Glazer 1981; MacColl and Guard-Friar 1987). The phycobiliproteins are among the most abundant cellular components when these organisms grow at low light intensities in the presence of adequate nutrients. Their strong distinctive colors are readily detected with the naked eye.

Upon cell lysis, phycobiliproteins are released into solution and fluoresce brilliantly. Their spectroscopic properties made a strong impression on early biologists and chemists of the 19th century. For much of

the past hundred years, it has been an article of faith that the phycobiliproteins of prokaryotes and eukaryotes have a common evolutionary origin and that they are valuable signposts in tracing the origins of algal chloroplasts. With the recent explosion of knowledge in algal cell biology, the growing availability of nucleic acid sequences, the substantial data on the structure, function and assembly of the phycobiliproteins, and extensive information on bilin diversity, it is now profitable to revisit the question of the interrelationships of cyanobacterial, rhodophytan, and cryptophytan phycobiliproteins.

Origins of rhodophytan and cryptophytan chloroplasts

Oxygen appeared at significant concentrations in the Earth's atmosphere about 2.3 billion years ago (Hayes 1983), long before the appearance of eukaryotic cells in the fossil record. Thus oxygenic photosynthesis evolved in prokaryotes. At first, photosynthesis was acquired by eukaryotes through the endocytosis of a photosynthetic bacterium. The prokaryotic endosymbiont was then reduced to a chloroplast, a semi-autonomous organelle. It is still debatable whether all chloroplasts are derived from a single (monophyletic) origin (e.g. Cavalier-Smith 1992), or whether they have polyphyletic origins (e.g. Raven 1970).

In the red and green algae, the chloroplast is enclosed solely by a two-membrane envelope (Gibbs 1981a). The inner chloroplast membrane is inferred to represent the endosymbiont's plasma membrane, while the outer membrane may either be derived from the outer membrane of the endosymbiont or the phagocytic vacuole membrane of the host cell (Cavalier-Smith 1987; Whatley and Whatley 1981). In a scenario supported by numerous nucleic acid sequence data, a cyanobacterial endosymbiont (or an organism closely related to present day cyanobacteria) was the precursor of the red and green algal chloroplasts (Morden et al. 1992). Yet, red and green algal chloroplasts have different major light-harvesting complexes. In the former, much of the light-harvesting is accomplished by phycobiliproteins, in the latter by chl *a/b*-containing proteins. Bryant (1992) has proposed that the precursor prokaryote was endowed with both antenna systems, and that the differences between red and green algal chloroplast antennae reflect loss of one or the other system sometime after the endosymbiotic events. In support of this view, it is seen that modern cyanobacteria include not only a majority of strains with phycobiliproteins as light-harvesting pigments, but also organisms with a chl *a/b* antenna, present in different ecosystems (*Prochlorococcus*, *Prochlorothrix*, *Prochloron*), that appear widely distributed within the cyanobacterial radiation (Urbach et al. 1992; Palenik and Haselkorn 1992). An extant cyanobacterium with both types of antenna systems has yet to be described.

The cryptomonads are a small group of unicellular biflagellated algae found in both marine and freshwater habitats (Gantt 1979). In contrast to red algae, the cryptomonads are believed to have acquired their chloroplast by a secondary endosymbiotic event, the phago-

cytosis of a rhodophyte-like ancestor. This hypothesis is strongly supported by cytological, genetic, and biochemical evidence.

The cryptomonad chloroplasts are surrounded by four membranes. The inner pair of membranes is homologous to the two-membrane chloroplast envelope described above for red and green algal chloroplasts. The two outer membranes have been designated the 'chloroplast endoplasmic reticulum' (CER). The inner CER membrane may represent the plasma membrane of the eukaryotic endosymbiont, while the outer CER membrane is believed to be derived from the internal membrane system of the host. The outer CER membrane is usually continuous with the nuclear envelope and bears eukaryotic ribosomes on its surface (Gibbs 1981a). The cryptomonad chloroplast has a significant space between the inner and outer pairs of membranes. This region, the periplastidal compartment, contains a 'nucleomorph', a small double membrane-bounded, DNA-containing body, 0.5 μm in width and about 1 μm long (Greenwood et al. 1977), believed to be the vestigial nucleus of the phototrophic eukaryotic endosymbiont (Gillott and Gibbs 1980). In situ hybridization studies indicate that the nucleomorph is a transcriptionally active eukaryotic nucleus (McFadden 1990). The periplastidal compartment also contains 80S ribosomes, scattered vesicles and tubules, and numerous prominent starch granules (Gibbs 1981b). A probe specific for prokaryotic rRNA labelled the chloroplast ribosomes in *Chroomonas caudata*, but did not label those in the periplastidal space (McFadden 1990). The periplastidal compartment does not contain mitochondria or dictyosomes (Golgi bodies). Within the chloroplast proper, the thylakoids are organized into extended lamellae consisting of paired thylakoids. These thylakoids are not tightly stacked as they are in higher plant grana, but have a gap of 3 to 8 nm between the members of a pair (Dodge 1973).

Sequencing of the small subunit ribosomal RNAs (SSU rRNAs) of the nuclear, mitochondrial, nucleomorph, and plastid genomes of *Pyrenomonas salina* confirmed the chimeric nature of the cryptomonad cell and provided insights into its origins. The sequence of the nuclear SSU rRNA clusters with those of green algae (Eschbach et al. 1991a), as does the sequence of the mitochondrial SSU rRNA (Maier 1992). A nucleomorph SSU rRNA is phylogenetically related to red algal nuclear-encoded SSU rRNA (Maier et al. 1991). Finally, the plastid SSU rRNA sequence also clusters with those of red algae (Maerz et al. 1992).