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Marine algae and land plants share conserved phytochrome signaling systems

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Phytochrome photosensors control a vast gene network in streptophyte plants, acting as master regulators of diverse growth and developmental processes throughout the life cycle. In contrast with their absence in known chlorophyte algal genomes and most sequenced prasinophyte algal genomes, a phytochrome is found in *Micromonas pusilla*, a widely distributed marine picoprasinophyte (<2 μm cell diameter). Together with phytochromes identified from other prasinophyte lineages, we establish that prasinophyte and streptophyte phytochromes share core light-input and signaling-output domain architectures except for the loss of C-terminal response regulator receiver domains in the streptophyte phytochrome lineage. Phylogenetic reconstructions robustly support the presence of phytochrome in the common progenitor of green algae and land plants. These analyses reveal a monophyletic clade containing streptophyte, prasinophyte, cryptophyte, and glaucophyte phytochromes implying an origin in the eukaryotic ancestor of the Archaeplastida. Transcriptomic measurements reveal diurnal regulation of phytochrome and bilin chromophore biosynthetic genes in *Micromonas*. Expression of these genes precedes both light-mediated phytochrome redistribution from the cytoplasm to the nucleus and increased expression of photosynthesis-associated genes. Prasinophyte phytochromes perceive wavelengths of light transmitted farther through seawater than the red/far-red light sensed by land plant phytochromes. Prasinophyte phytochromes also retain light-regulated histidine kinase activity lost in the streptophyte phytochrome lineage. Our studies demonstrate that light-mediated nuclear translocation of phytochrome predates the emergence of land plants and likely represents a widespread signaling mechanism in unicellular algae.

phytoplankton | light harvesting | transcriptomics | marine ecology | light signaling evolution

Phytochromes perform critical regulatory roles in land plants, fungi, and bacteria (1–4). Expansion of the phytochrome gene family has occurred during evolution of plants, in which phytochromes optimize photosynthesis and regulate developmental progression, e.g., seed germination, leaf and stem expansion, reproduction, and seed dispersal (1, 5). Consisting of multiple domains, including a conserved photosensory core input module (PCM) (Fig. 1) and a histidine kinase-related output module (HKM), plant phytochromes share similarities with two-component signaling (TCS) systems widespread in bacteria (6). In plants, light sensing by phytochromes relies on a covalently bound linear tetrapyrrole (bilin) chromophore that is synthesized within plastids (7), the organelle for eukaryotic photosynthesis (see, e.g., refs. 8, 9). Bilin photoisomerization triggers reversible interconversion between red and far-red absorbing states (10) initiating downstream signaling events associated with translocation into the nucleus (11, 12). Phytochromes thereby transduce light signals into biochemical outputs that shape overall organismal responses (1, 13).

Although plant phytochromes control vast, complicated gene networks, their origin, evolution, and ancestral signaling mechanisms remain uncertain (14–17). Similarities between streptophyte (land plants and charophyte algae) and cyanobacterial phytochromes, such as shared red/far-red photocycles, shared bilin chromophores, and identical protein–chromophore linkages (10), have been considered indicative of cyanobacterial origins via endosymbiotic gene transfer (EGT) (14, 16, 18). In this scenario, EGT of cyanobacterial phytochromes occurred during or after the primary endosymbiosis event that gave rise to the Archaeplastida approximately 1 billion years ago, whereby an engulfed cyanobacterium became the plastid (8, 9). The Archaeplastida ancestor then diverged to form three major extant photosynthetic groups: Viridiplantae (streptophyte, prasinophyte, and chlorophyte algae, as well as land plants), Rhodophyta (red algae), and Glaucophyta.

Significance

Phytochromes are photosensory signaling proteins widely distributed in unicellular organisms and multicellular land plants. Best known for their global regulatory roles in photomorphogenesis, plant phytochromes are often assumed to have arisen via gene transfer from the cyanobacterial endosymbiont that gave rise to photosynthetic chloroplast organelles. Our analyses support the scenario that phytochromes were acquired prior to diversification of the Archaeplastida, possibly before the endosymbiosis event. We show that plant phytochromes are structurally and functionally related to those discovered in prasinophytes, an ecologically important group of marine green algae. Based on our studies, we propose that these phytochromes share light-mediated signaling mechanisms with those of plants. Phytochromes presumably perform critical acclimative roles for unicellular marine algae living in fluctuating light environments.

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See Commentary on page 15608.

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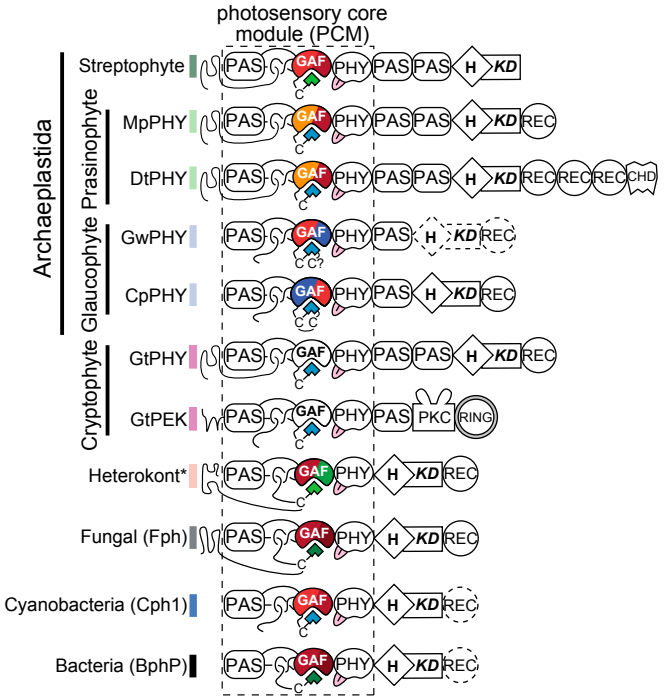

Fig. 1. Domain structures of phytochrome proteins. The N-terminal photosensory core module (PCM) of phytochromes is composed of PAS, GAF, and PHY domains (dashed box). Colors on the chromophore binding GAF domains correspond to those of the two reversibly photointerconverting states of each phytochrome where known or as described here. C-terminal output modules of phytochromes from all Archaeplastida lineages typically contain one or two PAS domains adjacent to histidine kinase modules (HKM). Lack of C-terminal receiver (REC) domains in streptophyte phytochromes contrasts with their presence in prasinophyte, glaucophyte, and cryptophyte phytochromes. Structurally distinct phytochrome eukaryotic kinase (PEK) hybrids are present in the cryptophyte alga *Guillardia theta*. The *Ectocarpus siliculosus* photocycle shown here (asterisk) may not be representative of other heterokont phytochrome photocycles. Domain names: CHD, cyclase homology domain; GAF, cGMP phosphodiesterase/adenylate cyclase/FhlA; H/KD, HisKA and H-ATPase-c domains comprising the HKM; PAS, Per/Arnt/Sim; PHY, phytochrome; PKC, protein kinase catalytic domain; REC, response regulator receiver; and RING, really interesting new gene. Taxonomic assignments (colored bars) follow color-coding used in Fig. 2. Dashed outlines indicate domains that are not always present.

In addition to land plants, some fungi, heterokont algae and glaucophyte algae, possess phytochromes (3, 17, 19, 20). However, many photosynthetic eukaryotes with sequenced genomes do not, such as the rhodophytes *Porphyridium purpureum*, *Cyanidioschyzon merolae*, *Pyropia yezoensis*, and *Chondrus crispus*, the diminutive picoprasinophyte (green) algal species *Ostreococcus* spp. and *Bathycoccus prasinos*, and the model green algae (chlorophytes) *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Volvox carteri*. This patchwork distribution of phytochromes in extant eukaryotes has been an obstacle to establishing plausible evolutionary scenarios and to understanding early functional roles.

Prasinophyte algae retain some characteristics of the land plant progenitor that are absent from chlorophyte algae and several nonvascular plants (21). The prasinophytes are composed of diverse lineages that as a group branch adjacent to the chlorophyte algae (21–23). Together, prasinophytes and chlorophytes form a sister group to the streptophytes. Here, we expand genomic resources for prasinophyte, chlorophyte, and glaucophyte algae through sequencing and assembling transcriptomes from multiple independent lineages. Phylogenetic analyses of new predicted and experimentally verified phytochrome proteins provide insights into the origins of Archaeplastida phytochromes. Using sequence confirmation methods, RNA-seq and immunochemical analyses, we document the expression of phytochrome and photosynthesis-related genes across a diurnal light–dark cycle for the prasinophyte *Micromonas*, a marine algal genus found from tropical to Arctic ecosystems (21). Together with biochemical and localization analyses, these studies reshape our understanding of plant phytochrome evolution and reveal light-mediated phytochrome signaling mechanisms in unicellular algae.

Results

Phytochrome Domain Structure and Evolutionary Relationships. We sequenced transcriptomes from algae with informative evolutionary positions relative to plant ancestry. These include representatives from six of the seven prasinophyte classes and several other Archaeplastida algae (*SI Appendix*, Table S1). Phytochromes were not found in the two *Chlamydomonas* species examined, *Chlamydomonas chlamydogama* and *Chlamydomonas leiostraca*, as is the case for published chlorophyte genomes. Full-length phytochrome transcripts were present in five prasinophyte lineages, specifically classes I, II (*Dolichomastix tenuilepis* and *Micromonas pusilla*), III, IV, and VI (*SI Appendix*, Fig. S1), as well as in the glaucophyte *Gloeochaete wittrockiana*. Phytochrome RNA-seq transcript assemblies were affirmed using RACE and PCR for multiple taxa (*SI Appendix*, Tables S2 and S3). Additionally, using immunoblot analysis and mass spectra, the *M. pusilla* phytochrome gene (*MpPHY*) was shown to encode a 1,850-amino-acid polypeptide (MpPHY) (*SI Appendix*, Fig. S2). These results demonstrate that prasinophyte phytochrome genes encode significantly larger proteins than those of plants, which lack C-terminal TCS receiver (REC) domains (Fig. 1 and *SI Appendix*, Table S3). These high-quality sequences as well as those of the more derived cryptophyte alga *Guillardia theta* were used to reconstruct the evolutionary history of eukaryotic phytochromes.

Phylogenetic reconstructions using maximum likelihood and Bayesian methods showed that glaucophyte phytochrome sensors are the earliest branching members within a strongly supported clade (91% bootstrap support, one posterior probability) containing prasinophyte, cryptophyte, and streptophyte PCM sequences (Fig. 2 and *SI Appendix*, Fig. S3). Prasinophyte phytochromes form a sister group to land plants (90% bootstrap support, one posterior probability), whereas cryptophyte phytochromes diverge earlier (see *Discussion*). Fungal and heterokont phytochromes appear to have a distinct origin from the Archaeplastida PCMs (Fig. 2 and *SI Appendix*, Fig. S3). Finally, cyanobacterial phytochromes group among bacteria, apart from those of plants and other Archaeplastida taxa. In contrast, application of the same phylogenetic methods to PCM sequences available before our study provided a maximum likelihood topology where cyanobacterial sequences were basal to Archaeplastida taxa, but lacked statistical support at key nodes (*SI Appendix*, Fig. S4). The reconstruction based on our broader taxonomic sampling (Fig. 2 and *SI Appendix*, Fig. S3) clearly supports a common origin of Archaeplastida PCMs, distinct from that of cyanobacterial phytochromes.

Prasinophyte, cryptophyte, and streptophyte phytochrome HKM origins are also monophyletic (*SI Appendix*, Fig. S5), akin to PCM results. Because only 142 HKM residues (encompassing both HisKA and HATPase-c domains) were appropriate for phylogenetic analysis, overall relationships could not be resolved. However, the HKM phylogeny supports neither acquisition from cyanobacteria nor multiple horizontal gene transfer (HGT) events from bacteria (24) (*SI Appendix*, Fig. S5). Streptophyte phytochrome HKMs typically lack a canonical His autophosphorylation site found in functional TCS histidine kinases (6). By contrast, critical residues for histidine kinase catalytic function are present in those of prasinophytes, glaucophytes, and

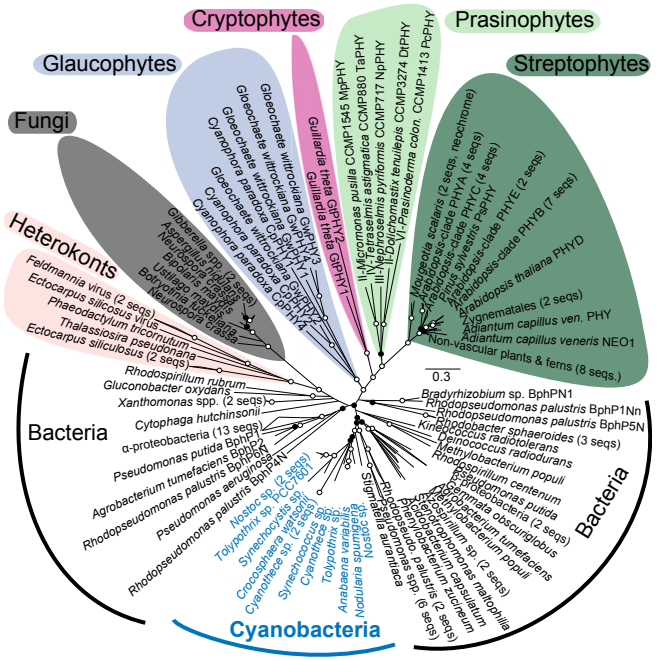

Fig. 2. Evolutionary analyses establish common ancestry of phytochromes from Archaeplastida (and cryptophyte) lineages and support presence in early eukaryotes. Evolutionary relationships are based on maximum likelihood (ML) analyses of phytochromes from 128 representative taxa using 407 homologous positions in the N-terminal PAS–GAF–PHY region. Colored backgrounds indicate eukaryotic sequences. Cyanobacterial sequences are in blue text. Collapsed streptophyte clades are named according to *Arabidopsis thaliana* (if present) but also include other taxa (*SI Appendix*, Fig. S3). Plastids in cryptophyte and heterokont algae were likely attained through independent secondary endosymbiosis with red algae (23), unlike Archaeplastidal plastids, which are thought to have arisen from a single primary endosymbiosis event with cyanobacteria (8, 9). Placement of cryptophyte PCMs within the Archaeplastida could therefore represent a red algal version recruited via EGT from the secondary plastid ancestor (although absent from sequenced red algal genomes) or contributions from a putative green algal forebear implicated in its genome composition (8). The tree is unrooted. Support is indicated by open circles (≥90%, ML; ≥0.9 posterior probability, Bayesian) or black circles (≥75%, ML; ≥0.9 posterior probability, Bayesian).

cryptophytes. The phylogenic analyses indicate that the origin of the glaucophyte phytochrome HKM is distinct from that shared by prasinophyte, cryptophyte, and streptophyte phytochromes (*SI Appendix*, Fig. S5). Underscoring frequent replacement of phytochrome output domains, we observed that cryptophytes possess two types of phytochromes, one of which has a eukaryotic serine/threonine kinase output domain rather than an HKM (Fig. 1). Taken together, our analyses suggest that plant-type phytochrome structure and function were established early in the Viridiplantae, concomitant with the loss of phycobiliprotein antennae that have been retained in glaucophytes.

Expression and Signaling Mechanisms. In view of the close evolutionary relationship of prasinophytes and streptophytes (Fig. 2), we investigated whether their phytochromes share a common signaling mechanism, i.e., light-dependent nuclear translocation, a hallmark of plant phytochrome signaling (25). Genetic systems are not available for the lineages harboring the newly discovered phytochromes. A series of RNA-seq and protein analyses were therefore performed on tightly synchronized mid-exponential growth *M. pusilla* cells, over a light:dark (diel) cycle. Under these conditions, the bulk of cells in the population was entrained to the same cell cycle phase at each harvest time point (Fig. 3*A*). Using monospecific antibodies raised against recombinant PCM and REC domains of MpPHY, we detected expression of the full-length protein in these cultures (*SI Appendix*, Fig. S2). MpPHY protein levels were constant throughout the diel (Fig. 3*A*). However, MpPHY accumulation in the nucleus was higher during the light period (Fig. 3*B*), demonstrating that redistribution to the nucleus occurs throughout the day as *Micromonas* replicates its genome, but has yet to divide (Fig. 3*A*). These results indicate that light-dependent nuclear translocation of phytochrome predates divergence of streptophytes and prasinophytes.

To examine light-mediated gene expression in *M. pusilla*, we profiled diel transcriptional responses using deep coverage directional paired-end RNA-seq. Unexpectedly, diel variation of *MpPHY* transcript abundance was pronounced (Fig. 3*C*) even though MpPHY protein abundance was relatively stable (Fig. 3*A*). In contrast to the light-mediated nucleus accumulation of MpPHY protein, maximal *MpPHY* transcript accumulation preceded that of most photosynthesis and tetrapyrrole synthesis genes (Fig. 3*D* and *SI Appendix*, Fig. S6 and Table S4). Notable exceptions were genes for both plastid-targeted ferredoxin-dependent bilin reductases (FDBRs) phycourobilin synthase (PUBS) and phycocyanobilin:ferredoxin oxidoreductase (PCYA), bilin chromophore biosynthetic enzymes that catalyze conversion of biliverdin to phycourobilin (PUB) and phycocyanobilin (PCB), respectively (26). Expression patterns of *MpPUBS* and *MpPCYA* differed, suggesting distinct roles in bilin-dependent signaling pathways. *MpPCYA* had relatively high abundance across time points with insignificant variations (*SI Appendix*, Table S4, $P > 0.05$). In contrast, *MpPUBS* exhibited a sharp predawn peak ($T3_R$) and significant fold-changes ($P < 0.01$ or $P < 0.001$) over time, similar to the pattern of *MpPHY* expression (Fig. 3*C*). This coordinated predawn expression peak implicates a clock-regulated, bilin-signaling pathway similar to the phytochrome-independent system proposed to anticipate the diurnal dark-to-light transition and the increase in photosynthesis-derived oxygen levels in *C. reinhardtii* (27).

Light Detection and Histidine Kinase Activities. We expressed the confirmed MpPHY PCM sequence in bilin-producing *Escherichia coli* cells. Unlike the characteristic red/far-red spectrum of plant phytochromes (5, 10), MpPHY exhibits an orange/far-red photocycle (Fig. 4*A*) with a blue-shifted, orange-absorbing dark state. This result is consistent with those obtained for other prasinophytes (20). In view of the shared domain structure of prasinophyte and streptophyte phytochromes (excepting the REC domain in the former), we undertook experiments to test whether the prasinophyte sensors exhibit histidine kinase catalytic activity. Attempts to express full-length MpPHY in *E. coli* yielded little soluble protein that could not be purified. However, recombinant expression of phytochrome from *D. tenuilepis* proved robust, enabling isolation of preparative quantities of a nearly full-length (DtPHY-ΔL) holoprotein lacking the three REC and nucleotide cyclase domains at the C terminus (Fig. 1). The photochemical properties of DtPHY-ΔL are similar to those of the MpPHY holoprotein (Fig. 4 *A* and *B*) and are nearly identical with the PCM-only version (DtPHY-PCM) reported recently (20). Recombinant DtPHY-ΔL exhibited light-regulated autophosphorylation activity at a level comparable with that of the cyanobacterial phytochrome Cph1 (Fig. 1) from *Synechocystis* sp. PCC 6803 (Fig. 4*C*). In contrast with Cph1, phosphorylation of the light-activated Pfr state of DtPHY-ΔL exceeded that of the orange-absorbing Po dark state. This light-regulated kinase activity was not observed for the H927Q mutant DtPHY-ΔL holoprotein lacking the conserved histidine autophosphorylation site, despite retention of wild-type spectral activity (Fig. 4 *B* and *C*). Taken together, these experiments demonstrate that DtPHY, and by extension prasinophyte phytochromes likely function as light-activated histidine kinases.

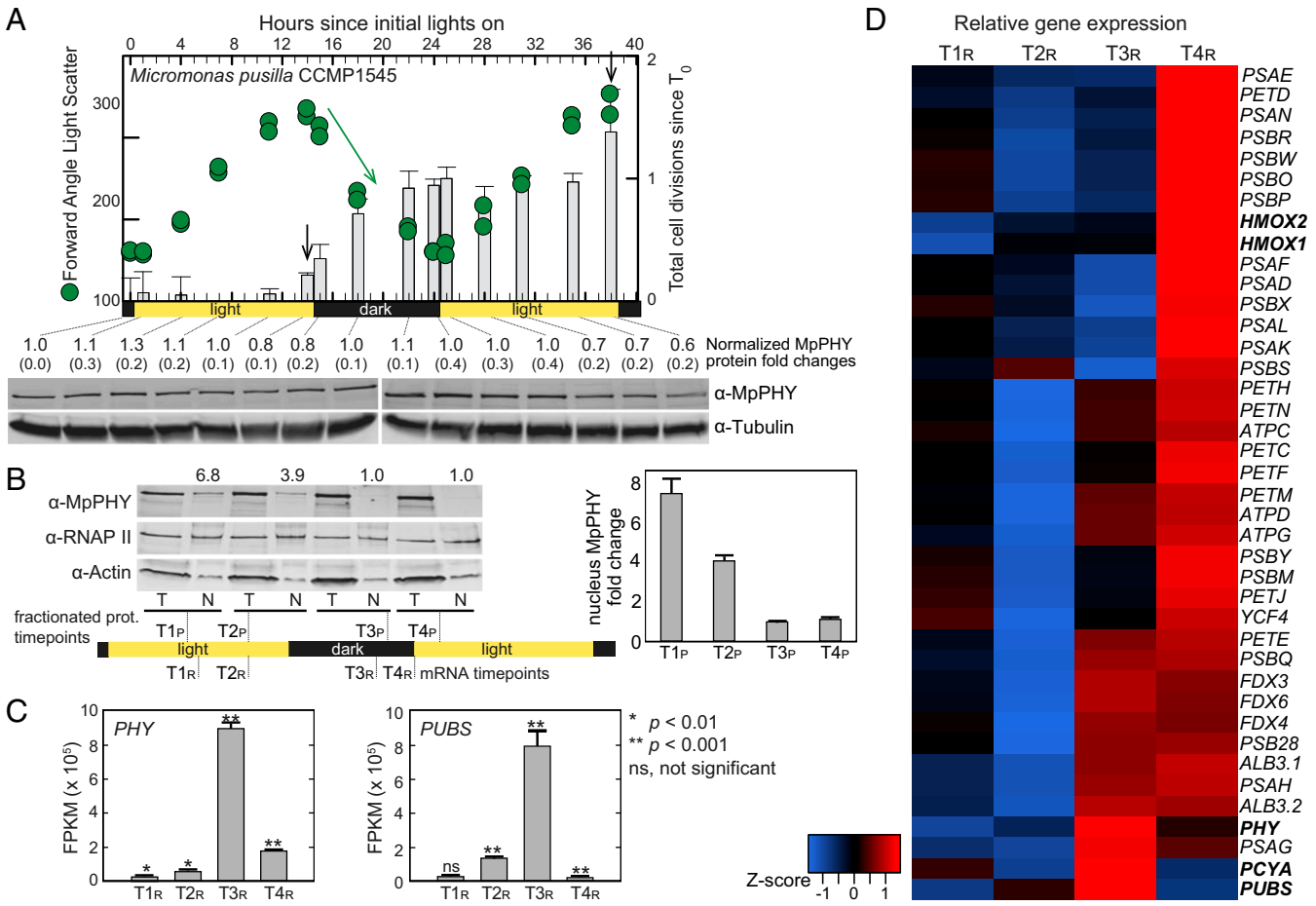

Fig. 3. Synchronized *M. pusilla* cells exhibit strong predawn phytochrome gene expression, preceding most photosynthesis genes and phytochrome protein translocation to nucleus. (*A*) *Micromonas* cells in mid-exponential growth exhibit synchronized division once per day. Cell size (green circles, represented as bead-normalized mean forward angle light scatter, FALS) increases throughout the photoperiod as cells prepare to divide and decreases (green arrow) once division begins at the onset of night (black arrow). Total cell divisions (bars) are shown since the start of the experiment. Division progresses into predawn hours and a second round commences at the end of the day 2 photoperiod (second black arrow). Immunoblot quantitation of MpPHY protein shows little variation from the first measurement (0.5 h before lights on), as determined from biological triplicates and normalized against alpha-tubulin (reported as fold change ± SD). (*B*) Immunoblot analysis of total (T) and nucleus-localized (N) MpPHY during the light period ($T1_P$ and $T2_P$), the subsequent dark period ($T3_P$), and the following morning ($T4_P$). Numbers over lanes indicate nucleus-localized MpPHY protein fold changes relative to $T4_P$, the earliest light period time point (as done in *A*) and normalized against RNA polymerase II (RNAP II). Bars and error bars represent the mean and SD of technical duplicates, respectively. (*C*) *MpPHY* and *MpPUBS* transcript abundances over the diel. Bars represent average quartile normalized fragments per kilobase of transcript per million mapped reads (FPKM) from biological triplicates and error bars represent the SD. "R" in the sample name indicates RNA time points. *T* tests between adjacent time points show significance (*, ** represent significance for comparisons with the preceding time point; symbols over $T1_R$ data represent a test between $T4_R$, just as lights came on, and $T1_R$). (*D*) *Z*-score analysis of *MpPHY*, heme oxygenases (*HMOX1* and *HMOX2*, responsible for initial chromophore synthesis steps), FDBRs, and photosynthesis-related genes (the latter in nonbold font). Relative change from mean transcript levels (log transformed) in negative (blue) or positive (red) directions is shown for each gene across time points. Upper-quartile normalized FPKM (± SD) are provided in *SI Appendix*, Table S4.

Discussion

Phytochrome gene transfer from an engulfed cyanobacterium that engendered the first eukaryotic plastid is a prominent hypothesis for the origin of plant phytochromes. Indeed, overall evolutionary relationships suggested by analysis of previously available PCM sequences lend support to this assertion (*SI Appendix*, Fig. S4) (14, 16). Furthermore, other cases of cyanobacterial EGT have been established in land plants (9). It also has been hypothesized that similarities between extant cyanobacterial and streptophyte phytochromes reflect convergent evolution (15). Our results establish a single Archaeplastida phytochrome clade, separate from that of extant cyanobacteria and indicate that an ancestral plant-like phytochrome evolved in the Viridiplantae before the divergence of streptophytes, prasinophytes, and chlorophytes.

A Eukaryotic Origin of Archaeplastida Phytochromes? Our results suggest that phytochromes were acquired in the last common ancestor of the Archaeplastida at, before, or soon after the primary cyanobacterial endosymbiosis and before the diversification of the major Archaeplastida lineages. We favor the hypothesis that a phytochrome PCM was present in the genome of the prephotosynthetic eukaryotic host that gave rise to the Archaeplastida (Fig. 2 and *SI Appendix*, Figs. S3 and S5). Alternatively, lateral transfer of a phytochrome gene (from another bacterial lineage) to cyanobacteria followed by EGT (9) of that phytochrome is also possible. However, available cyanobacterial genomes show no traces of such an event. We were only able to recover a topology suggestive of cyanobacterial origins using lower taxonomic sampling in a maximum likelihood reconstruction, but it lacked statistical support at the critical nodes (*SI Appendix*, Fig. S4). Moreover, cyanobacteria were not basal to the

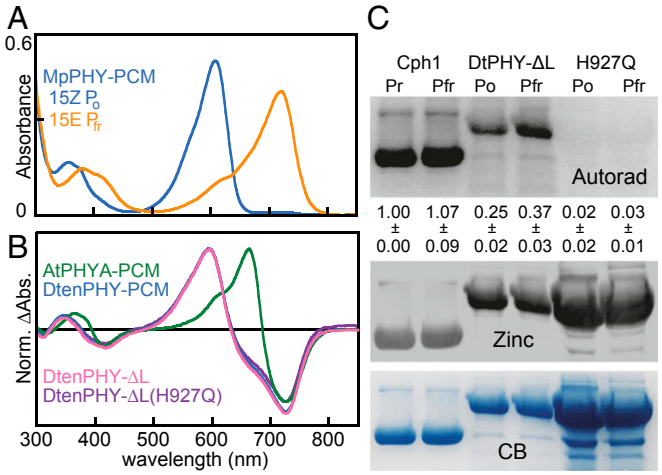

Fig. 4. Spectral properties and light-regulated protein kinase activities of recombinant prasinophyte phytochrome. (*A*) Spectral properties of the PCM of *M. pusilla* phytochrome (MpPHY-PCM). (*B*) Spectra of *D. tenuilepis* (DtPHY-PCM) and *A. thaliana* phytochrome A (AtPHYA-PCM) PCMs. Presence of a histidine kinase domain following the PCM (DtPHY-ΔL, DtPHY-ΔL H927Q) does not change *D. tenuilepis* phytochrome spectral properties (the three spectra overlap). The ΔL truncation of DtPHY lacks all three REC and CHD domains (Fig. 1), as does DtPHY-ΔL H927Q, which also lacks the conserved histidine autophosphorylation site. (*C*) Comparative kinase analysis of dark-adapted states of DtPHY-ΔL (Po), DtPHY-ΔL with single mutation (H927Q), and *Synechocystis* Cph1 (Pr) and their respective far-red absorbing Pfr states. *Top*, *Middle*, and *Bottom* represent autoradiograph, zinc blot, and Coomassie blue-stained images of the transblotted proteins (5 μg per lane). Numbers indicate kinase activity relative to Cph1 Pr by normalization of each sample against the zinc blot signal. Technical duplicates were analyzed.

Archaeplastida in the related Bayesian reconstruction. Increased taxonomic sampling provides a more unified topology between these phylogenetic methods, with statistical support at multiple nodes (Fig. 2). These results indicate that the last common ancestor of the Archaeplastida had a phytochrome of distinct origin from that of extant cyanobacteria.

Placement of cryptophyte phytochromes within the Archaeplastida is not surprising. *G. theta* whole genome analyses show significant red and green algal contributions to this derived algal lineage, making a green algal origin through EGT plausible (8). Although a red algal phytochrome origin could also be possible, phytochrome has not been observed in the limited set of rhodophyte genomes sequenced to date. In contrast to cryptophyte phytochromes, heterokont and fungal phytochromes are more distant from those of the Archaeplastida. Hence, we cannot dismiss the possibility of independent, possibly bacterial, origins for heterokont and fungal phytochromes from those of Archaeplastida and cryptophyte phytochromes.

It is clear that several Archaeplastida algal groups as well as cyanobacteria have lost phytochromes. This is true for chlorophytes, many class II prasinophytes (Mamiellophyceae; *SI Appendix*, Fig. S1), all rhodophytes sequenced to date, and for marine picocyanobacteria, such as *Prochlorococcus*. The patterns observed indicate these losses occurred as multiple independent events. Differential loss of genes encoding particular functions is considered a common mechanism behind specialization (28). The loss of phytochromes may reflect niche specialization, the sufficiency of other photoreceptors [e.g., phototropin, cryptochrome, rhodopsins (29), UVR8 (30), or phytochrome-independent bilin-based sensors (27)], some of which serve overlapping functions with phytochromes in plants (1, 13), and/or the evolution of new photoacclimative systems (31). The extent of overlap in niches occupied by various clades within each Mamiellophyceae genus is not well understood (32). Those that have lost phytochrome are picoplanktonic (≤2 μm diameter; *SI Appendix*, Fig. S1). Redundant or deleterious antagonistic functions could explain phytochrome losses in *Ostreococcus* and *Bathycoccus*, which have extremely reduced overall gene content, almost 2,000 less than their relative *Micromonas* (21, 33). Nevertheless, some *Micromonas* clades have also lost phytochrome. Thus, the drivers behind independent losses within the Mamiellophyceae genera are particularly intriguing in terms of how they might connect to niche differentiation. Because Mamiellophyceae algae have smaller genomes (13–22 Mb) than chlorophytes (46–138 Mb) (34), and reside in different ecosystems, the drivers behind loss events may be unrelated and remain an open question.

Evolution of Phytochrome Signaling. Our studies identify prasinophyte-like phytochromes as representing an ancestral state to plant phytochromes in PCM, dual PAS (Per/Arnt/Sim) repeat, and HKM regions, making modern prasinophyte phytochromes an attractive system for comparative studies with those of streptophytes. Multiple phytochrome output domain replacements have occurred in streptophyte lineages (35, 36) and streptophyte HKMs appear more highly derived than those of prasinophytes and other eukaryotic TCS (24). Moreover, plant phytochromes, which lack phospho-accepting REC domains and often the conserved histidine autophosphorylation site, can exhibit serine/threonine kinase activity (37). This contrasts with prasinophyte phytochromes, which retain ancestral TCS histidine kinase activity. Prasinophyte and streptophyte phytochrome families both exhibit light-dependent kinase activities, also contrasting with the light-inhibited kinase activity of Cph1 (38, 39). The shared signaling properties of prasinophyte and streptophyte phytochromes correlate well with the success of the chlorophyll-based light harvesting Viridiplantae lineage.

Phytochromes are also widespread in nonphotosynthetic organisms, including bacteria and fungi (3, 4, 16) (Fig. 2). In most cases, these proteins use biliverdin IXα (BV) as chromophore. BV is ubiquitous, owing to the widespread distribution of heme oxygenases used in heme detoxification and degradation (40), excepting obligate anaerobes (7). Bilin-based sensors such as phytochromes are well suited to integrate both light and oxygen signals because bilin biosynthesis is oxygen dependent (18). It is therefore plausible that the eukaryotic ancestor of the Archaeplastida lineage already possessed a BV-binding phytochrome light sensor before engulfing the cyanobacterium that became the plastid. Such prephotosynthetic eukaryotes may have used phytochromes for integrating environmental light and oxygen signals to induce photoprotective pathways, e.g., at dawn when oxygen evolution increases due to activity of nearby photosynthetic organisms. Indeed, *C. reinhardtii* uses a nonphytochrome-based retrograde bilin signaling system to anticipate diel oxidative stress during daylight (27). Ancestral phytochrome photosensors for light- and oxidative-stress anticipation, therefore, may represent an important innovation to entrain the circadian clock and to optimize cyclic light energy storage during daytime and utilization at night.

A Spectral Range Tuned for Aquatic Environments. The presence of phytochromes in multiple marine algae is surprising because red and far-red wavelengths are attenuated rapidly in seawater (41). However, the *Micromonas* phytochrome shows a photocycle better suited for life in aquatic environments, with a blue-shifted dark state detecting wavelengths attenuated less strongly in seawater than those detected by streptophyte phytochromes. Whereas spectral responses of phytochromes from important marine taxa, such as diatoms (17), remain uncharacterized, similar responses have recently been shown for a number of other algae, extending even into the blue (20). Thus, spectral tuning of phytochromes appears in eukaryotic algae from different

lineages, and even within specific groups such as glaucophytes and prasinophytes.

Our results have important implications for understanding marine phytoplankton, which as a whole are responsible for ~50% of global CO_2 uptake (42). We have also expanded prasinophyte genomic resources from one class (class II/Mamiellophyceae; composed of taxa that have reduced genomes) (21, 33) to six of seven total classes, as well as resources for other algae. The widespread primary producer *M. pusilla* provides a simplified model system to address the adaptive role(s) played by this photoreceptor family. It also provides a platform for investigating physiological and ecological consequences of phytochrome gene loss in other *Micromonas* species, related phytoplankton, and chlorophyte algae. Our findings underscore ancestral aspects of plant phytochrome signaling and photosensory adaptations for an aquatic lifestyle. The widespread occurrence and diversity of phytochromes in plants, heterokonts, cryptophytes, and prasinophytes provide new impetus for studies to understand adaptation and acclimation of major primary producers to the solar cycle.

Methods

Algal strains were grown under a 14:10 h light:dark cycle, monitored by flow cytometry or fluorometry, and harvested in exponential phase for DNA, RNA, or protein analyses. Paired-end Illumina sequencing [directional for *M. pusilla* Culture Collection of Marine Phytoplankton strain 1545 (CCMP1545)] was performed on 14 prasinophyte, chlorophyte, and glaucophyte strains; for 13 of these, transcriptome assemblies were constructed, which rendered total contig numbers between 5,937 and 34,476. Expression in *M. pusilla* CCMP1545 was analyzed using standard RNA-seq approaches, Northern/Western blotting, and mass spectrometry; light-regulated distribution of phytochrome was analyzed by subcellular fractionation. The photosensory PCM regions of *M. pusilla*, *D. tenuilepis*, and *Arabidopsis thaliana* phytochromes, and the PCM-containing dual PAS HKM output region of *D. tenuilepis* phytochrome were expressed in *E. coli* for photocycle measurements. For phylogenies, protein and nucleotide sequences were obtained from the transcriptomes generated here and GenBank, aligned using MAFFT and masked using MUST. Phylogenies were constructed using maximum likelihood and Bayesian methods with support computed using 1,000 ML bootstraps and posterior probabilities. Details are in *SI Appendix, Materials and Methods*.

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