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Clocks in Algae

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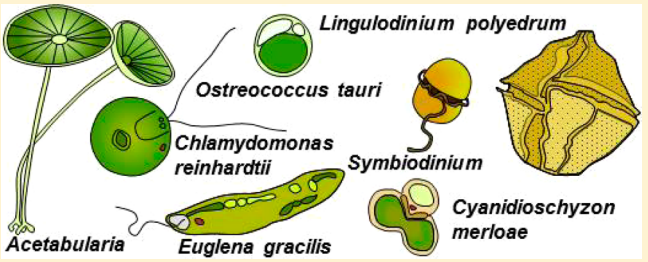
ABSTRACT: As major contributors to global oxygen levels and producers of fatty acids, carotenoids, sterols, and phycocolloids, algae have significant ecological and commercial roles. Early algal models have contributed much to our understanding of circadian clocks at physiological and biochemical levels. The genetic and molecular approaches that identified clock components in other taxa have not been as widely applied to algae. We review results from seven species: the chlorophytes *Chlamydomonas reinhardtii*, *Ostreococcus tauri*, and *Acetabularia* spp.; the dinoflagellates *Lingulodinium polyedrum* and *Symbiodinium* spp.; the euglenozoa *Euglena gracilis*; and the red alga *Cyanidioschyzon merolae*. The relative simplicity, experimental tractability, and ecological and evolutionary diversity of algal systems may now make them particularly useful in integrating quantitative data from "omic" technologies (e.g., genomics, transcriptomics, metabolomics, and proteomics) with computational and mathematical methods.



Algae represent one of the largest polyphyletic groups in the eukaryotic domain and vary greatly in morphology and ecology.[1] A feature of most algae is that they possess plastids derived from an endosymbiotic event between a cyanobacterium and a heterotrophic eukaryote ~1.5 billion years ago.[2] This event gave rise to three distinct groups of algae (glaucophytes, red algae, and green algae), with land plants arising from the green algal group. Through subsequent secondary and tertiary endosymbiotic events, many differing lineages of algae later emerged.[3] Circadian rhythms have been observed in a number of algal species spanning the breadth of these diverse groups. Circadian rhythms coordinate biological processes with the 24 h rotation of the Earth, ensuring that physiological conditions at different phases of the daily cycle are optimal for an organism to develop, grow, survive, and proliferate. The benefit of circadian timekeeping is that it allows an organism to anticipate periodic changes in the environment and prepare an appropriate response. For autotrophs that use light to produce energy and fix carbon, correct circadian timing is competitively advantageous to ready the photosynthetic machinery in advance of dawn.[4] Indeed, circadian rhythms have been observed in a range of processes in algae, including cell division, photo- and chemotaxis, bioluminescence, and protein synthesis.[5,6] In this paper, we use examples from the green and red algal lineages and describe the initial observations of circadian rhythms in emerging algal model organisms, report the recent model algae that have contributed to our understanding of the circadian clock and its control at genomic, transcriptomic, proteomic, and metabolomic levels, and discuss the experimental and mathematical tools available for researching circadian clocks in algae. We do not include brown algal species as fewer molecular and "omic" approaches have been used to investigate circadian rhythms in brown algae. Known clock components, outputs, and modulators in the algal species reviewed here are summarized in Figure 1.

The circadian clock system is conventionally conceived in terms of input pathways, the central oscillator, and the output pathways (Figure 2). The input pathways communicate timing information from the environment to the central oscillator, thereby setting the phase of the rhythm. Common inputs are the light to dark transitions imposed by day/night cycles and also daily changes in temperature. Once synchronized with the environment, the central oscillator is responsible for generating the 24 h rhythm that controls the rhythms of biological processes via the output pathways. In plants, these rhythmic processes include gene expression, protein phosphorylation, chloroplast movement, stomatal opening, leaf movement, and flowering.[7] The *Arabidopsis thaliana* circadian clock model has been extensively studied and forms the reference system for the clock mechanisms in the green lineage.[8,9] The central oscillators of all organisms comprise a set of transcriptional regulatory proteins that control the expression of their cognate genes, directly or indirectly, through a series of multiple interlocked transcriptional/translational feedback loops (TTFLs).[10] A significant literature covers the principal DNA-binding components of the *A. thaliana* clock, notably, the myb-related proteins [LATE ELONGATED HYPOCOTYL (LHY), CIRCADIAN CLOCK ASSOCIATED1 (CCA1), and LUX ARRHYTHMO (LUX)/PHYTOCLOCK 1 (PCL1)] and the pseudoresponse regulator family (PRR1−PRR9), which are included in current mathematical models of the system.[11,12] Work by T. Kondo and colleagues revealed that circadian rhythms in the cyanobacterium *Synechococcus elongatus* can be

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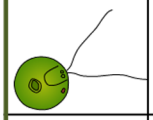
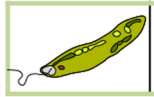
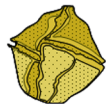
		Species	Genome size	Photoreceptors	Clock components	Clock outputs	Clock modulators	Refs
Chlorophyta		*Acetabularia* spp.	(C) *A. acetabulum* ~4,411 ESTs.	AR, ARII.		Photosynthetic activity, chloroplast shape and migration, rhythms of *A. acetabulum* P230 *A. cliftonii* P200, P130 synthesis.		33, 34, 37, 38
		*Chlamydomonas reinhardtii*	(N) 112 Mbp; 16, 709 genes. (C) 203.8 kb; 99 genes. (M) 16.0 kb.	CPH1 (plant CRY), aCRY (animal CRY), NPH1 (phototropin), HK-Rhodopsin.	ROC15, ROC40, ROC66, ROC 75, ELF4, EPR1, COP1 and TOC1 homologues.	UV sensitivity, photosynthesis, chloroplast metabolism, phototaxis, chemotaxis, cell division, adhesion, transcription rates [B1], chloroplast DNA supercoiling.	CHLAMY1 (subunits C1 and C3), XRN1, CK1, CK2, SGG, PDI2, PRX2.	6, 67, 68, 72, 82
		*Ostreococcus tauri*	(N) 12.6 Mbp; 7, 989 genes	LOV-HK, CPFs, HK-Rhodopsin.	CCA1, TOC1.	Cell division, photosynthesis, lipid metabolism, PRX.	CK1, CK2, GSK3, Ca^{2+}, multiple pharmacological agents.	69, 81, 92, 101, 102
Euglenoidea		*Euglena gracilis*	(C) 143, 170 Mbp.	PAC, putative blue and red light photoreceptors.		Cell cycle progression, UV radiation resistance.	PKA and PKB regulation of cAMP.	17, 20, 21, 23, 24
Dinoflagellata		*Lingulodinium polyedrum* (formerly *Gonyaulax polyedra*)	(N) 189.5 Gbp.	Putative blue and red light photoreceptors.	PP1 phosphatase.	Bioluminescence, aggregation, photosynthesis, cell division, protein synthesis, metabolism.	Gonyauline, creatine, nitrate, multiple protein kinase, phosphatase and synthesis inhibitors.	5, 41–44
		*Symbiodinium* spp. microadriaticum	(N) 616 Mbp; ~42, 000 genes.	Putative CRY1s, PHY and rhodopsin.		Karyokinesis, cytokinesis, motility, photosynthesis, HSP90 rhythmic gene expression.		120, 121, 123
Rhodophyta		*Cyanidioschyzon merolae*	(N) 16.5 Mbp; 5, 331 genes.	Putative CPF-derived CRY.		Cell cycle progression, phosphorylation of E2F.		116, 117

Figure 1. Summary of algal models used in circadian research. Examples of model species from four algal phyla are described in terms of known clock components, circadian outputs, modulators of the clock, and photoreceptors. Nuclear (N) and chloroplast (C) genome information is as of April 2014. Blue text denotes photoreceptor and clock components with either known sequence or functional data, whereas black text denotes where both are known.

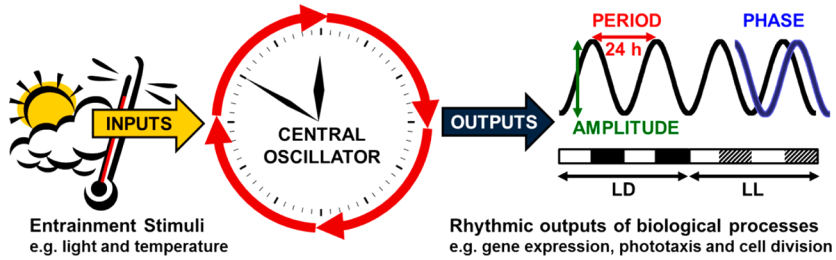


Figure 2. Simplified schematic of the circadian system. Entrainment stimuli via input pathways synchronize the circadian oscillator with the environment. Timing signals from the oscillator produce 24 h circadian rhythms of biological processes through output pathways. Common terms in chronobiology, period, amplitude, and phase are depicted. White and black boxes indicate light and dark (LD) diurnal intervals. White and hatched boxes show free-running conditions in constant light (LL), where day and night imposed by entrainment are known as "subjective day" and "subjective night", respectively.

governed by the sequential phosphorylation of three proteins, KaiA–KaiC, meaning that a nontranscriptional oscillator (NTO) can also drive circadian rhythmicity,[13] though even this NTO is coupled to transcriptional feedback in the intact cell. Though the Kai proteins are lacking in eukaryotes, circadian rhythms of peroxiredoxin (PRX) oxidation (discussed further below) persist in the absence of transcription and have been observed across eukaryotic taxa.[14] Therefore, in addition to the TTFL, a nontranscriptional oscillator (NTO) can also drive circadian rhythmicity.[15]

PIONEER ALGAL MODELS IN CIRCADIAN BIOLOGY

During the mid-20th Century, circadian rhythms were observed in the unicellular organisms *Euglena gracilis* and *Acetabularia* spp. and in the dinoflagellate *Lingulodinium polyedrum* (formerly known as *Gonyaulax polyedra*). The first measurements demonstrated circadian rhythms of phototaxis in *E. gracilis*, and this was soon followed by reports of temperature compensation in *L. polyedrum* bioluminescence rhythms and persistent rhythms of photosynthesis in enucleated *Acetabularia* cells.[16] Since these initial findings, some progress has been made in characterizing the molecular mechanisms of these circadian clocks. Sequence data for the chloroplast genomes of *Acetabularia* spp. and *E. gracilis* are available.[17,18] *L. polyedrum*

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has an exceptionally large genome with high-copy number tandem gene arrays; however, an extensive gene catalogue comprising ~75K contigs from RNA sequencing is now available.[19] It was, rather, the relative ease of dynamic time series studies in these unicellular species that allowed insightful and lasting contributions to the circadian field.

E. gracilis. In *E. gracilis*, for example, detailed physiological studies suggest that two independent circadian rhythms regulate cell cycle progression. The first control mechanism ensures that in photoautotrophic cell cultures of *E. gracilis*, the commitment to cell cycle progression is determined by the sensitivity to photoinduction in a circadian-phase-dependent manner. Cell cycle is arrested in the dark, but G2-phase cells can progress to mitosis only when they are photoinduced at subjective dusk.[20] The second mechanism gates the population growth by preventing G2 cells from entering mitosis during the day.[21] It was not established whether these were under the control of the same circadian oscillator. The signaling molecule cyclic adenosine monophosphate (cAMP) is thought to be involved in the circadian regulation of the *E. gracilis* cell cycle. Bimodal circadian oscillations of cAMP levels were observed,[22] and a continuous elevation of cAMP resulted in arrhythmic cell division.[23] cAMP signaling was proposed to be mediated by two cAMP-dependent protein kinases, PKA and PKB, at subjective dawn and dusk, respectively.[23] A blue light-dependent photoactivated adenylate cyclase (PAC) photoreceptor, comprising two homologous α and β subunits, has been identified in *E. gracilis*.[24] Each PAC has two catalytic domains and two blue light receptors using FAD (BLUF) domains, primarily found in prokaryotes. Photoactivation of *E. gracilis* PAC expressed in *Xenopus laevis* oocytes, HEK293 cells, and *Drosophila melanogaster* directly modulates intracellular cAMP levels such that targets of cAMP (e.g., PKA) are activated.[25] PAC has not yet been linked to the clock but is a candidate for a circadian photoreceptor. Action spectra from the photoinduction of *E. gracilis* cells indicate that further photoreceptors, including a phytochrome, may be involved in photoperiodism.[26]

***Acetabularia* spp.** *Acetabularia* is an organism of great historical significance in circadian biology. Extensive and robust circadian rhythms of photosynthesis have been demonstrated in enucleated *Acetabularia* cells, which persist in the presence of nuclear and organelle RNA synthesis inhibitors, because of highly stable cytoplasmic mRNAs.[27−29] Daily rhythms of photosynthesis in the absence of nuclear transcription challenged the early paradigm that a transcription−translation oscillator mechanism is essential to elicit these rhythms. Indeed, since these findings in *Acetabularia*, oscillator mechanisms independent of transcriptional−translational timekeeping have been widely identified: the Kai oscillator in *S. elongatus*; post-translational regulation of transcriptional−translational oscillator components in mammals, insects, fungi, and plants; and rhythms of PRX oxidation in human red blood cells and in the marine alga *Ostreococcus tauri*.[15]

From the 1960s to the mid-1980s, the H. G. Schweiger and T. Van den Driessche laboratories made a number of contributions to circadian biology in *Acetabularia* and in the process developed a method to stably transform the alga.[30−32] Their research described circadian rhythms of photosynthetic output and chloroplast migration; they and other groups found that the timekeeping mechanism resided in the nucleus of the cell.[33,34] Others, however, have argued that the nucleus has no role in the generation of circadian rhythms as rhythms of photosynthesis persisted in enucleated cells but that the nucleus may communicate phase information.[27] Much later followed a report that the nucleus plays no role in phase communication, in that cells receiving opposite light/dark (LD) entrainment from the nucleus-containing "rhizoid" half of the alga ultimately exhibit highly variable phases of rhythms between replicates.[35]

The photoreceptor rhodopsin, containing the chromophore retinal (a form of vitamin A), is responsible for vision in animals. Variants of these photoreceptors known as microbial rhodopsin are distributed widely across microorganisms.[36] Rhodopsin has long been postulated as the photoreceptor for *Acetabularia*, and in 2006, a rhodopsin was identified and named AR, followed by the identification of a homologue designated ARII, both of which function as light-driven H^+ pumps.[37,38] At present, there is no functional evidence indicating that this rhodopsin resets the clock, but it remains a promising candidate photoreceptor for circadian entrainment. These findings suggest a possible method by which light can enter the circadian system of *Acetabularia*, but in the absence of a defined molecular oscillator, we cannot know precisely how light drives this system. Research on the *Acetabularia* circadian clock dallied at the threshold of the molecular age but perhaps fell out of favor because of the long life cycle, labor-intensive culturing, and lack of genomic information compared to other algal systems.[30] It remains to be seen whether there will be a resurgence in this particular area of study.

L. polyedrum. Fundamental concepts concerning temperature compensation mechanisms and the effect of pharmacological inhibitors on circadian phase and period have arisen from early discoveries made in *L. polyedrum*. Over the past 60 years, outputs of the circadian system in *L. polyedrum* have been well-established.[5] Endogenous bioluminescence was its most distinctive feature, rhythms of which persist for more than 30 cycles in constant light (LL), acting as a natural reporter. J. W. Hastings and colleagues did much to establish this system, uncovering both canonical and unusual circadian behaviors, such as apparent timekeeping in cells made overtly arrhythmic at 12 °C.[39] B. M. Sweeney observed temperature compensation, the robustness of the circadian period at a range of constant temperatures, and rationalized it as a balance of period-lengthening and -shortening responses to temperature.[40] In a mixed population of *L. polyedrum* in which the bioluminescences of two cultures were out of phase, the peaks of bioluminescence merged after 10 days.[5] This phenomenon was not observed in cultures in which the medium was regularly replaced, suggesting that a chemical present in the media is associated with the changes in circadian period that allowed the cultures to synchronize. An endogenous cyclopropane carboxylic acid (termed "gonyauline") was identified in *L. polyedrum* extracts and caused period shortening.[41] Synthetic gonyauline and other compounds such as creatine had similar effects on circadian period, accelerating the clock by up to 5 h.[42] *L. polyedrum* treated with protein kinase inhibitors [6-dimethylaminopurine (6-DMAP) and staurosporine], protein phosphatase inhibitors (okadaic acid, cantharadin, and calyculin), and protein synthesis inhibitors (anisiomycin, cycloheximide, puromycin, and strepimidone) showed phase shifts and/or an altered free-running period, suggesting that protein phosphorylation has a role in regulating the circadian clock.[43] Levels of the tryptophan derivative melatonin, which is a rhythmic hormone in vertebrates, also cycle in *L. polyedrum* on a daily basis, with rapid onset upon darkness. Melatonin has been proposed to integrate circadian timing information with

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photoperiodism and temperature to regulate seasonal rhythms,[44] though the hypothesis has not received support recently.

Circadian rhythms can also be observed in dinoflagellate swimming behavior known as aggregation. Under certain experimental conditions, the free-running periods and phase response curves following dark and light pulses are different for aggregation and bioluminescence, suggesting they are under the control of two distinct oscillators, at least within the culture but presumably within single cells.[45,46] The aggregation oscillator is sensitive to blue and red light, whereas the bioluminescence oscillator is predominantly sensitive to blue light.[46] Under constant conditions of blue light with increasing intensity, there is a shorter period in bioluminescence, whereas the opposite is true for an increasing intensity of red light.[47] The opposing effects on the circadian rhythm suggest that two photoreceptors or pigments contribute to light input in this system. Taken together with the varying light sensitivities of the two oscillators, the photoreceptors may act differentially upon each oscillator mechanism.

Chlamydomonas reinhardtii. The green alga *C. reinhardtii* has long been a favorable model for circadian biology because of several measurable circadian-regulated physiological outputs, such as photo- and chemotaxis, cell division, and cell adhesion. Maximal levels of phototaxis in nondividing cultures of *C. reinhardtii*, maintained in constant dark (DD), and in dividing cultures, maintained in either 12 h/12 h light/dark (LD) or LL, were phased to the day/subjective day with a period of 24 h.[48] V. Bruce isolated mutants from a mutagenesis screen based on rhythmic phototaxis, exhibiting short period (~21 h) and long period (~28 h) phenotypes at 22 °C.[49] Crosses using long period mutants, designated "*period*" *per-1* to *per-4* (not homologous to mammalian and fly *per* genes), revealed that the period lengthening effect is additive, with single, double, triple, and quadruple averaging periods of ~27, 31, 35, and 40 h, respectively, in DD.[50] Circadian rhythms of *C. reinhardtii* phototaxis were also tested in space, where there was no influence of the exogenous entrainment cues imposed by the Earth's 24 h day/night cycles.[51] The wild type and a short period mutant kept the expected circadian periods, in space and on the ground, confirming that circadian rhythms are endogenous in origin and do not depend on cryptic entrainment cues. *C. reinhardtii* demonstrates circadian rhythms of chemotaxis to ammonium, peaking in the subjective night in cycles of LL; however, a maximal level of ammonium uptake is phased to subjective dawn, also in a circadian-dependent manner.[52] In phasing ammonium sourcing and ammonium uptake 6 h apart, *C. reinhardtii* might optimally find, acquire, and metabolize nitrogen before shifting into a phototactic state for efficient photosynthesis.

Cell division in *C. reinhardtii* is also gated by the circadian clock and is timed to coincide with the beginning of subjective night.[53] Interestingly, *C. reinhardtii* cells exhibit circadian-dependent variations in sensitivity to ultraviolet (UV) radiation, the effects of which are detrimental to nuclear DNA replication; UV sensitivity is also maximal at subjective dusk.[54] Commonly known as the "escape from light" hypothesis, circadian clocks might have conferred an early adaptive advantage by temporally segregating cellular processes that were attenuated by light into the subjective night phase.[55] The algal studies outlined above, and others in the same vein, were seminal in establishing and developing the field of chronobiology. Understanding the mechanisms of timing, in contrast, depended upon molecular and genetic tools.

ALGAE AS MODELS IN THE ERAS OF MOLECULAR BIOLOGY AND "OMICS"

Prior to work in *C. reinhardtii*, molecular analysis of circadian regulation was most advanced in *L. polyedrum*. The crucial, rhythmic bioluminescence is produced by the interaction between a unique circadian-controlled luciferase (LCF) and its substrate luciferin-binding protein (LBP). LCF and LBP are localized to scintillons, organelles that are pocketed in the vacuolar membrane, and interact upon an influx of protons.[56] Circadian control of daily protein synthesis of LCF, LBP, scintillons, and many other *L. polyedrum* proteins, e.g., glyceraldehyde-3-phosphate dehydrogenase (GAPDH), occurs at the translational, rather than transcriptional, level from a constant pool of long-life mRNA that is viable at all times of the circadian cycle.[57−59] A protein factor binds to a 22-nucleotide UG-repeat site within the 3′-untranslated region (3′-UTR) of *lbp* mRNA in a circadian-dependent manner.[60] The protein, termed CCTR (circadian-controlled translational regulator), is proposed to function as a clock-controlled repressor that prohibits *lbp* mRNA translation during the day by increasing binding at the end of subjective night, which coincides with a decrease in the level of LBP synthesis. Three proteins with RNA-binding domains that are rhythmically phosphorylated in LD have also been identified, but it would be necessary to examine the phosphoproteome under constant conditions to evaluate the role of these proteins in circadian timekeeping in *L. polyedrum*.[61]

C. reinhardtii. *C. reinhardtii* had been an early genetic model for circadian studies, supporting the identification of a handful of mutant lines with altered circadian rhythms.[49] M. Mittag later transferred her studies of circadian translational regulation from *L. polyedrum* to identify a similar mechanism in *C. reinhardtii*.[62] A *C. reinhardtii* CHLAMY1 binds to a UG-repeat region of the *L. polyedrum* 3′-UTR *lbp* sequence in a circadian-dependent manner, analogous to the (unidentified) *L. polyedrum* CCTR. CHLAMY1 consists of two subunits, C1 and C3, which are involved in the control of the period and phase of *C. reinhardtii*, respectively.[63] Phosphorylation of C1 and transcriptional control of *c3* expression are temperature-dependent.[64] An E-box element in the promoter region of *c3* is required for the circadian expression and temperature-dependent upregulation of *c3* as well as the coregulation of C3 by C1.[65] C3 interacts strongly with the exoribonuclease XRN1 at the beginning of the night or at low temperatures (18 °C), and knockouts of XRN1 result in low-amplitude rhythms of bioluminescence.[66,67] This suggests that XRN1 has a role in regulating transcript metabolism of clock-relevant mRNAs, which maintain robust circadian rhythms in *C. reinhardtii*, by preventing their degradation at night.

The availability of full *C. reinhardtii* nuclear (111 Mbp), chloroplast, and mitochondrial genome sequences allows a molecular approach to the analysis of the circadian clock. Putative clock genes have been identified from a library of *C. reinhardtii* insertional mutants that have defects in circadian period, phase angle, and amplitude.[67] "*Rhythms of chloroplast*" (*roc*) mutants that had severe effects on circadian rhythmicity were *roc15*, *roc40*, *roc66*, and *roc75*, the mRNAs of which all cycle in a circadian manner. The proteins encoded by these genes have conserved domains with *A. thaliana* proteins involved in the transcriptional−translational oscillator:

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ROC15 and ROC75 have glutamic acid-rich protein (GARP) domains shared by LUX and *ARABIDOPSIS* RESPONSE REGULATOR1 (ARR1); ROC40 has a Myb domain from LHY and CCA1; ROC66 has B-box and CCT domains from CONSTANS (CO) and CO-LIKE (COL) 1 and 9. *C. reinhardtii* also shares domain architecture with *A. thaliana* CONSTIUTIVE PHOTOMORPHOGENIC1 (COP1), EARLY-PHYTOCHROME-RESPONSIVE1 (EPR1), and *Mesembryanthemum crystallinum* EARLY FLOWERING4 (ELF4).[6] This study did not reveal *ROC* genes that were adequately similar to TIMING OF CAB EXPRESSION1 (TOC1), PRR7 and PRR9, or GIGANTEA (GI).[68] However, a putative *C. reinhardtii* homologue to TOC1 has been identified by sequence conservation in the receiver and CCT domains with plant TOC1 and PRRs.[69] Thus, functionally relevant clock components in *C. reinhardtii* share protein domains and rhythmic regulation with their homologues in *A. thaliana*. The genes for further *roc* mutants are now being identified, such as a protein N-terminal acetyltransferase, which may be required to maintain robust circadian rhythms.[70] Their roles within the *C. reinhardtii* molecular oscillator need to be further elucidated, and experimental tools are available for this task.

The repertoire of photoreceptors that might mediate light input signaling to the circadian clock starts from the observed conservation in *C. reinhardtii* of photoreceptors such as cryptochromes (CRYs), and a phototropin (PHOT).[6] The sequences of blue light sensitive CRYs are highly homologous with those of DNA photolyases but do not retain this function; higher-plant CRYs regulate flowering time and development,[71] whereas animal CRYs function as transcriptional repressors in the clock, with or without a photoperceptive role. It is now understood that *C. reinhardtii* does not possess a red light-absorbing phytochrome, but instead an atypical animal-like cryptochrome (aCRY) that absorbs red (635 nm) and yellow (590 nm) light as well as blue (465 nm).[72] The aCRY response to blue or red light resulted in an upregulation of many genes involved in the light-harvesting complex, chlorophyll and carotenoid biosynthesis, nitrogen metabolism, and the circadian clock. In blue or red light, there is strong upregulation of the gene encoding the C3 subunit of CHLAMY1 and modest upregulation in the genes encoding the C1 subunit of CHLAMY1, ROC55, ROC66, CO, and CK1. Though the RNA levels of genes encoding both the CHLAMY1 subunits and various ROC genes are regulated by light, the consequences for circadian resetting are not yet understood.

The last potential blue/UV-A light sensitive photoreceptor is a PHOT, with interacting photoreceptive light−oxygen−voltage (LOV) domains that modulate the kinase domain.[73] PHOTs control stomatal opening, chloroplast migration, and phototropism in plants but have no direct effect on the circadian clock.[74] As other LOV domain proteins affect the circadian oscillator in higher plants,[75] there is the possibility of evaluating whether PHOT has an input into the circadian clock in *C. reinhardtii*.

Two light input mechanisms have been demonstrated. Proteasomal degradation of the DNA-binding ROC15 protein is involved in clock resetting upon input of blue, green, and particularly red light, in a circadian-phase-dependent manner.[76] *C. reinhardtii* has two isoforms of a plantlike cryptochrome, *Chlamydomonas* Photolyase Homologue1 (CPH1), both of which accumulate in the dark and are rapidly degraded by red or blue light-induced proteolysis.[77] *C. reinhardtii* strain CC-124 (a nitrate reductase 1 and 2 deficient WT strain) is highly sensitive to blue, green, and red light, and the circadian clock can be reset with a short light pulse.[78] An RNA interference strain with a reduction in the level of CPH1 showed increased sensitivity to blue light, suggesting that CPH1 functions as a negative modulator of the *C. reinhardtii* circadian clock resetting response.

It is interesting that while components of the transcriptional−translational circadian oscillator are not well conserved across eukaryotes, clock-related protein kinases and phosphatases are, thereby supporting a case for an ancestral circadian clock that is likely to involve phosphorylation-based signaling.[15] Clock-related protein kinases and phosphatases in *C. reinhardtii* are well-conserved with higher plants, fruit flies, and mammals. Notable examples include CASEIN KINASE 1 (CK1), CASEIN KINASE 2 (CK2), and SHAGGY (SGG), the GLYCOGEN SYNTHASE KINASE 3 (GSK3) fly orthologue;[6] 202 proteins (identified by more than two peptides) were identified by proteomics of the *C. reinhardtii* eyespot, including CK1.[79] Functional studies show that CK1 has major roles in hatching, flagellum formation, and circadian regulation of phototaxis. Phosphoproteomics of the *C. reinhardtii* flagellum has revealed targets of CK1, including the kinases GSK3 and MITOGEN ACTIVATED PROTEIN KINASE 7 (MAK7).[80] The ROS defense system has more recently attracted attention as a target for a distinct circadian oscillator, from work in *O. tauri* (below) and other systems. Peroxiredoxins detoxify ROS, notably hydrogen peroxide (H_2O_2). Oxidation of peroxiredoxin is a biomarker for post-translational circadian rhythmicity across eukaryotes, furthering the case for nontranscriptional circadian timekeeping.[81] PROTEIN DISULFIDE ISOMERASE 2 (PDI2), a protein involved in nascent protein folding, forms a complex with 2-CYS PEROXIREDOXIN (PRX2) in *C. reinhardtii*, which is enriched during subjective night.[82] Overexpression of PDI2 causes a slight lengthening of the period but a significant phase shift of the circadian rhythm of phototaxis, linking PRX to the canonical, transcriptional−translational oscillator mechanism.

The rapid culturing, simple genetic manipulation, and availability of mutant strains are just some of the plentiful attributes that have made *C. reinhardtii* a useful system for molecular genetics. The systematic genetic dissection of the circadian clock mechanism has begun with the identification of conserved plantlike oscillator components. The forward genetic approach is ongoing and could still reveal novel clock-relevant genes. Though plantlike in its capacity for chloroplast-based photosynthesis, *C. reinhardtii* shares genes with animals derived from the last common ancestor of plants and animals. The genome sequence of *C. reinhardtii* revealed that there are 706 encoded protein families shared with *Homo sapiens* but not with *A. thaliana* (and 1879 protein families shared with only *A. thaliana*), which are enriched for eukaryotic flagellum (cilium) functions that are not found in plants.[83] As mentioned previously, clock-relevant protein kinases are present in the flagella of *C. reinhardtii*.[80] In addition, class III guanylate and adenylate cyclases, which catalyze the synthesis of cyclic guanosine monophosphate (cGMP) and cAMP, respectively, are represented by 51 gene members in *C. reinhardtii*. These cyclases, found in animals but controversial in plants, serve as second messengers in signal transduction pathways and are required for circadian clock function in mammalian systems.[84,85] Though it has not been fully explored, there is a potential role for these animal-like components in the circadian system of *C. reinhardtii*, raising interesting questions about the

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evolution of circadian clocks. The availability of microarray, proteomic, and metabolomic data positions *C. reinhardtii* well in the era of systems biology.[80,86−88]

O. tauri. *A Reduced Plant Circadian Clock.* The smallest free-living eukaryote, *O. tauri*, is fast becoming a useful model organism because of its small, sequenced genome, encoding 7989 proteins, with minimal genetic redundancy.[89,90] The position of *O. tauri* in the earliest diverging class of the Chlorophyta, the Prasinophyceae, places this alga well for extrapolating hypotheses regarding higher plants in the green lineage.[1] The majority of differentially expressed genes in *O. tauri* cells exposed to a 12 h/12 h LD cycle are transcriptionally regulated at the light-to-dark transition, including genes involved in cell cycle, DNA replication, nuclear transcription, and photosynthesis.[91,92] The short culturing time, daily synchronized binary cell division, and the ability to perform stable transformation and utilize transcriptional and translational luciferase reporters for the clock make *O. tauri* an attractive experimental model for circadian biology.[69,93,94] Misexpression lines can be produced under a phosphate-inducible promoter.[69,94−96] The lack of a cellulose cell wall facilitates simple organelle enrichment and protein extraction, allowing large-scale analysis of the *O. tauri* proteome and phosphoproteome by mass spectrometry. In *O. tauri* grown in 12 h/12 h LD cycles, 27% of the predicted proteome was detected,[97,98] including phosphopeptides from proteins relevant to the circadian clock (CO, CRY1, LHY, TOC1, CK1, and GSK3). No forward genetic methods have been developed as yet, hampering the identification of clock components that lack homology to those of other organisms, but a gene knockout protocol using homologous recombination was recently published.[95]

F. Y. Bouget identified two major plant transcriptional regulators, TOC1 and CCA1, that are well-conserved in *O. tauri* at the respective PRR, CCT, and Myb domains.[69] No obvious homologues of other plant clock genes such as GI (ZEITLUPE), ZTL, and ELF3 and ELF4 exist, but there are representatives of the GARP and B-box transcription factor families, similar to *A. thaliana LUX* and *CO*, respectively.[69] Table 1 compares the components of the *O. tauri* and *A. thaliana* clocks. Circadian regulation of TOC1 and CCA1 has been observed at the transcriptional, RNA, and post-translational levels.[69,99,100] Light inputs also affect multiple processes, notably TOC1 protein degradation, resetting the clock and adjusting the waveforms of clock gene expression under different photoperiods. The level of expression of *TOC1* peaks at subjective dusk, similar to that of the *A. thaliana* homologue. The level of *TOC1* expression falls as that of CCA1 rises, consistent with CCA1's binding to the Evening Element in the TOC1 promoter to repress *TOC1*, as in *A. thaliana*.[69] However, the level of expression of *O. tauri CCA1* peaks earlier during the subjective night and begins decreasing by dawn, unlike that of its plant homologue that peaks just after dawn (Figure 3). Taken together, the circadian oscillator in *O. tauri* is considerably reduced relative to that of *A. thaliana*, but TOC1 and CCA1 do not function alone in the clock mechanism.

Two classes of photoreceptor have been identified and characterized in *O. tauri*. LOV-histidine kinases (LOV-HKs) are evolutionarily distinct from other LOV domain-containing proteins, i.e., phototropins, and are found in prokaryotes and in certain algal groups, including a single gene in *O. tauri*.[101] Photochemical analyses showed that the LOV-HK protein behaved in a manner similar to that of plant phototropins, with a maximal absorption at 450 nm. Transcripts of *O. tauri LOV-HK* are strongly rhythmic in LD cycles, peaking at dawn. A translational reporter for LOV-HK shows its peak signal during the middle of the day in both LD and LL, indicating that LOV-HK is circadian-regulated. Under constant blue or red light, overexpression and downregulation of *LOV-HK* caused arrhythmia of CCA1 in a luciferase reporter line, suggesting that the role of LOV-HK in the clock is not blue light-dependent. Thus, LOV-HK is a candidate clock component that also integrates multiple light cues.

In addition to LOV-HKs, the *O. tauri* genome has five potential blue light-sensing proteins of the cryptochrome/photolyase family (CPFs) but lacks plantlike CRYs.[102] The CPF family contains the groups, cyclobutane pyrimidine dimer (CPD) photolyases (including OtCPF3-4), 6-4 photolyases (including OtCPF1), and the *Drosophila*, *Arabidopsis*, *Synechocystis*, and human (DASH) cryptochromes (including

Table 1. Clock Components Present in *A. thaliana* and *O. tauri*

	A. thaliana	*O. tauri*
clock components		
LHY/CCA1	2	1
RVE family	5	0
TOC1 and PRR family	4	1
GI	1	0
LUX and GARP family	56	2
ELF3	1	0
ELF4	4	0
ZTL	3	0
clock-relevant kinases		
CK1	12	1
CK2α	4	1
CK2β	4	1
GSK3	10	1
photoreceptors		
CRY	2	0
CPF	3	5
PHY	5	0
PHOT	2	1
LOV-HK	0	1
HK-rhodopsin	0	1

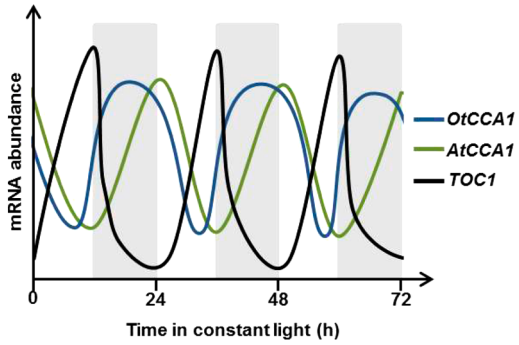

Figure 3. Illustration of *CCA1* and *TOC1* transcript expression patterns. In *O. tauri* and *A. thaliana*, the level of expression of *TOC1* peaks at subjective dusk (gray), falling as that of *CCA1* rises. *O. tauri CCA1* has an earlier and broader peak than *A. thaliana CCA1*. The level of *O. tauri CCA1* starts decreasing before subjective dawn (white), whereas that of *A. thaliana CCA1* peaks just after dawn. Figure modified from that of ref 69.

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OtCPF2). Characterization of *O. tauri* CPF1 and CPF2 reveals that both proteins retain their photolyase function for DNA damage repair, a property considered to be lacking in true CRYs. mRNA levels of *CPF1* and *CPF2* are rhythmic in LD. Only *CPF1* levels rise in anticipation of dawn and remain rhythmic under LL. Period lengthening in *CPF1* knockdown antisense lines suggests this photolyase might also control the clock mechanism. Interestingly, it can inhibit CLOCK:BMAL transcription in mammalian cells, in a manner akin to that of mammalian CRY.[102]

A Universal Marker for Circadian Rhythms. J. S. O'Neill, A. J. Millar, and colleagues demonstrated that *O. tauri* is capable of 24 h timekeeping without transcription, recalling the earlier observations in enucleated *Acetabularia* and in *S. elongatus*.[81] Rhythms of transcription and translation of *CCA1* reporters that persist in LL were abolished in DD. Luciferase expression collapsed, and all transcription in *O. tauri* stopped. Several independent lines of evidence demonstrated that rhythms were maintained. When the algae were returned to LL, rhythms of bioluminescence were restored at a phase that reflected the prior oscillation rather than only resetting upon transfer to light.

The nontranscriptional oscillation was revealed more directly through the persistent rhythms of peroxiredoxin oxidation in DD, which are resistant to inhibitors of cellular transcription, translation, and proteasome function.[14,81,100,103] In the light, the canonical transcription-translational circadian clock and the proposed nontranscriptional oscillator are coupled: the PRX rhythm is then sensitive to proteasome inhibitors and mutations in the transcriptional clock, though it may also be less robust.[14,100,103] The two clocks become uncoupled in DD, providing a unique experimental feature whereby both clock systems can be tested in the same cell type under different conditions. The circadian period in LL is affected by pharmacological inhibitors that target mammalian clock proteins absent in *O. tauri*; this may be taken either as evidence of unknown target proteins in the transcriptional clock of the alga or as evidence of additional, shared drug targets in the nontranscriptional oscillator that controls PRX in mammals, algae, and likely other domains of life.[14,81]

Among these pharmacological results, the ubiquitous effects of protein kinase inhibitors and early data on the effect of a CK1 inhibitor on the PRX rhythm suggest that protein kinases likely contribute to nontranscriptional timekeeping.[81] Kinases that modulate the transcriptional regulators of the circadian clock through phosphorylation are remarkably well conserved across eukaryotes.[15] The minimal genomic palette of *O. tauri* might make this unknown clock system easier to identify than in more complex organisms. Recent phylogenetic analysis of the *O. tauri* kinome revealed a reduced set of 133 protein kinase genes (compared to >1000 in *A. thaliana*) that still includes almost all eukaryotic protein kinase families, sometimes with remarkable sequence conservation.[104] Conserved phosphorylation sites have been identified for *O. tauri* CK1 and CK2, signaling components that are involved with the transcriptional/translational loops of the circadian clock.[81,104,105] In addition, CK2 phosphorylation motifs in *O. tauri* CCA1 are conserved with those in *A. thaliana*.[104] Given that land plants and *O. tauri* share a common algal ancestor, it is surprising that *O. tauri* CK1 is more closely related to CK1 isoforms from *Homo sapiens* than to CK1 isoforms of *A. thaliana*. Nonetheless, this pattern of relatedness was repeated in several kinase families. CK1 has yet to be involved in the circadian system of plants; however, it does contribute to circadian timekeeping in *O. tauri*.[105] Overexpression of CK1 lengthens the period of the circadian clock, as do several CK1 inhibitors.[81,105]

Phosphoproteomic analysis of the CK1-overexpressing *O. tauri* lines revealed phosphosites of significant differential abundance, and it was observed that many phosphorylation motifs conserved with the *H. sapiens* CK1ε isoform were upregulated. This suggests that *O. tauri* CK1 acts on the clock in a manner similar to the way CK1ε acts on the *H. sapiens* clock. The naturally occurring *tau* mutation of CK1 in the Syrian hamster shortens the circadian period; however, overexpressing the equivalent mutant protein in *O. tauri* causes period lengthening, similar to that of wild-type CK1.[106] There is clearly a role for kinases in the *O. tauri* clock, but identifying the clock-relevant targets and how they fit together in the canonical and nontranscriptional timekeeping mechanisms is one current challenge.

A Simpler Target for Modeling Circadian Clocks. The reduced genetic redundancy and tractability of reporter gene assays of *O. tauri* make it a useful model for applying mathematical approaches to understand circadian timing.[69] Models of *TOC1* and *CCA1* in a simple two-gene feedback loop have been developed with three levels of biochemical detail, each of which can reproduce a broad set of experimentally determined clock dynamics under different conditions.[107−109] In the simplest models, all processes were modeled with input from the photoperiod or a single light input represented regulation of the TOC1 protein under a more limited set of conditions.[107,108] The former approach suggested that circadian regulation of the light inputs conferred robust timing. In the latter approach, a three-component "repressilator" model in which TOC1 functions as a repressor matched the data for CCA1 and TOC1 reporters as well as or better than the two-gene model. Indeed, recent data show that *A. thaliana* TOC1 is a repressor;[110,111] the other two models assume that *O. tauri* TOC1 is an activator of *CCA1*. The feasibility of the three-gene model can be understood intuitively, from the fact that the level of CCA1 protein expression can fall well before the level of *TOC1* expression rises (Figure 3). In the two-gene model, the level of *TOC1* RNA should rise as soon as levels of its repressor CCA1 fall. In the three-gene alternative, the observed interval leaves time for expression of a putative inhibitor of *CCA1*, which is then repressed by the rising level of TOC1.

The most detailed model has five light inputs, each of which has a unique contribution to the dynamics of the clock model.[109] Light signaling can be circadian-modulated and photoperiod-dependent because of the interactions of other clock components in this model, rather than being specified separately by the modeler. Theoretical arguments suggest that flexible timing by circadian clocks enhances the capacity for an organism to respond to multiple, clock-relevant changes in the environment.[111] The original proposal suggested that flexibility arose from the number of feedback loops in the clock mechanism,[111] which would be limited in *O. tauri* because of the few clock components in its reduced genome. However, the alga's rhythms were clearly timed flexibly under different light conditions; moreover, the detailed, single-loop model reproduced this regulation, so it need not arise from components that are missing from the model, such as the nontranscriptional oscillator.[109] Mathematical analysis showed that flexibility of the *O. tauri* oscillator model was achieved through the multiple light inputs acting on the clock components, whereas in *A.*

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thaliana, flexibility is governed more by the complexity of the loop architecture.[112] Consistent with the light dependence of the algal clock, it resets very rapidly after a change in photoperiod, whereas transient cycles are observed while the many loops of the *A. thaliana* clock realign to new phase relationships.[112,113]

A Tractable Model System. Understanding the biological functions of large genomes is inevitably complicated. Comparative analysis of plant and algal systems may help to reveal their benefits and limitations in circadian timing, as an example of dynamic biological regulation. Taken together, *O. tauri* is emerging as a tractable model for systems biology, which is also of ecological relevance. Phytoplankton sustains the marine food web, accounting for around half of global carbon fixation. *O. tauri* serves as a relevant model for aquatic ecology and experimental evolution and has been used to measure physiological changes in response to changing climate conditions, such as elevated levels of CO_2.[114]

A REDUCED RED ALGAL MODEL FOR CIRCADIAN BIOLOGY

Cyanidioschyzon merolae shares many of the attributes that make *O. tauri* a useful model organism. *Cy. merolae* has a small 16.7 Mbp, sequenced genome and is readily transformable, with a cell cycle that is well synchronized to LD cycles giving daily binary cell division.[115] Recently, cell division of *Cy. merolae* has been shown to be regulated by the circadian clock during the subjective night and is determined by whether the cells have reached a threshold level of photosynthetic growth.[116] The retinoblastoma tumor suppressor pathway regulates cell cycle at the G1/S transition in eukaryotic cells. *Cy. merolae* encodes components of this system, including cyclins, cyclin-dependent kinases (CDKs), and E2F. E2F is a transcriptional activator of S-phase genes, and the time-dependent phosphorylation of E2F advances the G1/S transition during the subjective night, occurring even in the absence of cytosolic translation and in DD.[116] The cell cycle becomes uncoupled from the circadian clock in lines expressing a phosphomimic of E2F, and these cells undergo an increase in oxidative stress. Circadian gating of cell cycle progression is proposed to protect *Cy. merolae* from photosynthesis-derived ROS.

Little is known about how light signals affect the *Cy. merolae* system, but similar to *O. tauri*, CPFs are thought to be involved.[117] Chloroplast gene expression and mitochondrial gene expression are regulated by light cues and the cell cycle.[118] Eighteen genes with transcript profiles, which were highly responsive to light and which then correlated with the transcript profile of the light-responsive gene *psbD*, had a gradual predawn induction, suggesting that they are circadian-controlled. The expression of *psbD*, which encodes the D2 protein of photosystem II (PSII), is controlled by the nuclear-encoded sigma factor *SIG2* that allows binding of RNA polymerase to promote *psbD* transcription. Sigma factors have an important role in communicating timing information from the nucleus to regulate the circadian rhythms of chloroplast gene expression in plants.[119]

CIRCADIAN REGULATION IN CORAL–ALGAL SYMBIONTS

A fascinating area of algal chronobiology is how timekeeping is synchronized in symbiotic organisms, where each organism in the partnership contributes its own clock system. This is widely relevant to eukaryotes, as it likely also occurred before the ancestral chloroplast (and possibly the mitochondrion) lost its circadian clocks to the nucleus. Scleractinian coral (Cnidaria) forms the basis for the entire coral reef ecosystem, and the photosynthetic algal dinoflagellate *Symbiodinium* spp. are found in the endodermal tissue of the coral gastrovascular cavity.[120] In exchange for carbohydrates, the coral provides *Symbiodinium* with essential nutrients. Cell division and motility cycle diurnally in LD in *Symbiodinium microadriaticum*; in LL and DD, both motility and cytokinesis are under circadian regulation.[121] Photosynthetic processes such as oxygen evolution, PSII yield, and concentrations of pigments are rhythmic under constant conditions in both free-living *Symbiodinium* and *Symbiodinium* associated with the coral.[122,123] Circadian rhythmicity can also be observed in transcripts of *OXYGEN EVOLVING ENHANCER1* (*OEE1*), required for water molecule oxidation in PSII, peaking during the subjective day. *Symbiodinium* as a model for symbiotic circadian biology is in its early stages, so molecular components of the core oscillator have yet to be determined. Photosynthesis in *Symbiodinium* means that the coral tissue is hyperoxic by day and hypoxic by night. In response to cues from *Symbiodinium*, expression levels of genes that have a function in oxidative stress, such as *ferritin-H*, *heme-binding protein 2* (*HeBP2*), and *catalase*, peak during the day in the coral *Acropora millepora* under LD cycles.[124] Genes encoding molecular chaperones such as HEAT SHOCK PROTEIN 90 (HSP90) are rhythmic under both diurnal and constant conditions. HSP90 has been linked to the circadian clock by regulating the autophosphorylation of GSK3, and inhibition modulates the circadian period.[81,104] The coral–algal association provides another platform upon which to investigate circadian regulation of stress protection and reinforces the link between the cellular redox state and the circadian clock.[14]

The periodicity of oxygen evolution in free-living *Symbiodinium* is preserved under constant conditions under blue or red light.[123] In LD and under all light conditions tested, the period was approximately 24 h. Under the same light conditions but in LL, the clock was slightly faster in high blue light irradiance (50 μmol m^{-2} s^{-1}) or in low red irradiance (25 μmol m^{-2} s^{-1}). When in association with the coral *Stylophora pistillata* under free-running conditions, the alga has longer and shorter periods in high irradiance of red and blue light, respectively. Two putative CRYs and a phytochrome (PHY) have been identified in *Symbiodinium*, and the mRNA level of each of these potential photoreceptors exhibits rhythmicity under LD and LL conditions. A method for creating mutant lines of *Symbiodinium* has not been established, so the exact roles of CRYs and PHY in mediating the input of light into the circadian clock have yet to be fully elucidated.[123]

CONCLUSIONS

For more than half a century, algae have served as experimental models used to understand the mechanism of circadian timekeeping. Organisms such as *E. gracilis*, *Acetabularia* spp., and *L. polyedrum* were easy to use and had measurable physiological characteristics that reflected the outputs of circadian rhythms. As molecular and "omic" technologies advanced, green algae such as *C. reinhardtii*, and more recently *O. tauri*, have enhanced our understanding of the molecular basis of circadian regulation. The ability to perform genetic manipulation and the availability of genomic information were pivotal in identifying candidate genes for the circadian oscillator

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in these organisms. Validating the function of clock-associated genes in dynamic time series will require genetic manipulation and transcript, protein, and metabolic data. Metabolic profiling of algae is becoming increasingly important, as they offer a rich diversity of compounds that have economic value for food and pharmaceutical industries, biofuel production, and public health.[125] The simplicity of some algal systems offers particular advantages for the investigation of the circadian regulation of metabolic networks.

In this review, we have described both historical and contemporary model organisms that have been used in circadian research spanning the different algal groups. Algae are a diverse and extensive group of organisms, and it is worth noting that copious data sets are accumulating from less extensively studied algal species, such as time series microarray data from the dinoflagellates *Pyrocystis lunula* and *Karenia brevis* under several conditions.[126,127] Redox regulation of clock-relevant protein kinase genes such as the GSK3 orthologue SHAGGY and cAMP-dependent kinase was observed in *P. lunula*, furthering the case for evolution of an endogenous timekeeping mechanism under the selection pressures imposed by the growing cellular presence of free radicals.[14,126] The ecological relevance of some algal groups, especially in the marine environment, makes them more interesting than laboratory model species. However, one of the limitations regularly encountered in high-throughput "omics" screens is the identification of genes and proteins with unknown function. More functional characterization would reduce the amount of relevant information, for example, in circadian time series studies, which is poorly used because of incomplete annotation. Here, the small genomes of some algal species offer significant advantages, which must now be combined with better technologies for genetic manipulation and phenotyping. Better consolidation of all the information coming from circadian research in algae will help to determine common links between timekeeping mechanisms in algal species as well as identifying missing information that can direct future research to unique clock mechanisms within the diversity of algal genomes. This will aid our understanding of the molecular evolution of clocks, perhaps uncover a universal core oscillator, and inform pressing research areas such as renewable energy and the response of the biosphere to global change.

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ABBREVIATIONS

3′-UTR, 3′-untranslated region; 6-DMAP, 6-dimethylaminopurine; aCRY, animal-like cryptochrome; AR, *Acetabularia* rhodopsin; ARR1, *Arabidopsis* response regulator1; B-box, B-box-type zinc finger domain; BLUF, blue light-using FAD; cAMP, cyclic adenosine monophosphate; CCA1, circadian clock associated1; CCGs, clock-controlled genes; CCT, Constans, CO-like, and TOC1; CCTR, circadian-controlled translational regulator; CDK, cyclin-dependent kinase; cGMP, cyclic guanosine monophosphate; CK1, casein kinase 1; CK2, casein kinase 2; CO, Constans; COL, Constans-like; COP1, constiutive photomorphogenic1; CPD, cyclobutane pyrimidine dimer; CPF, cryptochrome/photolyase family; CPH1, *Chlamydomonas* photolyase homologue1; CRY, cryptochrome; DASH, *Drosophila*, *Arabidopsis*, *Synechocystis*, human; DD, constant dark; ELF, early flowering; EPR1, early-phytochrome-responsive1; FAD, flavin adenine dinucleotide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GARP, glutamic acid-rich protein; GI, Gigantea; GSK3, glycogen synthase kinase 3; HeBP2, heme-binding protein 2; HSP90, heat shock protein 90; LBP, luciferin-binding protein; LCF, luciferase; LD, light/dark; LHY, late elongated hypocotyl; LL, constant light; LOV, light−oxygen−voltage; LOV-HK, light−oxygen−voltage histidine kinase; LUX, LUX Arrhythmo; MAK7, mitogen-activated protein kinase 7; Myb, myeloblastosis; NTO, nontranscriptional oscillator; PAC, photoactivated adenylate cyclase; PAS, period circadian protein/aryl hydrocarbon receptor nuclear translocator protein/single-minded protein; PCL1, Phytoclock 1; *per*, *period*; PDI2, protein disulfide isomerase 2; PHOT, phototropin; PHY, phytochrome; PKA, protein kinase A; PKB, protein kinase B; PRR, pseudo response regulator; PRX, peroxiredoxin; PRX2, 2-Cys peroxiredoxin; PSII, photosystem II; ROC, Rhythms Of Chloroplast; ROS, reactive oxygen species; RVE, Reveille; SGG, Shaggy; SIG2, sigma factor 2; TOC1, timing of cab expression1; TTFLs, transcriptional−translational feedback loops; UV, ultraviolet; VLC-PUFAs, omega-3 very long chain polyunsaturated fatty acids; WT, wild type; XRN1, exoribonuclease1; ZTL, Zeitlupe.

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