Long-distance signaling in plants: Graft transmission of a floral stimulant derived from *CONSTANS*

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Abstract: Photoperiod is perceived by leaves, and in many species influences the transition to reproductive growth through long-distance signaling. *CONSTANS* (*CO*) mediates between photoperiod perception and the transition to flowering in *Arabidopsis thaliana*. To test its role in long-distance signaling, *CO* was expressed from a promoter specific to the companion cells of minor veins in mature leaves. Expression in these tissues at the inception of the translocation stream accelerated flowering at the apical meristem under non-inductive (short-day) conditions. Grafting plants with different flower-timing phenotypes demonstrated that minor-vein expression of *CO* substituted for photoperiod in generating a mobile flowering signal. Our results suggest that a *CO*-derived signal(s), fits the definition of the hypothetical floral stimulant, florigen.

Introduction: In addition to transporting nutrients, the phloem transports signals that coordinate growth and differentiation with events in leaves. These signals influence diverse responses including host/pathogen interactions, tuberization, nodulation, the transition to flowering, and flower morphology.

The leaves of many species perceive the duration of day/night cycles and transmit a signal to promote the transition to reproductive growth at the apical meristem (Fig. 1). Despite decades of effort, the identity of this signal, commonly called florigen, remains elusive (Colasanti and Sundaresan, 2000).

Figure 1: Signals originating in leaves traffic through the phloem to coordinate growth and development.

Long days stimulate flowering in Arabidopsis. *CONSTANS* (*CO*) functions downstream of photoperiod perception in the flowering pathway (Fig. 2; Suarez-Lopez et al, 2001), however it is not clear if *CO* acts over long-distances by generating a phloem mobile signal, or acts locally in the meristem by responding to one. We report here that *CO* expression in the minor veins of mature leaves produces a phloem mobile signal that induces flowering at the meristem.

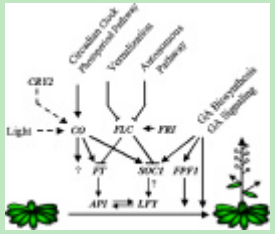
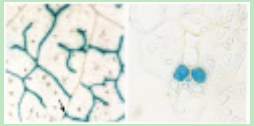


Figure 2: Genetic pathways influencing the transition to reproductive growth in Arabidopsis. From Mouradov *et al.*, (2002), copyright ASPB, used with permission.

Results: The phloem translocation stream begins in the minor veins of mature leaves. A *galactinol synthase* promoter from *Cucumis melo* (*CmGaS1*) is highly active in the minor veins of readily transformed species and is an ideal tool for studying long-distance transport (Fig. 3; Haritatos *et al*, 2000).



CmGaS1-GUS in *N. tabacum*

CmGaS1-GUS in *A. thaliana*

Figure 3: The *CmGaS1* promoter drives expression in the minor vein companion cells of tobacco and Arabidopsis. Expression is absent from larger veins (arrows), and immature tissues.

The assay for transport of a floral stimulant is simple: the gene of interest (*CO*) is expressed from the *CmGaS1* promoter in Arabidopsis grown under non-inductive conditions. Accelerated flowering indicates the production of a phloem-mobile floral stimulant (Fig. 4).

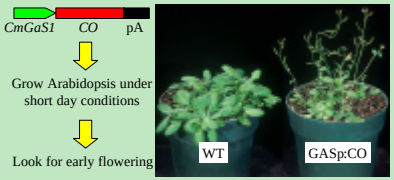


Figure 4: Assay for long-distance signaling – early flowering in short days

A CO-GUS fusion confirmed that expression was in the minor veins and not the meristem (Fig. 5). Therefore, the *CO*-derived stimulant was transported from leaves.

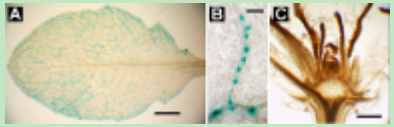


Figure 5: GUS activity from a CO-GUS fusion. (A) Staining in the minor veins. (B) CO is a transcription factor and CO-GUS localizes to companion cell nuclei. (C) Absence of staining in the meristem.

Graft transmission is a recognized property of florigen, and plants with different flower-timing phenotypes were grafted (Fig. 6). *CmGaS1-CO* in stock plants complemented the *co-1* mutation in scion plants, which normally flower late and are insensitive to day length. Control grafts using *co-1* plants as stock and scion flowered concurrently with ungrafted *co-1* plants (Fig. 7).

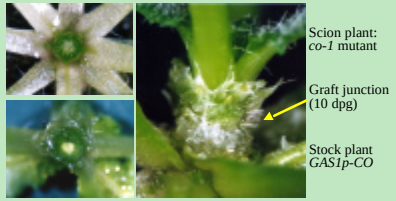
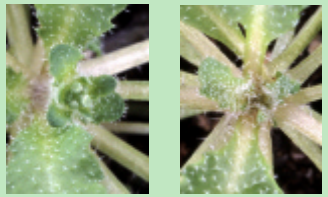


Figure 6: Grafting in *Arabidopsis*. Upper left, prepared stock; lower left, prepared scion; right, healed graft junction (arrow) ten days post grafting. Genotypes are as marked.



Scion: *co-1* / Stock: *CmGaS::CO*

Scion: *co-1* / Stock: *co-1*

Figure 7: *CONSTANS* generates a graft-transmissible signal that accelerates the transition to flowering.

Conclusions: We demonstrate that *CO* generates a phloem-mobile and graft-transmissible floral stimulant. Recent work localizing *CO* expression to vascular tissues strengthens our findings (Takada and Goto, 2003). Further work with genes downstream of *CO* will help identify florigen.

Future Efforts: This work shows the value of a minor vein promoter for studying long-distance signaling. Future efforts will use the *CmGaS1* promoter to drive cDNA libraries in transgenic plants for high-throughput identification of mobile signals that influence diverse responses.

Graduate student research opportunities are available.

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