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

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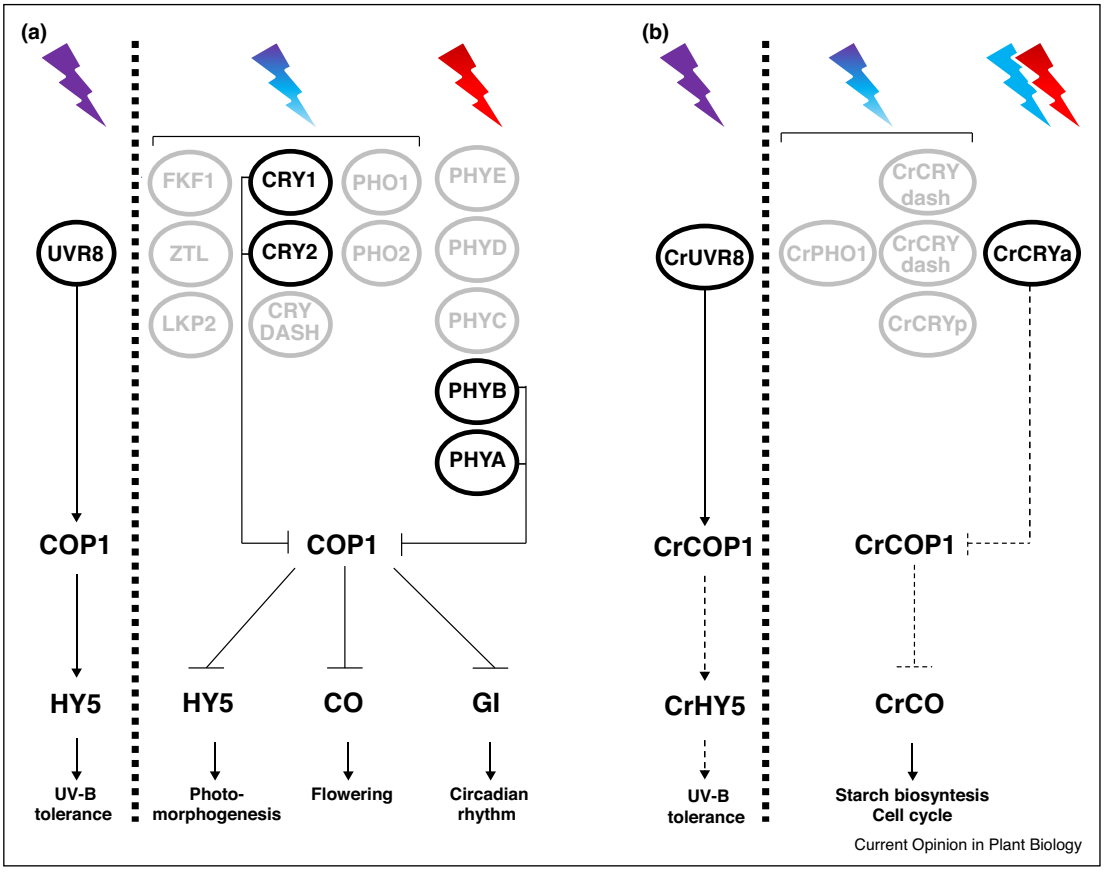
Figure 1



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.

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 HYPOCOTYL 5 (HY5) and CONSTANS (CO) for ubiquitination and degradation, suppressing photomorphogenesis and flowering respectively [20,21]. Photoactivated 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

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 *crcrya* mutant [15], suggesting a possible CrCO activation by CrCRYa. Besides, UV-B perception and signalling in *Chlamydomonas* is mediated by CrUVR8 that interacts with CrCOP1, although CrHY5 implication has not been demonstrated [14••]. RING-finger E3 ligases homologues 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.

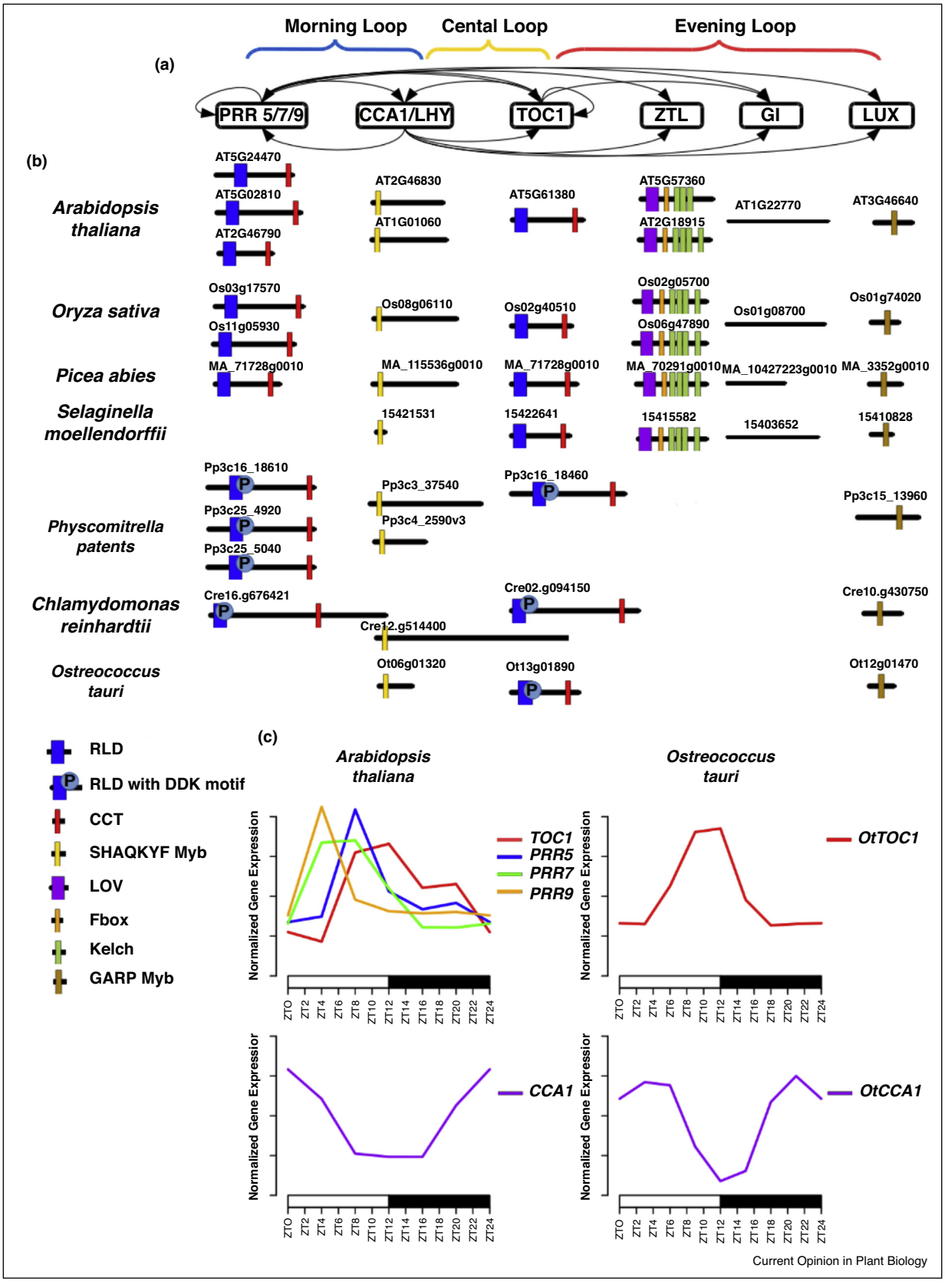
Three interlocked feedback loops have been identified in the model species *Arabidopsis*; morning, central and evening loops (Figure 2a) [26]. The key gene in the morning loop is *CIRCADIAN CLOCK-ASSOCIATED 1* (*CCA1*) that codes for a MYB transcription factor [27•] with a conserved N-terminal SHAQKYF 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 *REVEILLE* (*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 (*OtTOC1*) 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 *OtTOC1* 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

Figure 2



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 proteins in *Physco-mitrella*, so that these proteins could form a complex functionally equivalent to ZTL [33]. Alternatively, a closer inspection of the RLD domains in the *PRR* genes in *Physcomitrella* and *Ostreococcus* has revealed a potential phosphoacceptor DDK motif that is not present in the *PRR*s of land plants. This could indicate that these PRRs form part of a Hist-Asp phosphorelay system. In fact, Histidine kinases containing an N-terminal LOV domain has been identified in *Ostreococcus*, where it has been shown to respond to blue light, bind flavin and have a circadian function [38]. Therefore, these LOV-HKs could possibly take the role of ZTL in *Physcomitrella*, *Chlamydomonas* and *Ostreococcus*.

The evening loop includes also *LUX ARRHYTHMO*, *ELF3* and *ELF4* that repress circadian gene's night expression [39]. *LUX* homologs have been found in all plant taxa examined but no functional characterization is described. Potential *ELF3/4* orthologues have been iden-tified in *Physcomitrella* [33] and other land plants [40] but there seem to be no orthologues in *Chlamydomonas* [41] or *Ostreococcus*. Therefore, it seems that the central CCA1/LHY and TOC1 loop was established very early in microalgae and the subsequent loops and additional con-trol levels took place during the course of the evolution of land plants. This way, the more complex the clock, the more responses can be extracted from it to control new physiological processes, such as the above mentioned temperature compensation exerted by GI [33]. This could explain how land plants acquired much more versatile control of the external conditions, enhancing their plasticity and capacity to colonize new aerial niches.

Evolution of the photoperiod pathway

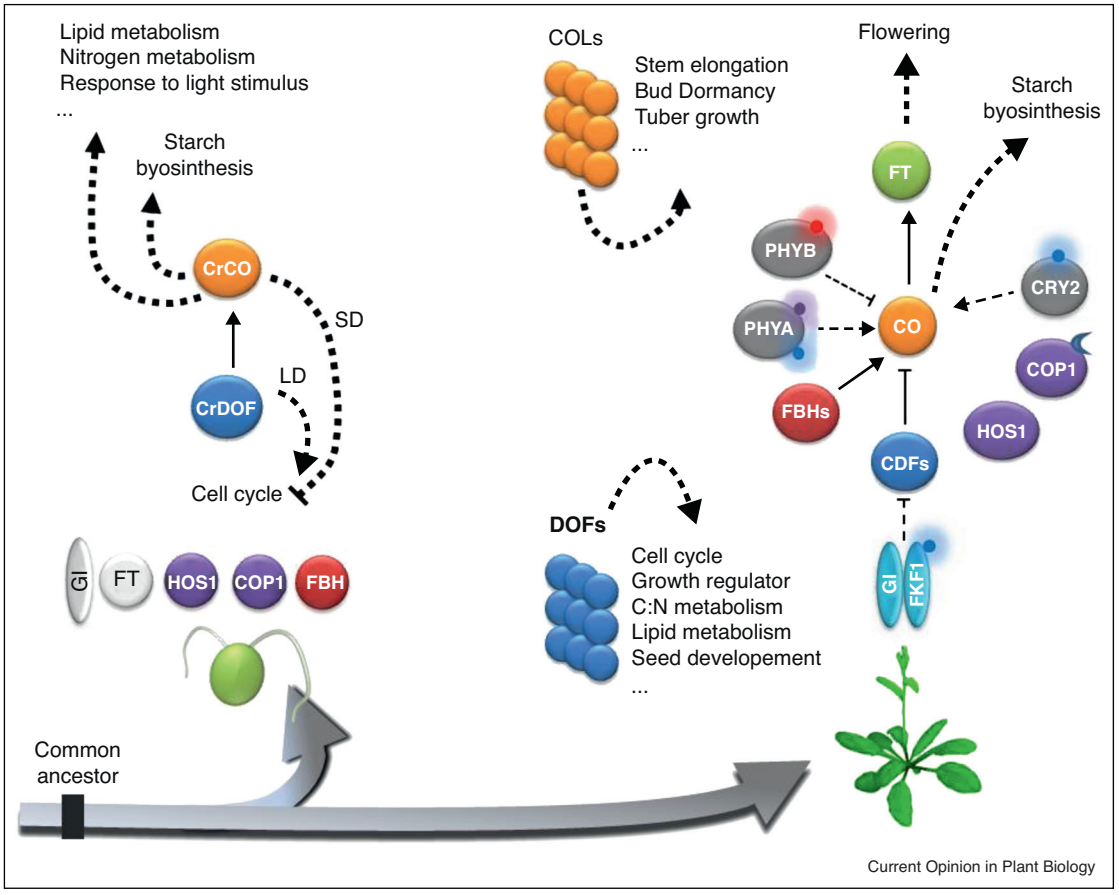
The long day (LD) *A. thaliana* plant and its photoperiodic control of flowering time has been the model for day length sensing studies. In *Arabidopsis* CO protein repre-sents a central hub that controls flowering in the proper season (Figure 3, right). Briefly, *CO* expression is regu-lated by circadian clock and photoperiodic inputs through GI-FKF1, the CDFs and FLOWERING BHLHs (FBHs). CO protein stability is controlled by photore-ceptors, E3 ubiquitin ligases [42] and through the inter-action with other proteins [43,44]. These complex regu-latory layers allow the correct expression of the florigen *FLOWERING LOCUST* (*FT*) gene that promotes flowering.

However, there are diverse and specific photoperiodic flowering regulatory strategies. In the short day (SD) plant rice, CO homologue functions as an inhibitor in LD and activator in SD [45], while another COL sup-presses flowering regardless of day length [46]. In *Med-icago* 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 gener-ating 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 DOFs regulators have coevolved from 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 effects 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 its homologue functions in *Arabidopsis* when ectopically expressed; *CrCO* inducing flowering time while *CrDOF* delays it [18•,57•]. Curiously, *COL1* (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, CCT, Myb, LOV, Fbox and Kelch) are color-coded identified on the table on the left.
(c) Conservation of the expression profiles of *CCA1*, *PRR9/7/5* and *TOC1* from *Arabidopsis thaliana* (left) and *OtCCA1* and *OtTOC1* from *Ostreococcus tauri* (right) in 24 hours experiments at 12 hours light/12 hours dark photoperiod. *CCA1* and *OtCCA1* present similar expression profiles peaking at dawn with a trough at dusk. *OtTOC1* and *TOC1* exhibit similar expression patterns peaking at dusk with a trough at dawn.

Figure 3



Evolution of the photoperiod pathway elements from a common ancestor.
In *Arabidopsis* (right) the main elements of flowering photoperiod pathway are shown. Grey and purple proteins represent phytocromes and E3 ubiquitine ligase proteins, respectively. Straight and dash arrows show transcriptional and post-transcriptional regulation, respectively. Colored circles represent light quality. In *Chlamydomonas* (left) CrCO and CrDOF are the first known elements involved in photoperiodic signaling (there is no evidence of GI and FT proteins presence in green algae). Although *HOS1*, *COP1* and *FBHs* orthologues are present in the *Chlamydomonas* genome, no functional characterization has been done. The big family of COLs and DOFs in angiosperms has plant specific functions. However, other functions are shared with the *Chlamydomonas* proteins (dash lines).

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 OBP1 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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