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Phytochrome Evolution in 3D: Deletion, Duplication, and Diversification

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SUMMARY

Canonical plant phytochromes are master regulators of photomorphogenesis and the shade avoidance response. They are also part of a widespread superfamily of photoreceptors with diverse spectral and biochemical properties. Plant phytochromes belong to a clade including other phytochromes from glaucophyte, prasinophyte, and streptophyte algae (all members of the Archaeplastida) and those from cryptophyte algae. This is consistent with recent analyses supporting existence of an AC (Archaeplastida + Cryptista) clade. AC phytochromes have been proposed to arise from ancestral cyanobacterial genes via endosymbiotic gene transfer (EGT), but most recent studies instead support multiple horizontal gene transfer (HGT) events to generate extant eukaryotic phytochromes. In principle, this scenario would be compared to the emerging understanding of early events in eukaryotic evolution to generate a coherent picture. Unfortunately, there is currently a major discrepancy between the evolution of phytochromes and the evolution of eukaryotes; phytochrome evolution is thus not a solved problem. We therefore examine phytochrome evolution in a broader context. Within this context, we can identify three important themes in phytochrome evolution: deletion, duplication, and diversification. These themes drive phytochrome evolution as organisms evolve in response to environmental challenges.

Keywords

endosymbiosis; evolution; light harvesting; photosynthesis; shade avoidance

I. INTRODUCTION

Modern agriculture relies on planting crop species at high density to maximize yield for a given amount of arable land (Fedoroff *et al.*, 2010). However, high-density planting means that individual plants can shade each other, triggering competition (Casal, 2013). Plants detect such competition via light quality and react by derepression of the shade avoidance response, with important agricultural consequences (Casal, 2013; Ballaré & Pierik, 2017). In 1959, the photoreceptor controlling this process was identified as phytochrome (Butler *et al.*, 1959). Phytochrome detects the presence of competition by measuring the ratio of photosynthetically active red light to photosynthetically inactive far-red light, a ratio that varies due to harvesting of red light by the competitor (Fig. 1). Phytochrome also functions as a master regulator of photomorphogenesis to control germination, flowering, and almost

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everything in between. Thousands of genes are activated or repressed in the model plant species *Arabidopsis thaliana* in response to phytochrome signaling (Rockwell *et al.*, 2006; Hu *et al.*, 2009; Franklin & Quail, 2010; Strasser *et al.*, 2010; Chen & Chory, 2011; Hu *et al.*, 2013), so phytochrome research remains important both for plant biology and for photobiology in general. Signal transduction by phytochrome is a complex field in its own right, but it is clear that activation of plant phytochromes by red light triggers translocation to the nucleus (Nagatani, 2004).

Canonical plant phytochromes are large (ca. 1100 amino acids) photoreceptors that have a conserved domain architecture including a three-domain photosensory core module (PCM, Fig. 2) near the N-terminus. The PCM has PAS (Fig. 2), GAF, and PHY domains that adopt a conserved knotted structure (Wagner *et al.*, 2005; Essen *et al.*, 2008; Yang *et al.*, 2008; Burgie *et al.*, 2014a). In this knot (Fig. 2B), an N-terminal extension upstream of the PAS domain passes through a loop internal to the GAF fold (Essen *et al.*, 2008; Yang *et al.*, 2008). Light perception by phytochrome requires a linear tetrapyrrole (bilin) chromophore derived from heme, and plant phytochromes adopt a red-absorbing C5–*Z,syn* C10–*Z,syn* C15–*Z,anti* configuration in the dark-adapted state (P_r, Fig. 1C). Light absorption triggers a 15,16–photoisomerization reaction that generates a far-red-absorbing C5–*Z,syn* C10–*Z,syn* C15–*E,anti* photoproduct (P_{fr}, Fig. 1C) which can regenerate P_r upon light absorption. 15,16–photoisomerization underlies the signature red/far-red reversibility of phytochrome action in plant biology. P_{fr} can also decay to P_r in the absence of light (dark or thermal reversion). The stability of P_{fr} is thus determined by the light environment, the presence of possible binding partners that modulate the rate of dark reversion, the widely varying rate of dark reversion among different phytochromes, and the temperature at which the light-independent reaction is occurring. Most phytochromes follow this pattern, but bathyphytochromes instead adopt the *15E* P_{fr} form as the dark-stable state with a *15Z* photoproduct (Rockwell *et al.*, 2006). Typically dimeric, phytochromes are found in plants (canonical plant phytochromes), bacteria, fungi, and diverse eukaryotic algae (Fig. 3A). These diverse phytochromes share the knotted PCM fused to different C-termini in different organisms (see below).

Photoconversion thus requires a bilin with a 15,16–double bond (Murphy & Lagarias, 1997). Three suitable chromophore precursors have been reported to date (Fig 4). Canonical plant phytochromes utilize phytochromobilin, or PΦB, as the chromophore precursor (Lagarias & Rapoport, 1980; Rockwell *et al.*, 2006). PΦB is autocatalytically attached to a conserved Cys residue in the GAF domain (the GAF Cys: Fig. 2B). Phytochromes from streptophyte algae, sister to land plants, also have this Cys residue but instead synthesize phycocyanobilin, or PCB (Wu *et al.*, 1997; Rockwell *et al.*, 2017). Both of these phycobilins are synthesized from heme via two steps (Fig. 4). Heme oxygenase (HO) first converts heme to biliverdin IXα (BV, Fig. 4). In the second step, the ferredoxin-dependent bilin reductase (FDBR) HY2 converts BV into PCB or PΦB (Frankenberg *et al.*, 2001; Kohchi *et al.*, 2001; Rockwell *et al.*, 2017). PΦB synthesis from BV is a 2-electron reduction, whereas PCB synthesis is a 4-electron reduction (Fig. 4). PCB is also used by some bacterial phytochromes (Yeh *et al.*, 1997; Jaubert *et al.*, 2007), including cyanobacterial ones, but these organisms use the FDBR PcyA rather than HY2. FDBRs are ubiquitous in photosynthetic eukaryotes (Rockwell & Lagarias, 2017), with other family members (PEBA

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and PEBB) producing phycoerythrobilin for use in bilin-based light-harvesting systems (Glazer, 1985; Frankenberg *et al.*, 2001). Eukaryotic PCYA enzymes are found in glaucophyte, prasinophyte, and chlorophyte algae, defined as in (Duanmu *et al.*, 2014), as well as some rhodophytes and cryptophytes (see below). HY2 is currently only known from streptophytes (Fig. 3A). Eukaryotic FDBRs are plastid localized (Kohchi *et al.*, 2001; Duanmu *et al.*, 2013), and it is not known how bilin moves to the cytosol for assembly with apophytochrome. This process could be even more complicated in algae which acquired photosynthesis via secondary endosymbiosis (Fig. 3B).

However, not all phytochromes require reduced phycobilins synthesized by FDBRs. Phytochromes from nonphotosynthetic and anoxygenic phototrophic bacteria incorporate BV as chromophore precursor (Bhoo *et al.*, 2001; Giraud *et al.*, 2002), requiring only HO for chromophore biosynthesis. Such proteins are often designated bacteriophytochromes. These proteins retain the knotted PAS-GAF-PHY PCM, but BV is covalently attached to a conserved Cys residue upstream of the knot (the BV Cys: Fig. 2B). This Cys is absent in canonical plant phytochromes, but some phytochromes from cyanobacteria and ochrophytes (photosynthetic stramenopiles) have both Cys residues. The authentic chromophore composition of such cases is currently unknown. Distantly related cyanobacteriochrome photoreceptors (CBCRs, see below) typically contain the conserved GAF Cys residue and incorporate PCB as initial chromophore precursor (Ishizuka *et al.*, 2011; Rockwell *et al.*, 2012a), but exceptional cases using a different Cys residue for attachment (Fushimi *et al.*, 2016) or ligating BV to the GAF Cys (Narikawa *et al.*, 2015; Fushimi *et al.*, 2019) are also known. BV is also incorporated as chromophore by fungal phytochromes and by some phytochromes from cyanobacteria and ochrophytes (Blumenstein *et al.*, 2005; Froehlich *et al.*, 2005; Quest *et al.*, 2007; Fortunato *et al.*, 2016). Thus, there is no known case in which phytochrome incorporates a chromophore other than BV or a reduced phycobilin derived from BV. The photosensory function of phytochrome thus requires oxygen for synthesis of BV.

Photoconversion triggers structural changes in the dimeric configuration of phytochrome and refolding of a conserved “tongue” loop in the PHY domain (Fig. 2A) that contacts the chromophore-binding pocket in the GAF domain (Burgie *et al.*, 2014b; Takala *et al.*, 2014; Burgie *et al.*, 2016). These structural responses to photoconversion provide different exposed surfaces for protein-protein interaction that appear to be generally conserved in knotted phytochromes and to modulate activity of C-terminal “output” domains. Plant phytochromes translocate from the cytosol to the nucleus upon photoconversion (Nagatani, 2004), where they modulate gene expression by interacting with phytochrome-interacting factors (PIFs), a family of basic helix-loop-helix (bHLH) transcription factors (Franklin & Quail, 2010; Chen & Chory, 2011; Pham *et al.*, 2018). Upon interaction with phytochrome, PIFs are phosphorylated and targeted for degradation by the proteasome in a complex process involving multiple protein kinases, multiple ubiquitin ligases, the COP1/SPA complex, and other factors (Pham *et al.*, 2018).

Light-dependent nuclear accumulation of phytochrome has also been observed in the prasinophyte alga *Micromonas pusilla* (Duanmu *et al.*, 2014), so it seems likely that at least some aspects of phytochrome signal transduction are conserved in Viridiplantae

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(prasinophytes + streptophytes, Fig. 3A) and perhaps in other eukaryotes. However, there are cyanobacterial members of the phytochrome superfamily that lack the knotted 3-domain PCM (see below). We can thus define a superfamily of phytochrome photoreceptors by the presence of a bilin-binding GAF domain, with three families: knotted phytochromes (PAS-GAF-PHY), knotless phytochromes (GAF-PHY), and CBCRs (GAF-only). CBCRs do not have a PHY domain and consequently exhibit different structural changes upon photoconversion (Burgie *et al.*, 2013; Narikawa *et al.*, 2013; Lim *et al.*, 2014; Lim *et al.*, 2018).

The importance of canonical plant phytochromes for agriculture and the diversity of the phytochrome superfamily beg the question of phytochrome evolution. Where did phytochrome originate? How and when did plants acquire phytochrome? What drives the evolution of phytochrome? Recent studies have shown that the evolution of phytochrome is a complex process, and it is not currently possible to reconcile recent studies of phytochrome evolution with our understanding of eukaryotic evolution. We will summarize the present state of affairs, highlight areas of ongoing uncertainty, and attempt to place phytochrome evolution within the larger contexts of phytochrome photobiology and eukaryotic evolution. We argue that phytochrome has crossed from bacteria to eukaryotes more than once, with one of these transfers giving rise to modern plant phytochromes and with none of those transfers originating within cyanobacteria. We identify three themes driving phytochrome evolution: deletion, duplication, and diversification.

II. PHYTOCHROME ORIGINS: IT ALL STARTS IN BACTERIA

Both PAS and GAF domains are found in other proteins, and the PHY domain also adopts a GAF fold (Essen *et al.*, 2008; Yang *et al.*, 2008). However, it is not currently possible to identify a clear sister lineage to the phytochrome GAF domain. In the absence of an outgroup, we can consider the prerequisites for the rise of functional phytochrome photoreceptors. Light perception and signal transduction by known phytochromes requires a bilin chromophore that can undergo 15,16–photoisomerization (Murphy & Lagarias, 1997) and hence requires oxygen and HO.

Are there ancient alternatives? Alternative chromophores include heme, heme precursors such as porphyrin, and phyllobilins derived from chlorophyll breakdown (Kräutler, 2014). Phyllobilins seem to be a later innovation in photosynthetic organisms, but ancestral phytochromes evolved in cells containing heme and porphyrins. Phytochrome variants are known to bind porphyrins in recombinant expression systems (Fischer *et al.*, 2005; Wagner *et al.*, 2008), so there may have been an early stage in phytochrome evolution in which phytochromes bound porphyrins but did not carry out photoisomerization. It may also be possible to make suitable bilins in the absence of oxygen. Parallel oxygen-dependent and oxygen-independent pathways are known for the synthesis of heme (Dailey *et al.*, 2017). Alternative oxygen-dependent heme breakdown pathways have also been discovered (Matsui *et al.*, 2013; Nambu *et al.*, 2013), as has an oxygen-independent pathway (LaMattina *et al.*, 2016). The oxygen-independent pathway is not widespread, implying a recent origin, and the resulting anaerobilin product is thought to lack a 15,16–double bond. Nevertheless,

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this finding raises the possibility that suitable bilins could be made by cells in the absence of oxygen.

In speculating on the origins of phytochrome, it is also important to consider phytochrome in the absence of bilin. There are known functions for canonical plant phytochromes in the cytosol prior to photoconversion (Paik *et al.*, 2012; Hughes, 2013), so similar functions could have been carried out by apophytochrome or by phytochrome with bound porphyrin. Phytochrome function in the absence of HO has also been reported in the anoxygenic photosynthetic bacterium *Rhodopseudomonas palustris* (Fixen *et al.*, 2014), so either the apoprotein is biologically functional in this system or there is a labile alternative chromophore that was lost during purification. *R. palustris* also has an unusual phytochrome that has apparently lost light sensing altogether and functions as a redox-sensing histidine kinase, regulating kinase activity via redox-sensitive disulfide bonds (Vuillet *et al.*, 2007). We speculate that early ancestors of phytochromes could have bound different tetrapyrroles or had some other sensing function. Unfortunately, we have no evidence for oxygen-independent BV synthesis. Similarly, the achromatic phytochrome of *R. palustris* is proposed to have evolved from photosensory ancestors (Vuillet *et al.*, 2007), so it is not a reliable basis for inferring an ancestral achromatic phytochrome.

We thus cannot reliably infer the features of a phytochrome before the advent of HOs. We therefore begin with a photosensory phytochrome having a knotted PCM and a bound BV chromophore; in other words, we postulate a founder phytochrome resembling a modern bacteriophytochrome. One can instead argue that it would be simpler to begin with an isolated bilin-binding GAF domain similar to a modern CBCR. However, knotless phytochromes and CBCRs are only known from cyanobacteria. Moreover, the knotted PCM is much more widespread and therefore seems more plausible as the ancestral state. BV-containing phytochromes also are more widespread in both prokaryotes and eukaryotes (Table 1), and BV binding is less widespread in CBCRs than in knotted phytochromes (Narikawa *et al.*, 2015). BV itself can be made from ubiquitous heme with a single, widespread enzyme, whereas FDBRs are largely confined to photosynthetic organisms (Table 1).

We therefore view evolution of diverse PCMs having the GAF Cys, lacking the knot and PAS domain, and lacking both PAS and PHY domains as subsequent events. Known pathways for BV synthesis require HO and hence would have evolved after oxygen levels were high enough. Heme and porphyrin can promote formation of reactive oxygen species in the presence of light and oxygen (photosensitization), and HO is more widespread than phytochrome in both prokaryotes and eukaryotes (Table 1). HO may thus have provided a means for living cells to suppress this toxic combination. HO generates BV, carbon monoxide, and free iron as reaction products, permitting iron scavenging (Ratliff *et al.*, 2001; Fujii *et al.*, 2004; Gisk *et al.*, 2012; Duanmu *et al.*, 2013). Coupling BV to a photoreceptor would provide an elegant means for bacteria to use a single pathway for both sensing and detoxifying the toxic combination of light and oxygen.

What were these bacteria? The use of light-harvesting phycobiliproteins by cyanobacteria (Glazer, 1985) also requires HO and FDBRs; hence, cyanobacteria would possess the

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requisite chromophore precursors. However, recent phylogenetic analyses recover knotted cyanobacterial phytochromes as a derived clade within other bacterial phytochromes (Duanmu *et al.*, 2014; Li *et al.*, 2015). Moreover, heterotrophic bacteria would also find it advantageous to avoid the toxic cocktail of light, oxygen, and tetrapyrroles. Such organisms might well use HO for detoxification under hyperoxia or redox stress, like modern mammals, and hence would also be suitable candidates. We still must acknowledge the important caveat that an ancestral cyanobacterial lineage of phytochromes may have been completely supplanted by extant examples, but current evidence implicates heterotrophic or anoxygenic phototrophic bacteria as the origin of phytochrome because of the close relationships between their phytochromes and knotted cyanobacterial phytochromes (Duanmu *et al.*, 2014; Li *et al.*, 2015). Such bacteria lack FDBRs, and many possess phytochrome operons comprised of heme oxygenase (BphO) and bacteriophytochrome (BphP) genes (Auldridge & Forest, 2011). These operons could have provided a selective advantage by enabling adaptive responses to diurnal changes in light and oxygen levels as their cyanobacterial neighbors carried out photosynthesis. We envisage that phytochromes were subsequently co-opted by cyanobacteria, either for similar responses or for regulation of the newly emerging family of light-harvesting phycobiliproteins. Phycobiliproteins greatly expanded the wavelength range for cyanobacterial photosynthesis (Glazer, 1985), but they are metabolically expensive and hence could have been regulated by phytochrome early in cyanobacterial evolution in the same way that modern regulation of phycobiliproteins is carried out by CBCRs (see below).

Under these assumptions, the PCM would have subsequently transferred to eukaryotes. Canonical plant phytochromes are part of a large clade (Fig. 3C) also including phytochromes from prasinophyte, cryptophyte, and glaucophyte algae (Duanmu *et al.*, 2014; Li *et al.*, 2015; Kooss & Lamparter, 2017); hereafter, we designate these as AC phytochromes for Archaeplastida + Cryptista (Burki *et al.*, 2016). Phytochromes from ochrophytes and fungi have also been characterized (Blumenstein *et al.*, 2005; Froehlich *et al.*, 2005; Rockwell *et al.*, 2014; Fortunato *et al.*, 2016). More recent transcriptomic studies (Keeling *et al.*, 2014; Johnson *et al.*, 2019) have revealed the presence of phytochromes in labyrinthulomycetes (a nonphotosynthetic stramenopile lineage, Fig. 3A), raising the possibility that phytochrome was present in stramenopiles before acquisition of photosynthesis in the ochrophyte clade (Derelle *et al.*, 2016). Current studies do not support a single lineage of eukaryotic phytochromes (Duanmu *et al.*, 2014; Li *et al.*, 2015). Instead, there is a robust AC phytochrome clade and a possible phylogenetic relationship between ochrophyte and fungal phytochromes (Duanmu *et al.*, 2014), but there is no association between AC phytochromes and other eukaryotic phytochromes (Fig. 3C). It has been proposed that cyanobacterial sequences are sister to AC phytochromes (Kooss & Lamparter, 2017), implying that AC phytochromes are derived from cyanobacterial ancestors via endosymbiotic gene transfer (EGT). However, analyses with more extensive sampling instead place knotted cyanobacterial phytochromes as a derived, independent clade (Duanmu *et al.*, 2014; Li *et al.*, 2015). Current studies do not rule out the possibility of genetic drift in extant cyanobacterial sequences or the possibility that an earlier cyanobacterial phytochrome lineage has been supplanted in modern prokaryotes. However, under the assumption of an ancestral bacteriophytochrome, both current statistically robust studies indicate that

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phytochromes would have transferred into eukaryotes at least twice: once to give rise to the AC phytochrome lineage and at least once more to give rise to other eukaryotic phytochromes (Fig. 3C).

Within the AC clade, glaucophyte phytochromes are the first lineage to diverge from other AC phytochromes (Fig. 3C), followed by cryptophyte phytochromes. Prasinophyte and streptophyte phytochromes form a clade, as do these organisms (Duanmu *et al.*, 2014; Wickett *et al.*, 2014; Li *et al.*, 2015). However, the placement of cryptophyte phytochromes does not match the placement of these algae within the AC clade (Fig. 3A and C). Early evolution of the AC clade is not fully understood (Burki *et al.*, 2016; Strassert *et al.*, 2019). Recent studies provide evidence that rhodophytes diverged first within Archaeplastida, with glaucophytes as sister to Viridiplantae (Lax *et al.*, 2018; Price *et al.*, 2019). This result has gained further support with the very recent discovery of the Rhodelphidia, nonphotosynthetic flagellates sister to rhodophytes (Gawryluk *et al.*, 2019). Cryptophytes thus would be derived members of the Cryptista (sister to Archaeplastida within the AC clade, Fig. 3A), having acquired photosynthesis via secondary endosymbiosis of a rhodophyte (Fig. 3B). However, this pattern is in fundamental conflict with the evolution of AC phytochromes: cryptophyte phytochromes are recovered as sister to those from Viridiplantae (Fig. 3C). Future studies will be needed to resolve this discrepancy. However, our current level of knowledge is sufficient to identify three themes that play a major part in the evolution of phytochrome: deletion, duplication, and diversification.

III. DELETION: LIFE WITHOUT PHYTOCHROME

Tracing the evolution of phytochromes in eukaryotes presents challenges similar to that of doing so for other proteins potentially acquired from prokaryotes. Unless the protein in question is ubiquitous in eukaryotes, it is not straightforward to distinguish between multiple introductions to eukaryotes and a single introduction with subsequent losses. Invoking deletions minimizes the number of transfers to eukaryotes that would be required to explain the distribution of eukaryotic phytochromes; thus, one could envision a single introduction of PHY into eukaryotes with multiple subsequent losses. Might phytochrome have been present in the ancestral eukaryote? The possible relationship between stramenopile and fungal PHY could support such a model but could also be explained by horizontal gene transfer (HGT). Indeed, streptophyte phytochromes provide an example of HGT: one lineage of land plant phytochromes, the neochromes, i transferred from bryophytes to ferns and then within ferns via HGT (Li *et al.*, 2014). Neochromes are chimeric phytochrome-phototropin fusions retaining the phytochrome PCM that are confined to hornworts, ferns, and streptophyte algae of the Zygnemataceae. All neochrome lineages descended from a single ancestral PCM, but the algal and hornwort/fern C-termini have distinct origins (Li *et al.*, 2015). This may implicate additional HGT events. Therefore, the barrier preventing multiple, independent transfers of phytochrome from bacteria to eukaryotes via HGT may be intellectual rather than evolutionary.

However, this reasoning does not preclude loss of phytochrome during evolution. A simple analysis of the distribution of phytochrome in recent transcriptomes and genomes make it clear that phytochrome loss does occur. Phytochromes from diatoms, phaeophyte algae

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(kelps or brown algae), and giant viruses infecting phaeophytes (Schroeder *et al.*, 2009) form a clade (Duanmu *et al.*, 2014; Li *et al.*, 2015), implicating a single ancestral phytochrome in ochrophytes. Phytochromes are present in the diatoms *Thalassiosira antarctica*, *T. miniscula*, *T. pseudonana*, *T. rotul*a, and *T. weissflogii* but are absent in *T. oceanica* (Armbrust *et al.*, 2004; Lommer *et al.*, 2012; Johnson *et al.*, 2019). This situation could arise due to multiple HGT events after initial introduction into ochrophytes, but as many as four would be required in *Thalassiosira* alone. It thus seems less complex to imagine a middle ground combining fewer HGT events with subsequent loss of phytochrome in *T. oceanica* and other cases.

Could phytochrome loss resolve the conundrum posed by cryptophyte phytochromes? It is clear that rhodophytes have undergone substantial genome reduction (Collén *et al.*, 2013; Qiu *et al.*, 2017), so the absence of phytochromes in extant rhodophytes may be part of this process. Indeed, there is evidence for loss of FDBRs during evolution of rhodophyte and cryptophyte algae: polyextremophilic *Cyanidioschyzon* spp. contain only PCYA (Rockwell & Lagarias, 2017; Rossoni *et al.*, 2019), whereas many other rhodophytes instead contain PEBA and PEBB (Rockwell & Lagarias, 2017). Cryptophytes have rhodophyte-derived secondary plastids (Fig. 3B), and most cryptophytes contain only PEBA and PEBB. Some cryptophytes also contain PCYA derived from *Cyanidioschyzon* PCYA (Rockwell & Lagarias, 2017), but such cryptophytes do not form a clade. Therefore, either a subset of mesophilic cryptophytes acquired PCYA from *Cyanidioschyzon* via two or more HGT events or all three FDBRs were present in the ancestor to the cryptophyte plastid. The latter scenario implies *independent* loss of one or more of these enzymes from rhodophytes and cryptophytes *after* establishment of secondary endosymbiosis in cryptophytes. A similar process would provide an admittedly speculative explanation for the close relationship between cryptophyte phytochromes and those from Viridiplantae, but in this case one would assume that ancestral rhodophyte phytochromes were transferred to the cryptophyte nucleus via EGT but were lost in extant rhodophytes.

Even so, the distribution of phytochromes in extant eukaryotes is not consistent with an ancestral eukaryotic phytochrome. Eukaryotic phytochromes are confined to three major eukaryotic assemblages (MEA): stramenopiles belong to the SAR or TSAR supergroup (Strassert *et al.*, 2019), Archaeplastida and Cryptista belong to the AC supergroup (Burki *et al.*, 2016), and Fungi belong to the Amorphea (Fig. 3A). Moreover, phytochrome-containing taxa are in clades that are well nested within Amorphea, AC, and SAR/TSAR (Fig. 3A). Ancient HGT among those lineages could explain the possible relationship between fungal and stramenopile phytochromes (Duanmu *et al.*, 2014), but AC phytochromes are a distinct lineage. We therefore conclude that deletion of phytochromes is a frequent occurrence during evolution, but that this does not support an ancestral eukaryotic phytochrome. Instead, phytochrome has crossed from bacteria into eukaryotes at least twice. After these transfers, there have been deletion events in some lineages, but there have also been duplications.

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IV. DUPLICATION: MANY PHYTOCHROMES FROM ONE

Even though many organisms lack phytochrome altogether, others have large numbers of phytochrome photoreceptors. *Arabidopsis* and related mustards have five phytochromes, but one of these (PHYD) is a late duplication found only in the flowering plant family Brassicaceae (Mathews, 2006; Mathews & McBreen, 2008). The model kelp *Ectocarpus siliculosus* has four phytochromes (Cock *et al.*, 2010), as do some *Hemiselmis* strains (Keeling *et al.*, 2014; Johnson *et al.*, 2019). Remarkably, phytochrome gene families have apparently undergone much greater expansion in glaucophytes, with as many as eleven members of this family in *Cyanophora paradoxa* (Price *et al.*, 2019). Such duplication is not limited to eukaryotes; indeed, *R. palustris* has six phytochromes, serving as sensors for light and redox conditions (Giraud *et al.*, 2002; Giraud *et al.*, 2005; Vuillet *et al.*, 2007; Fixen *et al.*, 2014). Cyanobacteria can contain a lavish panoply of photoreceptors, as shown by the more than twenty members of the phytochrome superfamily found within the genome of *Nostoc punctiforme* (Rockwell *et al.*, 2015a). Hence, duplication of phytochrome genes is clearly an important factor in phytochrome evolution.

What can duplication tell us about the evolution of canonical plant phytochromes? Remarkably, multiple duplication events occurred during evolution of plant phytochrome itself (Fig. 3D). In a recent study (Li *et al.*, 2015), Mathews and colleagues discovered unexpectedly high levels of phytochrome diversity in the streptophyte algal clades that are the closest relatives of land plants (Mesostigmatales, Klebsormidiales, Desmidiales, Coleochaetales, and Zygnematales). Land plant phytochromes are members of the streptophyte *PHY1*/2 lineage (Fig. 3D), and all members of this lineage share the canonical plant phytochrome domain architecture (see below). Within this clade, the gene phylogenies generally match that of the organisms (Wickett *et al.*, 2014), although there is evidence of early duplications and subsequent losses (Li *et al.*, 2015). Remarkably, two additional phytochrome lineages were found outside this lineage but within the larger clade of streptophyte phytochromes. These are the *PHYX1* and *PHYX2* genes, which apparently occur only in the Coleochaetales and Zygnematales (Fig. 3D). These organisms are more derived orders of streptophyte algae (Wickett *et al.*, 2014), so the placement of PHYX1 and PHYX2 is at odds with the organismal phylogeny. This discrepancy provides further evidence of gene duplication and subsequent loss. Canonical plant phytochromes thus arose via duplication in streptophyte algae that also contained *PHYX1* and *PHYX2*, with subsequent deletion of *PHYX1* and *PHYX2* in most streptophytes (Li *et al.*, 2015). PHYX1 and PHYX2 proteins have a different C-terminal architecture than those of canonical plant phytochromes (see below), so we speculate that the master regulator function of plant phytochromes arose with or after the duplication event establishing the canonical plant phytochrome lineage. Subsequent duplications in land plants (Fig. 3D) have given rise to ‘light-stable’ PHYN/A and ‘light-labile’ PHYP/B/D/E canonical plant phytochrome lineages (Mathews, 2006; Li *et al.*, 2014; Li *et al.*, 2015), allowing the generation of a range of responses to different light environments and illustrating the link between duplication and diversification.

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V. DIVERSIFICATION: A CONSERVED PCM IN DIFFERENT CONTEXTS

In most phytochromes, the PCM is fused to variable C-termini, with an N-terminal PCM that could simplify formation of the knot. Prokaryotic phytochromes do not have large N-terminal extensions, with the noteworthy exception of hybrid Ppr and Ppd photosensors from some purple bacteria (Kyndt *et al.*, 2005; Kyndt *et al.*, 2007; Kyndt *et al.*, 2010). In these proteins, an N-terminal PYP (photoactive yellow protein) domain precedes the PCM (Fig. 5); it is not clear whether the knot forms in this case, but the PYP domain is known to affect the photosensory properties of the PCM (Kyndt *et al.*, 2007). Many eukaryotic phytochromes have longer extensions N-terminal to the knot (Fig. 5). For example, streptophyte and prasinophyte phytochromes have a Ser/Thr rich region upstream of the knot that possesses sites for autophosphorylation and inter-domain contacts (Rockwell & Lagarias, 2006; Han *et al.*, 2010a; Velazquez Escobar *et al.*, 2017). This region is thought to be unstructured and can be removed without affecting photoconversion or knot formation, but it is required for proper function *in vivo* (Uenaka & Kadota, 2007; Han *et al.*, 2010b). Fungal and stramenopile phytochromes have even longer extensions of unknown function which similarly lack recognizable structural elements (Rockwell *et al.*, 2006). Glaucophytes contain phytochromes with shorter N-termini similar to those of prokaryotic phytochromes, but they also contain uncharacterized sequences with longer N-terminal extensions that show sequence similarity to basic helix-turn-helix DNA-binding domains (Price *et al.*, 2019). Thus, regions of phytochrome that lack universal conservation or recognizable domains have diversified and potentially acquired distinct biological functions.

C-terminal to the PCM, phytochromes from different organisms have different domain architectures. Bacterial phytochromes typically have a PCM associated with a functional histidine kinase bidomain (i.e., containing the phosphoacceptor His residue), as in the well-characterized model phytochrome Cph1 from *Synechocystis* sp. PCC 6803 (Hughes *et al.*, 1997; Yeh *et al.*, 1997). Some examples, such as Anacy_3347 from the cyanobacterium *Anabaena cylindrica* PCC 7122, also have a C-terminal response regulator (REC) domain (Fig. 5). Others, such as EPX62760 from the myxobacterium *Cystobacter fuscus* DSM 2262 and ADC61659 from the purple bacterium *Allochromatium vinosum* DSM 180^{T}, have a PAS domain between the PHY domain and the His kinase module, again with or without a C-terminal REC domain (Fig. 5). In another variation, the genome of the cyanobacterium *Microcoleus* sp. PCC7113 encodes a large protein with an N-terminal knotted PCM and C-terminal histidine kinase bidomain linked by multiple PAS domains and a single CBCR (Fig. 5). There are also examples linking the phytochrome PCM to other bacterial signal transduction pathways; e.g., the α-proteobacteria, *R. palustris* and *Bradyrhizobium* sp. strain ORS278, contain phytochromes with C-terminal S-boxes implicated in protein-protein interactions (Giraud *et al.*, 2002). Moreover, some phytochromes and hybrid Ppd photoreceptors have a C-terminal GGDEF domain (Fig. 5) that can synthesize the bacterial second messenger cyclic-di-GMP (Kyndt *et al.*, 2005; Ryu & Gomelsky, 2014).

C-terminal domain architectures also vary in eukaryotic phytochromes. Stramenopile and fungal phytochromes have functional histidine kinase bidomains at the C-terminus, typically followed by C-terminal REC domains (Fig. 5). Glaucophyte GPS (Glaucophyte phytochrome sensor) proteins have a single PAS domain followed by a functional histidine

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kinase bidomain, sometimes with a C-terminal REC domain (Duanmu *et al.*, 2014). Cryptophyte phytochromes are associated with two different termini (Fig. 5). In one of these, the PCM is followed by a single PAS domain, a eukaryotic protein kinase domain, and a RING domain (putative E3 ubiquitin ligase). Such proteins have been termed PEKs for phytochrome eukaryotic kinase (Duanmu *et al.*, 2014), but little is known about their properties and functions. In other cryptophyte phytochromes, the PCM is followed by a pair of PAS domains followed by a histidine kinase bidomain and a REC domain. This architecture is also shared by prasinophyte phytochromes, and the phosphoacceptor His is present in both (Li *et al.*, 2015). Some prasinophyte phytochromes have three REC domains and a possible nucleotide cyclase domain after the histidine kinase module (Fig. 5). One such protein, DtenPHY1 from *Dolichomastix tenuilepis* CCMP3274, was nevertheless shown to exhibit light-regulated autophosphorylation of the His residue as a recombinantly expressed truncation (Duanmu *et al.*, 2014).

The domain architecture shared by conventional cryptophyte and prasinophyte phytochromes has a long N-terminal extension and a PCM followed by two PAS domains, a functional histidine kinase, and a REC domain (Fig. 5). This C-terminal architecture also is apparently ancestral to that of canonical plant phytochromes. The PAS repeat does not derive from the N-terminal PCM PAS domain, and the C-terminal histidine kinase is not descended from those of bacterial phytochromes (Duanmu *et al.*, 2014). This architecture matches that of some PHYX1 proteins from streptophyte algae; while others lack the REC domain (Li *et al.*, 2015). PHYX1 is the earliest-diverging lineage of streptophyte phytochromes (Fig. 3D), implicating the presence of phytochrome with this domain architecture in the common ancestor of prasinophytes and streptophytes. The REC domain is absent in the next streptophyte lineage to diverge, PHYX2 (Fig. 5). Interestingly, PHYX1 lacks a number of conserved residues required for chromophorylation and/or photoconversion (Li *et al.*, 2015), so it may not function as a photoreceptor. However, PHYX1 and PHYX2 seem to be found together in algal transcriptomes. It thus seems plausible that these two proteins may function as an obligate heterodimer, which would explain the correlated deletion of both phytochromes. Similarly, it is known that Arabidopsis PHYC primarily functions as a heterodimer with PHYB (Clack *et al.*, 2009).

Like streptophyte PHYX1, PHYX2 retains the phosphoacceptor His residue (Fig. 5). That residue was subsequently lost in the streptophyte PHY1/2 lineages, a large clade including phytochromes from early-diverging streptophyte algae and canonical plant phytochromes (Wickett *et al.*, 2014; Li *et al.*, 2015). The resulting C-terminus retains the PAS repeat of cryptophyte and prasinophyte phytochromes and contains both of the structural regions found in a functional histidine kinase, but canonical plant phytochromes lack the phosphoacceptor His residue (Fig. 5). Although such phytochromes exhibit Ser-Thr kinase activity (Yeh & Lagarias, 1998), the functional significance of this activity remains a point of ongoing debate (Ni *et al.*, 2017; Pham *et al.*, 2018). It is also possible that this controversy does not have a single answer. For example, some phytochromes may function to phosphorylate a subset of PIFs, but recruit other protein kinases in other cases.

Further phytochrome diversification occurred in streptophytes as they began to colonize land environments. For example, the moss *Ceratodon purpureus* contains an unusual

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phytochrome somewhat similar to a PEK, with a C-terminal eukaryotic protein kinase domain apparently replacing the histidine kinase-related output region (Thummler *et al.*, 1992). As noted above, some algae and plants contain chimeric neochrome photoreceptors first discovered in ferns (Suetsugu *et al.*, 2005). In neochromes, the phytochrome PCM is fused to the flavin-based blue light receptor phototropin, resulting in a photoreceptor which regulates chloroplast dynamics in response to red light via the Ser/Thr kinase domain of phototropins. The evolution of canonical plant phytochromes thus involves repeated duplication to give rise to PHYX1, PHYX2, and canonical plant phytochrome, diversification with loss of the REC domain followed by loss of the phosphoacceptor His residue, and deletion of PHYX1 and PHYX2 in the earliest-diverging streptophyte lineages. Overall, a key driving force for phytochrome diversification is the placement of the photosensory PCM into different protein contexts over the course of evolution.

VI. DIVERSIFICATION: UNTYING THE RED/FAR-RED KNOT

Canonical plant phytochromes use the knotted, three-domain PCM to photoconvert between red- and far-red sensing states. The first phytochromes characterized from algae and cyanobacteria exhibited very similar red/far-red photocycles (Kidd & Lagarias, 1990; Hughes *et al.*, 1997; Yeh *et al.*, 1997), as did phytochrome from a member of the sister clade to all other known streptophytes, the streptophyte *Mesostigma viride* (Wickett *et al.*, 2014; Rockwell *et al.*, 2017). Bacteriophytochromes exhibited similar photocycles (Davis *et al.*, 1999; Giraud *et al.*, 2002), but these proteins are red-shifted relative to plant phytochromes because their BV chromophore has a longer conjugated system (Fig. 4). Such photocycles are also seen in BV-utilizing fungal and diatom phytochromes (Blumenstein *et al.*, 2005; Froehlich *et al.*, 2005; Fortunato *et al.*, 2016). Conventional thinking holds that such phytochromes are representative of the entire family, implying that phytochrome photosensing measures the red/far-red ratio and requires a knotted three-domain PCM.

Modern research has overturned this view. Many cyanobacteria contain knotless phytochromes (also termed PAS-less or Cph2 phytochromes; Fig. 6). In such proteins, the N-terminal extension for the knot and the PAS domain are absent, yielding a two-domain GAF-PHY PCM (Wu & Lagarias, 2000; Anders *et al.*, 2013). The large loop in the GAF domain is still present, even though there is no N-terminal extension for knot formation, and the red/far-red photocycle is quite similar to those of knotted phytochromes (Fig. 7). In the only available GAF-PHY structure for a knotless phytochrome, that for Cph2 from *Synechocystis* sp. PCC 6803 (Anders *et al.*, 2013), the PHY domain adopts a slightly different fold relative to knotted cases of known structure (Fig. 6B–C). Interestingly, stramenopile phytochromes have a PHY domain apparently more closely related to those of knotless phytochromes than to those of other knotted phytochromes (Duanmu *et al.*, 2014; Li *et al.*, 2015), possibly indicating a similar structural adaptation. Deletion of this domain has produced varying results: some knotless phytochromes require the PHY domain for efficient formation of the photoproduct state, but others do not (Wu & Lagarias, 2000; Ulijasz *et al.*, 2008; Gan *et al.*, 2014).

There are also knotted phytochromes that violate the red/far-red paradigm. Exceptions to this paradigm were first noted in phytochromes from the anoxygenic photosynthetic bacteria

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R. palustris and *Bradyrhizobium* sp. strain ORS278 (Giraud *et al.*, 2005; Jaubert *et al.*, 2007). Subsequently, *N. punctiforme* was shown to encode a very unusual phytochrome termed NpF1183 or TP1 (Rockwell *et al.*, 2011). NpF1183 exhibits a violet-sensing dark state (peak absorption at 392 nm); photoconversion yielded an orange-absorbing species that underwent slow thermal conversion to a red-absorbing state (598 and 670 nm, respectively). This trichromatic behavior requires a second Cys residue immediately C-terminal to the GAF Cys. A similar tandem-Cys phytochrome from the glaucophyte *C. paradoxa* was shown to pair a blue-absorbing dark state with a conventional far-red sensing photoproduct (Rockwell *et al.*, 2014). The phytochrome GwitGPS1, from the glaucophyte *Gloeochaete wittrockiana*, instead combines a conventional red-absorbing dark state with a novel blue-absorbing photoproduct state (Rockwell *et al.*, 2014). Phytochromes from prasinophyte alga were also found to go beyond the expected red/far-red photocycle. All prasinophyte phytochromes characterized to date form a normal far-red absorbing *15E* photoproduct, but this is associated with variable *15Z* dark-adapted states absorbing yellow, orange, or red light ranging from 584 to 648 nm (Rockwell *et al.*, 2014).

Perhaps the most surprising result to come out of these studies is the detection of blue or violet by phytochromes, because their chromophores intrinsically absorb red to green wavelengths of light (Lamparter & Michael, 2005; Rockwell *et al.*, 2012a; Narikawa *et al.*, 2015). The tandem Cys arrangement of NpF1183 was critical in answering this question. Replacement of the second Cys with Ala in NpF1183 and introduction of this residue into Cph1 demonstrated that the second Cys is necessary and sufficient for detection of short-wavelength light (Rockwell *et al.*, 2011). Such tandem-Cys phytochromes are found in cyanobacterial genomes, glaucophyte transcriptomes, and transcriptomes from cryptophytes of the genus *Hemiselmis* (Keeling *et al.*, 2014; Johnson *et al.*, 2019). The tandem-Cys motif is absent in GwitGPS1, but this protein and related proteins from *G. wittrockiana* have a different conserved Cys residue proximal to the bilin-binding pocket (Rockwell *et al.*, 2014). Thus, all known phytochromes detecting ultraviolet to blue wavelength light (370–470 nm) also have a second Cys residue. The function of such second Cys residues has been well established in CBCRs.

CBCRs have only been found in cyanobacteria and were discovered long after plant phytochromes (Butler *et al.*, 1959; Kehoe & Grossman, 1996; Ikeuchi & Ishizuka, 2008; Fushimi & Narikawa, 2019). CBCRs have a bilin-binding GAF domain homologous to that of the phytochrome PCM (Fig. 6D–E) that includes the GAF Cys residue (Fig. 2). No other domains are required for folding, initial chromophore binding, covalent attachment, or photoconversion (Ikeuchi & Ishizuka, 2008). Indeed, one CBCR lineage consists of only an isolated bilin-binding GAF domain, yet it exhibits a conserved blue/orange photocycle (Rockwell *et al.*, 2015b). Other CBCR domains can be found as one or more photosensory components of larger proteins, or even as long tandem arrays (Fig. 7). A number of CBCR subfamilies have been described to date via *in vitro* characterization and phylogenetic analysis (Hirose *et al.*, 2008; Narikawa *et al.*, 2008; Rockwell *et al.*, 2011; Rockwell *et al.*, 2012a; Rockwell *et al.*, 2012b; Narikawa *et al.*, 2014; Rockwell *et al.*, 2015a; Rockwell *et al.*, 2015b; Fushimi *et al.*, 2016; Rockwell *et al.*, 2016). These proteins exhibit an astonishing array of photocycles, with peak absorption for dark-adapted CBCRs ranging from circa 380–740 nm (ultraviolet to far-red) and that for their photoproducts ranging from

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circa 430–660 nm (blue to red). This diversity is achieved through several tuning mechanisms, including chromophore deprotonation, shortening of the bilin π system via isomerization at C5, and trapping of twisted intermediates (Ishizuka *et al.*, 2011; Rockwell *et al.*, 2012a; Hirose *et al.*, 2013; Lim *et al.*, 2018; Wiebeler *et al.*, 2019).

Studies of CBCRs have also elucidated the basis for detection of violet or blue light. Every known CBCR photostate detecting light in the UV to blue (330–470 nm) requires a second Cys residue. In two such cases, it has been established that this residue forms a second thioether linkage to the C10 atom of the bilin chromophore in the blue- or violet-absorbing state (Ishizuka *et al.*, 2011; Rockwell *et al.*, 2011; Burgie *et al.*, 2013; Lim *et al.*, 2014). This second linkage shortens the conjugated system and blue-shifts the peak absorption. Given the presence of second Cys residues in all phytochromes known to sense this wavelength range, it seems likely that a similar tuning mechanism is at work in those proteins.

VII. DIVERSIFICATION: SPECIALIZED CIRCUITS AND MASTER REGULATORS

Our view of phytochrome has been shaped by its importance in development and shade avoidance for crop plants such as wheat and rice, annuals that grow rapidly from small seeds under the right conditions (Mathews, 2006; Casal, 2013). Plants that take different strategies, such as conifers, can behave differently during early development (Mathews, 2006; Mathews & Tremonte, 2012). Little is known about the function of canonical phytochromes in early-diverging streptophyte algae, much less about the function of PHYX1 and PHYX2. Phytochrome accumulates in the nucleus in response to light in the prasinophyte alga *Micromonas pusilla* as well as in flowering plants, so it seems likely that some aspects of phytochrome signaling are broadly conserved in Viridiplantae (Duanmu *et al.*, 2014).

However, the biological function of phytochromes has clearly diversified from its ancestral state. Fungal phytochrome signaling proceeds not through PIFs but via the white collar complex and the HogA/Hog1 MAP kinase pathway (Yu *et al.*, 2016). In cyanobacteria, a knotless phytochrome has been transferred to diverse cyanobacteria as part of a genomic island that permits growth in far-red light (Gan *et al.*, 2014). This island induces a profound red shift in both light-harvesting and photosynthetic apparatus, with induction of ~900 genes and repression of ~2000 genes. Core subunits of photosystem I, photosystem II, and phycobilisomes are replaced by members of this 21-gene island. This far-red acclimation response depends on the function of the knotless phytochrome RfpA and its cognate response regulators (Zhao *et al.*, 2015). *R. palustris* also uses phytochromes to regulate expression of light-harvesting pigments via dedicated signal transduction pathways involving histidine kinases and protein-protein interactions (Giraud *et al.*, 2002; Giraud *et al.*, 2005). In both cases, a small number of transcription factors are involved rather than the panoply of PIFs found in flowering plants (Pham *et al.*, 2018).

We are also beginning to learn about the function of CBCRs (Wiltbank & Kehoe, 2019). The first known regulatory photobiological response for any cyanobacterium was complementary chromatic acclimation (CCA), described in the early 20th Century (Gaidukov, 1902). Over ninety years later, CCA was shown to be controlled by the green/red CBCRs RcaE and CcaS

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via dedicated histidine kinase pathways (Kehoe & Grossman, 1996; Hirose *et al.*, 2008; Hirose *et al.*, 2013). Other CBCRs are phototaxis sensors, as has been verified for SyPixJ1/TaxD in *Synechocystis* sp. PCC 6803 (Yoshihara *et al.*, 2000; Bhaya *et al.*, 2001), $PixJ_{Se}$ in *Synechococcus elongatus* UTEX 3055 (Yang *et al.*, 2018), and PtxD/NpF2164 in *N. punctiforme* (Campbell *et al.*, 2015). These proteins contain one or more CBCR domains and a C-terminal methyl-accepting chemotaxis protein (MCP) domain. Others, like UirS/PixA, have C-terminal histidine kinases and modulate negative phototaxis via a phosphotransfer cascade (Narikawa *et al.*, 2011; Song *et al.*, 2011). In *Thermosynechococcus elongatus* and *T. vulcanus*, three CBCRs with GGDEF and/or EAL domains regulate cyclic-di-GMP levels in response to blue and green light, resulting in a switch between motile and sessile lifestyles (Enomoto *et al.*, 2014; Enomoto *et al.*, 2015). A CBCR from *Microcoleus* sp. PCC 7113 (cPAC or Mic7113_2205) has also been found to act as a light-regulated adenylate cyclase both *in vitro* and upon heterologous bacterial expression (Blain-Hartung *et al.*, 2018).

We can thus see that the phytochrome superfamily encompasses far more than canonical plant phytochromes, with a complex, ongoing interplay between deletion, duplication, and diversification during evolution of life. Extant diversity has been shaped by a drive toward smaller knotless phytochromes and CBCRs in cyanobacteria, by spectral diversification from the ancestral red/far-red photoreceptor, by fusion to diverse domains in prokaryotes and eukaryotes, and by acquisition of different biological functions. Indeed, canonical plant phytochromes and PIFs seem to be the exception rather than the rule in phytochrome signaling. The greater diversity of algal phytochromes may also reflect the greater environmental and ecological diversity of eukaryotic algae compared to land plants.

VIII. AND NOW A WORD FROM THE LETTER E: ENVIRONMENT

The three themes of phytochrome evolution all interact with the environment of the organisms in which phytochromes are evolving, even though we may not understand the interactions. For example, deletion and diversification of phytochrome do not always correlate with photosynthesis. In one ochrophyte lineage, the absence of phytochrome roughly correlates with loss of photosynthesis (Beisser *et al.*, 2017). By contrast, at least one non-photosynthetic *Cryptomonas* strain retains phytochromes (Keeling *et al.*, 2014; Johnson *et al.*, 2019). Photosynthetic organisms that have selected for small cell size and streamlined genomes sometimes lack phytochrome, as in the picoprasinophyte *Ostreococcus* but not in the related *Micromonas pusilla* (Palenik *et al.*, 2007; Duanmu *et al.*, 2014). Duplication and diversification of phytochromes and CBCRs seems roughly correlated with biological complexity in cyanobacteria. For example, there are only five CBCRs in *T. elongatus*, each of which has a single photosensory domain (Ikeuchi & Ishizuka, 2008), whereas *N. punctiforme* has 21 such proteins (Rockwell *et al.*, 2015a). Many of these proteins have multiple photosensory domains in tandem, for a total of 41 phytochromes or CBCRs in the genome. *N. punctiforme* also exhibits much more complexity than *T. elongatus*: it exhibits CCA, nitrogen fixation, and differentiation of vegetative cells into three distinct, mutually exclusive cell types (Campbell *et al.*, 2007; Hirose *et al.*, 2010).

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Diversification of phytochromes and CBCRs is likely driven by environmental factors. For example, consider competition between land plants and soil cyanobacteria. Land plants compete for sunlight via the shade avoidance response (Fig. 1). Given that the conifer *Sequoia sempervirens* can exceed 115 m in height, soil cyanobacteria cannot compete using a similar vertical response. However, CCA allows them to use green light that plants cannot, providing a successful alternative in shaded environments. CCA requires metabolically expensive remodeling of the light-harvesting apparatus (Kehoe & Gutu, 2006), so there is a clear selective advantage for regulating CCA with photosensors such as CcaS and RcaE. Photosynthetic organisms living in a water column face a different set of environmental challenges. Red and far-red light penetrate poorly in water, and there is thought to be some correlation between peak dark-state absorption of algal phytochromes and the depths at which those algae are encountered (Forest, 2014; Rockwell *et al.*, 2014). Factors other than light can affect phytochrome function as well. The thermal instability of the photoproduct state confers a second sensory function upon phytochromes: dark reversion is a light-independent thermal reaction, so it proceeds with a pronounced temperature dependence in the presence or absence of light. The amount of P_{fr} that is present is thus dependent on both light and temperature, permitting phytochromes to exhibit different temperature responses depending on their dark reversion rates. Consistent with this, phytochromes are now known to function as temperature sensors in both prokaryotes and eukaryotes(Njimona & Lamparter, 2011; Njimona *et al.*, 2014; Jung *et al.*, 2016; Legris *et al.*, 2016). Some CBCRs have even more unstable photoproducts and serve as broad-spectrum sensors of light intensity (Rockwell *et al.*, 2012b). Although the evolutionary trajectory of this transition is not known, these observations demonstrate the extent to which the phytochrome superfamily provides photosensors for almost every color and purpose imaginable.

IX. CONCLUSION

Given the biological, photochemical, and ecological complexity of the phytochrome superfamily, phytochrome presents far more than a particularly vexatious exercise in phylogenetic inference. Indeed, each duplication and each fusion to a new C-terminus is a discrete evolutionary event with potential biological implications. Phytochromes are but one piece of a larger jigsaw puzzle for any organism: in some cases, they may be left in the box when the puzzle is complete, leading to their deletion, but in others they are critical. Deletion, duplication, and diversification have all played a part in shaping extant phytochromes as a whole and canonical plant phytochrome as a special case. In the future, we may expect new insights from improved sampling of biological diversity, from studies of phytochrome signaling mechanisms, from studies of chromophore biosynthesis, and from studies of bilin-based light harvesting systems. We also hope to learn more about the roles phytochromes play in the adaptation of photosynthetic organisms to changing environments brought on by transient breezes, daily and annual changes in solar irradiance, and the long-term effects of climate change. Sixty years after the first description of canonical plant phytochromes, the future of phytochrome science remains bright.

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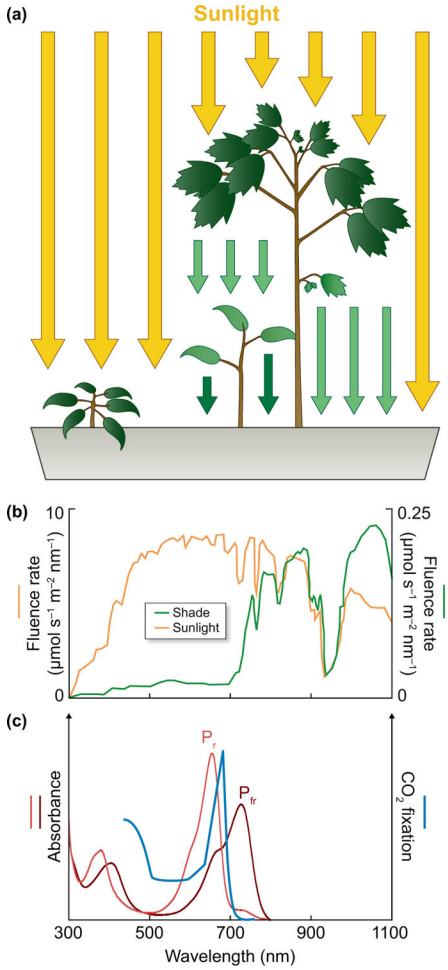

Figure 1. Phytochrome and shade sensing.

(a) Plants growing in direct sunlight or under shade from competitors are exposed to different light environments, leading to distinct morphology and pigment development. The plant on the left is in direct sunlight, but the lower plant on the right is shaded by a competing plant of a different species. Scattering is omitted for clarity. (b) A competing canopy depletes light for shaded plants. In the case of tropical rain forests, attenuation is approximately 40-fold for the near-infrared and greater for photosynthetically useful red light. Units are μmol s^{-1} m^{-2} nm^{-1} for both axes. The red:far-red ratio (R:FR) is 1.25 in direct sunlight and 0.31 in shade (Lee & Graham, 1986). (c) The action spectrum for carbon fixation in the green alga *Hydrodictyon* is compared to absorption spectra for the red-absorbing P_r and far-red-absorbing P_{fr} photostates of wheat phytochrome C (Raven, 1969; Chen *et al.*, 2014).

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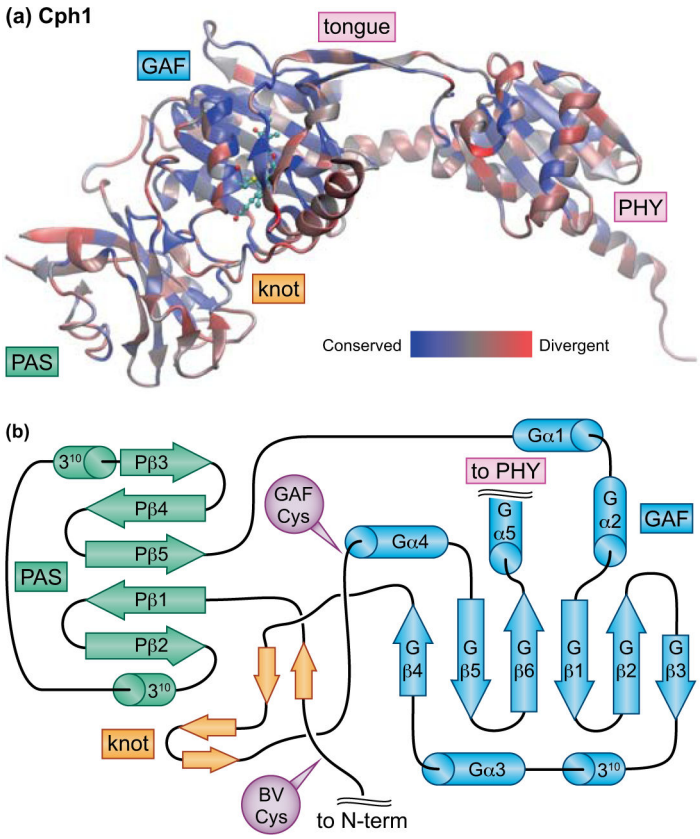

Figure 2. Structure of the knotted phytochrome PCM.

(a) Cyanobacterial phytochrome Cph1 from *Synechocystis* sp. PCC 6803 (Essen *et al.*, 2008) is shown color-coded by sequence similarity, calculated using a published sequence alignment (Rockwell *et al.*, 2006) and the BLOSUM62 matrix as implemented in homolmapper (Rockwell & Lagarias, 2007). (b) Topology cartoon for the knotted PAS-GAF region of the PCM with the knot in orange, the PAS domain in green, and the GAF domain in blue. Cys residues used for chromophore ligation are indicated in purple. Secondary structure elements are numbered from the N-terminus of each domain for the PAS and GAF domains, with the domain indicated by P and G, respectively. 1-turn 3^{10} helices and short β-strands in the knot are indicated but not numbered. Domain names: GAF, cGMP phosphodiesterase/Adenylate cyclase/FhlA; PAS, Per/ARNT/Sim; PHY, phytochrome-specific. BV, biliverdin; N-term, N-terminus; tongue, conserved insertion loop specific to the PHY domain.

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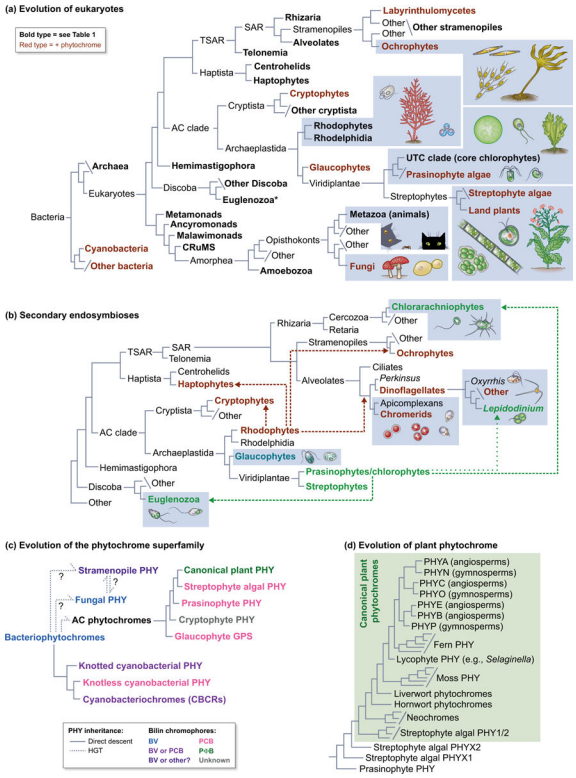

Figure 3. Phytochrome and the evolution of eukaryotes.

(a) A simplified working view of the tree of life is shown, drawing on recent studies (Duanmu *et al.*, 2014; Wickett *et al.*, 2014; Burki *et al.*, 2016; Derelle *et al.*, 2016; Brown *et al.*, 2018; Lax *et al.*, 2018; Gawryluk *et al.*, 2019; Strassert *et al.*, 2019). Bold type indicates taxa assessed for phytochrome and bilin biosynthesis in Table 1. Lineages containing at least some organisms in which phytochromes are present are indicated in red. Representative organisms are shown for selected lineages (not to scale; enclosed in dotted boxes). Branch lengths are arbitrary. Asterisk, see legend to Table 1 for possible phytochromes in Euglenozoa. CRuMS, supergroup containing collodictyonids, rigifilids, and Mantamonas; AC clade, Archaeplastida + Cryptista; UTC clade, Ulvophyceae, Trebouxiophyceae and Chlorophyceae; TSAR, Telonemia + SAR; SAR, supergroup containing Stramenopiles, Alveolates, and Rhizaria. (b) Secondary endosymbioses in eukaryotes. Acquisition of photosynthesis in cryptophytes, haptophytes, ochrophytes, and photosynthetic alveolates (chromerids and dinoflagellates) occurred via secondary endosymbiosis of a rhodophyte (dashed red lines). Acquisition of photosynthesis in euglenozoa and chlorarachniophytes occurred via secondary endosymbiosis of prasinophyte or chlorophyte algae (green dashed lines). “Green dinoflagellates” of the genus *Lepidodinium* have replaced the ancestral rhodophyte-derived plastid via secondary endosymbiosis of a prasinophyte or chlorophyte alga (green dotted lines). Representative organisms are shown for selected lineages as in (a). Colors indicate plastid lineage; nonphotosynthetic plastids are not colored. (c) A schematic view of phytochrome evolution is shown. Colors indicate chromophore precursors incorporated by phytochromes from different taxa: BV only, blue; BV or PCB, purple; BV or unknown bilin, deep purple (Rockwell *et al.*, 2014); PCB, pink; PΦB, green; unknown, grey. Dashed lines indicate inferred HGT events. PHY, phytochrome; BV, biliverdin IXα;

PCB, phycocyanobilin; PΦB, phytochromobilin. (d) A schematic view of plant phytochrome evolution is shown, demonstrating that canonical plant phytochromes are part of a larger clade of streptophyte phytochromes using the nomenclature of (Li *et al.*, 2015).

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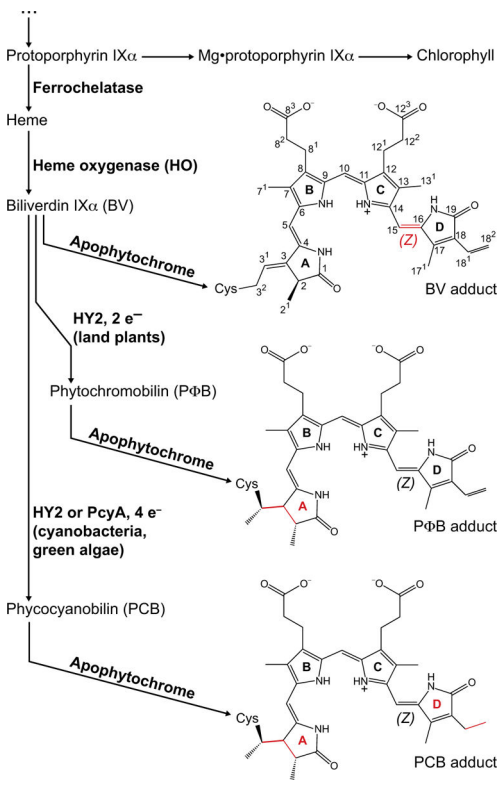

Figure 4. Biosynthesis of bilin chromophores.

The pathway from heme to biliverdin IXα (BV) and reduced phycobilins (PCB and PΦB) is shown. Free bilins are then assembled with apophytochrome to yield covalent adducts, examples of which are shown on the right in the C5–*Z,syn*, C10–*Z,syn*, C15–*Z,anti* configuration (Wagner *et al.*, 2005; Essen *et al.*, 2008; Burgie *et al.*, 2014a). The numbering system is indicated for BV. For simplicity, a single structure is shown; however, other BV adducts have been observed (Salewski *et al.*, 2013). For BV the photoactive 15,16–double bond is highlighted in red. For PCB and PΦB, sites of reduction relative to BV are highlighted in red. The degree of reduction relative to BV is indicated (e^-, electron).

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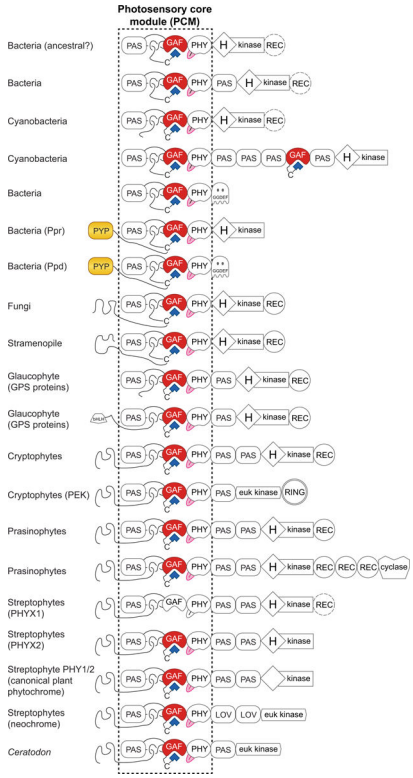

Figure 5. Diverse domain architectures in knotted phytochromes.

Jellybean domain diagrams are shown for diverse prokaryotic and eukaryotic phytochromes that use the canonical knotted PCM (Duanmu *et al.*, 2014; Li *et al.*, 2015). Bilin-binding GAF domains are red, the tongue region of the PHY domain is pink, bilin chromophores are shown as blue polygons, and chromophore-binding PYP domains are yellow. REC domains that are present in only some cases are dashed, as in PHYX1. Domain names: GAF, cGMP phosphodiesterase/Adenylate cyclase/FhlA; PAS, Per/ARNT/Sim; PHY, phytochrome-specific; (H)kinase, histidine kinase bidomain, with the presence of the His indicated by H; REC, response regulator receiver; RING, really interesting new gene; euk kinase, eukaryotic protein kinase; cyclase, eukaryotic adenylate/guanylate cyclase; GGDEF, diguanylate cyclase; PYP, photoactive yellow protein; bHLH, basic helix-loop-helix; LOV, PAS domain belonging to the light/oxygen/voltage lineage. Two-Cys phytochromes from cyanobacteria and glaucophytes are not indicated.

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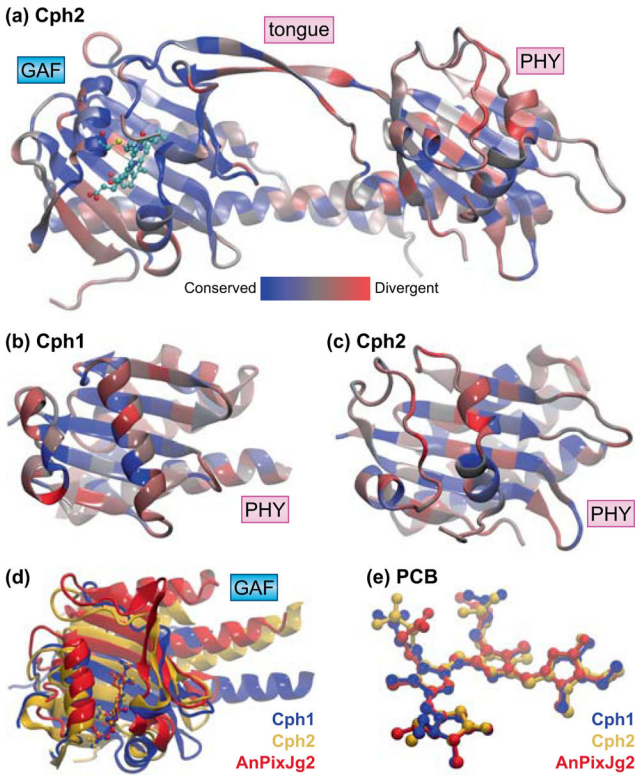

Figure 6. Structures of knotless phytochromes and CBCRs.

(a) The GAF-PHY PCM of Cph2 from *Synechocystis* sp. PCC 6803 is shown (Anders *et al.*, 2013), color-coded by sequence similarity using a published sequence alignment (Gan *et al.*, 2014) as in Fig. 2(a). (b) Detailed view of the PHY domain of Cph1, colored as in (a); the tongue region and the long helices connecting to adjacent domains are omitted for clarity. (c) Detailed view of the PHY domain of Cph2, colored as in (a) and presented as in (b). (d) The bilin-binding GAF domain of AnPixJg2 (red) from *Nostoc* sp. PCC 7120 (sometimes designated *Anabaena* sp. PCC 7120) is compared to that of Cph2 (gold) from (a) and that of Cph1 (blue) from Fig. 2 (Anders *et al.*, 2013; Narikawa *et al.*, 2013), with PCB coordinates used for superposition. (e) The PCB chromophores and ligated Cys residues are shown superposed for Cph1 (red), Cph2 (blue), and AnPixJg2 (grey). All three cases correspond to dark-adapted, red-absorbing photostates in the C5–*Z,syn* C10–*Z,syn* C15–*Z,anti* configuration. Domain names: GAF, cGMP phosphodiesterase/Adenylate cyclase/FhlA; PHY, phytochrome-specific. PCB, phycocyanobilin; tongue, conserved insertion loop specific to the PHY domain.

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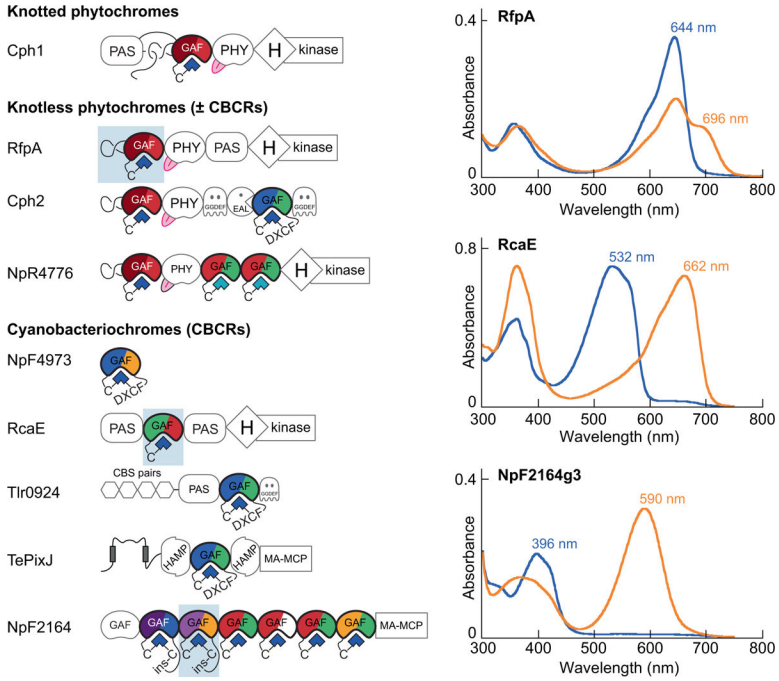

Figure 7. Diversification of the phytochrome superfamily in cyanobacteria.

Jellybean domain architectures and photocycles are shown for selected photoreceptors (Wu & Lagarias, 2000; Rockwell *et al.*, 2011; Rockwell *et al.*, 2012a; Rockwell *et al.*, 2012b; Savakis *et al.*, 2012; Hirose *et al.*, 2013; Gan *et al.*, 2014; Rockwell *et al.*, 2016). Photocycles are shown for heterologously expressed GAF-only constructs that are highlighted with blue shading in the domain architectures. In each case, the *15Z* configuration is colored blue and the *15E* configuration is colored orange; peak wavelengths and domain names are indicated. In the domain architectures, bilin-binding GAF domains are colored by photocycle, with the dark-adapted state on the left and the photoproduct on the right. Bilin chromophores are shown as blue polygons; domains that do not photoconvert (Rockwell *et al.*, 2012b) are indicated with a white “photoproduct” state. The bilin-binding GAF lineage defining the phytochrome superfamily is only a subset of all GAF domains, so non-photosensory GAF domains are indicated in white. Domain names: GAF, cGMP phosphodiesterase/Adenylate cyclase/FhlA; PAS, Per/ARNT/Sim; PHY, phytochrome-specific; (H)kinase, histidine kinase bidomain, with the presence of the His indicated by H; GGDEF, diguanylate cyclase; HAMP, histidine kinase/adenylate cyclase/methyl-accepting chemotaxis protein/phosphatase; CBS, cystathionine β-synthase domain; EAL, diguanylate phosphodiesterase; MA-MCP, methyl-accepting domain of methylated chemotaxis proteins. Vertical rectangles on TePixJ are predicted transmembrane helices. DXCF, ins-C are different second Cys residues.

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Table 1:

Distribution of Phytochromes and Bilins Across the Tree of Life[1]

Lineage	Examples	PHY	HO	FDBR	bilins
Archaea	*Pyrococcus,* Lokiarchaeota	−	−	−	none known
cyanobacteria	*Prochlorococcus, Nostoc*	±	+	+	BV, PCB, other
other bacteria	*Rhodopseudomonas, Escherichia coli*	±	±	±	±BV, ±PCB
metamonads	*Giardia, Trichomonas*	−	−	−	none known
ancyromonads	*Fabomonas, Nutomonas*	−	fragments[2]	−	±BV?
malawimonads[3]	*Malawimonas, Gefionella*	−	−	−	none known
CRuMs	*Rigifila, Collodictyon*	−	−	−	none known
Amoebozoa	*Paramoeba, Dictyostelium*	−	±	−	±BV
Fungi	yeast, molds, mushrooms	±	±	−	±BV
Metazoa	*Homo sapiens*, birds, bees (animals)	−	±	−	±BV
Euglenozoa	*Euglena, Eutreptiella*	fragments[4]	+	+	BV, PCB
other Discoba	*Leishmania, Trypanosoma*	−	−	−	none known
Hemimastigophora	*Hemimastix, Spironema*	−	−	−	none known
cryptophytes	*Guillardia, Hemiselmis*	+	+	+	BV, PCB, other
other Cryptista	*Roombia, Palpitomonas*	−	±	−	±BV
rhodophytes	*Galdieria,* laver, *Cyanidioschyzon*	−	+	+	BV, ±PCB, ±other
Rhodelphidia	*Rhodelphis*	−	−	−	none known
glaucophytes	*Cyanophora*	+	+	+	BV, PCB
prasinophyte algae	*Micromonas, Ostreococcus*	±	+	+	BV, PCB, ±other
UTC clade	*Chlamydomonas, Volvox*	−	+	+	BV, PCB
streptophyte algae	*Mesostigma, Chara, Klebsormidium*	+	+	+	BV, PCB, other
land plants	ferns, cacti, redwood trees, bananas	+	+	+	BV, PΦB, ±other
centrohelids	*Acanthocystis* (centroheliozoans)	−	±	−	±BV
haptophytes	*Pavlova, Emiliana, Phaeocystis*	−	+	+	BV, ?
Telonemia[3]	*Telonema*	fragments	−	−	BV?
Rhizaria	*Paulinella,* foraminiferans	−	±	±	±BV, ±PCB, ±?
alveolates	dinoflagellates, ciliates, *Plasmodium*	−	±	±	±BV, ±PCB, ±?
labyrinthulomycetes (slime nets)	*Aplanochytrium,* QPX pathogen	±	±	−	±BV
ochrophytes	diatoms, kelp	±	+	+	BV, ?
other stramenopiles	*Cafeteria, Phytophthora*	−	±	−	±BV

[1]Drawn from (Duanmu *et al.*, 2014; Keeling *et al.*, 2014; Li *et al.*, 2015; Brown *et al.*, 2018; Lax *et al.*, 2018; Gawryluk *et al.*, 2019; Johnson *et al.*, 2019; Strassert *et al.*, 2019). PHY, phytochrome. See Fig. 3A for an overview of eukaryotic evolution. Bilin composition is listed for cases where a phytochrome chromophore is known or can be inferred from the presence of PCYA or HY2. "Other," reduced bilins other than PCB or PΦB are known to be present. "?," FDBRs are present, but their reaction products are unknown. Fragments: short fragments are present in one or more transcriptomes. Such cases have not been phylogenetically analyzed.

[2]Short sequences closely related to heme oxygenases are present in the deposited raw sequence reads form the transcriptome of *Fabomonas tropica* NYK3C (SRA SRX3153020 in the Genbank Sequence Read Archive), so this organism may contain a heme oxygenase.

[3] Raw sequence reads for *Gefionella okellyi* strain 249 and *Telonema* isolate P-1 are available from the Genbank Sequence Read Archive (SRA SRX3152400 and SRA SRR7371268, respectively). Short sequences closely related to heme oxygenases are present in the latter, so *Telonema* may contain a heme oxygenase. However, it should be noted that the gene content of other members of these lineages may vary.

[4] Possible phytochrome sequences are present in one *Eutreptiella* transcriptome but have not been phylogenetically analyzed; similar sequences are not found in other genomes and transcriptomes from Euglenozoa. The provenance of these sequences is thus unclear.

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