Photoreceptor Regulation of Constans Protein in Photoperiodic Flowering

by

Valverde et. Al

Published in

Science 2004

Presented by

Boyana Grigorova

CBMG 688R

Feb. 12, 2007

Circadian Rhythms: The Clock Within

- Circadian rhythms: 24 hours
- Internal pacemaker (endogenous oscillator)
- Circadian rhythms exhibit period, phase, and amplitude
- In constant darkness, rhythms adjust in relation to solar time
- Endogenous oscillators synchronize to a true 24-hour period by the environmental cues such as light-to-dark transition at dusk, and the dark-to-light transition at dawn
- Endogenous oscillators set a physiological response to occur at particular time of day
- Zeitgebers

Photoperiodism: Monitoring Day Length

- Photoperiodism - the ability to detect day length
- Plants monitor day length by measuring the length of night
- Interruption of the dark period by a brief light treatment prevents flowering
- The Circadian Clock is Involved in Photoperiodic Timekeeping
- Control of Flowering by Photoperiodism is achieved by an oscillation of phases with different sensitivities to light
- Light is able to promote or inhibit flowering only if its presence coincides with the plant being in light-sensitive phase
- The leaf is the site of perception of the photoperiodic stimulus

Plants Can Be Classified by Their Photoperiodic Responses

- Short-day plants
- Long-day plants

Four Developmental Pathways For Flowering in Arabidopsis

Constans is a Key Gene in Flowering Pathway of Long Day Plants (also Arabidopsis)

The coincidence of light at the end of long day and high CO protein levels activated FT. FT interacts with the CO and LEAFY (LFY) genes to promote flowering
1. What is known?
- In Arabidopsis, Constans (CO) promotes flowering in long days.
- Co-promoter mRNA abundance is regulated by the circadian clock and accumulates late in the afternoon when long-day plants are exposed to light.
- CO activates transcription of Flower Locus T (FT) gene, which encodes a RAF-kinase inhibitor protein that promotes flowering.
- Flowering under LDs occurs because of coincidence between circadian-clock controlled transcription of CO and light-mediated posttranscriptional regulation.

2. Hypothesis:
CO is ubiquinated and degraded by the proteasome protein complex in the morning darkness, while stabilized by light towards dusk. Photoreceptors regulate CO stability and act antagonistically to generate daily rhythms in CO abundance. This layer of regulation refines the circadian rhythm of CO messenger RNA and is central to the mechanism by which day length controls flowering.

How is FT Expression Dependent on CO Abundance Under Light of Different Wavelengths?

FT Expression Under Different Light Regimes

Fig. 1: A, B, C: FT expression under different light regimes. W is white, B is blue, R is red, FR is far-red.

Conclusion
Posttranscriptional regulation of CO activity by blue and far-red light rapidly activates Floral Locus T (FT) transcription after transfer from darkness.

Does Exposure to Light Increase FT by Increasing CO protein Abundance?
**CO Protein Abundance Under Different Light Regimes**

Fig 2. (A) Confocal images of guard cells of 35S::GFP:CO plants exposed for 2 days to different light conditions. Green fluorescence represents GFP::CO; the red-yellow signal is plastid autofluorescence. Arrows indicate nuclei. (B) CO protein in nuclear extracts of 35S::CO plants after two-day light treatment analyzed by Western blotting. Early in short days used as control. (C) CO protein in nuclear extracts of co-8, wild-type, Ler, and 35S::CO plants exposed to two days blue light.

---

**Conclusion**

Under the light conditions tested there is a positive correlation between the abundance of CO protein and FT expression levels. There is a high expression of CO under blue-light conditions which might explain why plants flower extremely early under these conditions.

---

**Does CO Protein Abundance Correlate With FT Expression Under Diurnal Cycles Of Light and Dark?**

LD → Amount Luminescence → SD → Amount Luminescence

---

**GFP::CO Abundance Under LDs and SDs**

Fig 2D: Confocal images of guard cells of 35S::GFP:CO plants during 24 hours under LDs and SDs.

---

**GFP::CO Abundance Under LDs and SDs**

Fig 2D: Confocal images of guard cells of 35S::GFP:CO plants during 24 hours under LDs and SDs. The green signal represents GFP::CO, the red-yellow signal is plastid autofluorescence.

---

**Continued...**

---

**Luminescence and CO/ FT mRNA expression levels of FT::LUC**

35S::CO plants during 24 hours under LDs and SDs.

---

**Luminescence**

- **LD:** 100
- **SD:** 50

**FT::LUC**

- **LD:** 30
- **SD:** 25

**CO mRNA**

- **LD:** 20
- **SD:** 15

---

**Fig. S2:** FT expression under LDs and SDs in a 35S::CO background. Quantification of FT from the Northern blot data in Figure 1E.
Continued...

**Fig2E:** CO protein in nuclear extracts of 35S::CO plants during 24 hours under LDs (left) or SDs (right). Graph shows quantification of CO protein abundance as the mean of three LD and two SD experiments.

**Fig2F:** CO protein in nuclei of 35S::CO plants grown under LDs and harvested in the morning. CO analyzed by Western blotting, antibody to Histone 3A used as a control.

**Fig2G:** CO protein in nuclear extracts of wild-type (LER) plants grown under LDs or SDs of blue light. CO could not be detected under white light in WT plants.

**Conclusion**

The diurnal pattern of FT:LUC expression in 35S::CO plants grown under LDs and SDs closely follows the abundance of CO protein, suggesting that regulation of CO abundance is a major determinant of FT expression levels. Under LDs the diurnal rhythm in CO is similar in wild-type and 35S::CO plants, and its amplitude is reduced in both genotypes under SDs.

**Conclusion**

The diurnal pattern of FT:LUC expression in 35S::CO plants grown under LDs and SDs closely follows the abundance of CO protein, suggesting that regulation of CO abundance is a major determinant of FT expression levels. Under LDs the diurnal rhythm in CO is similar in wild-type and 35S::CO plants, and its amplitude is reduced in both genotypes under SDs.

**Is Constans Abundance Regulated by a Proteasome in Response to Light/Dark Transition?**

![Diagram](image)

**Effect of Proteasome on CO Stability**

**Fig. 3:** (A) CO protein in nuclear extracts of LD-grown 35S::CO plants during the first 4 hours of the dark period (upper panel). CO protein in nuclear extracts of 35S::CO plants after incubation for four hours with proteasome inhibitors (lower panel). (B) In vitro assay to detect ubiquitinated CO. Recombinant HisCO protein was added to cell free extracts of Ler plants grown for two days under continuous light or dark. CO (left) or proteins modified by ubiquitin (right) were detected by Western blotting. Either proteasome inhibitors or ATP were added.

**Conclusion**

The results of in vivo and in vitro experiments suggest that Constans is ubiquinated and degraded by the proteasome.

**Which Photoreceptors Regulate Flowering by Influencing CO Posttranscriptional Activation?**

- Phytochrome (phy) and cryptochrome (cry) photoreceptors regulate flowering and they do so through CO.

**Experimental setup to test which photoreceptors activate Constans posttranscriptionally:**

- 35S::CO phyA
- 35S::CO phyB
- 35S::CO cry1/cry2

**Measure FT mRNA abundance**
**Effect of photoreceptor mutations on FT mRNA abundance**

A Northern blot

**Fig 4A**: FT mRNA detected by Northern blotting. The 35S:CO plants carrying photoreceptor mutations were grown for 7 LDs and 2 additional days in continuous W, B, or FR. Northern blots probed with FT, CO, and TUB probes.

**Conclusions**

Constans function is enhanced by posttranscriptional regulation through the photoreceptors phyA, cry1 and cry2, which is in agreement with FT expression levels in otherwise wild-type plants carrying photoreceptor mutations.

**What are the Effects of Photoreceptors PhyA, Cry1/Cry2 and PhyB on CO Protein abundance?**

B Immunoblot

**Fig 4B**: CO protein detected in nuclear extracts of 35S::CO plants carrying photoreceptor mutations. Blots were probed with CO, H3A, or ESD4 antibodies.

**CO Stability in Photoreceptor Mutants and Flowering Times of Photoreceptor Mutants**

**Fig S3**: Effect of photoreceptor mutants on CO stability in blue and far-red light. The cry1/cry2 and phyA mutations cause a reduction in Constans protein stability under blue and far-red light.

**Table S1**: Flowering time of 35S::CO plants in different mutant backgrounds in SDs and Extended Short Days.

**Conclusion**

- phyB reduces CO abundance in Red light and during the morning
- Cry1/Cry2 stabilize CO protein during the morning and evening
- Cry2 promotes flowering by antagonizing the repressive phyB by targeting CO abundance
- phyA and cry2/cry1 promote flowering by stabilizing Constans

**Model of Constans Protein Regulation Related to mRNA Levels in Wild Type Plants**

**Fig 4E**: Arrows indicate stabilization/activation of the protein, and perpendicular lines illustrate degradation/inactivation. CO protein activity is restricted to the end of a LD.

**CO Stability in Photoreceptor Mutants and Flowering Times of Photoreceptor Mutants**

**Fig S3**: Effect of photoreceptor mutants on CO stability in blue and far-red light. The cry1/cry2 and phyA mutations cause a reduction in Constans protein stability under blue and far-red light.

**Table S1**: Flowering time of 35S::CO plants in different mutant backgrounds in SDs and Extended Short Days.