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Article Addendum

Evolutionarily conserved photoperiod mechanisms in plants

When did plant photoperiodic signaling appear?

José M. Romero and Federico Valverde*

Instituto de Bioquímica Vegetal y Fotosíntesis; Consejo Superior de Investigaciones Científicas y Universidad de Sevilla; Sevilla, Spain

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Day-length and the circadian clock control critical aspects of plant development such as the onset of reproduction by the photoperiodic pathway.[1] CONSTANS (CO) regulates the expression of a florigenic mobile signal from leaves to the apical meristem and thus is central to the regulation of photoperiodic flowering.[2] This regulatory control is present in all higher plants,[3] but the time in evolution when it arose was unknown. We have shown that the genomes of green microalgae encode members of the CONSTANS-like (COL) protein family. One of these genes, the *Chlamydomonas reinhardtii CO* homolog (*CrCO*), can complement the *co* mutation in Arabidopsis.[4] *CrCO* expression is controlled by the clock and photoperiod in Chlamydomonas and at the same time is involved in the correct timing of several circadian output processes such as the accumulation of starch or the coordination of cell growth and division. We have proposed that, since very early in the evolutionary lineage that gave rise to higher plants, CO homologs have been involved in the photoperiod control of important developmental processes, and that the recruitment of COL proteins in other roles may have been crucial for their evolutionary success.

Plants have adapted several physiological mechanisms to finely respond to external signals and coordinate the correct timing of important developmental processes.[5] This way, different diurnal and seasonal potentially deterring or optimal situations can be predicted and an adequate response prepared in advance. In the case of the control of reproduction, predicting the best time of the year to flower confers a fitness improvement directly related to seed productivity and thus is naturally selected as an important trait in plant evolution.[6] Plants and algae posses sophisticated mechanisms to measure time, such as a circadian clock or a photoperiod control to detect day-length and probably temperature.[7,8] In photoperiodic flowering, the protein CONSTANS plays a central role because it activates in the leaves the expression of *FLOWERING LOCUS T (FT)*. The small FT protein is able to move through the vascular bundles from the leaves to the apical meristem and trigger the program that transforms it into a reproductive meristem.[2] Eventually, these changes induce the production of the flower.

We have recently described[4] that in green microalgae the mechanism detecting photoperiod signals involves a *CO* homolog (*CrCO*) with a crucial role. *CrCO* expression is controlled by photoperiod and at the same time regulates several output processes of the clock such as starch accumulation or the onset of cell division.

Algal Genomes Contain CO Homolog Sequences

The recent sequencing of genomes from phylogenetically diverse microalgae allowed the identification of genes that encode homologs of proteins involved in circadian clock and developmental processes in higher plants.[9,10] In *C. reinhardtii*, a chlorophyte microalga who has been extensively studied as a simple model for plant-specific processes like photosynthesis or phototaxia,[11] we have identified several proteins that belong to the COL family. One of these proteins, which we have called CrCO, has the domain structure characteristic of CO.[12]

We also showed that *CrCO* transcript is regulated by photoperiod, but unlike in Arabidopsis the peak in expression of the gene always occurs during the light period and is actually higher in short days (SD, 8 h light–16 h dark) than in long days (LD, 16 h light–8 h dark). The protein production seems to closely follow the RNA expression so it seems that the control of *CrCO* in Chlamydomonas is simpler than that of CO in Arabidopsis.[13] Observation under the confocal microscopy of GFP fusion tags in onion epidermal cells shows that like CO, CrCO is probably localized in the nuclei, further confirming their physiological analogies.

*Correspondence to: Federico Valverde; Instituto de Bioquímica Vegetal y Fotosíntesis; CSIC—Universidad de Sevilla; Av Américo Vespucio 49; Sevilla 41092 Spain; Tel.: ++34.954489525; Fax: ++34.954460065; Email: federico@ibvf.csic.es

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Expression of *CrCO* in Plants Under Different Promoters Induces Early Flowering

When we expressed *CrCO* under the control of a constitutive (35S) promoter and transformed Arabidopsis *co* mutant plants, we were able to rescue the delay in flowering produced by the lack of *CO* function. These constructs also induced early flowering in two different wild type ecotypes of Arabidopsis, L*er* and col-0. Furthermore, some 35S::*CrCO* plants phenocopied several growth alterations that had also been described for CO overexpression such as club-like siliques and low polen production.[14]

Interestingly, when *CrCO* was expressed under the control of a phloem-specific promoter we were also able to accelerate flowering. This was not the case when *CrCO* was expressed under the control of a meristem-specific promoter. Thus, as in the case of CO, *CrCO* expression in the vascular tissues is enough and sufficient to activate flowering. In plants were *CrCO* was overexpressed we detected both the transcript for the transgene as well as the recombinant protein, except for the meristem-specific construct, were we could detect the transcript at high levels, but not the protein. In all cases, production of CrCO protein was followed by high levels of *FT* expression both in SD and in LD, which confirmed that CrCO was activating flowering via the canonical CO-FT module previously described for the induction of flowering in Arabidopsis.[15] We think that the capacity to activate flowering is due to a lesser functional specificity in the *CrCO* gene, so that it will be able to activate flowering in a wide range of plant species. To test whether this is the case we are starting to test the effect of *CrCO* overexpression in other plant model species like tomato and rice.

Several Output Processes from the Circadian Clock are Disrupted in Chlamydomonas by Modification of *CrCO* Expression

The function of CO described to date is to induce expression of *FT* in the vascular tissue of photosynthetic tissues and thus generate a signal that is transmitted to the meristem to activate flowering.[16] In microalgae, there is no clear *FT* homolog, neither a distance signal is needed to activate any developmental process. So, the question remained about the role of *CrCO* in Chlamydomonas. To answer this, *CrCO* overexpressor (*CrCO*ox) and *CrCO* suppressor (*CrCO*as) microalgal lines were produced and their effects on starch accumulation and cell division studied. Both *CrCO* suppression and overexpression had a drastic effect in the clock outputs analyzed thus showing that *CrCO* is important in the control of both processes. *CrCO* overexpression caused the asynchrony between cell growth and division. Synchronous growth is a common feature of algal cultures.[17] *CrCO*ox lines also showed lower accumulation of starch and different capacity to accumulate it during the day both in SD and LD. We further demonstrated that *CrCO*ox lines presented aberrant cell morphotypes that were probably caused by desynchronized growth and division. Furthermore, *CrCO*as lines had a deterred growth capacity, many of them never surviving the first rounds of duplication in restrictive (high light) conditions. It seemed then that, at least in Chlamydomonas, CrCO function was crucial for survival.

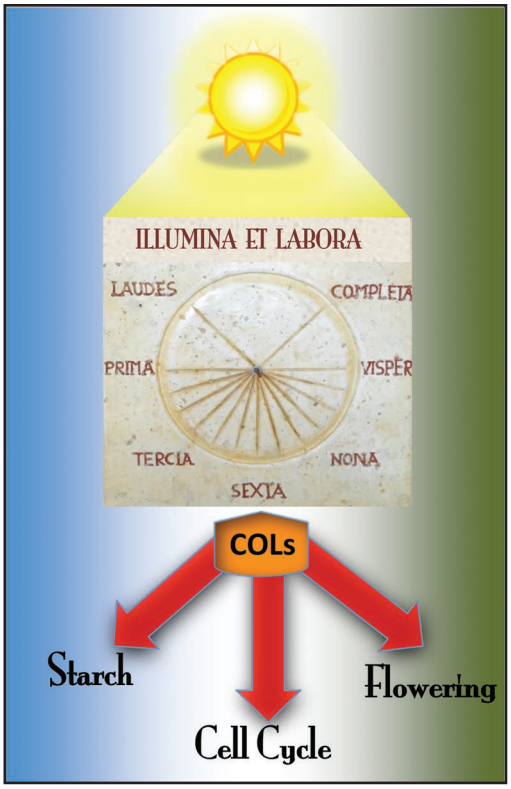

Figure 1. *ILLUMINA ET LABORA*. COL proteins receive signals from daylength and the circadian clock and are activated by light. During evolution they have developed roles from the regulation of starch synthesis and cell cycle (algae, blue side) to the control of flowering time (plants, green side).

The fact that in Chlamydomonas CrCO has a role in starch accumulation and cell division opens the possibility that this role might be maintained in higher plants CO (Fig. 1). It has been previously reported that starch accumulation is under circadian clock control,[18,19] the *gi* mutant showed elevated levels of starch[20] and the cell cycle might be controlled by the clock.[21] These observations suggest that the transition to flowering might be coordinated with cell division and carbon metabolism through outputs of the circadian clock, and CO arises as a putative integrator of all these processes (Fig. 1).

Conclusions

The functional complementation of CO by CrCO is remarkable for several reasons. Firstly, because it implies that the mechanisms that control daylength and clock dependent processes in higher plant are already present in microalgae. Secondly, because our results show that this process is crucial for algal growth and viability, and although CO mutation is not lethal in Arabidopsis, presumably due to function redundancy in the different CO homologs, all in all, the family of COL proteins may have important functions still concealed in higher plants. In the third case, it is remarkable that after the great evolutionary distance between CO and CrCO proteins and sharing only 27% identity in amino acid sequence, the function is still highly conserved. That means that the structure of CO and CrCO proteins shares a high degree

of homology, so that CrCO can substitute CO in the quaternary complex that is able to influence gene expression. Although the molecular mechanism controlling CrCO function in microalgae is still largely unknown, parallel proteomic studies in both CO and CrCO may give new lights into the function-structure of this group of proteins and help understand how photosynthetic organisms measure time in a daily and seasonal scale.

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