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Original article

Ferredoxin-dependent bilin reductases in eukaryotic algae: Ubiquity and diversity☆

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ABSTRACT

Linear tetrapyrroles (bilins) are produced from heme by heme oxygenase, usually forming biliverdin IXα (BV). Fungi and bacteria use BV as chromophore for phytochrome photoreceptors. Oxygenic photosynthetic organisms use BV as a substrate for ferredoxin-dependent bilin reductases (FDBRs), enzymes that produce diverse reduced bilins used as light-harvesting pigments in phycobiliproteins and as photoactive photoreceptor chromophores. Bilin biosynthesis is essential for phototrophic growth in *Chlamydomonas reinhardtii* despite the absence of phytochromes or phycobiliproteins in this organism, raising the possibility that bilins are more generally required for phototrophic growth by algae. We here leverage the recent expansion in available algal transcriptomes, cyanobacterial genomes, and environmental metagenomes to analyze the distribution and diversification of FDBRs. With the possible exception of euglenids, FDBRs are present in all photosynthetic eukaryotic lineages. Phylogenetic analysis demonstrates that algal FDBRs belong to the three previously recognized FDBR lineages. Our studies provide new insights into FDBR evolution and diversification.

1. Introduction

Tetrapyrroles are required for diverse metabolic processes essential to terrestrial life. Linear tetrapyrroles derived from heme (bilins) play important roles in the biology of photosynthetic organisms. Bilins are synthesized from heme via heme oxygenase (HO) to yield biliverdin IXα (BV, Fig. 1). In oxygenic photosynthetic organisms, BV is a substrate for ferredoxin-dependent bilin reductases (FDBRs: (Dammeyer and Frankenberg-Dinkel, 2008)) that generate a range of bilins by reducing different double bonds (Fig. 1). Cyanobacteria contain as many as three FDBRs: PcyA, PebA, and PebB. Phylogenetic analysis places these three enzymes into distinct lineages (Rockwell et al., 2017). Eukaryotic FDBRs characterized to date also belong to these three lineages. For example, HY2 enzymes from streptophytes are part of the PebB lineage, whereas PCYA from chlorophyte algae belongs to the PcyA lineage. PUBS from prasinophyte and streptophyte algae and nonvascular plants belongs to the PebA lineage (Rockwell et al., 2017). Thus, photosynthetic organisms can contain multiple FDBRs producing different reduced bilins, sometimes referred to as phycobilins or phytobilins.

The bilins produced by FDBRs play important roles in the photobiology of oxygenic photosynthetic organisms. Phycobilin chromophores are used by light-harvesting phycobiliproteins in cyanobacteria and eukaryotic glaucophyte, rhodophyte, and cryptophyte algae, frequently as components of large phycobilisome antennae. Phycobilisome-deficient mutant strains of *Synechococcus* sp. PCC 7002 are viable (Alvey et al., 2011). Land plants use phytochromobilin (PΦB, Fig. 1) as the chromophore for phytochrome photoreceptors. Plant phytochromes are critical master regulators for many processes, but plants lacking phytochrome are also viable (Hu et al., 2013). FDBRs would thus not be expected to be essential for oxygenic photosynthesis.

Surprisingly, there is evidence that FDBRs are essential for phototrophic growth. Mutant strains of *Synechococcus* sp. PCC 7002 lacking PcyA could only be obtained in the presence of heterologously expressed HY2 from *Arabidopsis thaliana* (Alvey et al., 2011). PcyA and HY2 produce structurally related phycocyanobilin (PCB) and PΦB, respectively (Fig. 1). There is also evidence that bilin biosynthesis is essential in the chlorophyte alga *Chlamydomonas reinhardtii* (Duanmu et al., 2013), which lacks phytochromes and phycobiliproteins but retains HO and PCYA. A mutant strain lacking *HMOX1,* encoding plastid HO, was unable to grow phototrophically. Addition of exogenous BV afforded partial rescue of *hmox1Δ* phenotypes (Duanmu et al., 2013). These results thus implicate essential functions for bilins in cyanobacteria and green algae.

A requirement for bilins in oxygenic photosynthesis would seem surprising given the intense research into this process for over a century. However, a requirement for bilins in oxygenic photosynthesis

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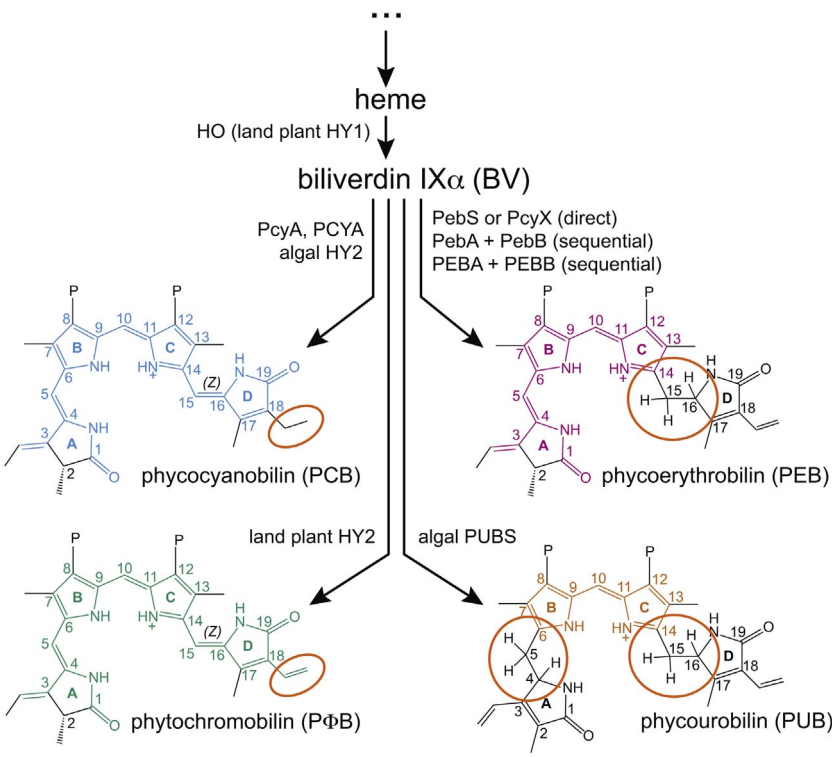

Fig. 1. Bilin biosynthesis by ferredoxin-dependent bilin reductases (FDBRs). After initial breakdown of heme by heme oxygenase (encoded by the *HY1* gene in streptophytes) to yield biliverdin IXα (BV), different FDBRs produce different bilins. PcyA and HY2 from streptophyte algae reduce BV to phycocyanobilin (PCB) in a 4-electron reduction, whereas HY2 from land plants reduces BV to phytochromobilin (PΦB) in a 2-electron reduction. PebA and PebB reduce BV to phycoerythrobilin (PEB) in a 2-enzyme, 4-electron reduction. The same reaction can be carried out by PebS or PcyX as 1-enzyme reactions. PUBS from Viridiplantae reduces BV to phycourobilin (PUB) in a 4-electron reduction. The chromophoric conjugated π systems of different bilins are colored, approximately matching their apparent visual colors. Orange, moieties whose structure differs in different bilins. P, propionate. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

implies the presence of HO and one or more FDBRs in all oxygenic photosynthetic organisms, including diverse algae (Fig. 2). Preliminary BLAST searches of algal transcriptomes (Keeling et al., 2014; Matasci et al., 2014) confirmed the presence of FDBRs in many algal lineages. In the current work, we use phylogenetic analysis to examine the distribution and diversification of FDBRs in photosynthetic organisms. The results confirm the presence of FDBRs in all algal lineages, with the possible exception of photosynthetic euglenids, and confirm that

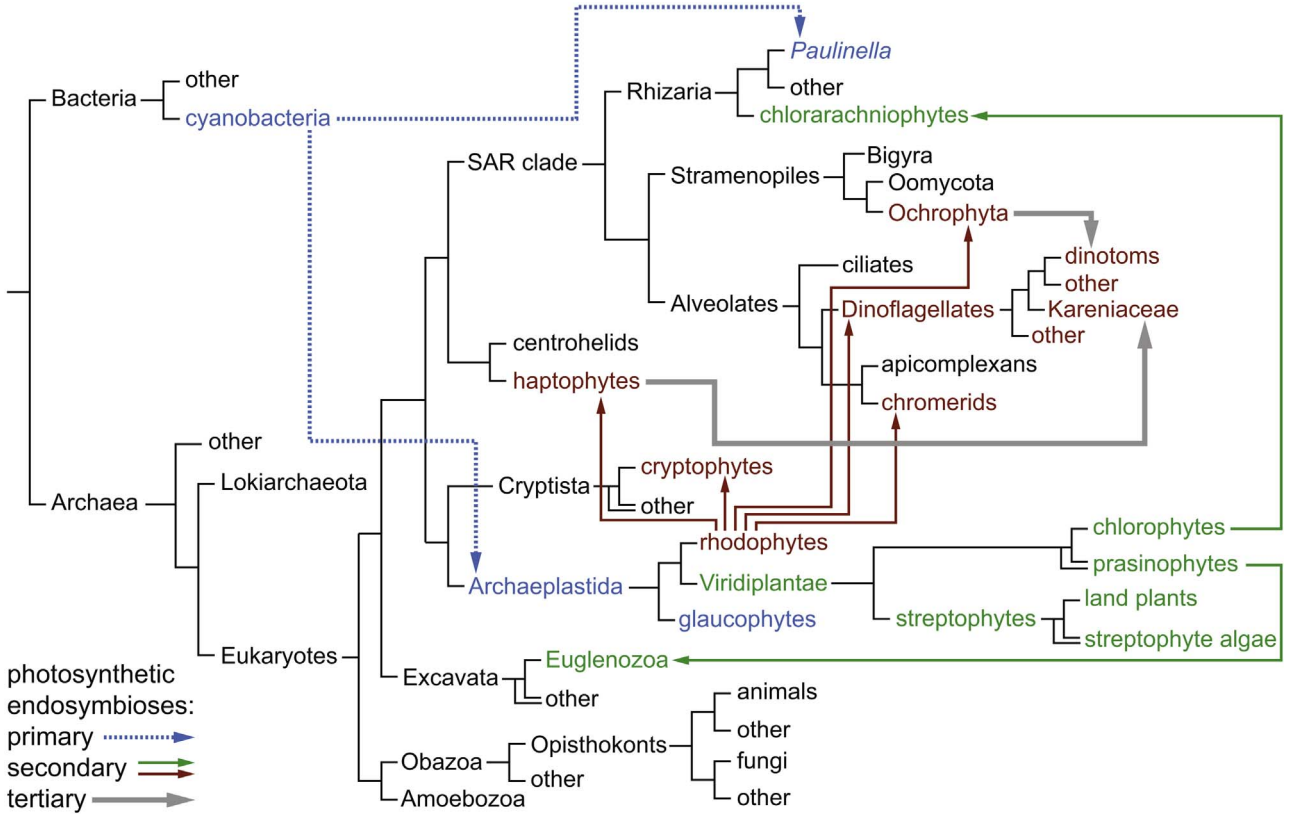

Fig. 2. Evolution of photosynthetic eukaryotes. A simplified view of the tree of life is shown, based on recent studies and assuming a monophyletic Archaeplastida for simplicity (Burki et al., 2016; Price et al., 2012; Spang et al., 2015). Primary (dashed blue lines), secondary (thin solid lines), and tertiary (thick grey lines) endosymbioses are shown. Oxygenic photosynthetic organisms are color-coded by light-harvesting strategy and plastid ancestry. Only endosymbioses resulting in creation of a photosynthetic, carbon-fixing organelle are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)