Commentary

Does CONSTANS act as a transcription factor or as a co-activator? The answer may be – yes

To ensure that reproduction occurs during optimal conditions, plants adapted to seasonal environments often coordinate their flowering in response to photoperiodic cues. Early plant biologists developed two longstanding mechanistic models to explain how plants recognize and respond to day length: the external coincidence model; and the florigen hypothesis (Kobayashi & Weigel, 2007). The former postulates that certain photoperiods induce flowering because a light-sensitive floral promoter is under the control of the circadian clock, such that it is only expressed during daylight hours in certain photoperiods. The latter model postulates the existence of a mobile signal that is produced under inductive photoperiods in the leaf and travels to the shoot apex where it promotes reproductive development. Recent molecular studies in *Aradiposis thaliana* have identified the genes that constitute the mechanistic bases for these models: *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*). Notably, these two mechanisms are connected because *CO* induces *FT* expression. The transcription of *CO* is regulated by the circadian clock, such that peak expression occurs late in the day under long days (e.g. 16 h light : 8 h dark), but after dark in short days (e.g. 8 h light : 16 h dark). Because the CO protein is only stable in the light, it only accumulates under inductive long days. The CO protein leads to the upregulation of *FT* in the vasculature of leaves; FT protein is then transported to the meristem to initiate reproductive development (Kobayashi & Weigel, 2007). A major unanswered question is exactly how CO functions to regulate *FT*. Although early circumstantial evidence suggested that CO might act as a typical transcription factor, more recent evidence indicates that CO may instead function as a co-activator. A report by Tiwari *et al.* in this issue of *New Phytologist* (pp. 57–66) provides fresh insight into this problem and suggests that CO actually has both transcription factor and co-activator activities.

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When CO was first identified, it was proposed to be a transcription factor because it contains two zinc-finger motifs that were originally thought to be homologous to those of the GATA transcription factors (Putterill *et al.*, 1995). B-box zinc-finger motifs were subsequently described, however, and it is now accepted that the CO zinc fingers are homologous to these domains that mediate protein–protein interactions, but not DNA binding, in other proteins (Khanna *et al.*, 2009). Nevertheless, several observations still supported the possibility that CO protein might function as a transcription factor with *FT* as a direct target. Overexpression of *CO* causes early flowering and increased *FT* expression; conversely, *co* mutants flower late and do not express *FT* during long days (Suárez-López *et al.*, 2001). Also, *FT* is the only gene upregulated in wild-type plants exposed to a single long day that is not also upregulated in *co* mutants exposed to a single long day (Wigge *et al.*, 2005). Finally, a screen for early CO targets had been conducted. A steroid-inducible version of CO was used to identify genes that are rapidly upregulated upon induction of CO activity and *FT* emerged as one of the few early targets identified in this screen (Samach *et al.*, 2000). Because this screen was performed in the presence of cyclohexamide, which blocks translation, the upregulation of *FT* by CO appears to be direct, at least in the sense that it does not require the synthesis of new proteins.

Despite the circumstantial evidence suggesting that CO acts directly to regulate *FT*, evidence that CO can actually bind *FT* promoter sequences has been lacking, and more recent studies have found evidence that CO may instead function as a co-activator. First in tomato and then in *A. thaliana*, *CO* homologs were determined to physically interact with components of the NUCLEAR FACTOR Y (NF-Y)/HEME ACTIVATOR PROTEIN (HAP) complex through their CONSTANS-CONSTANS LIKE-TIMING OF CAB 1 (CCT) motif (Ben-Naim *et al.*, 2006; Wenkel *et al.*, 2006; Cai *et al.*, 2007). Because this complex has been shown to bind DNA at CCAAT elements in yeast and mammalian systems, it was proposed that this complex recruits CO to the *FT* promoter where it acts as a co-activator. This model received further support when NF-YB/HAP3 proteins were demonstrated to bind the *FT* promoter *in vitro* (Kumimoto *et al.*, 2008). Thus, the pendulum had swung to favor CO acting as a co-activator in an NF-Y/HAP complex.

The new study from Tiwari and colleagues provides evidence that CO acts both as a typical transcription factor and as a co-activator (Fig. 1). First, consistent with a previous study of a tomato CO homolog (Ben-Naim *et al.*,

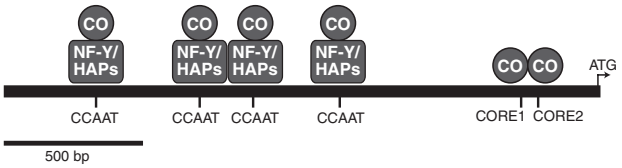

Fig. 1 Schematic drawing of the *FLOWERING LOCUS T* (*FT*) promoter. CONSTANS (CO) can bind the *FT* promoter directly at *CO*-responsive element (CORE) sites or through interactions with the NF-Y/HAP (NUCLEAR FACTOR Y/HEME ACTIVATOR PROTEIN) complex at CCAAT sites.

2006), the authors demonstrated that the glutamine-rich region of CO is sufficient to drive transcriptional activation in protoplasts. The authors then tested the ability of CO to activate transcription from a series of *FT* promoter fragments fused to a reporter gene. Notably, even when all the CCAAT motifs were deleted, CO still drove transcription above basal levels, indicating that CO could activate transcription independently of canonical NF-Y/HAP-binding sites. By further winnowing down the *FT* promoter to a minimal region responsive to CO activation, the authors were able to define two candidate CO-responsive elements (COREs). The importance of these CORE sequences was then confirmed by demonstrating that CO could activate transcription from minimal promoters containing multimerized CORE sequences, but not from mutated promoters containing several base changes in the COREs. To determine if this transcriptional activation might be a result of the direct binding of CO to CORE sequences, the authors performed *in vitro* gel-shift assays. These experiments showed that CO does indeed bind to COREs and that the CCT domain is responsible for DNA binding. In addition to demonstrating a novel NF-Y/HAP-independent role for CO in the activation of *FT*, Tiwari *et al.* also provide evidence for the importance of NF-Y/HAP in the activation of *FT* by CO. When multimerized and added to a minimal promoter, CORE sequences are sufficient to drive CO-dependent expression of *FT*; however, *FT* expression is further enhanced by the addition of a CCAAT sequence.

It is worth noting that the emerging model for gene regulation by CO and NF-Y/HAPs provides incredible potential for the formation of complexes with different specificities; Arabidopsis contains 17 CO-like (COL) proteins and 26 HAP3 and HAP5 proteins. Add to this variety the potential of CO and COL proteins to form homodimers or heterodimers and the regulatory plasticity of the system becomes vast. Tiwari and colleagues provide hints that differential affinities of CO/COL proteins for particular COREs may also shape gene-regulatory specificity. CO and COL15 produce similar levels of activation from reporter constructs containing CORE1 or CORE2. By contrast, COL9 shows little activation of CORE1, but strongly activates CORE2.

These results raise additional intriguing questions about the evolution of the COL family and the regulation of *FT* by CO. For instance, the finding that in some instances different COL proteins cannot bind the same CORE element is just as interesting as the finding that in some instances different COL proteins can bind the same CORE element. Competing interactions with different COL proteins that recruit different binding partners may foster regulatory innovation and complexity. In addition, this redundancy creates the opportunity for this regulatory interaction to experience developmental system drift (True & Haag, 2001), meaning that, over time, evolutionary distant COL proteins may replace one another as the predominant *FT* regulator. Such an evolutionary process may explain observations from tomato and morning glory suggesting that members of the COL family most closely related to CO in these organisms may not directly regulate *FT* (Ben-Naim *et al.*, 2006; Hayama *et al.*, 2007). The definition of a CORE sequence may also prove to be a boon for studies of natural variation. Regulatory variants in *FT* have been implicated in natural variation in flowering in several plant species, but the causal nucleotide changes have yet to be pinpointed, partly because of the absence of defined functional regulatory elements (e.g. Schwartz *et al.*, 2009; Takahashi *et al.*, 2009; Blackman *et al.*, 2010). Hence, polymorphisms in CORE-like sequences of *FT* homologs are genetic variants ripe for evaluation by functional studies, particularly in rice where variation in the CCT domain of a *CO* homolog is also associated with flowering time variation (Takahashi *et al.*, 2009).

Acknowledgements

B.K.B. is supported by NSF grant DBI-0905958. S.D.M. is supported by NIH grant GM075060.

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Key words: CCT (*CONSTANS-CONSTANS LIKE-TIMING OF CAB 1*) domain, CONSTANS, flowering, *FLOWERING LOCUS T*, HEME ACTIVATOR PROTEIN (HAP), NUCLEAR FACTOR Y (NF-Y), photoperiod.

Biotic and abiotic factors act in coordination to amplify hydraulic redistribution and lift

The possibility that water can flow from roots to the soil has been a major focus of attention for well over 70 yr in ecology and agronomy (Kramer, 1933) and much effort has been devoted to demonstrate its existence and to quantify its magnitude (Molz & Peterson, 1976). When the root-xylem tissue interfacing the soil has a hydraulic conductivity (K_r) well in excess of the soil hydraulic conductivity, the flow of water from a root to adjacent soil pore spaces can occur provided that the root water tension is smaller than the adjacent soil water tension. However, the idea that this mechanism may be part of a water-redistribution network induced by the rooting system as a plant strategy to buffer against prolonged droughts emerged later. It was shown that some species delay the onset of water stress by preferentially transporting soil water from the upper soil layers into deeper layers after rainfall and then releasing water from the deeper layers into the upper soil layers, where needed, via this rooting network (Mooney *et al.*, 1980). Hydraulic lift (HL), which occurs primarily at night, was coined to describe the latter mechanism (Caldwell *et al.*, 1998), while hydraulic redistribution (HR) is used to emphasize that the rooting system can distribute water passively within the soil profile from wet to dry soil layers. With the proliferation of sap-flow and stable isotope measuring techniques over the last two decades (Emerman & Dawson, 1996), evidence of HL has been reported for shrub, grasses and tree species, and for temperate, tropical and desert ecosystems (Caldwell *et al.*, 1998; Horton & Hart, 1998; Oliveira *et al.*, 2005). Despite the widespread evidence and voluminous data on the occurrence of HL and HR, three inter-related topics have resisted rigorous treatments to date: to what extent HR and HL confer advantages to the entire ecosystem in terms of carbon (C) gains (Emerman & Dawson, 1996); to what degree biotic and abiotic processes work in coordination to 'amplify' HR or HL; and given the reported magnitudes of HR and HL, how their effects can be represented in future generations of large scale C–water transport models, a topic now receiving attention in climate systems (Lee *et al.*, 2005). The study by Domec *et al.* (pp. 171–183), in this issue of *New Phytologist*, considered the first topic for a loblolly pine stand during months of normal and below-normal precipitation and 'fingerprinted' the effects of HR on tree transpiration, ecosystem water use and the key components of the C balance. Using combinations of long-term