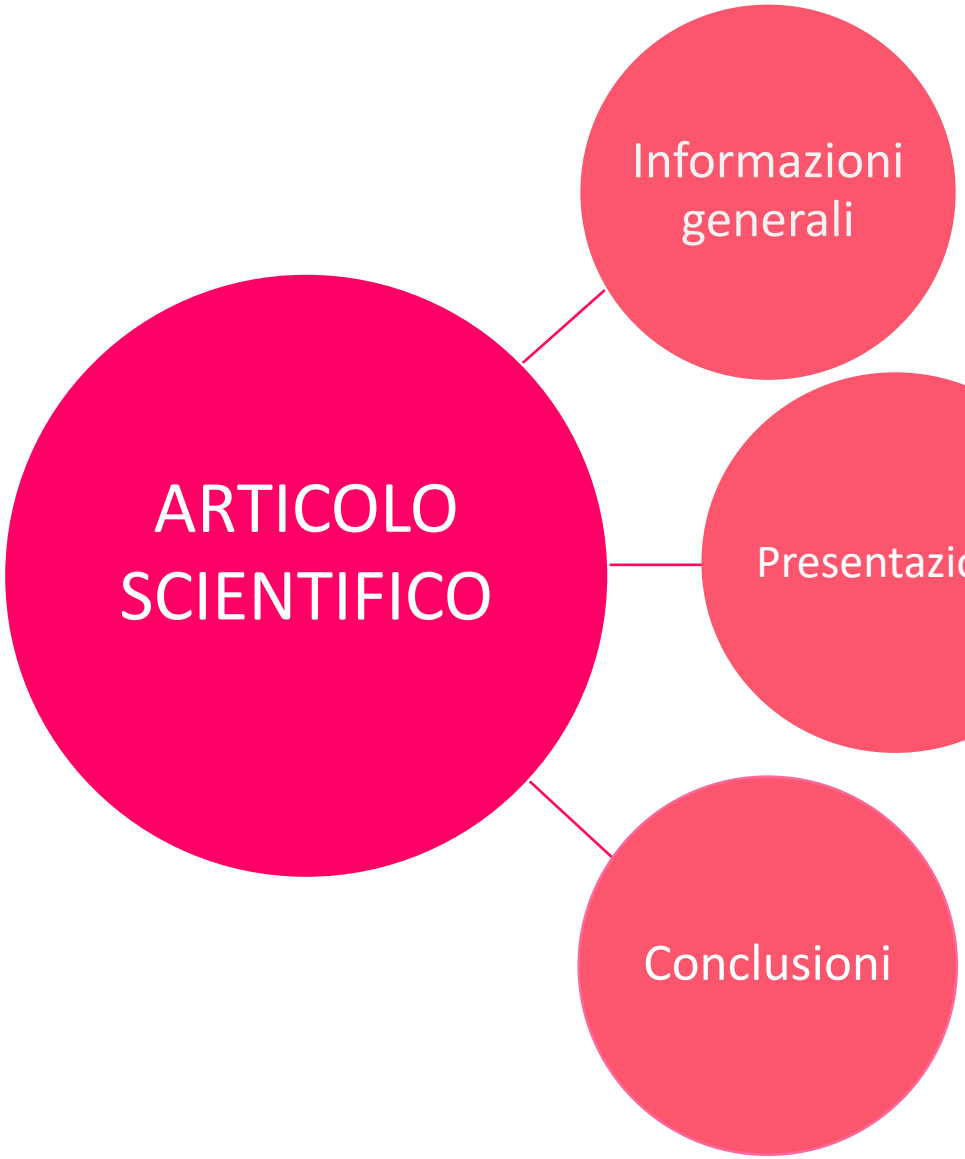
Mappa della lezione



- Cos'è e perché si usa?
- Struttura e utilizzo

- Affrontiamo insieme un articolo scientifico

- Take-home message

Chiara Longo, PhD
chiaralongo@uniroma1.it

Articolo scientifico: cos'è e perché si usa?

- il **mezzo di elezione** con cui gli scienziati comunicano tra di loro

- per condividere il proprio lavoro e risultati con gli altri scienziati ***research scientific paper,*** o per riesaminare ed organizzare i risultati di altri ***review***

- cruciali per il **progredire della scienza moderna**, dove il lavoro di uno scienziato si basa su quello degli altri

- devono essere **altamente leggibili,** cioè chiari, accurati e concisi: devono informare, non impressionare

Articolo scientifico: il peer reviewing

Peer review is designed to assess the validity, quality and often the originality of articles for publication

Its ultimate purpose is to maintain the integrity of science by filtering out invalid or poor-quality articles

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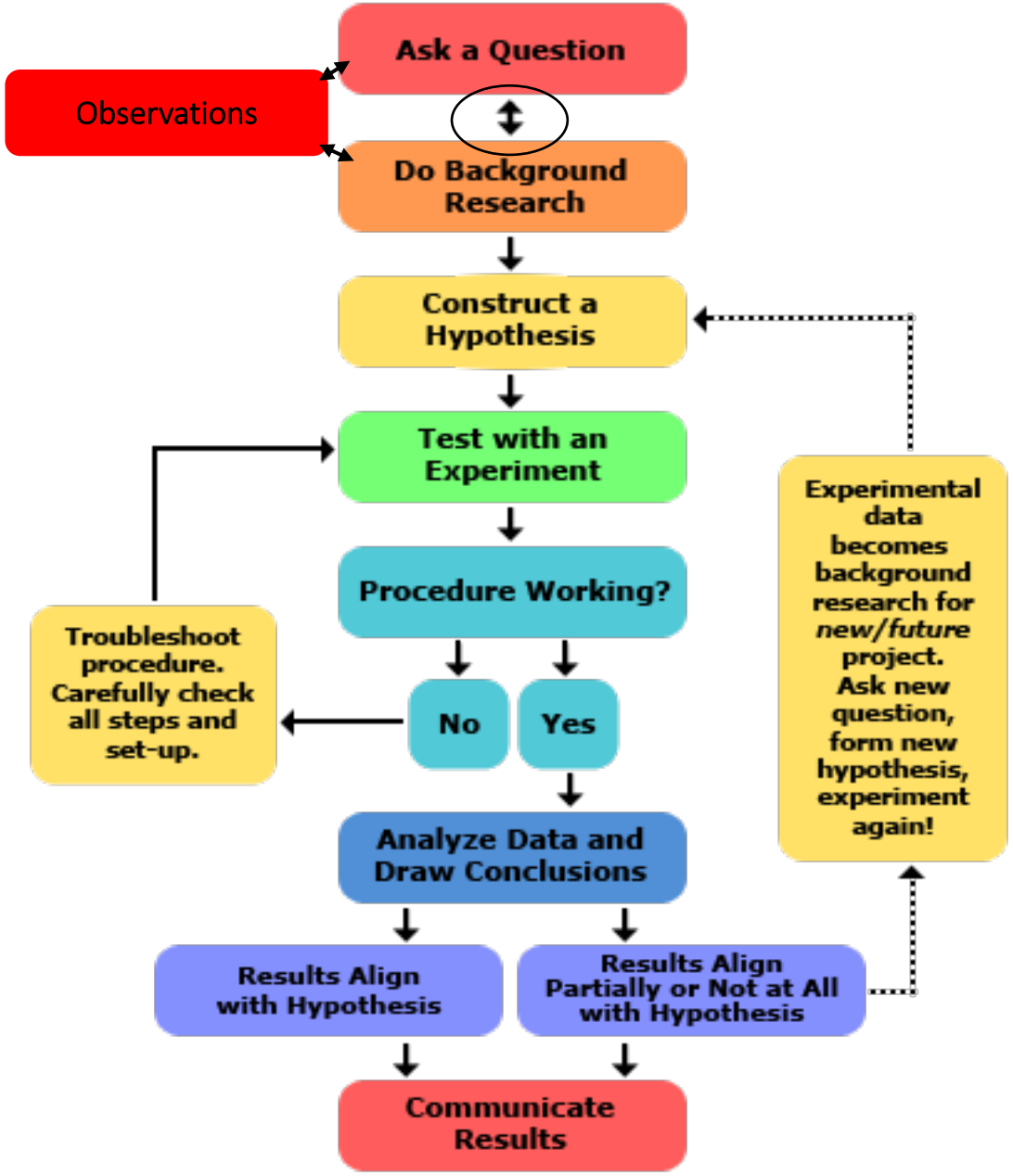
Informazioni generali

Articolo scientifico: la struttura

Abstract	In summary	Riassume motivazione (why), metodi (how), risultati (what they found) e conclusione (what it means)
Introduction	Why?	Introduce il contesto della ricerca (background): dal generale al particolare, fino alla domanda/e che si pongono gli autori (research question)
Methods	How?	Quali sono le metodologie adottate per rispondere alla research question?
Results	What?	Quale risposta/e gli autori trovano alla domanda/e che si sono posti?
Discussion/ Conclusion	So what?	Come si integrano i dati degli autori nel contesto della ricerca? Perché è importante?
References	Bibliografy	Elenco di articoli su cui si basa il lavoro: il lavoro di uno scienziato si basa sul lavoro di altri scienziati

From scientific method to a paper



Abstract

Author summary

Introduction

Results

Discussion

Materials and methods

Supporting information

Acknowledgments

References

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Informazioni generali

Articolo scientifico: come presentare un paper?

Dipende dal contesto, dal pubblico a cui è rivolto, dal tempo a disposizione.

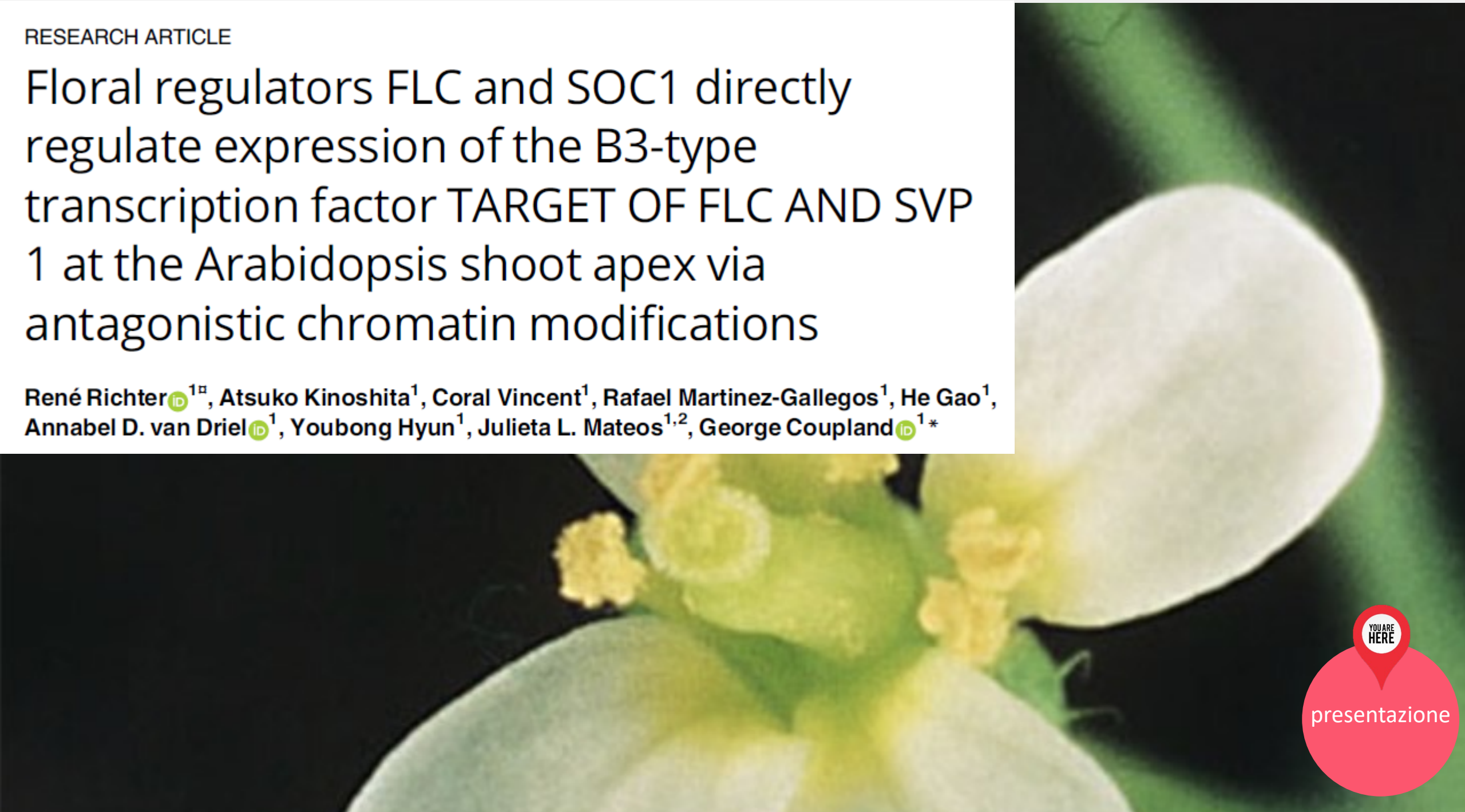
Si segue generalmente l'ordine Introduzione>Risultati>Conclusione, ma si può dividere i risultati in più parti (preferibilmente concettuali) e riportarli separatamente con introduzioni e conclusioni dedicate.

Prendete nota dei passaggi più importanti.
Schematizzate l'articolo
Provate a commentare le immagini dell'articolo insieme dopo averlo letto
Calcolate cosa dovete dire e quanto tempo avete

RESEARCH ARTICLE

Floral regulators FLC and SOC1 directly regulate expression of the B3-type transcription factor TARGET OF FLC AND SVP 1 at the Arabidopsis shoot apex via antagonistic chromatin modifications

René Richter[1¤], Atsuko Kinoshita[1], Coral Vincent[1], Rafael Martinez-Gallegos[1], He Gao[1], Annabel D. van Driel[1], Youbong Hyun[1], Julieta L. Mateos[1,2], George Coupland[1*]



Abstract

Integration of environmental and endogenous cues at plant shoot meristems determines the timing of flowering and reproductive development. The MADS box transcription factor FLOWERING LOCUS C (FLC) of *Arabidopsis thaliana* is an important repressor of floral transition, which blocks flowering until plants are exposed to winter cold. However, the target genes of FLC have not been thoroughly described, and our understanding of the mechanisms by which FLC represses transcription of these targets and how this repression is overcome during floral transition is still fragmentary. Here, we identify and characterize *TARGET OF FLC AND SVP1 (TFS1)*, a novel target gene of FLC and its interacting protein SHORT VEGETATIVE PHASE (SVP). *TFS1* encodes a B3-type transcription factor, and we show that *tfs1* mutants are later flowering than wild-type, particularly under short days. FLC and SVP repress *TFS1* transcription leading to deposition of trimethylation of lysine 27 of histone 3 (H3K27me3) by the Polycomb Repressive Complex 2 at the *TFS1* locus. During floral transition, after downregulation of *FLC* by cold, *TFS1* transcription is promoted by SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1 (SOC1), a MADS box protein encoded by another target of FLC/SVP. SOC1 opposes PRC function at *TFS1* through recruitment of the histone demethylase RELATIVE OF EARLY FLOWERING 6 (REF6) and the SWI/SNF chromatin remodeler ATPase BRAHMA (BRM). This recruitment of BRM is also strictly required for SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 9 (SPL9) binding at *TFS1* to coordinate RNAPII recruitment through the Mediator complex. Thus, we show that antagonistic chromatin modifications mediated by different MADS box transcription factor complexes play a crucial role in defining the temporal and spatial patterns of transcription of genes within a network of interactions downstream of FLC/SVP during floral transition.

The time of flowering transition
FLC represses floral transition
Reprimendo chi? Chi sono i suoi target?
Come? Con quali meccanismi?

Here, we identify *TFS1,* a novel target gene of FLC and its interacting protein SVP **I parte**

They repress *TFS1* leading to H3K27me3 deposition by the PRC2 at its locus
II parte

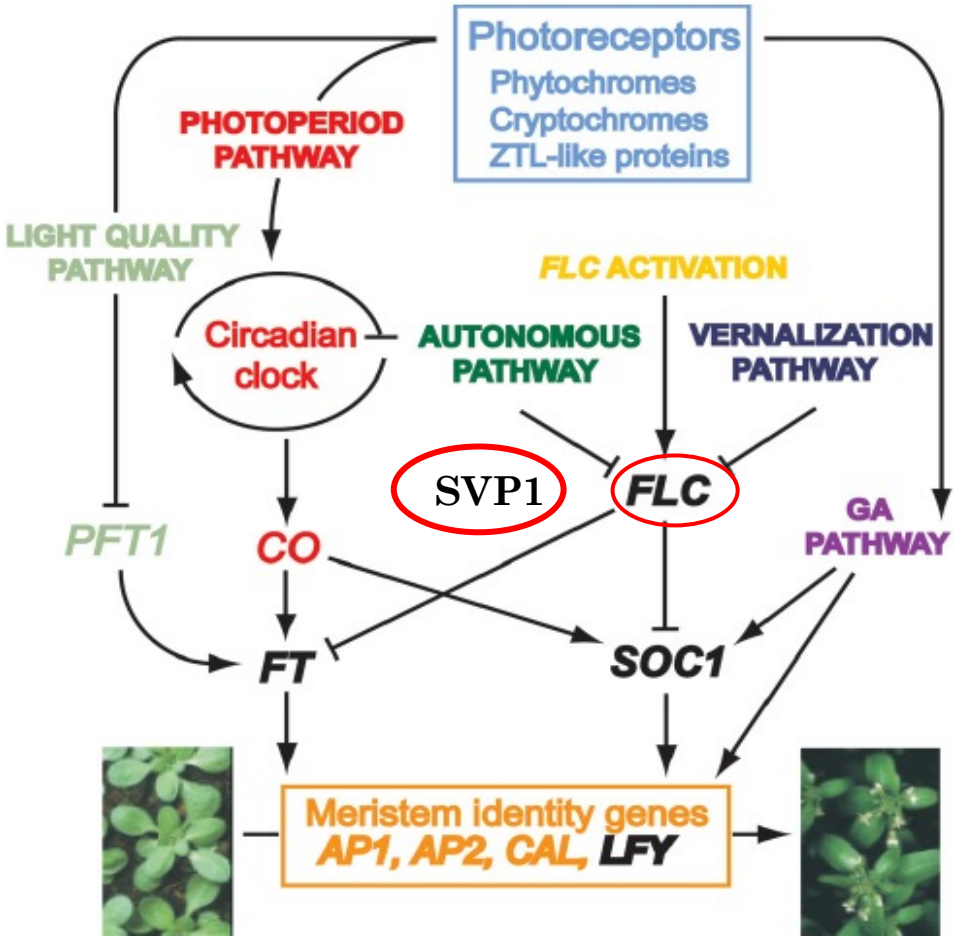
During floral transition*TFS1* transcription is promoted by SOC1. SOC1 opposes PRC2 through recruitment of the histone demethylase REF6 and the SWI/SNF chromatin remodeler BRAHMA (BRM)
II parte

Antagonistic chromatin modifications mediated by different MADS box TFs (FLC and SOC1) define temporal and spatial patterns of gene expression within a network of interactions downstream of FLC/SVP during floral transition

Introduzione:
Genetic control of flowering time in *Arabidopsis*

FLC and SVP1 integrate signals from several pathways

CO, a clock regulated gene, activates both FT and SOC1. FT and SOC1 activate meristem identity genes (from vegetative to reproductive development) at SAM.

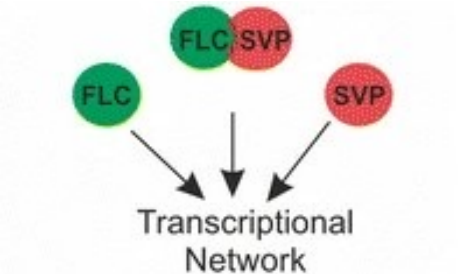


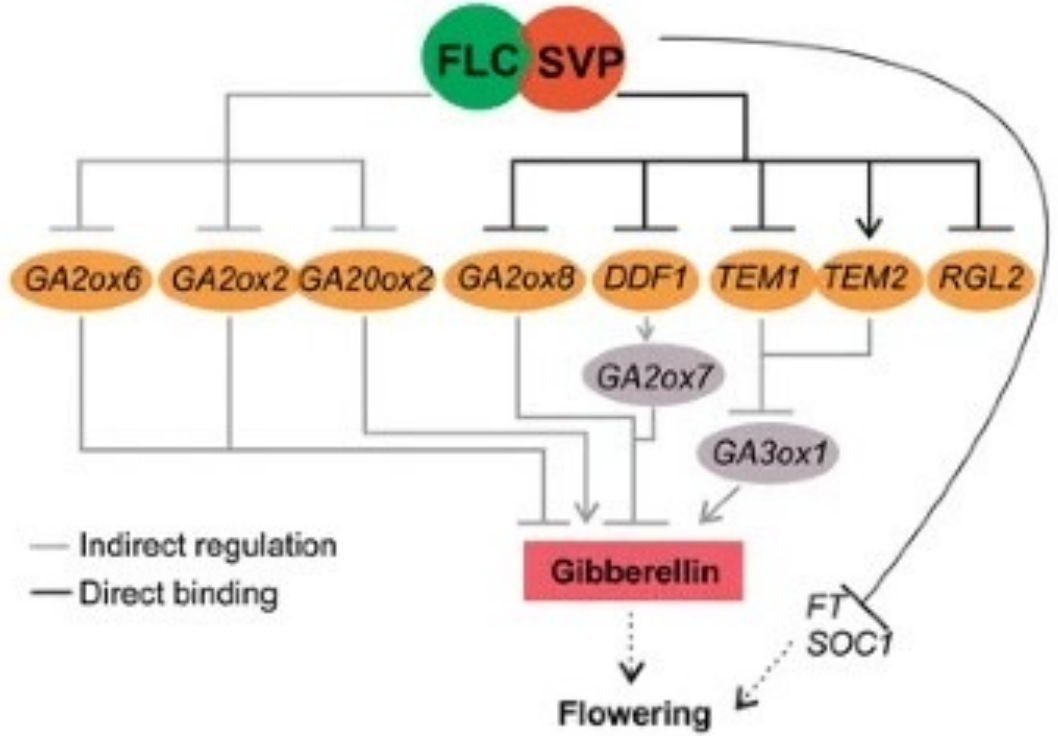
Introduzione: FLC e SVP

MADS-box TFs, key repressors of floral transition,

blocking flowering until plants are exposed to winter cold

- FLC interacts with SVP (SHORT VEGETATIVE PHASE) to repress genes that initiate flowering
- Their activity appears to involve modification of histones
- The targets of SVP-FLC complex include a higher proportion of genes regulating floral induction
- Activity of both TFs individually and as a complex on flowering studied through ChIP-Seq





Mateos et al., 2015

Introduzione: Le domande degli autori

Nevertheless, our understanding of how FLC influences the transcriptional network that controls floral transition and how it represses expression of its target genes is still fragmentary

Major Open Questions

- the target genes of FLC and SVP (at the shoot apex) – I PART
- the mechanisms by which FLC and SVP represses these targets – II PART
- the mechanisms by which these targets switch on during floral transition –II PART

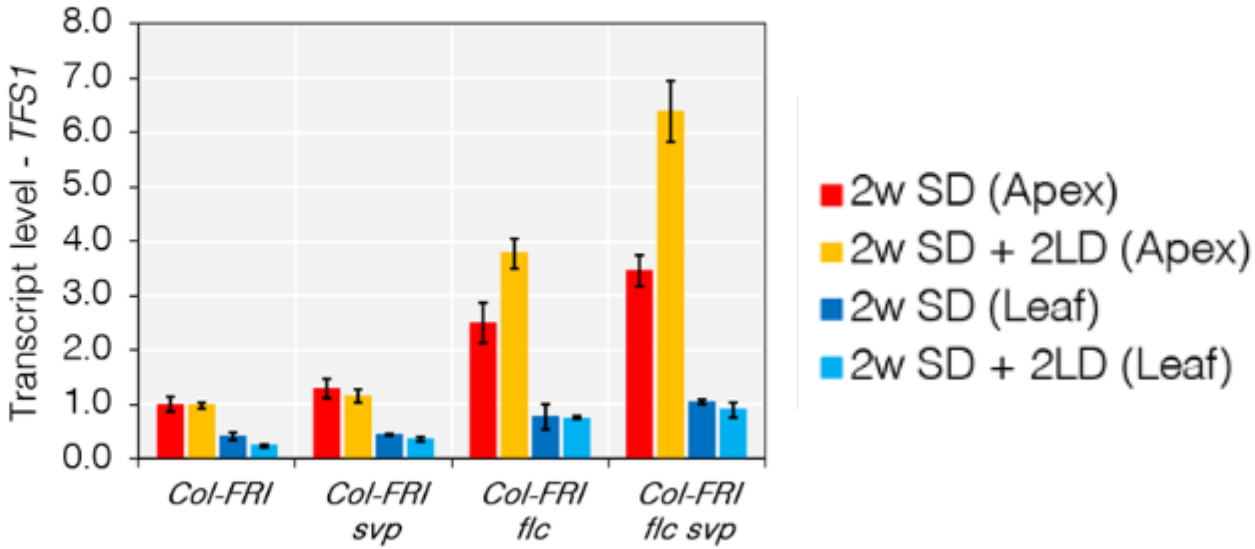
Risultati: Identificazione e caratterizzazione *TSF1*

a novel target gene of FLC and of SVP identified by analysis of previously published ChIP-Seq and tissue-specific RNA-Seq

atasets were examined searching for genes specifically expressed at the shoot apex and bound by FLC and SVP

Cross-referencing these datasets identified the gene encoding the B3-type transcription factor TARGET OF FLC AND SVP1 (TFS1), member of REPRODUCTIVE MERISTEM (REM) family

DAI DATI BIONFORMATICI ALLO STUDIO e
VALIDAZIONE GENETICO MOLECOLARE



TFS1 è sovrespresso in assenza di FLC ed in assenza di FLC e SVP
Specificamente negli SHOOT APEX
Particolarmente dopo uno switch SD to LD

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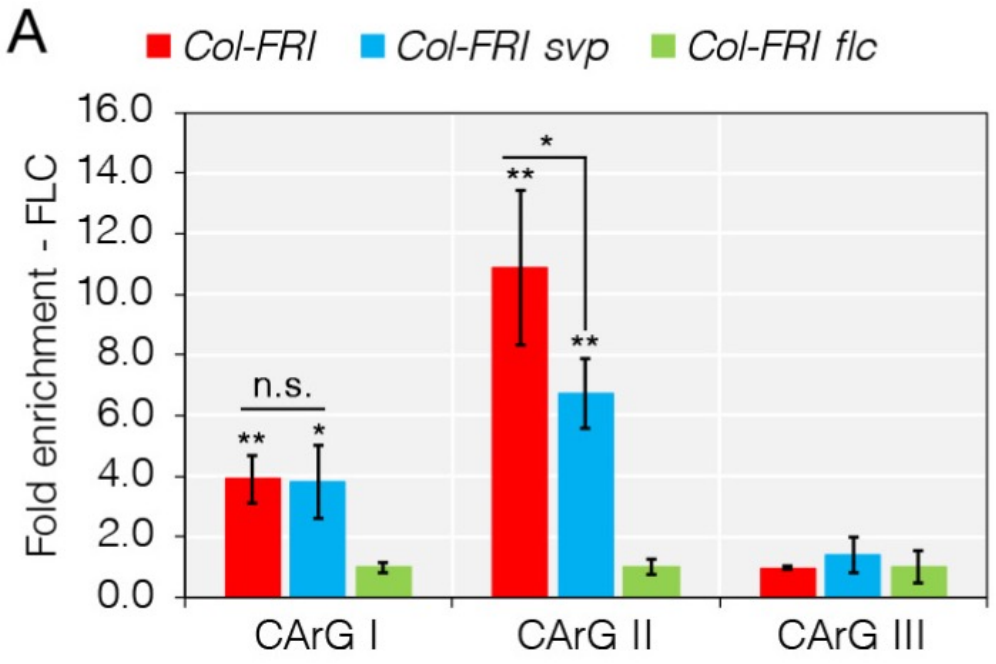
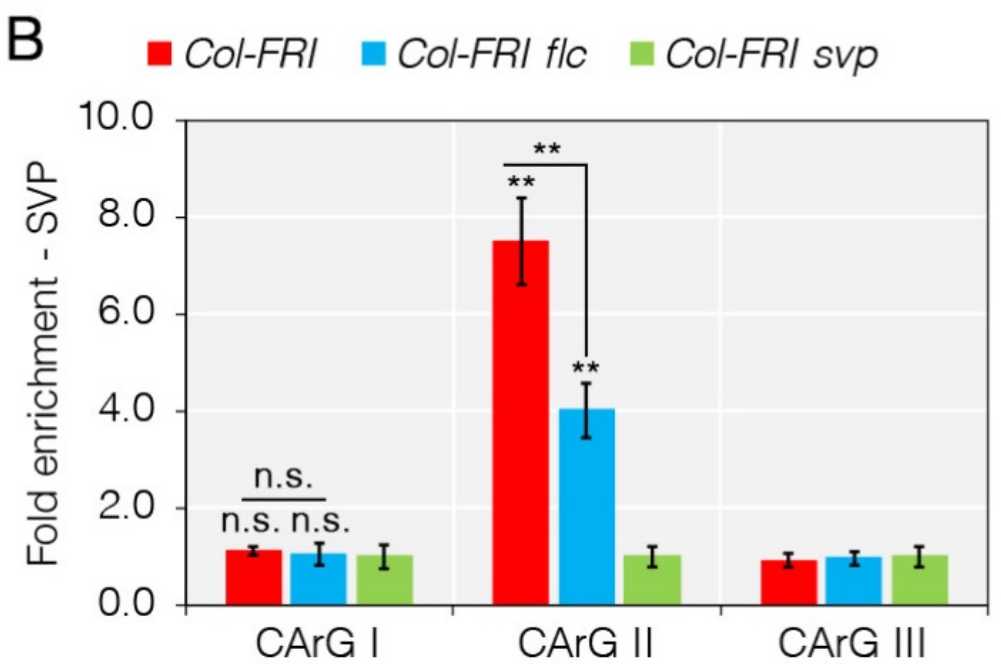
Risultati: FLC and SVP cooperate to repress *TFS1*

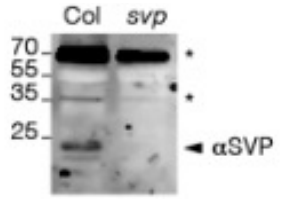
Enrichment of a fragment with the putative CArG-box II,3' end of *TFS1* detected after ChIP by qPCR
Mutual co-operation at *TFS1*: binding of FLC/SVP enhanced by the presence of both

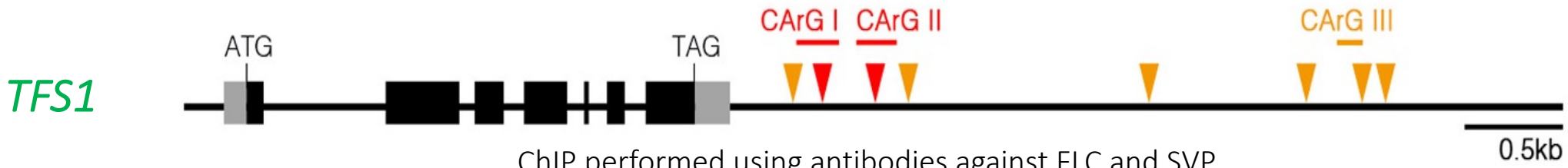
FLC



SVP





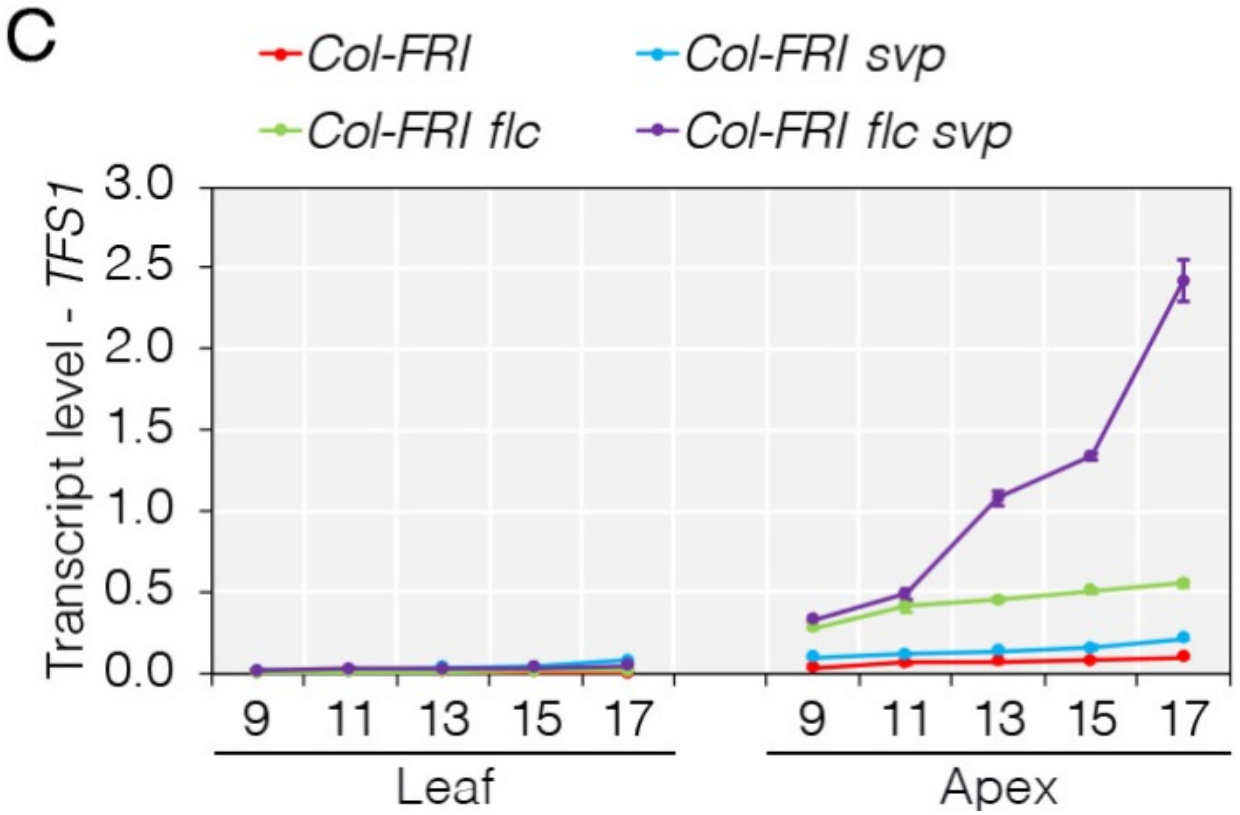


ChIP performed using antibodies against FLC and SVP

Values were scaled to set value of CArG-box I in *Col-FRI flc svp* to 1

Risultati: FLC and SVP cooperate to repress *TFS1*

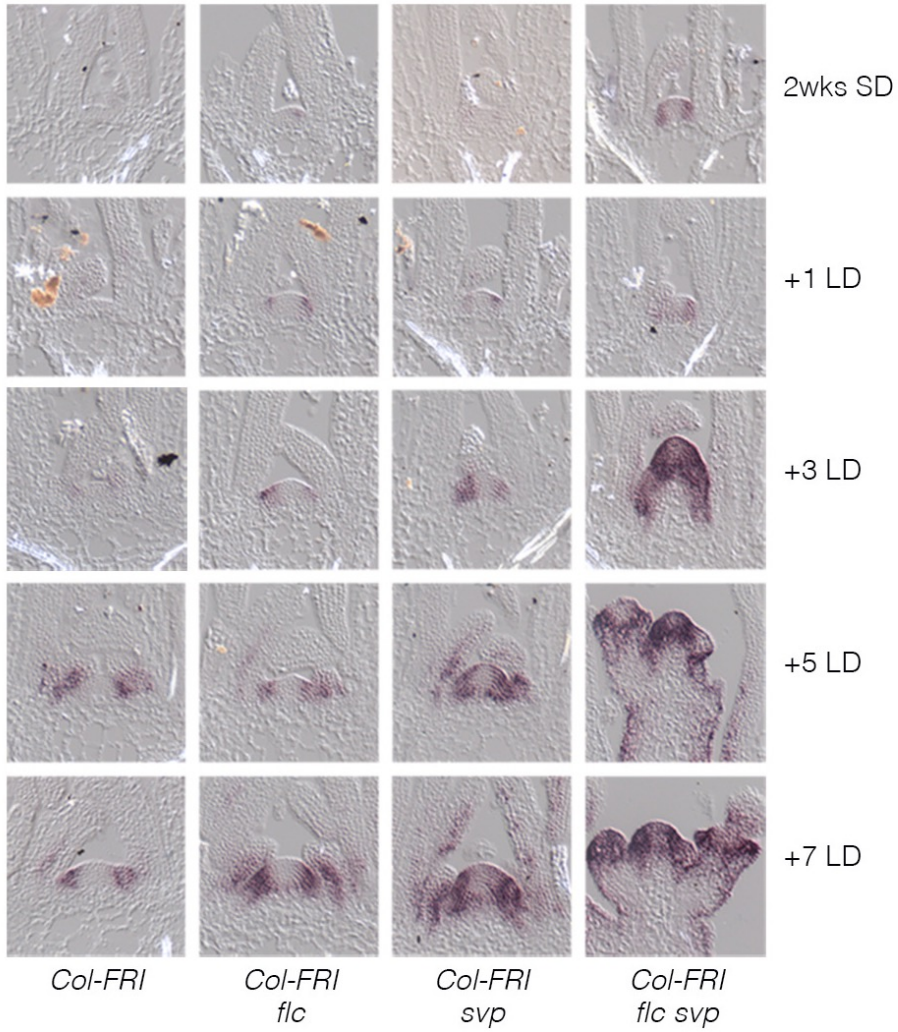
TFS1 transcription is induced during floral transition, while the timing and amplitude of its expression are modulated by SVP and FLC



Numbers on X- axis indicate number of long-days

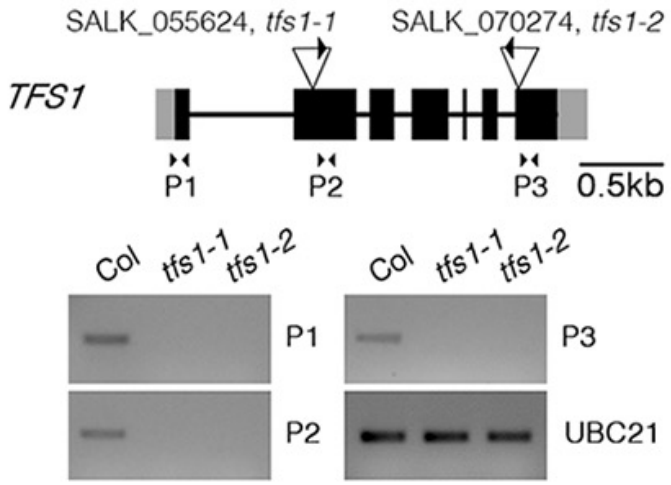
TFS1 is exclusively detected at the shoot apex, increased throughout a developmental and strongly increased in the double mutant

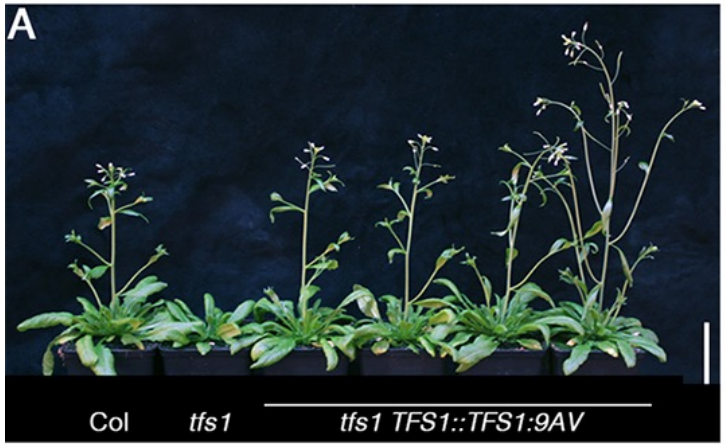
Spatial pattern of expression of *TFS1* assessed by *in situ* hybridization during floral transition

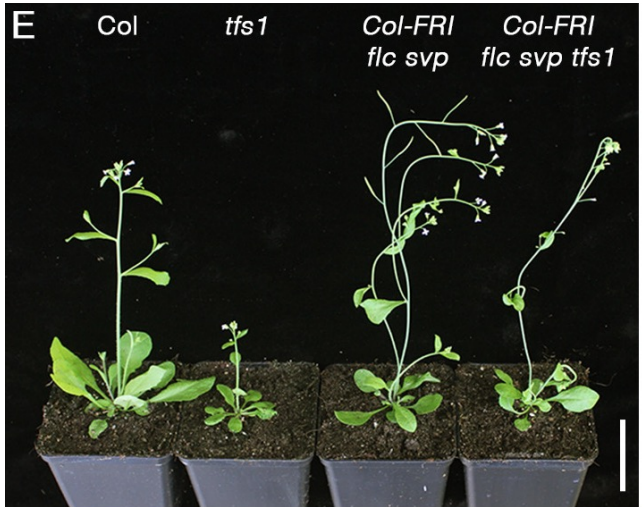


Risultati: *TFS1* is involved in promoting floral transition

Gene model for *TFS1* and T-DNA insertions







The *tfs1-1* mutant also delayed flowering in the *Col-FRI flc-3 svp-41* background
TFS1 acts downstream of FLC and SVP to promote flowering
(What does it mean EPISTATIC?)

Risultati : II PARTE

Major Open Questions

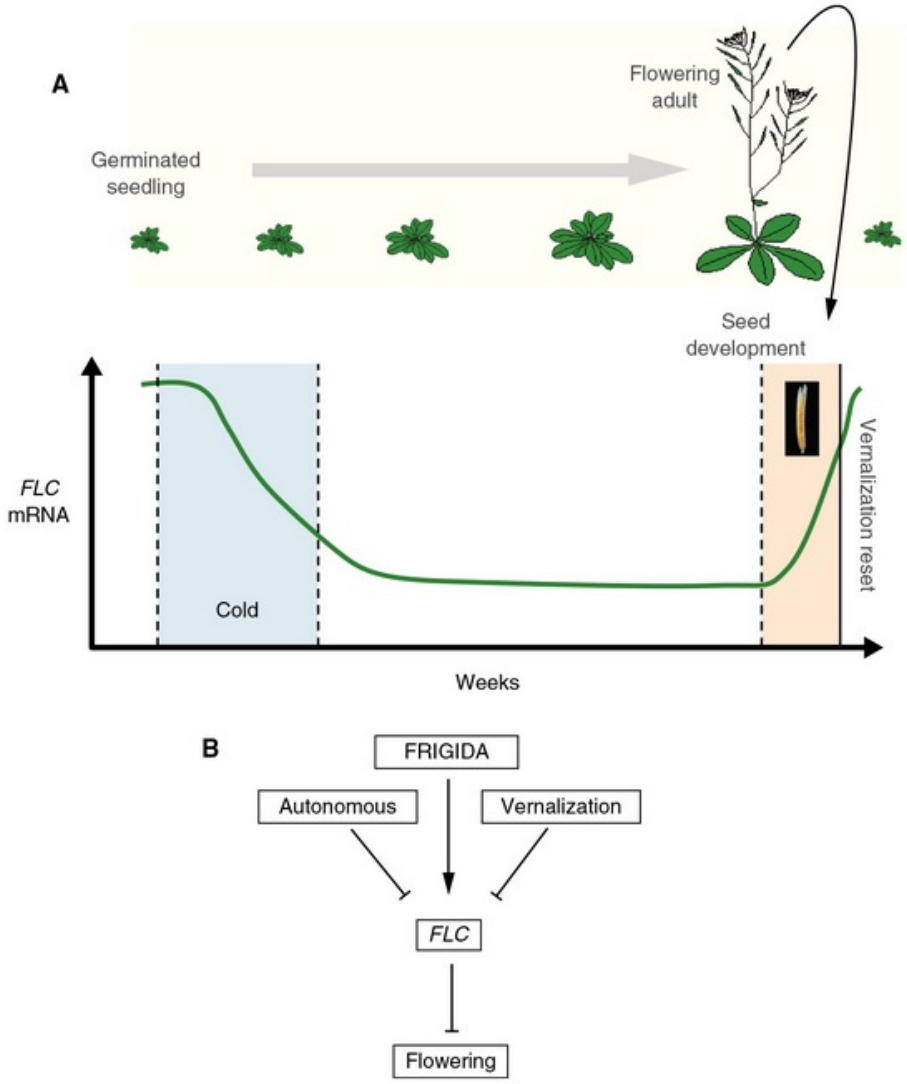
- the mechanisms by which FLC and SVP represses these targets
- the mechanisms by which these targets switch on during floral transition

MECCANISMI DI

CONTROLLO

EPIGENETICO

Risultati : II PARTE



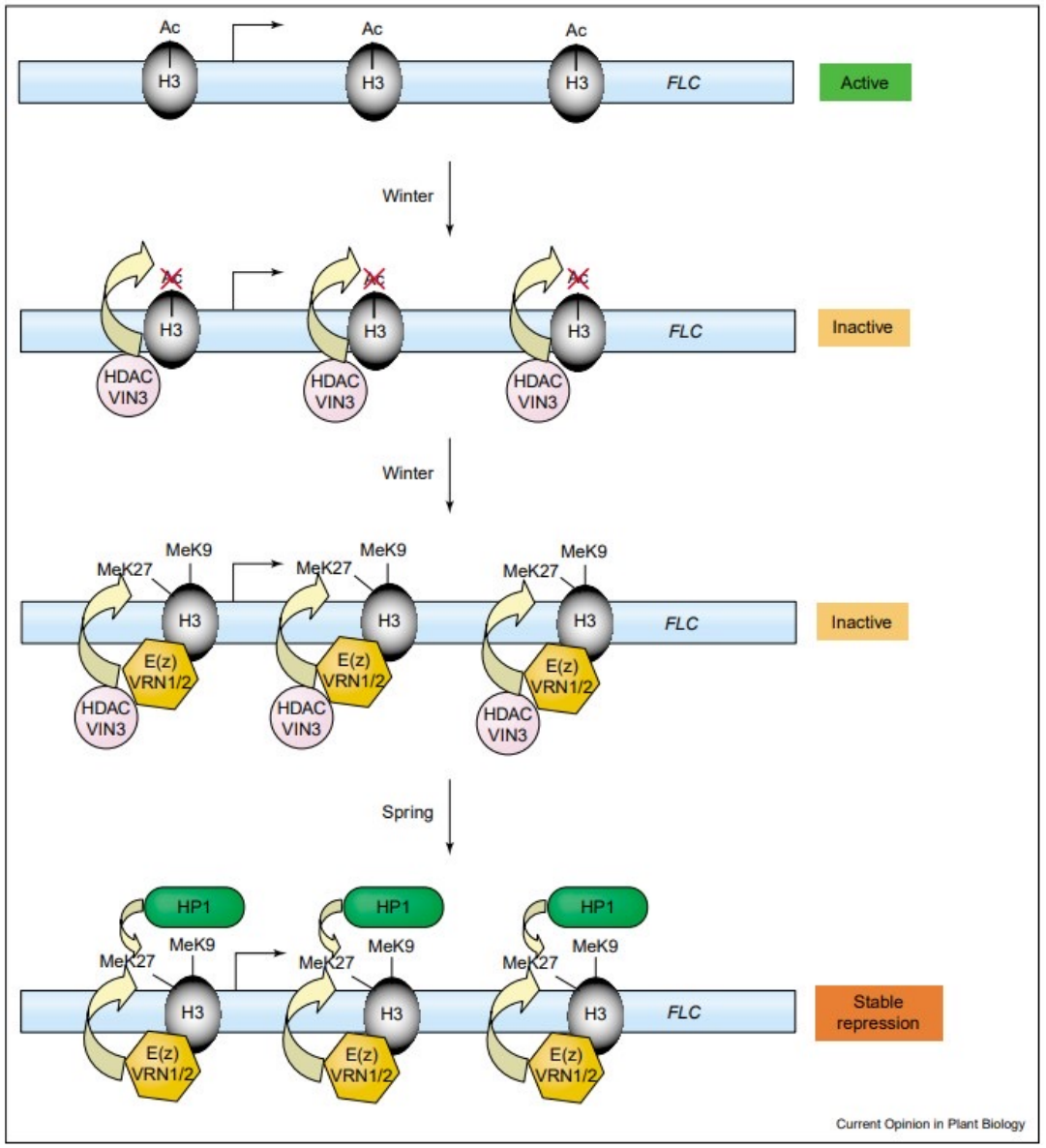
FLC is epigenetically controlled

FLC and *SVP* expression is silenced by PRC2

FLC highly expressed in seedlings. As plants perceive cold, the expression is quantitatively repressed, dependent on the length of cold experienced.

In spring, the repression is epigenetically maintained until seed development when it is reset.

Model of vernalization-mediated epigenetic silencing of *FLC*

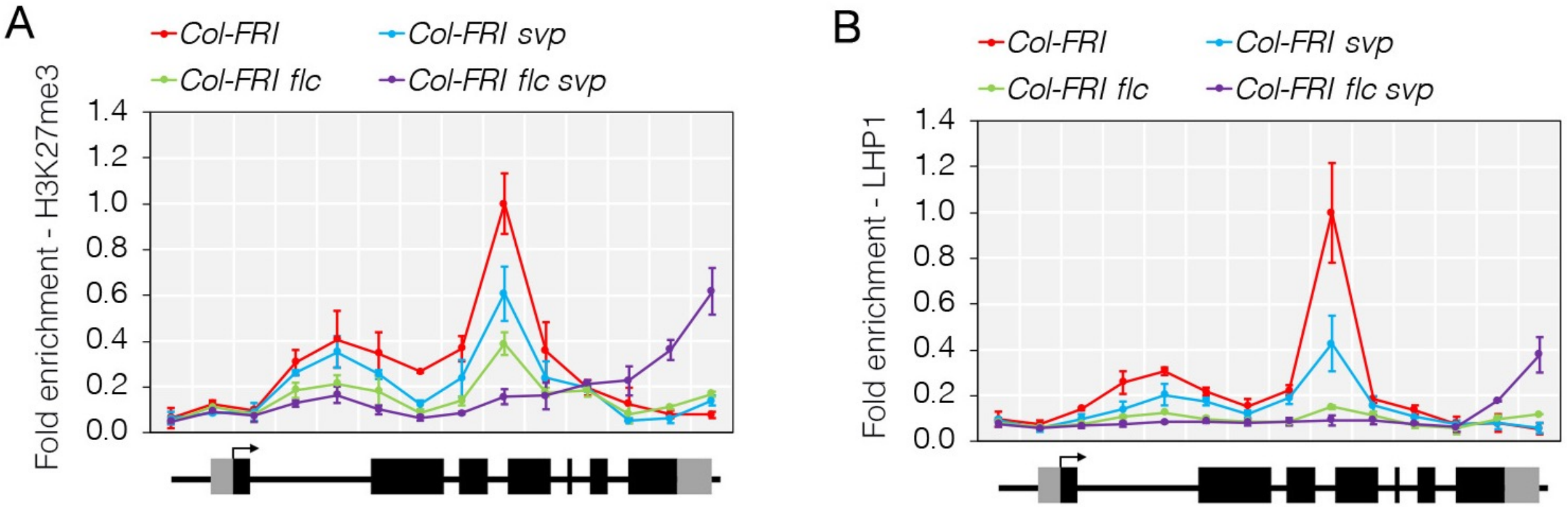


Winter: cold-induced expression of *VIN3* required for HDAC activity on *FLC* and PRC2 silencing

Spring: *VIN3* is no longer expressed, *FLC* repressed by PRC2 and HP1

RISULTATI: Is *TFS1* subjected to PRC-mediated regulation in a FLC/SVP dependent manner ?

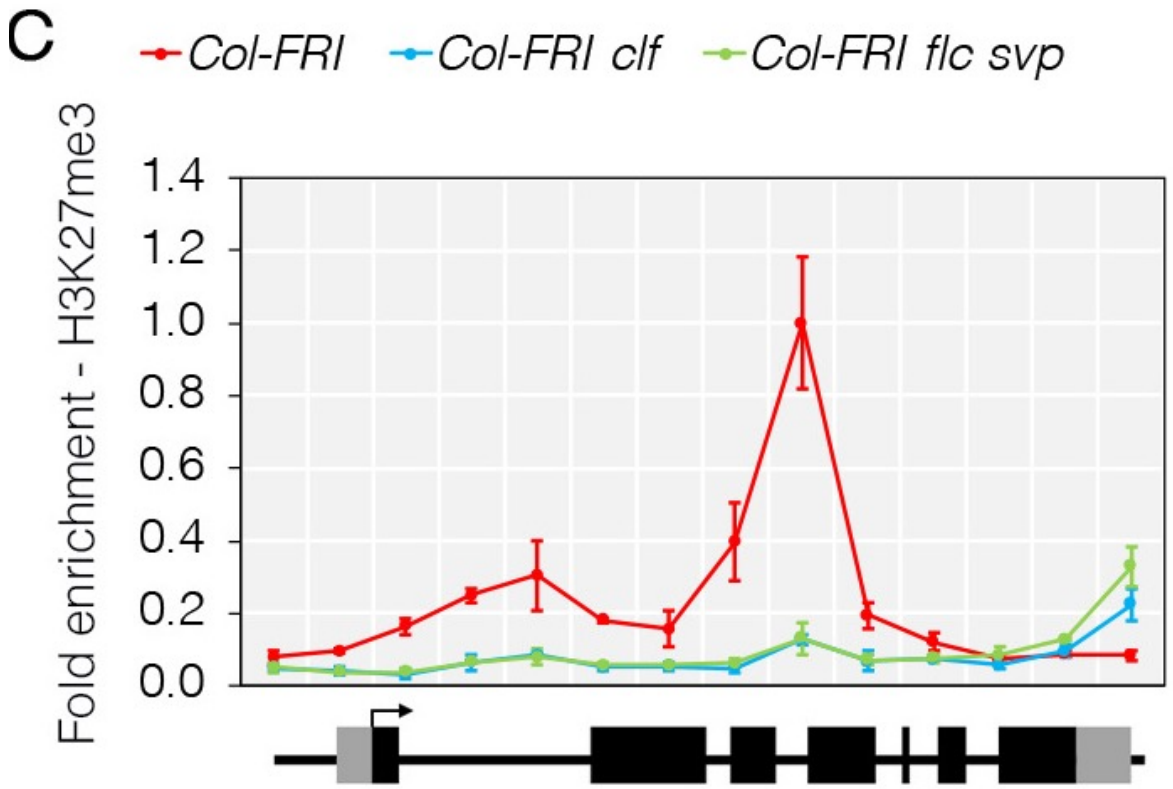
the activity of PRC2 at *TFS1* is dependent on FLC/SVP binding

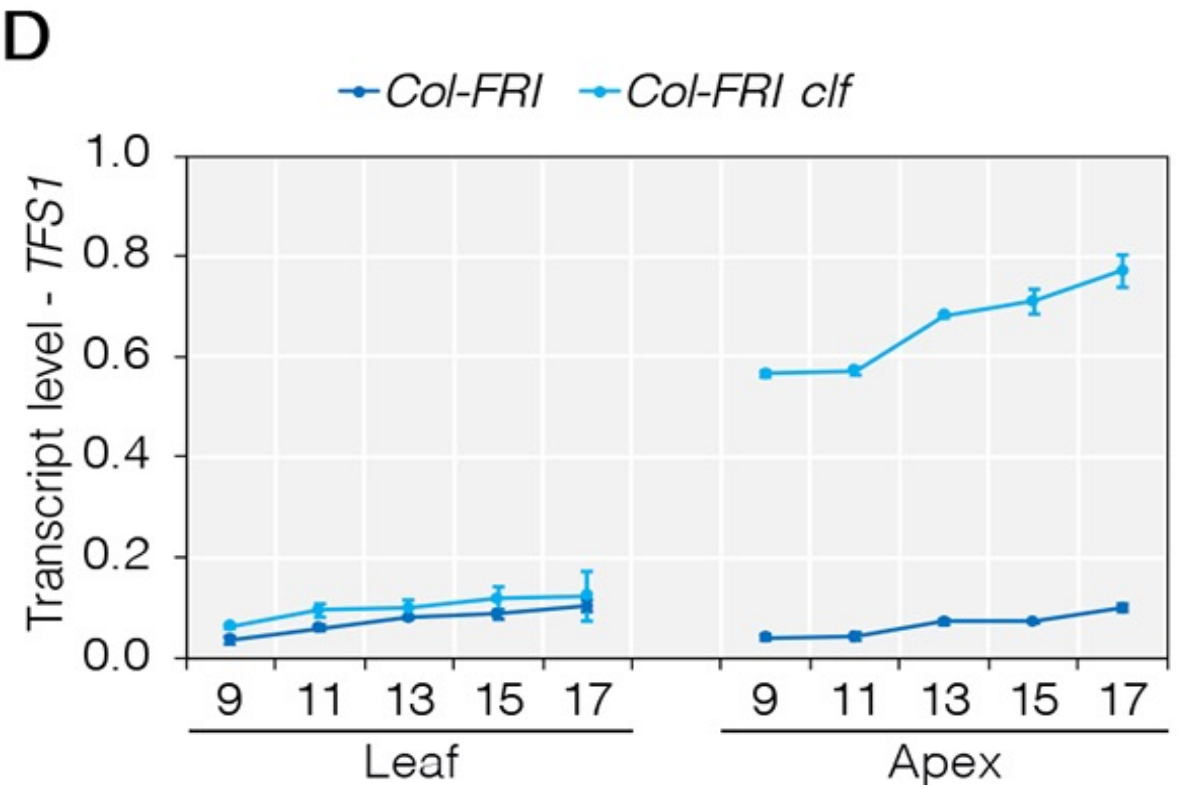


Binding of FLC and SVP to *TFS1* is associated with accumulation of H3K27me3 and LHP1

In *Col-FRI flc svp* plants an additional peak in H3K27me3 and LHP1

RISULTATI: PRC2 contributes to transcriptional repression of the FLC target *TFS1*



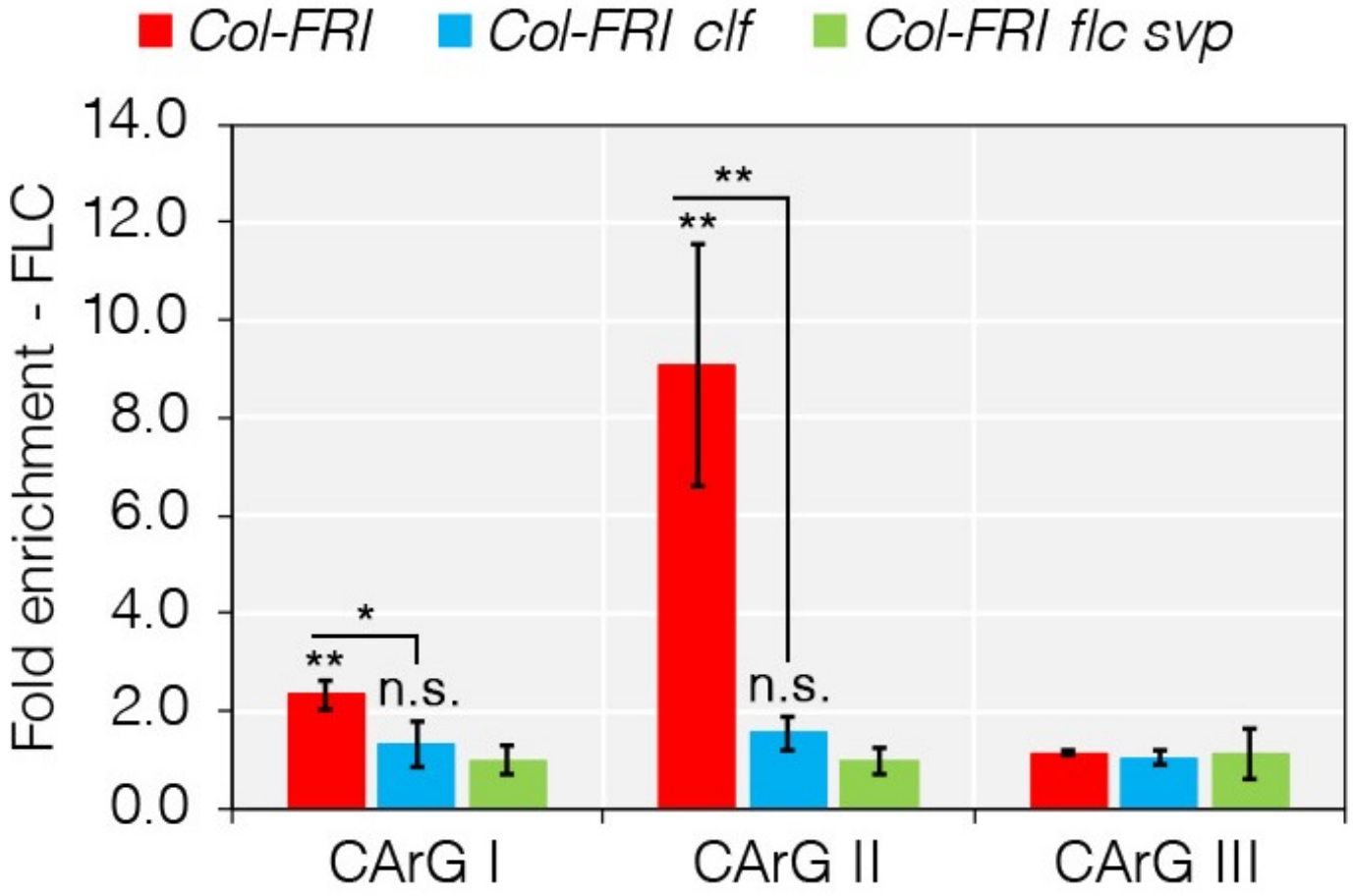


In *Col-FRI clf2* plants H3K27me3 level strongly decrease

Col-FRI clf-2 mutant plants expressed increased TFS1 mRNA levels

RISULTATI: PRC2 contributes to transcriptional repression of the FLC target *TFS1*

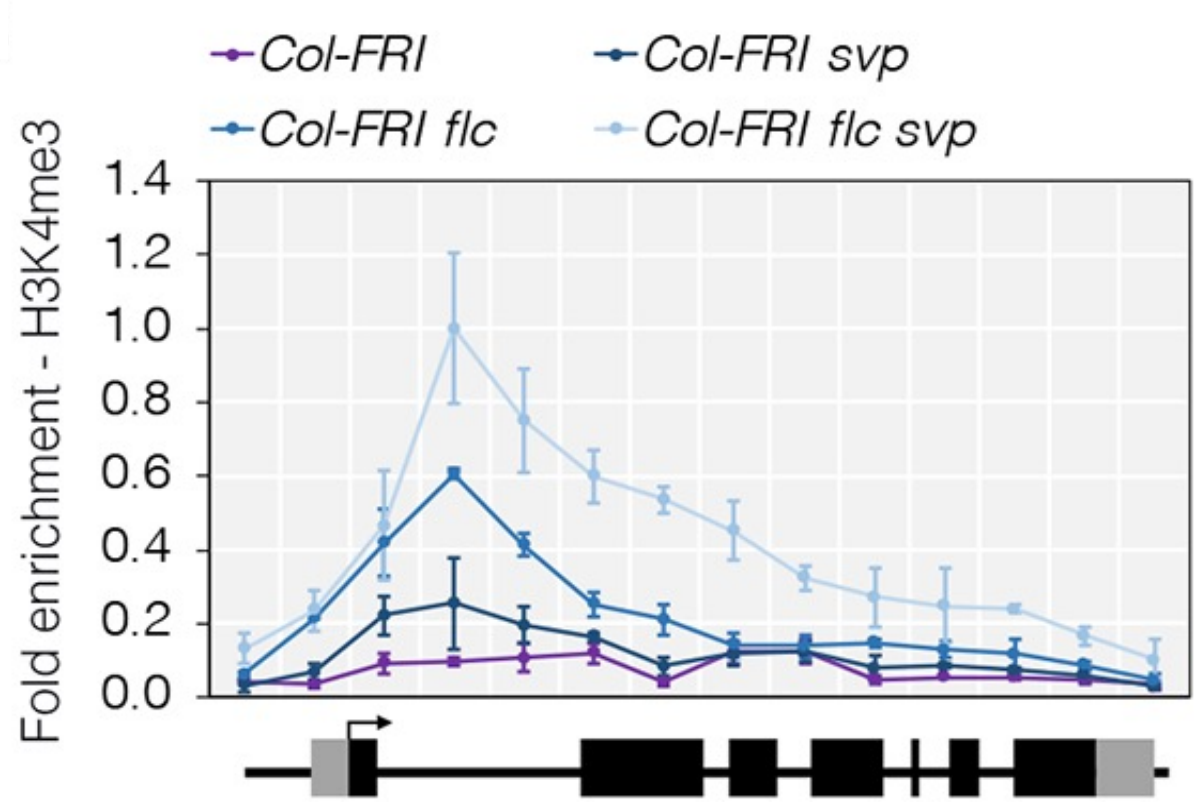
FLC binding requires and is sustained by PRC2 function



ChIP-qPCR –FLC: binding is strongly compromised in *Col-FRI clf-2*

Risultati: What about switch on during floral transition? H3K4me3 activation mark

Lack of *FLC* and *SVP* results in increased **H3K4me3** levels and *TFS1* expression



The dynamic of H3K27me3/H3K4me3 marks at *TFS1* correlates with *FLC* repression,

TFS1 induction and the transition to flowering

Risultati: What about switch on during floral transition?

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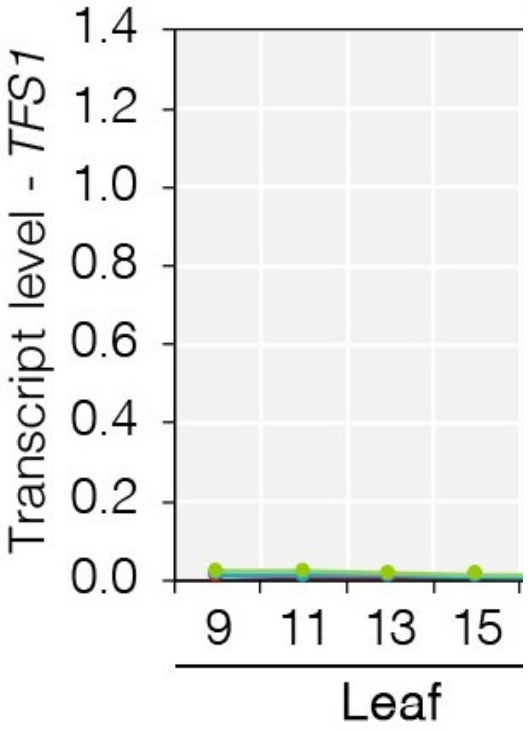

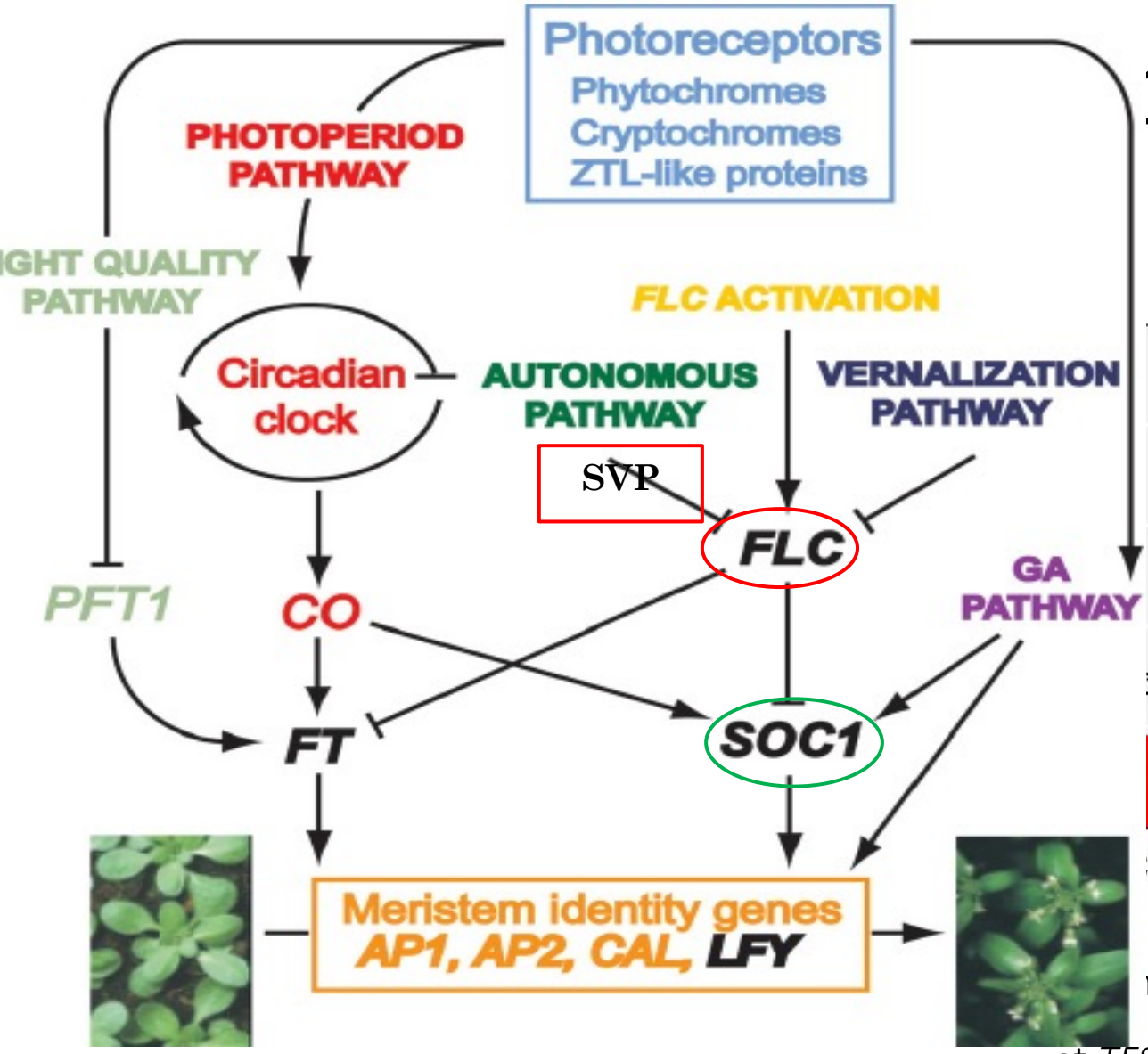

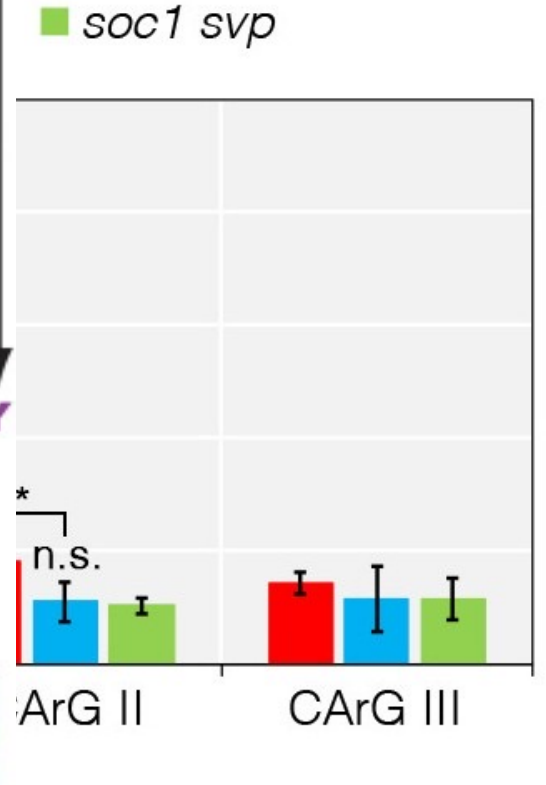

TFS1 mRNA levels i

ne CArG-box I (CArGI),

apices, and largely restored in *soc1-2 svp-41*

at *TFS1* 3' end

(*SUPPRESSOR OF OVEREXPRESSION OF CO 1*)

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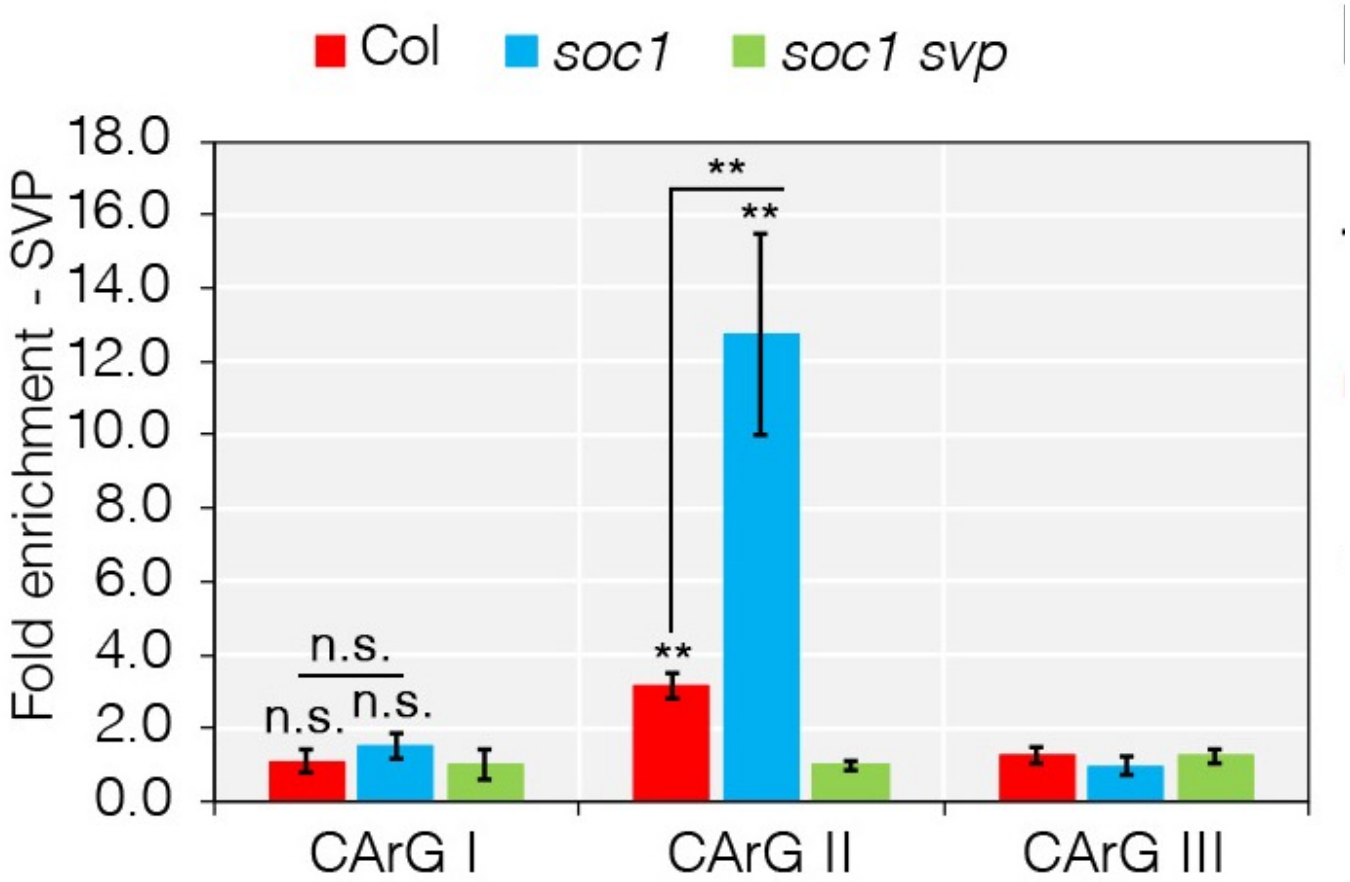
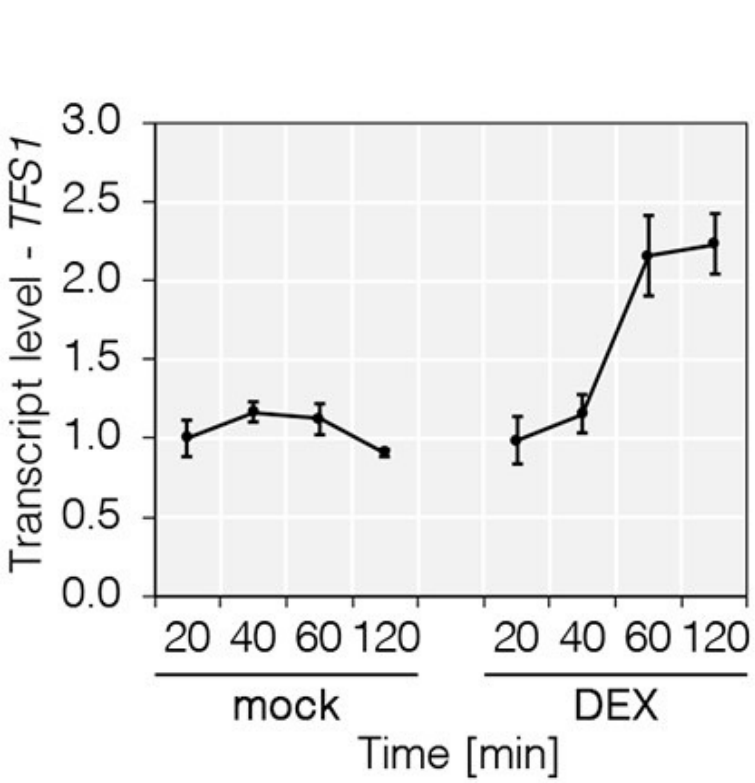
Risultati: SOC1 reduces SVP recruitment to *TFS1*



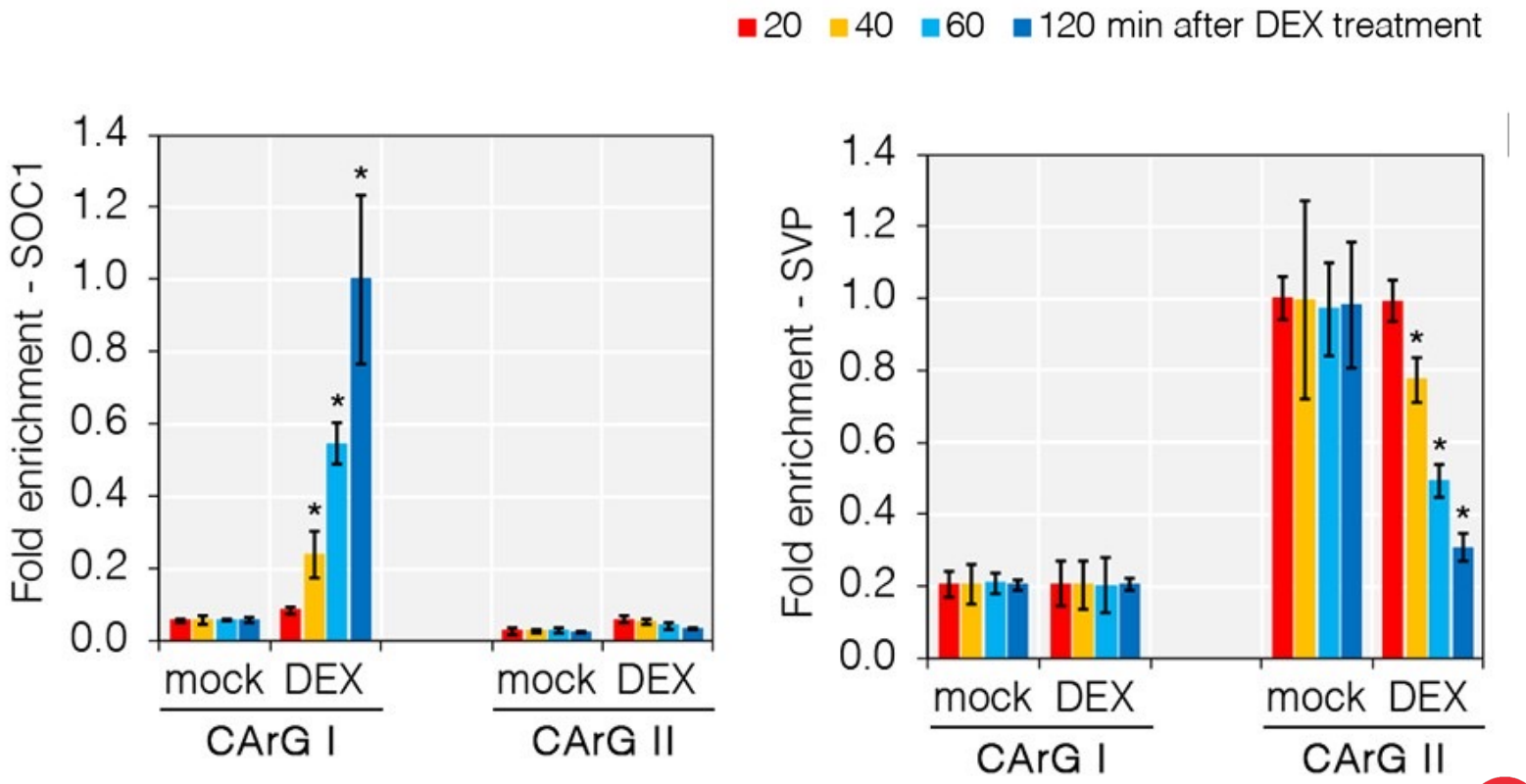
ChIP-qPCR - SVP: enrichment in the CArGbox II in *Col*, enhanced in *soc1-2*

Risultati: Is *TFS1* positively regulated by SOC1?
35S::SOC1:GR plants

DEX-induced nuclear translocation of SOC1:GR in 35S::SOC1:GR plants



increased *TFS1* expression



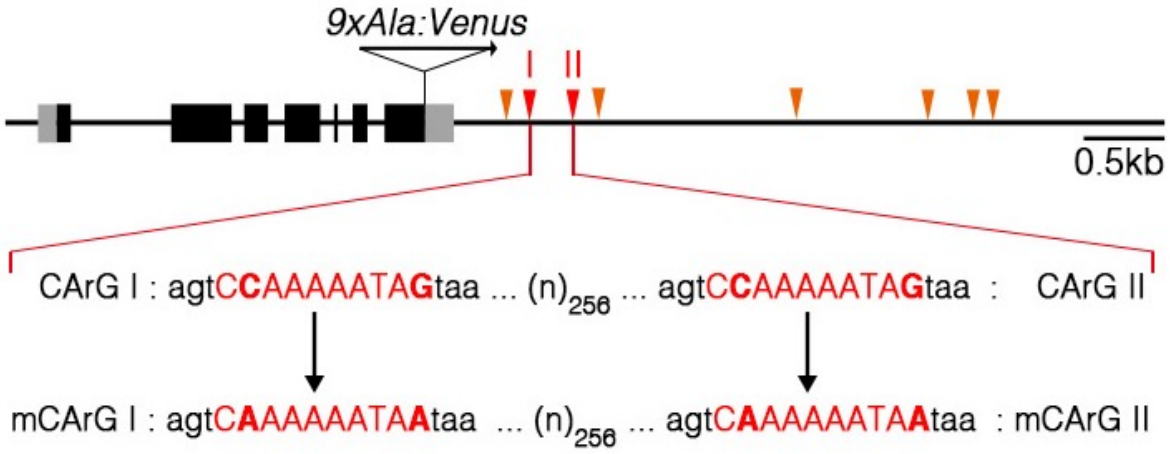
increased binding of SOC1 to CArGI

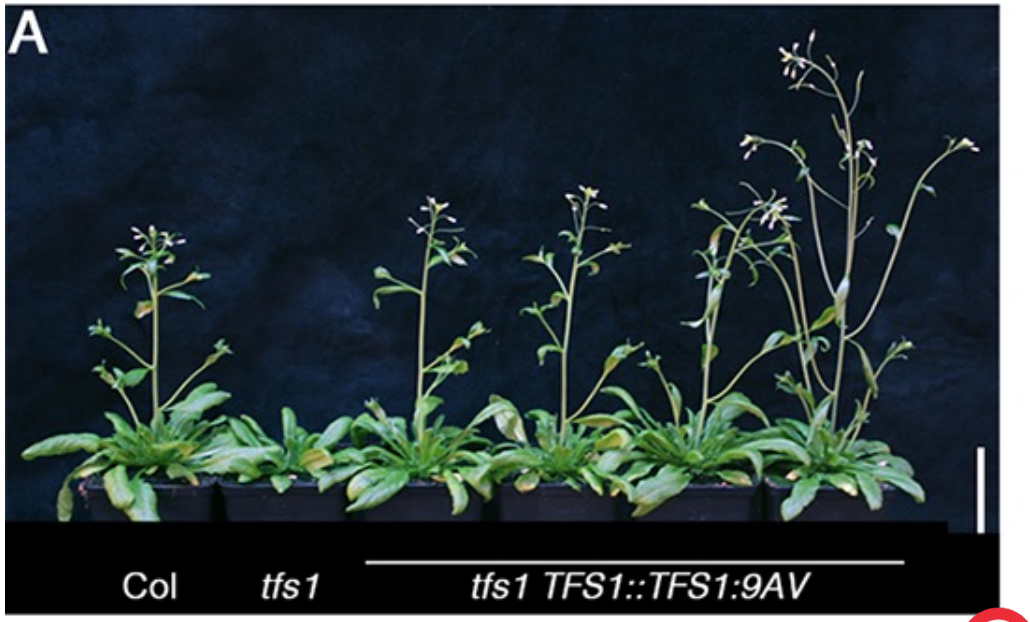
reduced binding of SVP to CArGII

Risultati:SOC1 activates *TFS1* through CArGboxI at 3'end of *TFS1*

TFS1::*TFS1*:*9xAla-Venus* (*TFS1*:: *TFS1*:*9AV*) gene fusion was constructed that contained the entire intergenic region flanking *TFS1* on the 5' and 3' sides

This gene fusion complemented the *tfs1-1* mutant



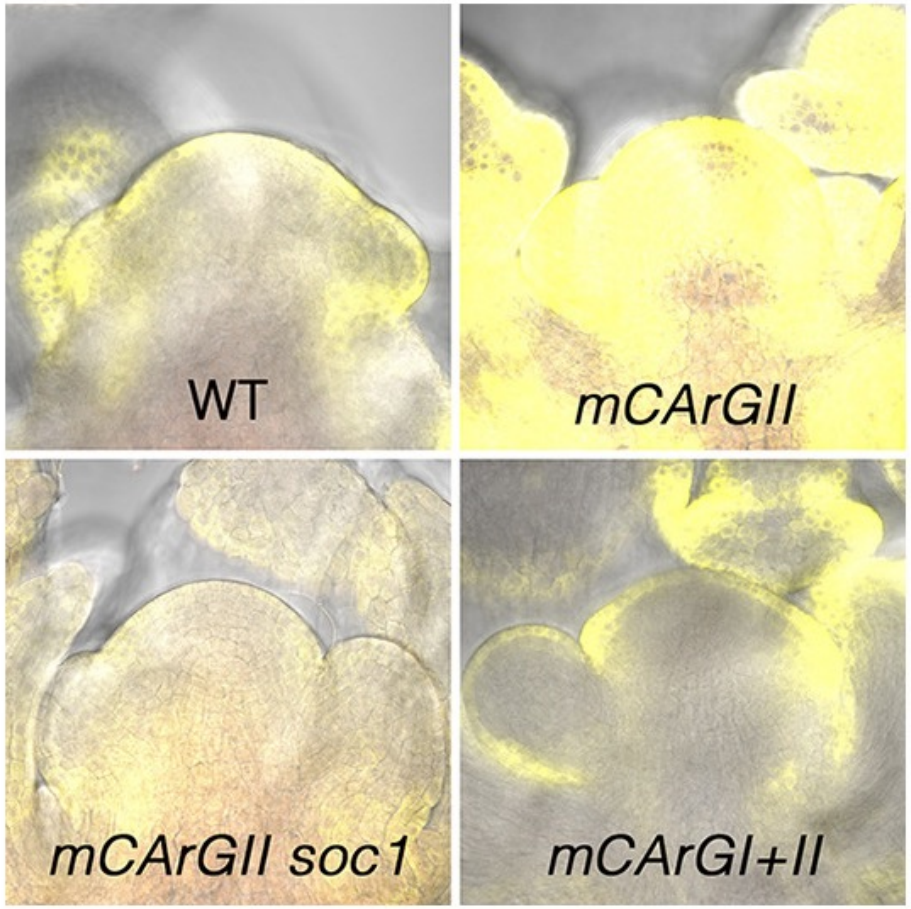


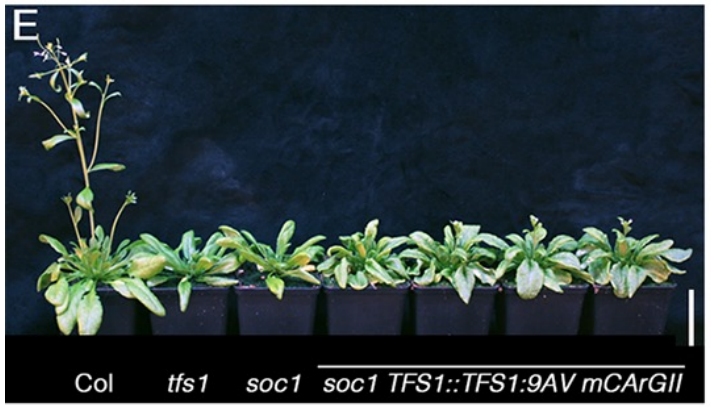
TFS1 genomic region and mutations introduced into CArG-boxes

CArG-box with consensus sequence CW6RG (red triangle) and CW6GG (orange triangle)

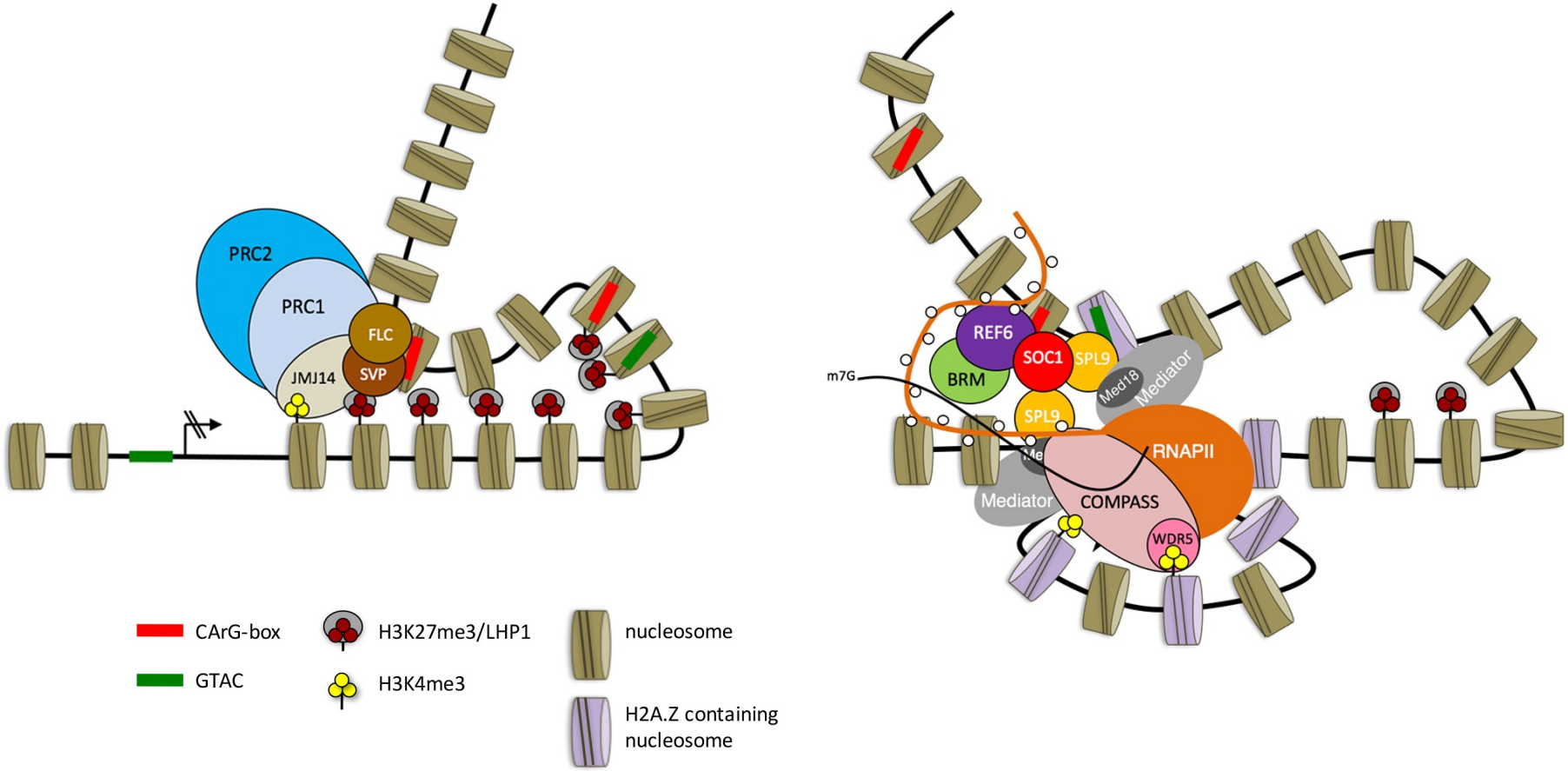
Risultati: SOC1 activates TFS1 through CArG box I at the 3’ end of TFS1

SOC1 activates and SVP represses transcription of TFS1 at least partly through binding to CArG-box I and CArG- box II, respectively



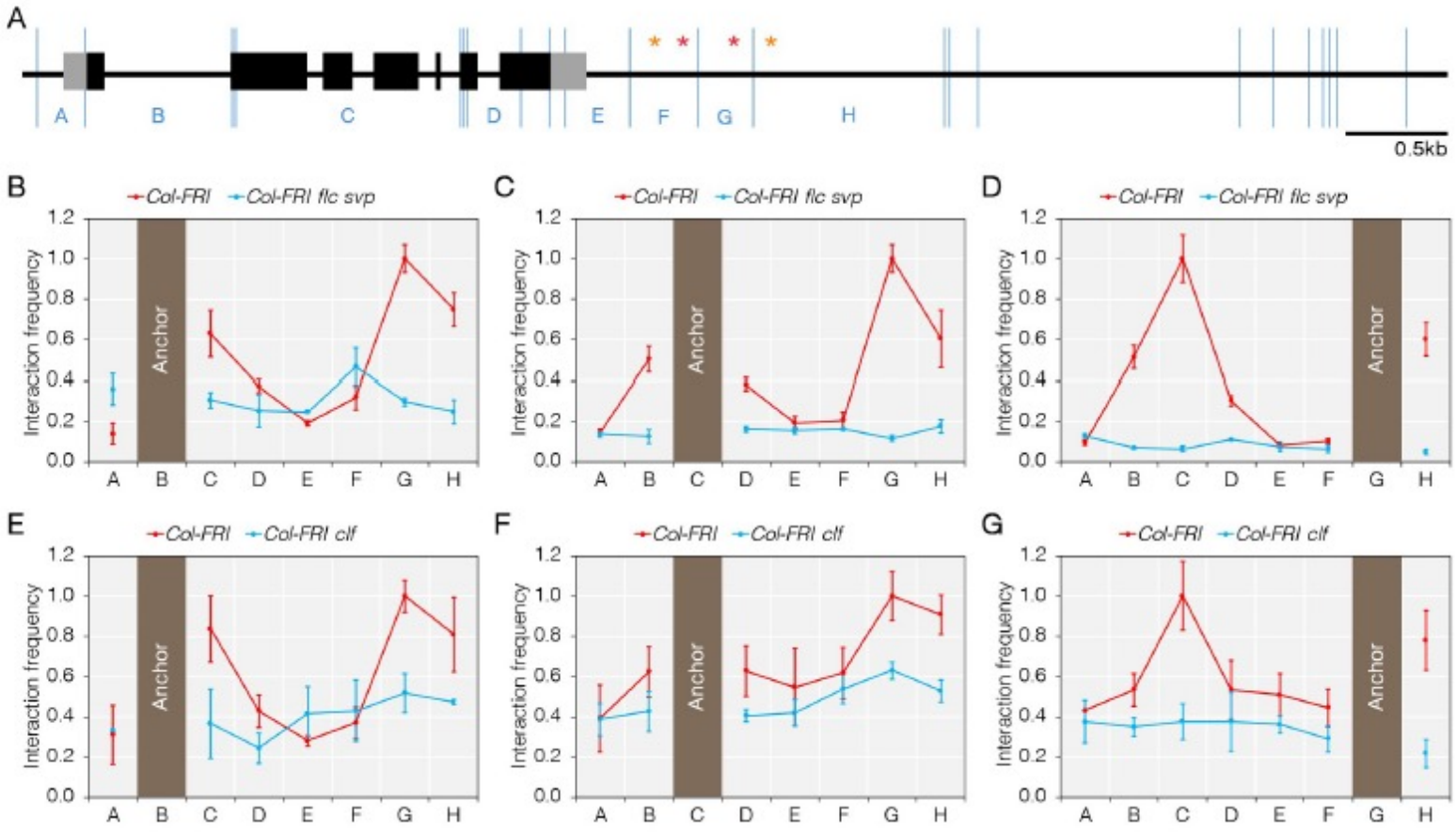


Discussion: Proposed model for *TFS1* regulation and function by different flowering pathways



- FLC and SVP mediated repression of *TFS1* requires PRC activity and a locked chromatin conformation.
- Activation of *TFS1* requires loop between 5’ and 3’ end and is mediated by the cooperativity between SOC1 and SPL9

FLC and SVP are associated with looping between the 3’ end of *TFS1* and gene body- tecnica 3C



Tecnica non affrontata nel Corso: private a leggere e capire l’utilizzo in generale e perchè è stata utilizzata.

Non scendete troppo nel dettaglio

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Conclusioni:

- FLC and SVP mediated repression of *TFS1* requires PRC activity and a locked chromatin conformation

- Activation of *TFS1* requires loop between 5’ and 3’ end and is mediated by the cooperativity between SOC1 and SPL9

How to present a paper…. Avete domande?

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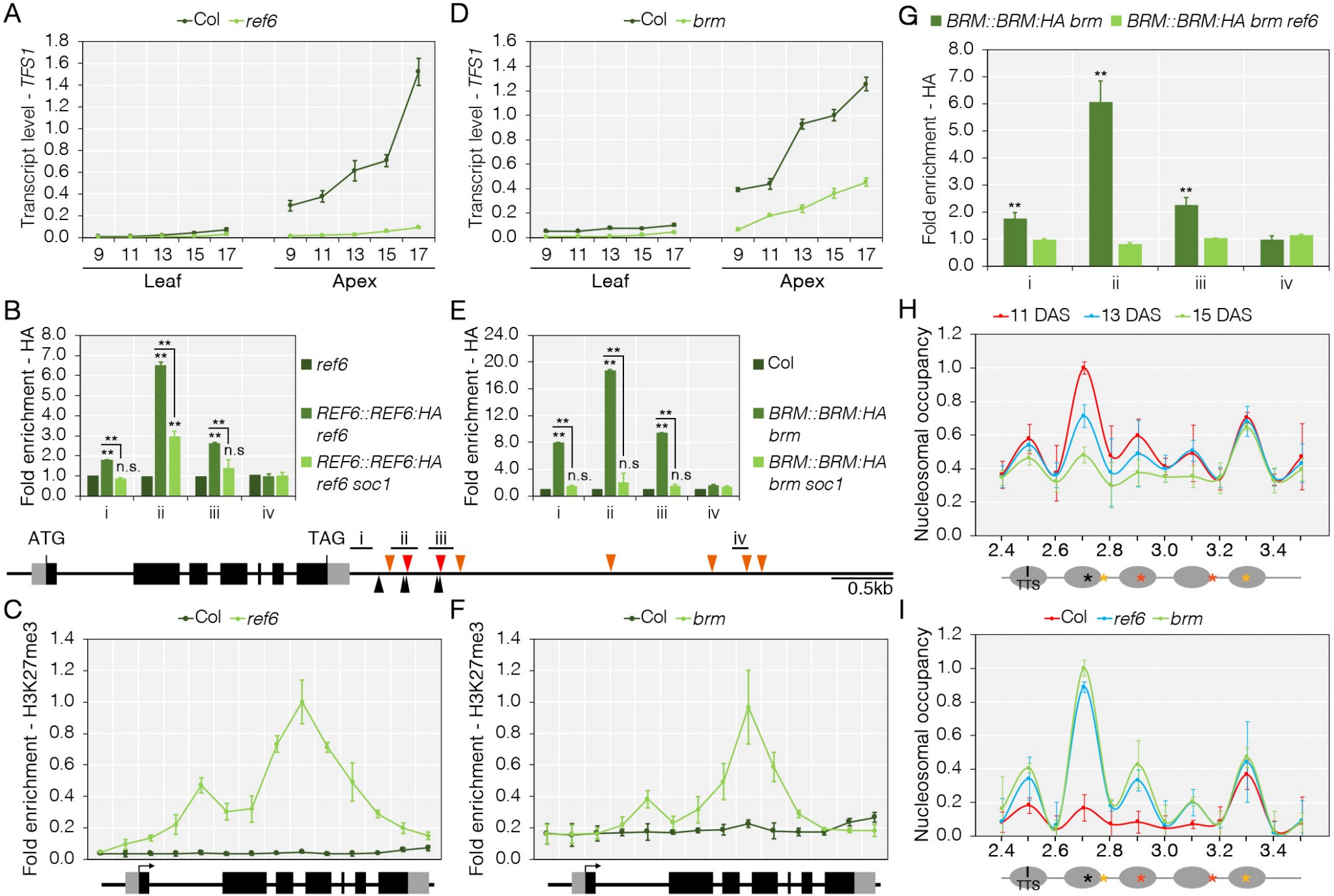
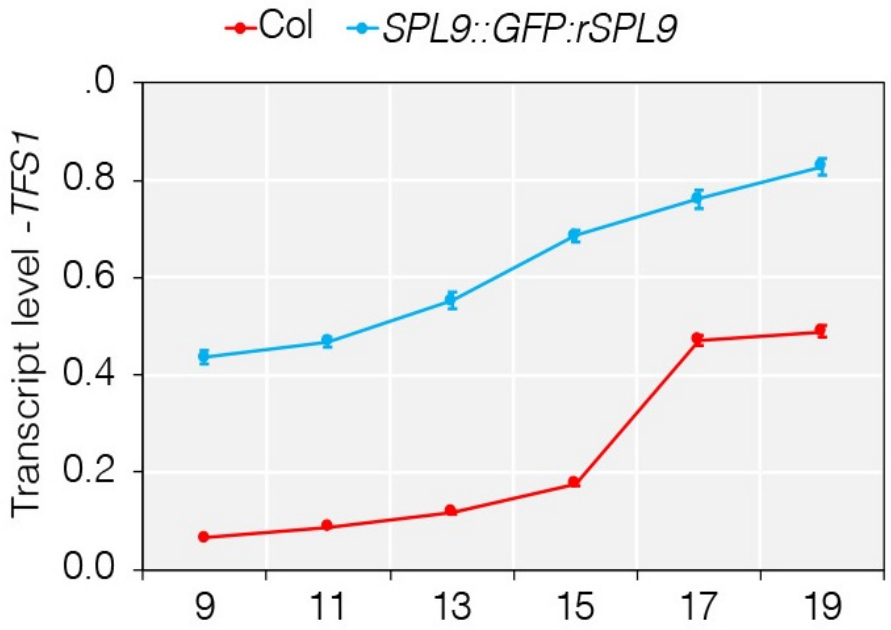

A
Col
ref6
Transcript level - TFS1
Leaf
Apex
D
Col
brm
Transcript level - TFS1
Leaf
Apex
G
BRM::BRM:HA brm
BRM::BRM:HA brm ref6
Fold enrichment - HA
B
ref6
REF6::REF6:HA ref6
REF6::REF6:HA ref6 soc1
Fold enrichment - HA
E
Col
BRM::BRM:HA brm
BRM::BRM:HA brm soc1
Fold enrichment - HA
ATG
TAG
0.5kb
H
11 DAS
13 DAS
15 DAS
Nucleosomal occupancy
TTS
C
Col
ref6
Fold enrichment - H3K27me3
F
Col
brm
Fold enrichment - H3K27me3
I
Col
ref6
brm
Nucleosomal occupancy
TTS

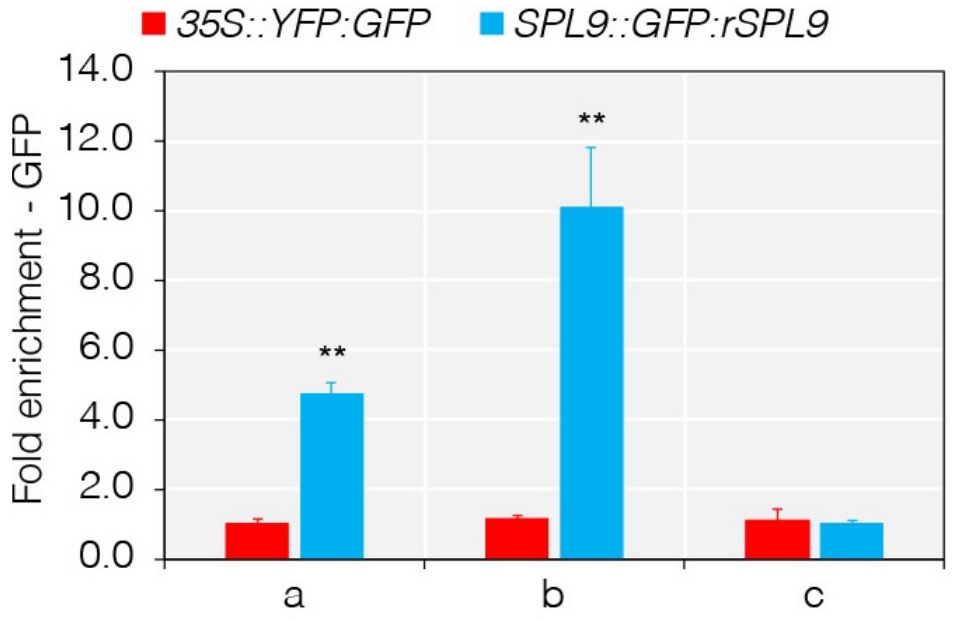
SPL9 cooperate with SOC1 in positively controlling *TFS1* expression

TFS1 spatial and temporal expression patterns is similar to those of *SPL9*

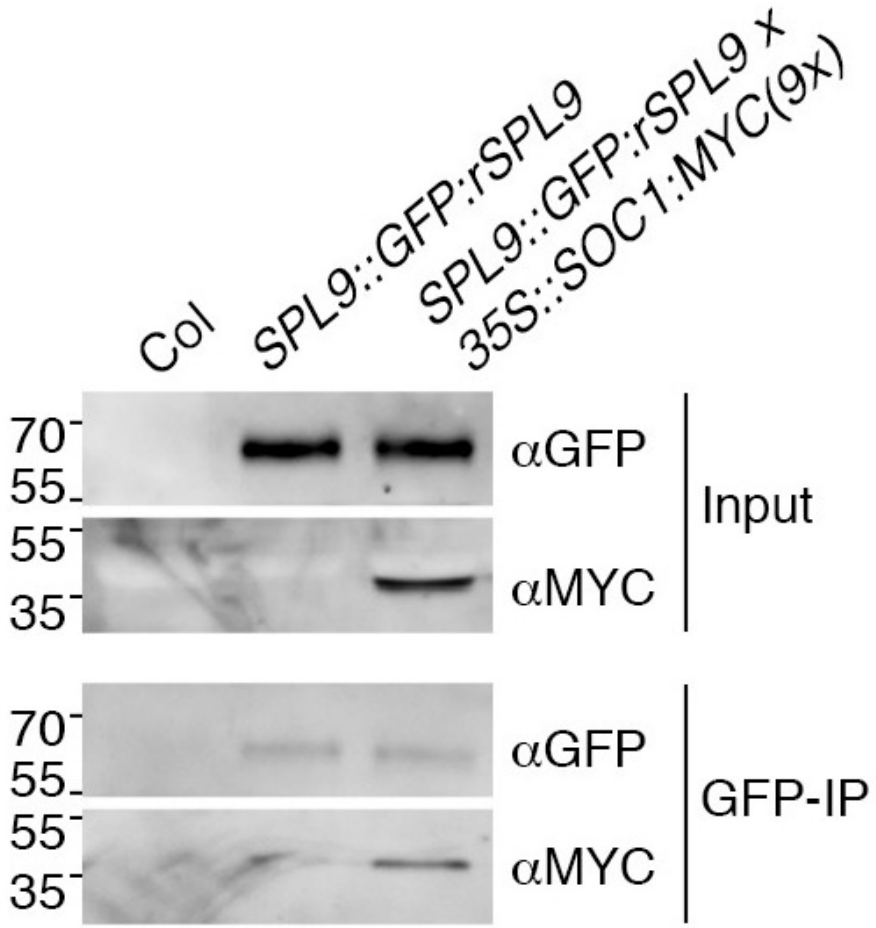
SPL9 is a TF binding to promoter of the floral meristem-identity genes



TFS1 in ***SPL9****::GFP:**r**SPL9*



ChIP-qPCR to test binding of GFP:rSPL9 at the 5’ and 3’ ends of *TFS1*



SPL9 physically interact with SOC1

co-IP of GFP:rSPL9 and SOC1:MYC(9x) was detected in protein extracts from shoot apical tissue of *SPL9*::*GFP*:*rSPL9 35S*::*SOC1*:*MYC(9x)* transgenic lines

Two distinct deletions in *FRI* are believed to confer early flowering in most of the rapid cycling accessions. The Columbia allele (*FRI-Col*) carries a 16 bp-deletion resulting in a premature stop codon and, thus, a truncated protein missing a part of the C-terminal [2],[14]. But the most frequent deleterious *FRI* mutation in nature is a 376 bp-deletion combined with a 31 bp-insertion in the promoter as observed in Landsberg *erecta* (*FRI-Ler*) [14],[21],[22]. This mutation disrupts the translational start but, due to a second alternative start codon, a short out-of-frame protein might be built [2]. The loss-of-function *FRI* alleles found in L*er* and Col are widely used as examples of positive selection towards rapid cycling accessions [26],[27].