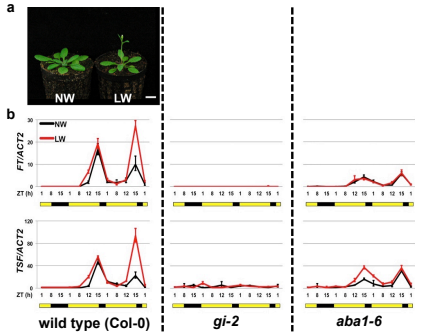
UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI BIOSCIENZE

Molecular Basis of the ABA Dependent Modulation of CONSTANS Activity in Drought Escape Response

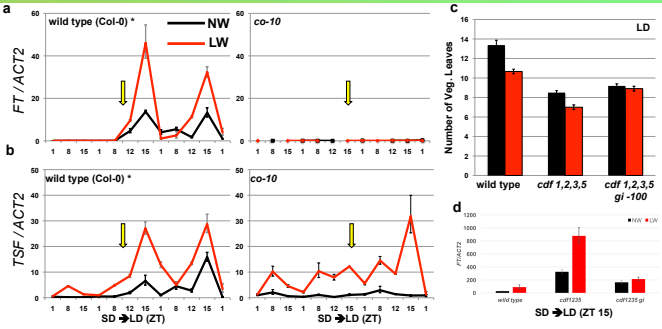
Alice Robustelli Test, Matteo Riboni, Sara Castelletti, Massimo Galbiati, Eirini Kaiserli#, Chiara Tonelli and Lucio Conti

 # Institute of Molecular, Cell & Systems Biology, University of Glasgow (UK)

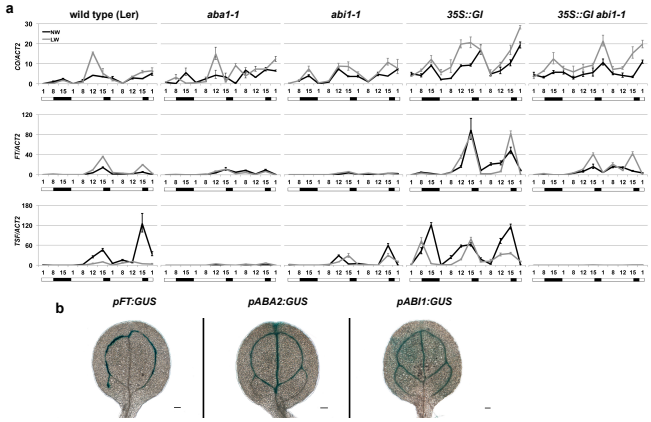
The drought escape (DE) response allows some plants to adaptively shorten their life cycle to make seeds before severe stress leads to death. In *Arabidopsis*, DE occurs under long day conditions (LDs), when photoperiod–stimulated GIGANTEA (GI) promotes the transcriptional activation of the florigen genes *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*). The phytohormone ABA participates in this process in an unknown manner, upstream of the florigen genes. A key question is how does ABA activate the florigen genes ? Here we use ABA signalling and photoperiod perception mutants to demonstrate that ABA transduces water status information upon the florigen gene *FT* by stimulating GI signalling and CONSTANS (CO) activity.



We have previously shown[1] that *Arabidopsis* wild-type plants low watering (LW) conditions promote flowering compared to normal watering (NW) (**a**) as a result of a drought-dependent upregulation of the florigen genes *FT* and *TSF*, specifically under LD conditions. No *FT* and *TSF* upregulation occurs in *gi* mutants at any time points, whereas in *aba1* mutants transcript accumulation of the florigen genes is reduced, especially under LW (**b**).

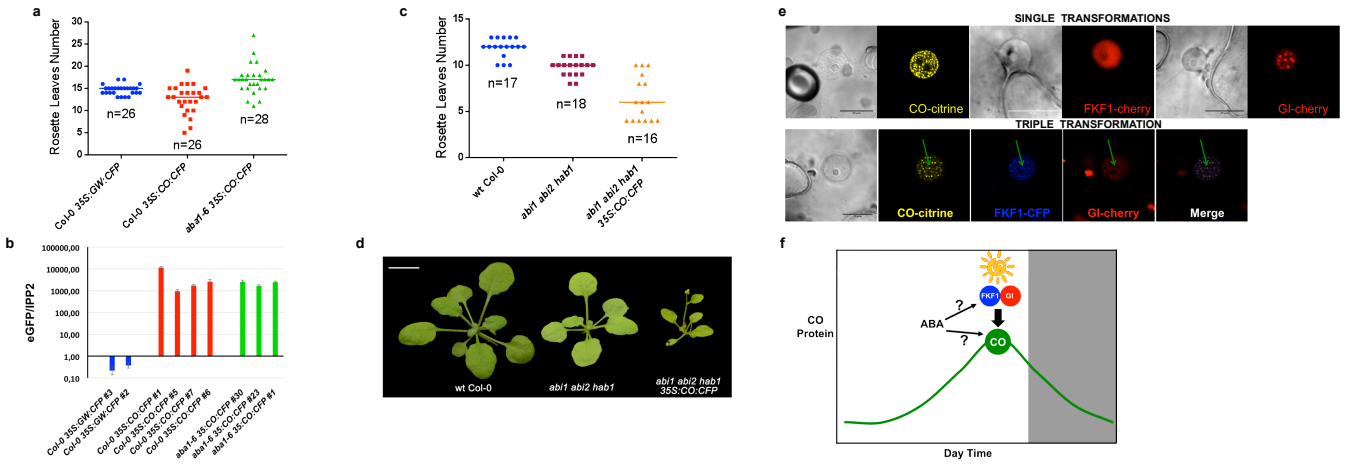


Unlike *FT*, *TSF* is upregulated in mutants of *co* under drought conditions (**a,b**). Mutants of *cdf*, characterised by increased levels of *CO* [2], produce a DE response comparable to wild type under LDs (**c**). Pointing to a specific role of GI in activating DE is the observation that *cdf gi* mutants are DE-insensitive under LDs. The pattern of *FT* upregulation reflects the DE response in these genotypes (**d**). We conclude that transduction of ABA signals onto *FT* promoter requires both photoperiod stimulated GI and CO.



We sought to elucidate the role of ABA upstream of *FT*, whether the canonical ABA signalling cascade could be involved [3] and whether ABA had any effect on GI/CO functions. As expected, in the L*er* wild-type background *FT* levels (but surprisingly not *TSF*) were increased under LW (**a**). Mutants impaired in ABA signalling (*abi1-1*) or with reduced ABA accumulation (*aba1-1*) displayed a general reduction in *FT*/*TSF* accumulation under LW. The pattern of *CO* transcript accumulation was unaffected in both ABA-defective backgrounds. Increased *FT*/*TSF* levels were apparent in *35S:GI* plants as a result of *CO* upregulation. Such phenotype was strongly suppressed in *35S:GI abi1-1* double mutants without a comparable decrease in *CO* accumulation. $ABI1_{promoter}$*:GUS* fusions revealed that *ABI1* expression overlaps with *FT* and the site of ABA biosynthesis in the phloem (**b**).

We conclude that ABA may stimulate a specific aspect of GI function responsible for *FT*/*TSF* genes transcriptional activation. ABA in conjunction with photoperiod-stimulated GI positively affect CO activity rather than *CO* transcript accumulation to promote *FT* transcriptional activation. We thus tested the hypothesis that ABA accumulation and signalling could be necessary for CO function.



Overexpression of *CO* under the *35S* promoter cannot not fully rescue the *aba1-6* flowering phenotype compared to the wild type (Col-0) in independent transgenic plants (**a,b**). In a complementary approach, when overexpressed in plants with enhanced ABA signalling (*abi1 abi2 hab1* [4]), *CO* causes an extreme early flowering phenotype in half of the T1 plants (**c,d**). These genetic data support the notion that ABA promotes CO activity and/or stabilization. CO and GI co-localize in the nucleus in association with the blue light receptor FKF1 (**e**). Since FKF1 promotes CO accumulation [5], ABA might affect the formation of the CO-GI-FKF1 complexes and thus CO accumulation. In summary, our data suggests a role for ABA in altering photoperiodic perception and signalling through modulation of CO function in a specific temporal window where GI and blue light photoreceptors (e.g. FKF1) are active (**f**).

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