Evolution of photoperiod sensing in plants and algae

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Measuring day length confers a strong fitness improvement to photosynthetic organisms as it allows them to anticipate light phases and take the best decisions preceding diurnal transitions. In close association with signals from the circadian clock and the photoreceptors, photoperiodic sensing constitutes also a precise way to determine the passing of the seasons and to take annual decisions such as the best time to flower or the beginning of dormancy. Photoperiodic sensing in photosynthetic organisms is ancient and two major stages in its evolution could be identified, the cyanobacterial time sensing and the evolutionary tool kit that arose in green algae and developed into the photoperiodic system of modern plants. The most recent discoveries about the evolution of the perception of light, measurement of day length and relationship with the circadian clock along the evolution of the eukaryotic green lineage will be discussed in this review.

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Introduction

Earth rotation around its axis and around the sun produces predictable day length (photoperiod) changes through the seasons that plants use, via sophisticated mechanisms, to measure time and take crucial physiological decisions [1]. Photoperiodism, or the ability to detect day length, is present in early photosynthetic eukaryotes so that algae can produce several photoperiod responses [2]. This way, during the green lineage evolution, photoperiodism pervaded into the major physiological systems, allowing them to predict the passing of the seasons and prepare plants for year-round predictable changing conditions.

The photoperiod sensing system involves a way to detect light (photoreceptors) and an internal system to measure time (circadian clock). In time they became so important for unicellular free living algae that for some marine picoeukaryotes 90% of its transcriptome is controlled by the clock [3]. However, more evolved and flexible species like modern plants, which developed the capacity to adapt to different environments, have reduced this number to less than 50% [4]. Paradoxically, more intertwined, complex systems allowed for a more independent response to external cues, thus permitting the colonization of ever demanding new niches and the acquisition of novel and complex physiological functions [5].

Photoreceptors evolution

Living organisms use a cluster of photoreceptors to measure the quality, quantity and direction of light to modulate physiological responses to changing lights [6]. This is particularly important for photosynthetic organisms that require light energy for photosynthesis and consequently to grow and develop. Photoreceptors can be divided into three groups according to the light quality they detect. Red and far-red lights are absorbed by phytochromes (PHys) while three types of photoreceptors perceive the blue/UV-A: Cryptochromes (CRYs), Phototropins (PHOTs) and three plant-specific LOV/F-box/Kelch-repeat proteins ZEITLUPE (ZTL), FLAVIN-BINDING KELCH REPEAT F-BOX (FKF), and LOV KELCH REPEAT PROTEIN 2 (LKP2). Finally, UV RESISTANCE LOCUS 8 (UVR8) was recently shown to be a UV-B photoreceptor [7]. Excellent reviews on plant and algae photoreceptor structure and function have recently been published [8-13].

The specific photoreceptor set has evolved across photosynthetic eukaryotes (Figure 1). In the chlorophyte model alga Chlamydomonas reinhardtii, UV-B light is detected by UVR8 while blue/UV-A is detected by one PHOT (pho1), two DASH (Drosophila, Arabidopsis, Synechocystis and Human) CRYs, one plant-like CRY (pCRY) and one animal-like CRY (aCRY). The latter can respond both to blue and red light [12,14,15]. In the fern Adiantum capillus-veneris four canonical plant PHYs have been
identified, two PHOTs and five CRYs [16]. Ferns include a specific neochrome, a chimeric photoreceptor consisting of an N-terminus PHY domain and several C-terminus PHOT domains that can sense both blue and red/far-red light to regulate chloroplast movement and phototropism [6]. However, UV-B photoreceptors have not been described in ferns, their absence justified by their growth habits under low-light angiosperm canopies. In the model plant Arabidopsis thaliana, red/far-red lights are detected by five PHYs (A-E), blue/UV-A by two PHOTs, three CRYs, and ZTL/FKF1/LKP2 proteins [6,11,13], while UV-B by a canonical UVR8 (Figure 1a). Increase in photoreceptors number and function during plant evolution has been related to fitness improvement [9].

In plants and algae, photoperiod regulates a number of processes including photomorphogenesis, growth, flowering, stress tolerance and circadian rhythms [16,17,18*]. In darkness, some of these pathways are inhibited by CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), a RING-finger E3 ubiquitin ligase. During the day, COP1 is inhibited by the photoreceptors allowing the activation of photoperiodic-dependent processes (Figure 1a). At night, COP1 interaction with SUPPRESSOR OF PHYTOCHROME A (SPA1) targets the transcription factors ELONGATED HYOCOTYL 5 (HY5) and CONSTANS (CO) for ubiquitination and degradation, suppressing photomorphogenesis and flowering respectively [20,21]. Photocactivated CRY1, CRY2 and PHYA directly bind SPA1 and inhibit the formation of COP1-SPA1 complex [19]. PHYB also promotes COP1-SPA1 dissociation and photomorphogenic development [22]. COP1 interaction with EARLY FLOWERING 3 (ELF3) induces degradation of GIGANTEA (GI), a circadian clock associated protein, process inhibited by CRY1/CRY2 in blue light [23]. Upon UV-B irradiation, UVR8

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**Figure 1**

Light signal transduction and photoperiodic regulation by COP1 in A. thaliana (a) and C. reinhardtii (b).

In A. thaliana, photoactivated CRY1, CRY2, PHYA and PHYB inhibit COP1 allowing accumulation of effectors and resulting in the specific light responses. Under UV-B, COP1 acts as positive regulator activating, among others, UVR8, HY5 and consequently upregulating UV-B tolerance genes. UV-B signal transduction is conserved in C. reinhardtii, although CRHY5 implication has not been investigated (dash lines). Arrows indicate positive regulation, while bars represent negative regulation. Low levels of CrCO expression observed in crcrya mutant suggest a conserved CRY-COP1-CO pathway, although CrCOP1 implication has not been described (dash lines). Photoreceptors not involved in COP1 regulation are shown in grey.

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monomerizes and interacts with COP1, promoting the expression of HY5, which is responsible for activation of UV-B responsive genes [24]. In Chlamydomonas cells grown under blue light, CrCO (Chlamydomonas CO homolog [18]) transcript levels are lowered in the crya mutant [15], suggesting a possible CrCO activation by CrCRYa. Besides, UV-B perception and signalling in Chlamydomonas is mediated by CrUV8 that interacts with CrCOP1, although CrHY5 implication has not been demonstrated [14**]. RING-finger E3 ligases homologous to COP1 can be also found in other microalgae. Therefore, it seems that a central role for COP1-like signalling mechanisms was already established in chlorophytes and evolved to the complexity found in modern plants.

Circadian clocks in algae and plants

Circadian clocks are molecular mechanisms that generate rhythmic or oscillating signals with a period of approximately 24 hours. They are ubiquitous systems present in almost all eukaryotic organisms, but in spite of the long evolutionary distance among them, they are composed of strikingly similar gene networks comprising intertwined positive and negative feedback loops [25]. These genes are not always orthologues, suggesting a convergent and independent evolutionary history. Recently, high throughput sequencing and genome-wide phylogenetics are starting to unveil an interesting evolution of the gene network underpinning the circadian clock composition in the green lineage.

Three interlocked feedback loops have been identified in the model species Arabidopsis: morning, central and evening loops (Figure 2a) [26]. The key gene in the morning loop is CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1) that codes for a MYB transcription factor [27*] with a conserved N-terminal SHAQKYF motif (Figure 2b). MYB family has gone through an intense process of amplification and functional diversification in the green lineage [28] and CCA1 homologues are present in every plant taxa, from the single copy gene OtCCA1 in Ostreococcus [29**] to the large gene family in Arabidopsis including CCA1, LATE ELONGATED HYPOCOTYL (LHY) and REV EILLE (RVE) 4, 6 and 8 [30]. All these homologues, with the exception of Chlamydomonas, exhibit the same expression pattern as Arabidopsis CCA1: A peak at dawn and a trough at dusk (Figure 2c).

ChIP-seq data has shown that CCA1 binds directly to the promoters of the other key genes in the morning loop, the PSEUDO-RESPONSE REGULATOR 9, 7 and 5 (PRR9/7/5) recognizing a specific DNA sequence called evening element [27] (Figure 2a). PRRs are repressors that contain in their N-terminus a receiver-like domain (RLD) similar to the one in RESPONSE REGULATORS involved in the His-Asp phosphorelay system, while in the C-terminus they present a CO, COL1 and TIME OF CAB EXPRESSION 1 (TOC1) domain (CCT). In Arabidopsis PRR9, PRR7 and PRR5 present a series of successive expression peaks at early morning, mid-day and afternoon respectively (Figure 2c) and their protein products bind to the same positions at the CCA1 promoter where a G-box can be found, repressing its expression during the entire day, and completing the positive/negative feedback morning loop [31].

TOC1 (PRR1) is directly repressed by CCA1 using the evening element located in its promoter. TOC1 in turn also acts as a repressor of CCA1 forming the central negative feedback loop [31]. PRRs homologues have been identified in all plant taxa. A single PRR gene was identified in the O. tauri genome (OiTOC1) presenting a similar expression profile as TOC1 and PRR5 (Figure 2c) and symmetric to the one in OtCCA1, suggesting that the central loop was already established in algae and is conserved across the entire eukaryotic green lineage [29**]. Additionally, an evening element has been found in the promoter of OiTOC1 providing supporting evidence. None of the two PRRs identified in Chlamydomonas follow similar expression profiles to any of the PRRs in Arabidopsis, which suggests a divergent evolution in Chlamydomonas in the regulation of circadian rhythms [32]. Four different PRRs were found in the Physcomitrella (a moss that seems to have derived from the direct evolutionary line of modern plants) genome. All these genes exhibit the same expression pattern, peaking at dusk with their troughs at dawn. No diversification in their peaking time points like the Arabidopsis PRR9/7/5 is observed, possibly because these genes are the result of very recent duplication events [33].

The third feedback loop is called the evening loop where TOC1 also plays a key role together with GI and ZTL (Figure 2). Both genes have been shown to be transcriptionally co-regulated directly by CCA1, PRRs and TOC1 [27*,31,34*]. In turn, GI and ZTL are known to form a complex involved in blue light and temperature sensing that induces TOC1 degradation by the 26S proteasome [35]. It is relevant to note that Arabidopsis ZTL homologue, FKF1, together with GI, mediates CYCLING DOF FACTORS (CDFs) degradation and the subsequent CO activation [36], a connection between the circadian clock and photoperiodic flowering. No potential GI homologue has been identified in microalgae and Physcomitrella, but it is present in other bryophytes such as Marchantia or Selaginella and the seed plants Picea and Oryza, suggesting that this gene is exclusive of land plants [33]. GI plays also a crucial role in the temperature compensation of the Arabidopsis circadian clock [37] and the defect in temperature compensation observed in Physcomitrella clock has been ascribed to the absence of GI [33]. ZTL presents three different protein domains: N-terminal LOV involved in blue light sensing, F-box that mediates protein ubiquitination, and multiple C-terminal Kelch domains involved in protein interactions. Similar to GI, ZTL homologs have only been identified in
Evolution of circadian clocks from algae to plants.

(a) Transcriptional network underpinning the circadian clock in *Arabidopsis thaliana* based on occupancy profiling by high throughput sequencing or ChIP-seq of CCA1, TOC1, PRR5, PRR7 and PRR9. Three different loops are identified, the morning loop constituted by CCA1 and PRR9/7/5,
land plants, although the three domains of ZTL have been separately identified in different protein homologs in Physcomitrella and Ostreococcus. This suggests that these proteins could form complex functionally equivalent ZTL homologs [33]. Alternatively, the close similarity of the RLD domain and the PRR genes in Physcomitrella and Ostreococcus has revealed a potential homolog of DDKmtorf that is not present in the PRR domain plants. This could indicate that these PRRs form part of a histidine-kinase transmembrane phosphorelay system. Indeed, histidine kinases containing an N-terminal LLOV domain have been identified in Ostreococcus and have shown an interaction with a flavin and have a circadian function [38]. Therefore, these LLOV kinases could possibly act on the ZTL in the Physcomitrella, Chlamydomonas, and Ostreococcus.

The evening loop includes CDFs, ELF3, and ELF4 that represent circadian genes with a LUX homolog [39]. LUX homologs have been found in all plant taxa examined, but no functional characterization is described. Potential ELF3/4 orthologues have been identified in Physcomitrella [33] and other land plants [40] but not in the moss Physcomitrella [41] or Ostreococcus. Therefore, it seems that the central clock of the Arabidopsis/LHCF1-TOC1 loop has been conserved for more than 500 million years and has subsequently evolved into a tetratricopeptide motif. The evolution of land plants. This way, the complex sequence can be inherited from the common ancestor and the physiological processes such as the temperature compensation in Arabidopsis have been conserved for more than 100 million years. Despite this, the plastidic and capacity to colonize new environments.

Evolution of the photoperiod pathway

The long-day (LD) Arabidopsis plant and its photoperiodic regulation of flowering time has been modeled for day length sensing studies. In Arabidopsis, CO protein is the primary regulator of flowering time and has been conserved for millions of years. In other plants, the CDFs and flowering timing tend to be different. In Arabidopsis, CO protein stability is controlled by photoreceptors, E3 ubiquitin ligases [42] and the interaction with other proteins [43, 44]. These complex regulatory layers allow the correct expression of flowering genes in Arabidopsis, controlling flowering time. However, there is diverse and specific photoperiodic flowering regulatory strategies. In the short day (SD) plant, CO homologues function as an inhibitor in LD and activator in SD [45], while another COL suppresses flowering regardless of the day length [46]. In Medicago and pea, CO seems to have no significant effect in flowering time and specifically in the latter, FT expression is controlled solely by a CDF gene [47, 48]. Nonetheless, evolution goes even further, altering flowering time at the species level by natural variation in the cis-regulatory elements of the CO promoter [49]. Moreover, a local adaptation of ecotypes to different flowering strategies depending on their geographic location has been described [50]. These examples and others [51–54] show how plants have adapted to optimize their reproductive timing.

Although the belief that CO is a widespread central element in the angiosperms flowering pathway is generating controversy [47, 55], the GI-CDFs–CO–FT core is highly conserved among distantly related flowering plants. In fact, it has been shown that COLs and Dof-FRs regulators have coevolved with single common algal ancestors, following the innovation, amplification and divergence model of gene evolution by duplication [56]. C. reinhardtii is considered to be the representative species of this common ancestor, in which a single copy gene of CO (CrCO) that controls the expression of a single copy Dof (CrDof) has been characterized [18, 57]. CrCO is a central hub involved in key physiological processes such as carbon metabolism and cell cycle, so that its expression is controlled by photoperiod and its mutation severely affects the capacity of the algae to synthesize starch and to synchronize cell division and growth [18, 56]. Furthermore, CrDof induces, unlike Arabidopsis, CrCO expression in SD by direct binding to its promoter. However, in LD, it represses cell cycle progression in a CrCO-independent way [57] (Figure 3). Surprisingly, both genes phenocopied their homologue functions in Arabidopsis when ectopically expressed: CrCO inducing flowering time while CrDof delays it [18, 57]. Curiously, CO1 (which shares 80% amino acid similarity with CO) overexpression produces no change in flowering time and this might reveal another evolutionary aspect: It must be the tertiary structure of the algal proteins what is conserved and recognized by the plant regulatory mechanisms to emulate its plant homologue function [56]. This does not seem to be an isolated event because a similar case was observed when tomato CDFs were expressed in Arabidopsis [58].

(Figure 2 Legend Continued) The central loop with TOC1 and CCA1, and finally, the evening loop formed by TOC1, GI, ZTL, and LUX. (b) Identification of orthologues circadian clock proteins in different plant taxa including gene ID from specific databases. Notice how proteins in the morning and central loops are conserved across the entire green lineage, while the key proteins in the evening loop, ZTL and GI, are only present from Selaginella on. Protein domains (RLD, CTT, Myb, LOV, FBox and Kelch) are color-coded identified on the table on the left. (c) Conservation of the expression profiles of CCA1, PRR5/7/8 and TOC1 from Arabidopsis thaliana (left) and Oryza sativa and Ostreococcus tauri (right) in 24 hours experiments at 12 hours light/12 hours dark photoperiod. CCA1 and OTC1A1 present similar expression profiles peaking at dusk with a trough at dawn. OTC1 and TOC1 exhibit similar expression patterns peaking at dusk with a trough at dawn.
In vascular plants, a great numbers of DOFs and COLs functions have been inherited from their common ancestor. For example, CO triggers starch biosynthesis and the DOF transcription factor OBPI controls cell cycle [59,60]. So, not only DOF-CO module has coevolved, but also a set of genes or key regulatory networks associated to these genes. Additionally, COLs and DOFs have acquired, throughout evolution, a wide repertoire of plant-specific light-dependent functions [5,61,62] leading to more complex organism with a higher photoperiod plasticity.

**Discussion**

Over evolutionary time, photosynthetic organisms have learnt to live and extract information from periodic changes in sunlight [1,2]. As the complexity of organisms increased, so did their capacity to respond to the environment and paradoxically, to become more independent from its rigours and more precise at taking crucial life decisions, such as the best time of the year to flower or the best time of the day to grow. The massive amount of information arising from comparative genomics projects is allowing us to understand photoperiodic sensing with an evolutionary perspective.

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**References and recommended reading**

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest


56. Demonstration of the recent origin of CO promoter polymorphism and how its natural variation regulates flowering time in different Arabidopsis thaliana accessions.


62. A recent demonstration of how the duplication of a core photoperiodic gene has a deep effect on the adaptation and flowering behaviour of a whole family of plants and the possible convergence of this function in different plant lineages.


65. By functional characterization of CrDOF in algae and plants, CO-DOF module is shown to be conserved throughout the green lineage evolution.


