An MRG-operated chromatin switch at *SOC1* attenuates abiotic stress responses during the floral transition

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Short title

SOC1 modulates stress responses during flowering.

Sentence summary

A chromatin switch coordinates flowering initiation with plant responsiveness to adverse conditions tuning down costly stress responses during flowering for optimal plant reproductive success

Author contributions

JAJ and MP designed the research. JB-G, AM and RP performed all the experimental approaches. JS designed the freezing tolerance analyses carried out and analyzed the resulting data. JB-G, JAJ and MP analyzed all the data and wrote the paper.

ABSTRACT

Plants react to environmental challenges by integrating external cues with endogenous signals to optimize survival and reproductive success. However, the mechanisms underlying this integration remain obscure. While stress conditions are known to impact plant development, how developmental transitions influence responses to adverse conditions has not been addressed. Here, we reveal a novel molecular mechanism of stress response attenuation during the onset of flowering in Arabidopsis. We show that Arabidopsis MORF-RELATED GENE (MRG) proteins, components of the NuA4 histone acetyltransferase (HAT) complex that bind trimethylated-lysine 36 in histone H3 (H3K36me3), function as a chromatin switch on the floral integrator *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) to coordinate flowering initiation with plant responsiveness to hostile environments. MRG proteins are required to activate *SOC1* expression during flowering induction by promoting histone H4 acetylation. In turn, SOC1 represses a broad array of genes that mediate abiotic stress responses. We propose that during the transition from vegetative to reproductive growth, the MRG-SOC1 module constitutes a central hub in a mechanism that tunes down stress responses to enhance reproductive success and plant fitness at the expense of costly efforts for adaptation to challenging environments.

INTRODUCTION

Plants often face unfavourable environmental conditions through their life cycle. To cope with them, plants have evolved to acquire complex mechanisms that either ameliorate the damaging effects of stress and increase tolerance, or accelerate the life cycle of the plant leading to an early reproductive phase in a response frequently known as escape. Stress perception and response involve intricate signalling networks that often entail substantial transcriptomic rearrangements (Asensi-Fabado et al., 2017; Haak et al., 2017; Baurle and Trindade, 2020). A paradigmatic example is the cold acclimation response of temperate plants, where low non-freezing temperature serves as an environmental cue for gene expression reprogramming to increase freezing tolerance (Barrero-Gil and Salinas, 2018). The C-REPEAT/DEHYDRATION RESPONSIVE ELEMENT BINDING FACTORS (CBFs) 1-3 and the plant hormone Abscisic acid (ABA) play key roles in this process (Eremina et al., 2016; Barrero-Gil and Salinas, 2018; Shi et al., 2018). Similar extensive transcriptomic adjustments also mediated by ABA signalling pathways (Harb et al., 2010) have been reported in response to drought. In addition, adaptation to suboptimal environments requires that plants also integrate external factors with endogenous cues to optimize developmental processes such as the floral transition. In this context, ABA accumulation triggered by mild drought conditions that compromise growth but not survival induce the expression of the floral integrator *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), accelerating flowering in a response of drought escape (Riboni et al., 2013). Remarkably, *SOC1* is also part of a cross-talk signalling pathway that negatively regulates cold response by inhibiting *CBF* expression (Seo et al., 2009).

Changes in the organization of chromatin and histone modifications are considered the interphase through which the environment interacts with the genome to promote alterations in gene expression (Lamke and Baurle, 2017; Chang et al., 2020). Acetylation on particular histone lysine (K) residues is reversibly controlled by both histone acetyltransferases (HATs) and histone deacetylases (Lee and Workman, 2007), and is essential for the regulation of gene expression in response to environmental stresses (Liu et al., 2016; Luo et al., 2017; Jiang et al., 2020). Histone modifications are recognized by “reader” proteins that contribute to modulate chromatin dynamics and to translate chromatin features into specific patterns of gene expression (Musselman et al., 2012). In Arabidopsis, for example, trimethylation of lysine 36 in histone H3 (H3K36me3) is recognized by two homologue proteins named MORF-RELATED

GENE 1 (MRG1) and MRG2 (Bu et al., 2014; Xu et al., 2014). These proteins are components of the Nucleosome Acetyl transferase of histone H4 (NuA4) HAT complex (Espinosa-Cores et al., 2020), and redundantly modulate the expression of the key floral integrator *FLOWERING LOCUS T* (*FT*) gene (Bu et al., 2014; Xu et al., 2014; Guo et al., 2020). However, the contribution of chromatin remodelling processes, and specifically MRG proteins, to the integration of stress adaptation with plant developmental progression remains virtually unknown. Here, we reveal an MRG-mediated chromatin mechanism that acts on the master flowering gene *SOC1* (Samach et al., 2000) to modulate abiotic stress responses depending on developmental signals. Our data suggest that this MRG-SOC1 regulatory module attenuates responsiveness of Arabidopsis plants to various stresses during the onset of flowering for optimal integration of development and adaptation to adverse environments.

RESULTS

MRG proteins are required for the *SOC1*-dependent downregulation of abiotic stress-responsive genes

To address the involvement of Arabidopsis MRG proteins on the regulation of gene expression and other physiological processes, we used two uncharacterized *MRG* mutant alleles, *mrg1-2* and *mrg2-4* (Supplemental Fig. 1A, B). Confirming previous observations (Bu et al., 2014; Xu et al., 2014; An et al., 2020; Guo et al., 2020), the floral integrator genes *FT* and *SOC1* were downregulated in *mrg1-2 mrg2-4* double mutant plants (Supplemental Fig. 1C), corroborating the role of MRGs in fine-tuning flowering responses specifically under long days (LD) conditions (Supplemental Fig. 1D, E). Our genetic analysis showed that *ft* mutations cause a modest, but significant, delay in the flowering time of *mrg1 mrg2* plants, whereas the combination of *soc1* mutations with *mrg1 mrg2* clearly enhances the late flowering phenotype of the double mutant (Fig. 1). These results show that the delay in flowering observed in *mrg1 mrg2* double mutant plants does not depend on a single floral integrator, suggesting that *MRG* genes influence flowering through the activity of both floral integrators. Furthermore, *MRG* function in the control of the floral transition shows a strong requirement on H3K36me3, a modification mediated by the histone methyl transferase SET DOMAIN GROUP 8 (SDG8) (Soppe et al., 1999; Zhao et al., 2005), since *sdg8* mutants fully suppress the late flowering phenotype of *mrg1 mrg2* mutant plants (Supplemental Fig. 2). These results are in line with the current model concerning the involvement of MRG

proteins in the regulation of floral transition (Bu et al., 2014; Xu et al., 2014; An et al., 2020; Guo et al., 2020).

Next, we performed a transcriptomic analysis on plants grown under LD photoperiod during the floral transition to examine the implication of Arabidopsis MRG proteins in the regulation of gene expression. We identified 552 differentially expressed genes (DEGs) in the *mrg1 mrg2* double mutant, of which 516 were induced and 21 were repressed (Supplemental Table 1). GO-term enrichment analysis revealed an over-representation of terms related to abiotic stress responses, including water deprivation, salt stress and hypoxia among upregulated genes (Fig. 2A; Supplemental Table 2). Intriguingly, among the genes induced in the *mrg1 mrg2* double mutant, we found a significant enrichment in direct targets of SOC1 (Fig. 2B). Indeed, SOC1 has been reported as a direct repressor of *CBF* genes, that regulate the tolerance to freezing temperatures (Seo et al., 2009) and a number of additional abiotic stress response mediators (Immink et al., 2012; Tao et al., 2012). Besides, a significantly high number of SOC1 and CBF-dependent genes were found differentially upregulated in *mrg1 mrg2* plants (Fig. 2C, D). Independent quantitative RT-PCR expression analyses confirmed the upregulation of several direct targets of SOC1 including *CBF2, WRKY33, RAV1* and *RAV2* (Immink et al., 2012; Tao et al., 2012) as well as different genes related with abiotic stress responses such as *ZAT10*, *SZF1*, *ABA2* and *COR15A* in *mrg1 mrg2* plants (Fig. 2E). Interestingly, the expression level for these genes in the *mrg1 mrg2 soc1* triple mutant is comparable to that observed in either *mrg1 mrg2* or *soc1* mutant plants, revealing no marked enhancement of expression upon the concurrent loss of function of these genes (Fig. 2E). The absence of additive effects in the *mrg1 mrg2 soc1* triple mutants supports that *MRG* genes and *SOC1* function in the same genetic pathway to control the expression of abiotic stress-responsive genes, although we cannot rule out that SOC1 could also perform MRG-independent roles in the control of these genes.

MRG2 protein binds *SOC1* chromatin and promotes H4 acetylation deposition in this locus during the floral transition

MRG proteins promote both *FT* and *SOC1* expression (Supplemental Fig. 1C), and at least MRG2 associates with the transcription factor CONSTANS (CO) to bind *FT* chromatin and activates its transcription under photoperiodic flowering-inducing conditions (Bu et al., 2014). Since FT is an activator of *SOC1* expression during the floral transition (Yoo et al., 2005), it is tempting to speculate that the decreased *FT*

expression caused by the loss of *MRG* function might be responsible for the reduced activation of *SOC1* expression in *mrg1 mrg2* plants. However, our genetic analysis showed that the function of *MRG* genes in the control of flowering initiation is not only dependent on *FT* (Fig. 1). Indeed, CO has been proposed to directly activate *SOC1* expression (Samach et al., 2000) and the absence of a functional *FT* does not completely suppress CO-mediated *SOC1* activation (Yoo et al., 2005), which also indicates that *SOC1* expression is not entirely dependent on *FT* function. Thus, we hypothesized that MRG proteins might directly bind *SOC1* chromatin. In turn, this floral integrator would control the expression levels of stress-responsive genes. To examine whether *SOC1* is a direct target of MRG proteins, we performed ChIP-PCR experiments using a *pMRG2::MRG2-YFP* transgenic line that fully complements the late flowering phenotype of *mrg1 mrg2* plants (Bu et al., 2014). Following immunoprecipitation with an α-GFP antibody, we observed a conspicuous enrichment of DNA corresponding to the regulatory region of *SOC1* in the *pMRG2::MRG2-YFP* transgenic line compared to WT plants (Fig. 3A-B), indicating that MRG2 directly binds *SOC1* chromatin. Consistent with the role of MRG proteins as H3K36me3 readers, this genomic region of the *SOC1* locus bears high levels of this histone modification (Bewick et al., 2016) (Fig. 3A). These observations suggest that MRG proteins directly and positively regulate *SOC1* expression.

The MRG2 protein was previously shown to be necessary for maintaining high H4 acetylation levels in the chromatin of *FT* to sustain its expression (Xu et al., 2014). Thus, we reasoned that MRG proteins might also regulate *SOC1* expression by modulating H4 acetylation levels. *SOC1* expression gradually increases from germination (Liu et al., 2008) but, according to our observations, MRG-dependent activation of *SOC1* is evidenced during the transition from vegetative to reproductive development (Fig. 3C). Indeed, the activation of *SOC1* is observed only in WT but not in *mrg1 mrg2* mutant plants between 8 and 12 days after sowing, the period when flowering commitment is taking place, as shown by the induction of the expression of floral meristem identity genes such as *APETALA 1 (AP1)* and *LEAFY (LFY)* (Fig. 3D). Therefore, we decided to monitor histone H4 acetylation levels in different regions of *SOC1* chromatin (Fig. 3A) in WT and *mrg1 mrg2* plants during the floral transition (days 8 and 12). ChIP experiments using an antibody against tetra-acetylated histone H4 (H4K5,8,12,16ac) revealed a pronounced increase of this mark around the genomic region of *SOC1* bound by MRG2 in WT plants during the initiation of flowering. In

contrast, H4ac levels remained steady in the *mrg1 mrg2* double mutant between 8 and 12 days after sowing, leading to significantly lower levels of this histone modification in mutant plants compared to WT at the latest developmental stage assessed (Fig. 3E). Remarkably, the intensity of H4 acetylation observed in WT and *mrg* mutants at the *SOC1* locus is consistent with the expression levels detected for this gene in these plants during the floral transition (Fig. 3C). Furthermore, during this phase of floral initiation, a conspicuous downregulation of diverse SOC1 direct target genes related to stress responses is observed in WT plants (Fig. 3F). Based on these observations, we concluded that MRG proteins mediate *SOC1* activation during the floral transition by promoting H4 acetylation levels at this locus, and cause a concomitant repression of stress-related genes (Fig. 3F).

Loss of *MRG* function increases abiotic stress tolerance in a *SOC1*-dependent manner

Since mutations in *MRG* genes increase the expression of genes involved in abiotic stress responses, we checked the tolerance of *mrg1 mrg2* mutants to different challenging environmental conditions. First, we assessed the basal freezing tolerance of two-week-old *mrg1 mrg2* mutants and WT plants. A significant increase in survival to freezing temperatures was observed in mutant plants compared with WT (Fig. 4A). Notably, the genetic relationship found between *mrg1 mrg2* and *soc1* mutants regarding the capacity to withstand freezing temperatures indicated that the increased tolerance displayed by *mrg1 mrg2* plants requires a functional *SOC1* gene (Fig. 4B). These results demonstrated that MRGs negatively regulate constitutive freezing tolerance and that this control relies, at least in part, on *SOC1* function. We also evaluated the ability of *mrg1 mrg2* double mutants to cope with drought and the genetic interaction between *MRG* and *SOC1* genes in modulating this trait. The data revealed that loss of *MRG* function results in increased tolerance to water deprivation, and, again, this negative regulation on drought tolerance mediated by MRG proteins displayed dependence on a functional *SOC1* gene (Fig. 4C). Finally, we wondered if these responses to abiotic stresses could be associated with an altered ABA responsiveness. The results showed that loss of *MRG* function rendered plants that were hypersensitive to ABA in a *SOC1*-dependent manner (Fig. 4D). Thus, we concluded that MRG proteins negatively regulate various abiotic stress responses, in part, by controlling *SOC1* expression, and possibly by modulating either ABA levels or signalling.

DISCUSSION

In this work we have explored the involvement of Arabidopsis MRG histone readers in the regulation of gene expression. We found that besides mediating the activation of key flowering genes like *FT* and *SOC1*, MRG proteins also control the expression of many abiotic stress-responsive genes. Furthermore, MRG-mediated repression of abiotic stress responses is dependent on the function of the floral integrator *SOC1*, a locus regulated by MRG proteins by directly binding to its chromatin and promoting histone acetylation during the floral transition. The contribution of chromatin remodelling processes to the coordination of stress adaptation with plant developmental progression remains practically unknown (Ma et al., 2020). We propose that the MRG-mediated remodelling of *SOC1* chromatin constitutes a central mechanism that tunes down stress responses during the floral transition likely to enhance reproductive success.

Previous works had established the central role displayed by MRG histone readers and the H3K36me3 epigenetic mark in promoting the expression of the key floral integrator gene *FT* through the deposition of acetylation on histone H4 in regulatory regions of this locus (Bu et al., 2014; Xu et al., 2014; Guo et al., 2020). Now we have found that MRG proteins are also involved in attenuating different abiotic stress responses, including drought and low temperature. This is consistent with recent reports showing that the histone methyl transferase SDG8 regulates a significant number of genes related to abiotic stress (Cazzonelli et al., 2014). In fact, another histone methyl transferase, SDG26, negatively regulates drought stress tolerance in a similar way to MRG proteins (Ma et al., 2013). Consistent with the involvement of SDG8 and SDG26 methyl transferases in the modulation of stress responses, H3K36me3 has been shown to play a key role in the adaptation of plants to fluctuating ambient temperature (Pajoro et al., 2017). The extreme phenotype of the *mrg1 mrg2 sdg8* triple mutant (Supplemental Fig. 2B, C) prevented us from assessing the possible genetic interaction of these genes in the context of abiotic stress responses. In any case, our results support the notion that reading of H3K36me3 through MRG proteins is a relevant mechanism in the control of the expression of abiotic stress-responsive genes.

Importantly, in this work we have established that MRG proteins tune down abiotic stress responses in a *SOC1*-dependent manner. Earlier research has established that the flowering promoting factor SOC1 decreases Arabidopsis tolerance to freezing

temperatures under conditions that favor the initiation of reproductive growth (Seo et al., 2009). Here, we report that the SOC1 repressive role on abiotic stress response is not restricted to low temperature but rather extends also to the reaction of Arabidopsis to other environmental challenges. For instance, we show that SOC1 acts to reduce the ability of this plant to cope with drought conditions. It is important to highlight that although previous studies had demonstrated a key role for SOC1 in a drought escape mechanism that accelerates flowering under conditions of water deficit (Riboni et al., 2013), here we are describing an entirely different mechanism that tunes down responses underlying the plant ability to cope with water shortage by enhancing drought tolerance mechanisms upon flowering initiation. Indeed, natural variation studies in drought stress responses in Arabidopsis have revealed a negative correlation between the capacity to increase survival to water deprivation and the ability to accelerate flowering and escape drought conditions, supporting the existence of a genetic trade-off between both mechanisms (McKay et al., 2003), and our results suggest that SOC1 function may represent an important determinant underlying this trade-off. Furthermore, we provide additional evidence indicating that both MRG and SOC1 proteins might be involved in controlling either ABA levels or downstream signaling pathways. Consistent with this hypothesis, SnRK2-substrate 1 (SNS1), a putative component of the HAT complex NuA4 that in yeast and mammals interacts with the homolog of MRG, is involved in ABA signaling in Arabidopsis (Umezawa et al., 2013).

Finally, we have demonstrated that the MRG histone readers promote histone acetylation at the *SOC1* locus during the floral transition, activating its expression. While various reports have shown the influence of abiotic stress signals in modulating developmental transitions, and specifically flowering time (Seo et al., 2009; Riboni et al., 2013), whether particular developmental stages exhibit differential adaptation capabilities to unfavorable environmental conditions is, in practice, unknown. This work has uncovered a developmental epigenetic switch that is timely activated during the floral transition with a bivalent involvement in triggering flowering and moderating stress responses. We interpret these findings as a novel plant chromatin-mediated mechanism that might operate under the control of MRG proteins to optimize reproductive success and fitness at the expense of costly efforts to adapt to challenging environmental conditions once flowering is initiated (Fig. 5). However, *SOC1* is a tightly regulated gene and MRG proteins are only one of the multiple factors at play controlling its expression, suggesting that additional transcriptional regulators could be

contributing to coordinate flowering and abiotic stress tolerance. Further studies will be necessary to fully unveil the intricate nature of the epigenetic mechanisms that integrate stress responses with plant developmental phase transitions as well as their contribution to Arabidopsis adaptive variation, but in view of these observations it is tempting to consider that chromatin dynamics at the *SOC1* locus could represent a driver for phenotypic plasticity in Arabidopsis.

MATERIALS AND METHODS

Plant materials, growth conditions, cold treatments and tolerance assays

All Arabidopsis mutant lines used in this study are in Columbia-0 (Col-0) background. The mutant alleles of *MRG1* and *MRG2* were named *mrg1-2* (SALK_089867) and *mrg2-4* (SAIL_317_F11), respectively, and were obtained from the Nottingham Arabidopsis Stock Centre (NASC, UK). Other mutants used have been previously described elsewhere: *ft-10* (Yoo et al., 2005*), sdg8-1* (Zhao et al., 2005) and *soc1-2* (Lee et al., 2000). Plants were grown at 21°C under LD photoperiods (16 hours of cool-white fluorescent light) or SD photoperiods (8 hours of cool-white fluorescent light) with photon flux of 110 μmol m^{-2} s^{-1}, in pots containing a mixture of organic substrate and vermiculite (3:1, v/v), or in Petri dishes containing 1/2x Murashige and Skoog medium supplemented with 1% (w/v) sucrose and solidified with 0.8 % (w/v) plant agar (GM medium).

Tolerance to freezing temperatures was determined in two-week-old plants grown on soil. The freezing treatment started by pre-incubating plants at 4°C for one hour followed by a gradual decrease of temperature at a rate of two degrees per hour to avoid intracellular freezing. Temperature drop stops at the indicated temperature, which is maintained for six hours followed by a gradual recovery of temperature at the aforementioned rate and incubation at 4°C for one hour before returning plants to normal growth conditions. Survival was scored after seven days.

To determine drought tolerance, one-week-old plantlets were either kept in a normal irrigation schedule (50-ml water for 21 cm^3 pots twice a week) or without any watering for fourteen days before resuming irrigation. Survival was scored after seven days.

ABA sensitivity assay

Seven-day old seedlings from the indicated genotypes grown on GM medium in LD conditions were transferred to GM medium supplemented with or without 20 µM ABA. Then seedlings were incubated for 9 additional days before taking pictures and measuring fresh weight.

Gene expression analysis

Plants were grown at 22°C for 12 days under LD photoperiod, taking samples from aerial tissue at Zeitgeber time (ZT) 8 for transcriptomic analysis, unless otherwise indicated. Total RNA was extracted using EZNA Plant RNA kit (Omega) following the manufacturer's protocol. RNA samples were treated with DNase I (Roche) to remove genomic DNA contamination. For RNA sequencing experiments, samples from three independent experiments were used to prepare three sequencing libraries for each genotype. RNA library preparation and sequencing was performed by the CRG - Centre de Regulació Genòmica (Barcelona, Spain), using Illumina HiSeq2000 technology. Approximately 45 million single-end 50-base reads per sample were generated and more than 90% of reads uniquely mapped to Arabidopsis TAIR10 reference genome using HISAT2 (Li et al., 2009). Differential expression analysis was performed using the DESeq2 module (Love et al., 2014) on SeqMonk v1.45 software (http://www.bioinformatics.babraham.ac uk/ projects/seqmonk/). To identify differentially expressed genes (DEGs) we set FDR≤ 0.05 and fold change ≥ 1.5 or ≤0.5 as cutoffs for any given DEG. Gene ontology (GO) enrichment analysis was performed on PANTHER (http://pantherdb.org/) using a Fisher's exact test corrected by a false discovery rate FDR < 0.05 as cutoff for a significantly enriched GO term. Interesting DEGs were validated by quantitative PCR (qPCR) assays as follows. For qPCR analysis, RNA samples from independent experiments were processed and analyzed separately. RNA was retro-transcribed using Maxima first strand cDNA synthesis kit (Thermofisher Scientific), and qPCRs were performed using LightCycler 480 SYBR Green I (Roche). Primers used for qPCR analysis are listed in Supplemental Table 3. The At4g26410 gene was used as a reference in all experiments (Czechowski et al., 2005). Fold change was calculated using the ΔΔCT method (Livak and Schmittgen, 2001).

Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) experiments were performed as described (Crevillen et al., 2019). Immunoprecipitated DNA was quantified by qPCR using the oligonucleotides described in Supporting Information (Supplemental Table 3). DNA enrichment was estimated as the fraction of immunoprecipitated DNA relative to input (% INPUT). We used the following antibodies: α-H4K5,8,12,16Ac (Merck-Millipore 06-598) and α-GFP (Invitrogen, A-6455).

Statistical analyses

Statistical analyses (ANOVA, Student´s t-test) were performed with GraphPad Prism software. Statistical significance of the overlap between two groups of genes was calculated using a hypergeometric test using Excel software.

ACCESSION NUMBERS

Sequence data related to this manuscript can be found in the Arabidopsis information portal (https://www.araport.org/) under the accession numbers *MRG1* (At4g37280), *MRG2* (At1g02740), and *SOC1* (At2g45660). Epigenomic data were retrieved from the Plant Chromatin State Database (http://systemsbiology.cau.edu.cn/chromstates/index.php). The complete genome-wide data from this publication were submitted to the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo/) under accession number GSE141135.

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FIGURE LEGENDS

Figure 1. *MRG* role in the regulation of floral induction is partially dependent on *FT* and *SOC1* function. Flowering time of *mrg1 mrg2 ft* (A) and *mrg1 mrg2 soc1* (B) triple mutants. Number of leaves at bolting in WT, and single *ft* and *soc1*, double *mrg1 mrg2* and triple *mrg1 mrg2 ft* and *mrg1 mrg2 soc1* mutant plants grown under LD. Statistical significance was calculated using one-way ANOVA with Tukey's correction for multiple comparisons and is denoted by different letters indicating $p < 0.05$. Box plots indicate the 25th and 75th percentiles of the data and the median is indicated by a line. Whiskers represent the minimum and maximum value. Individual data points are represented by black dots.

Figure 2. MRG and SOC1 proteins control the expression of a significantly high number of abiotic stress-responsive genes. A, Gene ontology term over-representation among differentially upregulated genes in *mrg1 mrg2* mutant plants. B, Overlap between genes upregulated in the *mrg1 mrg2* double mutant and SOC1 direct targets (Immink et al., 2012; Tao et al., 2012). C, Overlap between genes regulated by SOC1 (Seo et al., 2009) and genes upregulated in *mrg1 mrg2* mutant plants. D, Overlap between upregulated genes in the *mrg1 mrg2* double mutant and genes induced by CBF proteins (Jia et al., 2016; Zhao et al., 2016). E, Expression of key abiotic stress-responsive genes in WT and *soc1*, *mrg1 mrg2* and *mrg1 mrg2 soc1* mutants. Bars show the average of three independent experiments while error bars indicate the standard error of the mean (SEM, n=3 in all experiments). Significant differences were determined with a one-way ANOVA followed by Tukey's test ($p < 0.05$) and distinct groups are denoted by different letters. In B-D the number of genes of each dataset is indicated between parenthesis and the level of enrichment of each overlap along with the corresponding *P*-value is indicated below the Venn diagrams.

Figure 3. MRG2 binds *SOC1* chromatin promoting H4 acetylation. A, Schematic representation of *SOC1* locus indicating regions enriched in H3K36me3 identified in a ChIP-seq experiment (Bewick et al., 2016). Boxes indicate exons and lines indicate introns. Dark and light grey boxes correspond to untranslated regions (UTR) or coding sequences, respectively. Letters designate regions analyzed in ChIP-PCR experiments. B, ChIP performed using an α-GFP antibody on chromatin samples from *mrg1 mrg2* plants complemented with the specified construct. Untransformed plants were used as control. C, Expression of the floral integrator *SOC1* gene in 8-day or 12-day-old plants of the indicated genotypes. D, Upregulation of *LFY* and *AP1* expression during the

floral transition. Transcript levels of floral meristem identity genes in shoot-apical-meristem tissue from plants of the denoted age and phenotype. Bars show the average of three independent experiments and error bars represent SEM. Asterisks indicate significant differences ($p < 0.05$) determined by two-sided *t*-tests. E, ChIP experiments using an α-H4K5,8,12,16Ac antibody on chromatin samples from 8-day- and 12-day-old plants of the indicated genotypes. F, Expression of SOC1 direct targets in 8-day or 12-day-old WT plants. In B-E bars indicate the average of two (B) or three (C-E) independent experiments and error bars denote SEM. Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$) determined by two-sided t-tests. The retrotransposon Ta3 was used as a negative control (Johnson et al., 2002).

Figure 4. Loss of MRG function increases abiotic stress tolerance in a *SOC1*-dependent manner. A, Basal freezing tolerance of *mrg1 mrg2* as compared to WT. Two-week-old non-acclimated plants were exposed to the indicated freezing temperatures for 6 hours and survival was scored after 7 days of recovery at 22°C. Asterisks indicate significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) with WT determined by two-sided *t*-tests in four independent experiments. B, Freezing tolerance in WT, and *soc1*, *mrg1 mrg2* and *mrg1 mrg2 soc1* mutant plants. Two-week-old non-acclimated plants were exposed to -6°C for 6 hours and survival was scored after 7 days of recovery at 22°C. C, Drought tolerance in WT, and *soc1*, *mrg1 mrg2* and *mrg1 mrg2 soc1* mutant plants. Watering was withheld from one week-old plantlets for 14 days before resuming regular watering schedule. Plant survival was scored after 7 days. D, Sensitivity to ABA of WT and *soc1*, *mrg1 mrg2* and *mrg1 mrg2 soc1* mutants. One-week-old plantlets germinated on GM medium were transferred to petri dishes with GM medium in the presence or absence of 20 µM ABA. Fresh weight (FW) was measured after 7 days. Left panels show summarized data from four (A-C) or five (D) independent experiments. Right panels show representative plants from the indicated genotypes. Statistical significance in a one-way ANOVA test with Tukey's correction for multiple comparisons is denoted by letters above bars (different letters indicate an adjusted *p*-value $p < 0.05$). In all cases, bars indicate the average and error bars denote SEM.

Figure 5. Hypothetical working model showing how MRG-mediated chromatin acetylation at the *SOC1* locus coordinates the floral transition and abiotic stress responses. Reading of H3K36me3 by MRG proteins (brown oval) and subsequent remodeling of *SOC1* chromatin through H4 acetylation during floral transition activates

the transcription of this gene. The concomitant accumulation of SOC1 protein (purple rectangles) tunes down the magnitude of abiotic stress responses by repressing the transcription of stress-responsive genes.

SUPPLEMENTAL DATA

Additional supporting information may be found online in the Supporting Information tab for this report.

Supplemental Figure 1. Loss of *MRG* function delays flowering only under photoperiodic inductive conditions.

Supplemental Figure 2. *MRG* role in the regulation of floral induction depends on *SDG8*-mediated histone H3K36 trimethylation.

Supplemental Table 1. Transcriptomic analysis of *mrg1 mrg2* mutants through RNA-seq.

Supplemental Table 2. Gene Ontology terms overrepresented in MRG-regulated genes.

Supplemental Table 3. List of primers used in this study.

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An MRG-operated chromatin switch at *SOC1* attenuates abiotic stress responses during the floral transition

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Short title

SOC1 modulates stress responses during flowering.

Sentence summary

A chromatin switch coordinates flowering initiation with plant responsiveness to adverse conditions tuning down costly stress responses during flowering for optimal plant reproductive success

Author contributions

JAJ and MP designed the research. JB-G, AM and RP performed all the experimental approaches. JS designed the freezing tolerance analyses carried out and analyzed the resulting data. JB-G, JAJ and MP analyzed all the data and wrote the paper.

ABSTRACT

Plants react to environmental challenges by integrating external cues with endogenous signals to optimize survival and reproductive success. However, the mechanisms underlying this integration remain obscure. While stress conditions are known to impact plant development, how developmental transitions influence responses to adverse conditions has not been addressed. Here, we reveal a novel molecular mechanism of stress response attenuation during the onset of flowering in Arabidopsis. We show that Arabidopsis MORF-RELATED GENE (MRG) proteins, components of the NuA4 histone acetyltransferase (HAT) complex that bind trimethylated-lysine 36 in histone H3 (H3K36me3), function as a chromatin switch on the floral integrator *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) to coordinate flowering initiation with plant responsiveness to hostile environments. MRG proteins are required to activate *SOC1* expression during flowering induction by promoting histone H4 acetylation. In turn, SOC1 represses a broad array of genes that mediate abiotic stress responses. We propose that during the transition from vegetative to reproductive growth, the MRG-SOC1 module constitutes a central hub in a mechanism that tunes down stress responses to enhance reproductive success and plant fitness at the expense of costly efforts for adaptation to challenging environments.

INTRODUCTION

Plants often face unfavourable environmental conditions through their life cycle. To cope with them, plants have evolved to acquire complex mechanisms that either ameliorate the damaging effects of stress and increase tolerance, or accelerate the life cycle of the plant leading to an early reproductive phase in a response frequently known as escape. Stress perception and response involve intricate signalling networks that often entail substantial transcriptomic rearrangements (Asensi-Fabado et al., 2017; Haak et al., 2017; Baurle and Trindade, 2020). A paradigmatic example is the cold acclimation response of temperate plants, where low non-freezing temperature serves as an environmental cue for gene expression reprogramming to increase freezing tolerance (Barrero-Gil and Salinas, 2018). The C-REPEAT/DEHYDRATION RESPONSIVE ELEMENT BINDING FACTORS (CBFs) 1-3 and the plant hormone Abscisic acid (ABA) play key roles in this process (Eremina et al., 2016; Barrero-Gil and Salinas, 2018; Shi et al., 2018). Similar extensive transcriptomic adjustments also mediated by ABA signalling pathways (Harb et al., 2010) have been reported in response to drought. In addition, adaptation to suboptimal environments requires that plants also integrate external factors with endogenous cues to optimize developmental processes such as the floral transition. In this context, ABA accumulation triggered by mild drought conditions that compromise growth but not survival induce the expression of the floral integrator *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*), accelerating flowering in a response of drought escape (Riboni et al., 2013). Remarkably, *SOC1* is also part of a cross-talk signalling pathway that negatively regulates cold response by inhibiting *CBF* expression (Seo et al., 2009).

Changes in the organization of chromatin and histone modifications are considered the interphase through which the environment interacts with the genome to promote alterations in gene expression (Lamke and Baurle, 2017; Chang et al., 2020). Acetylation on particular histone lysine (K) residues is reversibly controlled by both histone acetyltransferases (HATs) and histone deacetylases (Lee and Workman, 2007), and is essential for the regulation of gene expression in response to environmental stresses (Liu et al., 2016; Luo et al., 2017; Jiang et al., 2020). Histone modifications are recognized by “reader” proteins that contribute to modulate chromatin dynamics and to translate chromatin features into specific patterns of gene expression (Musselman et al., 2012). In Arabidopsis, for example, trimethylation of lysine 36 in histone H3 (H3K36me3) is recognized by two homologue proteins named MORF-RELATED

GENE 1 (MRG1) and MRG2 (Bu et al., 2014; Xu et al., 2014). These proteins are components of the Nucleosome Acetyl transferase of histone H4 (NuA4) HAT complex (Espinosa-Cores et al., 2020), and redundantly modulate the expression of the key floral integrator *FLOWERING LOCUS T* (*FT*) gene (Bu et al., 2014; Xu et al., 2014; Guo et al., 2020). However, the contribution of chromatin remodelling processes, and specifically MRG proteins, to the integration of stress adaptation with plant developmental progression remains virtually unknown. Here, we reveal an MRG-mediated chromatin mechanism that acts on the master flowering gene *SOC1* (Samach et al., 2000) to modulate abiotic stress responses depending on developmental signals. Our data suggest that this MRG-SOC1 regulatory module attenuates responsiveness of Arabidopsis plants to various stresses during the onset of flowering for optimal integration of development and adaptation to adverse environments.

RESULTS

MRG proteins are required for the *SOC1*-dependent downregulation of abiotic stress-responsive genes

To address the involvement of Arabidopsis MRG proteins on the regulation of gene expression and other physiological processes, we used two uncharacterized *MRG* mutant alleles, *mrg1-2* and *mrg2-4* (Supplemental Fig. 1A, B). Confirming previous observations (Bu et al., 2014; Xu et al., 2014; An et al., 2020; Guo et al., 2020), the floral integrator genes *FT* and *SOC1* were downregulated in *mrg1-2 mrg2-4* double mutant plants (Supplemental Fig. 1C), corroborating the role of MRGs in fine-tuning flowering responses specifically under long days (LD) conditions (Supplemental Fig. 1D, E). Our genetic analysis showed that *ft* mutations cause a modest, but significant, delay in the flowering time of *mrg1 mrg2* plants, whereas the combination of *soc1* mutations with *mrg1 mrg2* clearly enhances the late flowering phenotype of the double mutant (Fig. 1). These results show that the delay in flowering observed in *mrg1 mrg2* double mutant plants does not depend on a single floral integrator, suggesting that *MRG* genes influence flowering through the activity of both floral integrators. Furthermore, *MRG* function in the control of the floral transition shows a strong requirement on H3K36me3, a modification mediated by the histone methyl transferase SET DOMAIN GROUP 8 (SDG8) (Soppe et al., 1999; Zhao et al., 2005), since *sdg8* mutants fully suppress the late flowering phenotype of *mrg1 mrg2* mutant plants (Supplemental Fig. 2). These results are in line with the current model concerning the involvement of MRG

proteins in the regulation of floral transition (Bu et al., 2014; Xu et al., 2014; An et al., 2020; Guo et al., 2020).

Next, we performed a transcriptomic analysis on plants grown under LD photoperiod during the floral transition to examine the implication of Arabidopsis MRG proteins in the regulation of gene expression. We identified 552 differentially expressed genes (DEGs) in the *mrg1 mrg2* double mutant, of which 516 were induced and 21 were repressed (Supplemental Table 1). GO-term enrichment analysis revealed an over-representation of terms related to abiotic stress responses, including water deprivation, salt stress and hypoxia among upregulated genes (Fig. 2A; Supplemental Table 2). Intriguingly, among the genes induced in the *mrg1 mrg2* double mutant, we found a significant enrichment in direct targets of SOC1 (Fig. 2B). Indeed, SOC1 has been reported as a direct repressor of *CBF* genes, that regulate the tolerance to freezing temperatures (Seo et al., 2009) and a number of additional abiotic stress response mediators (Immink et al., 2012; Tao et al., 2012). Besides, a significantly high number of SOC1 and CBF-dependent genes were found differentially upregulated in *mrg1 mrg2* plants (Fig. 2C, D). Independent quantitative RT-PCR expression analyses confirmed the upregulation of several direct targets of SOC1 including *CBF2, WRKY33, RAV1* and *RAV2* (Immink et al., 2012; Tao et al., 2012) as well as different genes related with abiotic stress responses such as *ZAT10*, *SZF1*, *ABA2* and *COR15A* in *mrg1 mrg2* plants (Fig. 2E). Interestingly, the expression level for these genes in the *mrg1 mrg2 soc1* triple mutant is comparable to that observed in either *mrg1 mrg2* or *soc1* mutant plants, revealing no marked enhancement of expression upon the concurrent loss of function of these genes (Fig. 2E). The absence of additive effects in the *mrg1 mrg2 soc1* triple mutants supports that *MRG* genes and *SOC1* function in the same genetic pathway to control the expression of abiotic stress-responsive genes, although we cannot rule out that SOC1 could also perform MRG-independent roles in the control of these genes.

MRG2 protein binds *SOC1* chromatin and promotes H4 acetylation deposition in this locus during the floral transition

MRG proteins promote both *FT* and *SOC1* expression (Supplemental Fig. 1C), and at least MRG2 associates with the transcription factor CONSTANS (CO) to bind *FT* chromatin and activates its transcription under photoperiodic flowering-inducing conditions (Bu et al., 2014). Since FT is an activator of *SOC1* expression during the floral transition (Yoo et al., 2005), it is tempting to speculate that the decreased *FT*

expression caused by the loss of *MRG* function might be responsible for the reduced activation of *SOC1* expression in *mrg1 mrg2* plants. However, our genetic analysis showed that the function of *MRG* genes in the control of flowering initiation is not only dependent on *FT* (Fig. 1). Indeed, CO has been proposed to directly activate *SOC1* expression (Samach et al., 2000) and the absence of a functional *FT* does not completely suppress CO-mediated *SOC1* activation (Yoo et al., 2005), which also indicates that *SOC1* expression is not entirely dependent on *FT* function. Thus, we hypothesized that MRG proteins might directly bind *SOC1* chromatin. In turn, this floral integrator would control the expression levels of stress-responsive genes. To examine whether *SOC1* is a direct target of MRG proteins, we performed ChIP-PCR experiments using a *pMRG2::MRG2-YFP* transgenic line that fully complements the late flowering phenotype of *mrg1 mrg2* plants (Bu et al., 2014). Following immunoprecipitation with an α-GFP antibody, we observed a conspicuous enrichment of DNA corresponding to the regulatory region of *SOC1* in the *pMRG2::MRG2-YFP* transgenic line compared to WT plants (Fig. 3A-B), indicating that MRG2 directly binds *SOC1* chromatin. Consistent with the role of MRG proteins as H3K36me3 readers, this genomic region of the *SOC1* locus bears high levels of this histone modification (Bewick et al., 2016) (Fig. 3A). These observations suggest that MRG proteins directly and positively regulate *SOC1* expression.

The MRG2 protein was previously shown to be necessary for maintaining high H4 acetylation levels in the chromatin of *FT* to sustain its expression (Xu et al., 2014). Thus, we reasoned that MRG proteins might also regulate *SOC1* expression by modulating H4 acetylation levels. *SOC1* expression gradually increases from germination (Liu et al., 2008) but, according to our observations, MRG-dependent activation of *SOC1* is evidenced during the transition from vegetative to reproductive development (Fig. 3C). Indeed, the activation of *SOC1* is observed only in WT but not in *mrg1 mrg2* mutant plants between 8 and 12 days after sowing, the period when flowering commitment is taking place, as shown by the induction of the expression of floral meristem identity genes such as *APETALA 1 (AP1)* and *LEAFY (LFY)* (Fig. 3D). Therefore, we decided to monitor histone H4 acetylation levels in different regions of *SOC1* chromatin (Fig. 3A) in WT and *mrg1 mrg2* plants during the floral transition (days 8 and 12). ChIP experiments using an antibody against tetra-acetylated histone H4 (H4K5,8,12,16ac) revealed a pronounced increase of this mark around the genomic region of *SOC1* bound by MRG2 in WT plants during the initiation of flowering. In

contrast, H4ac levels remained steady in the *mrg1 mrg2* double mutant between 8 and 12 days after sowing, leading to significantly lower levels of this histone modification in mutant plants compared to WT at the latest developmental stage assessed (Fig. 3E). Remarkably, the intensity of H4 acetylation observed in WT and *mrg* mutants at the *SOC1* locus is consistent with the expression levels detected for this gene in these plants during the floral transition (Fig. 3C). Furthermore, during this phase of floral initiation, a conspicuous downregulation of diverse SOC1 direct target genes related to stress responses is observed in WT plants (Fig. 3F). Based on these observations, we concluded that MRG proteins mediate *SOC1* activation during the floral transition by promoting H4 acetylation levels at this locus, and cause a concomitant repression of stress-related genes (Fig. 3F).

Loss of *MRG* function increases abiotic stress tolerance in a *SOC1*-dependent manner

Since mutations in *MRG* genes increase the expression of genes involved in abiotic stress responses, we checked the tolerance of *mrg1 mrg2* mutants to different challenging environmental conditions. First, we assessed the basal freezing tolerance of two-week-old *mrg1 mrg2* mutants and WT plants. A significant increase in survival to freezing temperatures was observed in mutant plants compared with WT (Fig. 4A). Notably, the genetic relationship found between *mrg1 mrg2* and *soc1* mutants regarding the capacity to withstand freezing temperatures indicated that the increased tolerance displayed by *mrg1 mrg2* plants requires a functional *SOC1* gene (Fig. 4B). These results demonstrated that MRGs negatively regulate constitutive freezing tolerance and that this control relies, at least in part, on *SOC1* function. We also evaluated the ability of *mrg1 mrg2* double mutants to cope with drought and the genetic interaction between *MRG* and *SOC1* genes in modulating this trait. The data revealed that loss of *MRG* function results in increased tolerance to water deprivation, and, again, this negative regulation on drought tolerance mediated by MRG proteins displayed dependence on a functional *SOC1* gene (Fig. 4C). Finally, we wondered if these responses to abiotic stresses could be associated with an altered ABA responsiveness. The results showed that loss of *MRG* function rendered plants that were hypersensitive to ABA in a *SOC1*-dependent manner (Fig. 4D). Thus, we concluded that MRG proteins negatively regulate various abiotic stress responses, in part, by controlling *SOC1* expression, and possibly by modulating either ABA levels or signalling.

DISCUSSION

In this work we have explored the involvement of Arabidopsis MRG histone readers in the regulation of gene expression. We found that besides mediating the activation of key flowering genes like *FT* and *SOC1*, MRG proteins also control the expression of many abiotic stress-responsive genes. Furthermore, MRG-mediated repression of abiotic stress responses is dependent on the function of the floral integrator *SOC1*, a locus regulated by MRG proteins by directly binding to its chromatin and promoting histone acetylation during the floral transition. The contribution of chromatin remodelling processes to the coordination of stress adaptation with plant developmental progression remains practically unknown (Ma et al., 2020). We propose that the MRG-mediated remodelling of *SOC1* chromatin constitutes a central mechanism that tunes down stress responses during the floral transition likely to enhance reproductive success.

Previous works had established the central role displayed by MRG histone readers and the H3K36me3 epigenetic mark in promoting the expression of the key floral integrator gene *FT* through the deposition of acetylation on histone H4 in regulatory regions of this locus (Bu et al., 2014; Xu et al., 2014; Guo et al., 2020). Now we have found that MRG proteins are also involved in attenuating different abiotic stress responses, including drought and low temperature. This is consistent with recent reports showing that the histone methyl transferase SDG8 regulates a significant number of genes related to abiotic stress (Cazzonelli et al., 2014). In fact, another histone methyl transferase, SDG26, negatively regulates drought stress tolerance in a similar way to MRG proteins (Ma et al., 2013). Consistent with the involvement of SDG8 and SDG26 methyl transferases in the modulation of stress responses, H3K36me3 has been shown to play a key role in the adaptation of plants to fluctuating ambient temperature (Pajoro et al., 2017). The extreme phenotype of the *mrg1 mrg2 sdg8* triple mutant (Supplemental Fig. 2B, C) prevented us from assessing the possible genetic interaction of these genes in the context of abiotic stress responses. In any case, our results support the notion that reading of H3K36me3 through MRG proteins is a relevant mechanism in the control of the expression of abiotic stress-responsive genes.

Importantly, in this work we have established that MRG proteins tune down abiotic stress responses in a *SOC1*-dependent manner. Earlier research has established that the flowering promoting factor SOC1 decreases Arabidopsis tolerance to freezing

253 temperatures under conditions that favor the initiation of reproductive growth (Seo et
254 al., 2009). Here, we report that the SOC1 repressive role on abiotic stress response is
255 not restricted to low temperature but rather extends also to the reaction of Arabidopsis
256 to other environmental challenges. For instance, we show that SOC1 acts to reduce the
257 ability of this plant to cope with drought conditions. It is important to highlight that
258 although previous studies had demonstrated a key role for SOC1 in a drought escape
259 mechanism that accelerates flowering under conditions of water deficit (Riboni et al.,
260 2013), here we are describing an entirely different mechanism that tunes down
261 responses underlying the plant ability to cope with water shortage by enhancing drought
262 tolerance mechanisms upon flowering initiation. Indeed, natural variation studies in
263 drought stress responses in Arabidopsis have revealed a negative correlation between
264 the capacity to increase survival to water deprivation and the ability to accelerate
265 flowering and escape drought conditions, supporting the existence of a genetic trade-off
266 between both mechanisms (McKay et al., 2003), and our results suggest that SOC1
267 function may represent an important determinant underlying this trade-off. Furthermore,
268 we provide additional evidence indicating that both MRG and SOC1 proteins might be
269 involved in controlling either ABA levels or downstream signaling pathways.
270 Consistent with this hypothesis, SnRK2-substrate 1 (SNS1), a putative component of
271 the HAT complex NuA4 that in yeast and mammals interacts with the homolog of
272 MRG, is involved in ABA signaling in Arabidopsis (Umezawa et al., 2013).
273 Finally, we have demonstrated that the MRG histone readers promote histone
274 acetylation at the *SOC1* locus during the floral transition, activating its expression.
275 While various reports have shown the influence of abiotic stress signals in modulating
276 developmental transitions, and specifically flowering time (Seo et al., 2009; Riboni et
277 al., 2013), whether particular developmental stages exhibit differential adaptation
278 capabilities to unfavorable environmental conditions is, in practice, unknown. This
279 work has uncovered a developmental epigenetic switch that is timely activated during
280 the floral transition with a bivalent involvement in triggering flowering and moderating
281 stress responses. We interpret these findings as a novel plant chromatin-mediated
282 mechanism that might operate under the control of MRG proteins to optimize
283 reproductive success and fitness at the expense of costly efforts to adapt to challenging
284 environmental conditions once flowering is initiated (Fig. 5). However, *SOC1* is a
285 tightly regulated gene and MRG proteins are only one of the multiple factors at play
286 controlling its expression, suggesting that additional transcriptional regulators could be

contributing to coordinate flowering and abiotic stress tolerance. Further studies will be necessary to fully unveil the intricate nature of the epigenetic mechanisms that integrate stress responses with plant developmental phase transitions as well as their contribution to Arabidopsis adaptive variation, but in view of these observations it is tempting to consider that chromatin dynamics at the *SOC1* locus could represent a driver for phenotypic plasticity in Arabidopsis.

MATERIALS AND METHODS

Plant materials, growth conditions, cold treatments and tolerance assays

All Arabidopsis mutant lines used in this study are in Columbia-0 (Col-0) background. The mutant alleles of *MRG1* and *MRG2* were named *mrg1-2* (SALK_089867) and *mrg2-4* (SAIL_317_F11), respectively, and were obtained from the Nottingham Arabidopsis Stock Centre (NASC, UK). Other mutants used have been previously described elsewhere: *ft-10* (Yoo et al., 2005*), sdg8-1* (Zhao et al., 2005) and *soc1-2* (Lee et al., 2000). Plants were grown at 21°C under LD photoperiods (16 hours of cool-white fluorescent light) or SD photoperiods (8 hours of cool-white fluorescent light) with photon flux of 110 µmol m^{-2} s^{-1}, in pots containing a mixture of organic substrate and vermiculite (3:1, v/v), or in Petri dishes containing 1/2x Murashige and Skoog medium supplemented with 1% (w/v) sucrose and solidified with 0.8 % (w/v) plant agar (GM medium).

Tolerance to freezing temperatures was determined in two-week-old plants grown on soil. The freezing treatment started by pre-incubating plants at 4°C for one hour followed by a gradual decrease of temperature at a rate of two degrees per hour to avoid intracellular freezing. Temperature drop stops at the indicated temperature, which is maintained for six hours followed by a gradual recovery of temperature at the aforementioned rate and incubation at 4°C for one hour before returning plants to normal growth conditions. Survival was scored after seven days.

To determine drought tolerance, one-week-old plantlets were either kept in a normal irrigation schedule (50-ml water for 21 cm^3 pots twice a week) or without any watering for fourteen days before resuming irrigation. Survival was scored after seven days.

ABA sensitivity assay

Seven-day old seedlings from the indicated genotypes grown on GM medium in LD conditions were transferred to GM medium supplemented with or without 20 µM ABA. Then seedlings were incubated for 9 additional days before taking pictures and measuring fresh weight.

Gene expression analysis

Plants were grown at 22°C for 12 days under LD photoperiod, taking samples from aerial tissue at Zeitgeber time (ZT) 8 for transcriptomic analysis, unless otherwise indicated. Total RNA was extracted using EZNA Plant RNA kit (Omega) following the manufacturer's protocol. RNA samples were treated with DNase I (Roche) to remove genomic DNA contamination. For RNA sequencing experiments, samples from three independent experiments were used to prepare three sequencing libraries for each genotype. RNA library preparation and sequencing was performed by the CRG - Centre de Regulació Genòmica (Barcelona, Spain), using Illumina HiSeq2000 technology. Approximately 45 million single-end 50-base reads per sample were generated and more than 90% of reads uniquely mapped to Arabidopsis TAIR10 reference genome using HISAT2 (Li et al., 2009). Differential expression analysis was performed using the DESeq2 module (Love et al., 2014) on SeqMonk v1.45 software (http://www.bioinformatics.babraham.ac uk/ projects/seqmonk/). To identify differentially expressed genes (DEGs) we set FDR≤ 0.05 and fold change ≥ 1.5 or ≤0.5 as cutoffs for any given DEG. Gene ontology (GO) enrichment analysis was performed on PANTHER (http://pantherdb.org/) using a Fisher's exact test corrected by a false discovery rate FDR < 0.05 as cutoff for a significantly enriched GO term. Interesting DEGs were validated by quantitative PCR (qPCR) assays as follows. For qPCR analysis, RNA samples from independent experiments were processed and analyzed separately. RNA was retro-transcribed using Maxima first strand cDNA synthesis kit (Thermofisher Scientific), and qPCRs were performed using LightCycler 480 SYBR Green I (Roche). Primers used for qPCR analysis are listed in Supplemental Table 3. The At4g26410 gene was used as a reference in all experiments (Czechowski et al., 2005). Fold change was calculated using the ΔΔCT method (Livak and Schmittgen, 2001).

Chromatin Immunoprecipitation

Chromatin Immunoprecipitation (ChIP) experiments were performed as described (Crevillen et al., 2019). Immunoprecipitated DNA was quantified by qPCR using the oligonucleotides described in Supporting Information (Supplemental Table 3). DNA enrichment was estimated as the fraction of immunoprecipitated DNA relative to input (% INPUT). We used the following antibodies: α-H4K5,8,12,16Ac (Merck-Millipore 06-598) and α-GFP (Invitrogen, A-6455).

Statistical analyses

Statistical analyses (ANOVA, Student´s t-test) were performed with GraphPad Prism software. Statistical significance of the overlap between two groups of genes was calculated using a hypergeometric test using Excel software.

ACCESSION NUMBERS

Sequence data related to this manuscript can be found in the Arabidopsis information portal (https://www.araport.org/) under the accession numbers *MRG1* (At4g37280), *MRG2* (At1g02740), and *SOC1* (At2g45660). Epigenomic data were retrieved from the Plant Chromatin State Database (http://systemsbiology.cau.edu.cn/chromstates/index.php). The complete genome-wide data from this publication were submitted to the Gene Expression Omnibus database (www.ncbi.nlm.nih.gov/geo/) under accession number GSE141135.

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FIGURE LEGENDS

Figure 1. *MRG* role in the regulation of floral induction is partially dependent on *FT* and *SOC1* function. Flowering time of *mrg1 mrg2 ft* (A) and *mrg1 mrg2 soc1* (B) triple mutants. Number of leaves at bolting in WT, and single *ft* and *soc1*, double *mrg1 mrg2* and triple *mrg1 mrg2 ft* and *mrg1 mrg2 soc1* mutant plants grown under LD. Statistical significance was calculated using one-way ANOVA with Tukey's correction for multiple comparisons and is denoted by different letters indicating $p < 0.05$. Box plots indicate the 25th and 75th percentiles of the data and the median is indicated by a line. Whiskers represent the minimum and maximum value. Individual data points are represented by black dots.

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the transcription of this gene. The concomitant accumulation of SOC1 protein (purple rectangles) tunes down the magnitude of abiotic stress responses by repressing the transcription of stress-responsive genes.

SUPPLEMENTAL DATA

Additional supporting information may be found online in the Supporting Information tab for this report.

Supplemental Figure 1. Loss of *MRG* function delays flowering only under photoperiodic inductive conditions.

Supplemental Figure 2. *MRG* role in the regulation of floral induction depends on *SDG8*-mediated histone H3K36 trimethylation.

Supplemental Table 1. Transcriptomic analysis of *mrg1 mrg2* mutants through RNA-seq.

Supplemental Table 2. Gene Ontology terms overrepresented in MRG-regulated genes.

Supplemental Table 3. List of primers used in this study.

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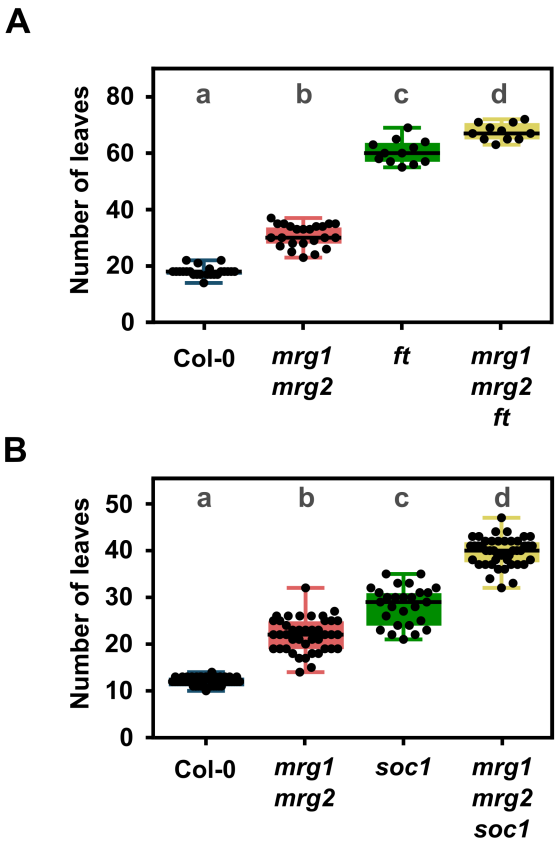

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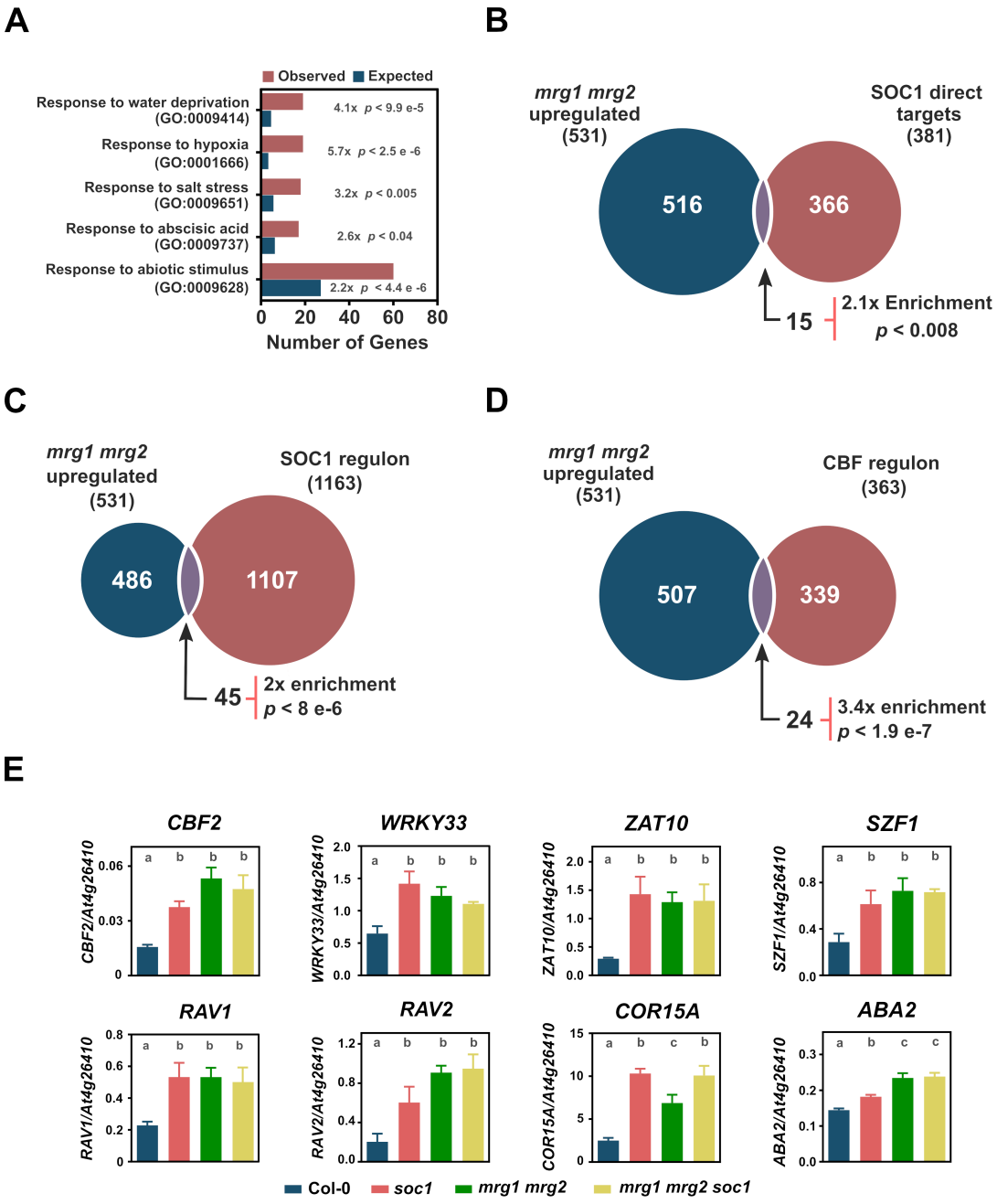

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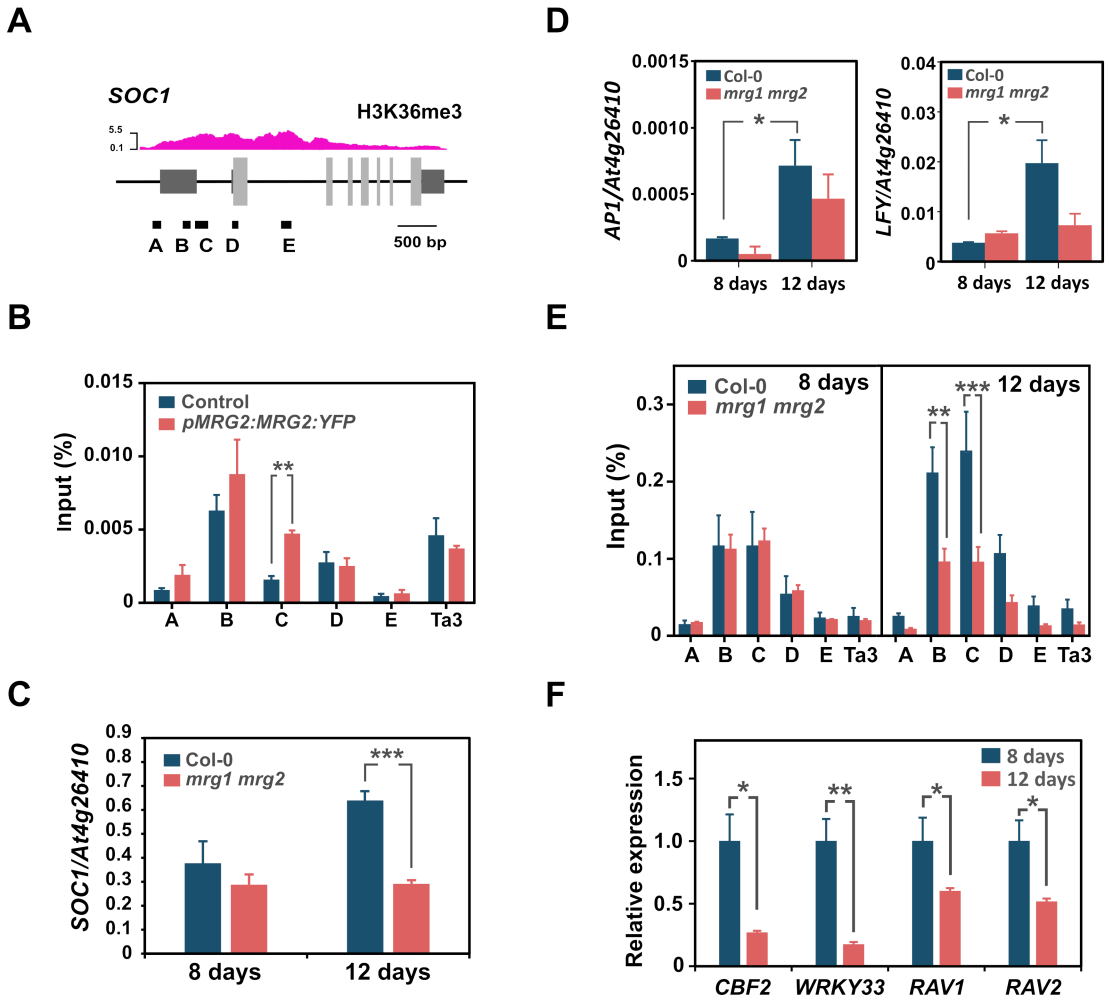

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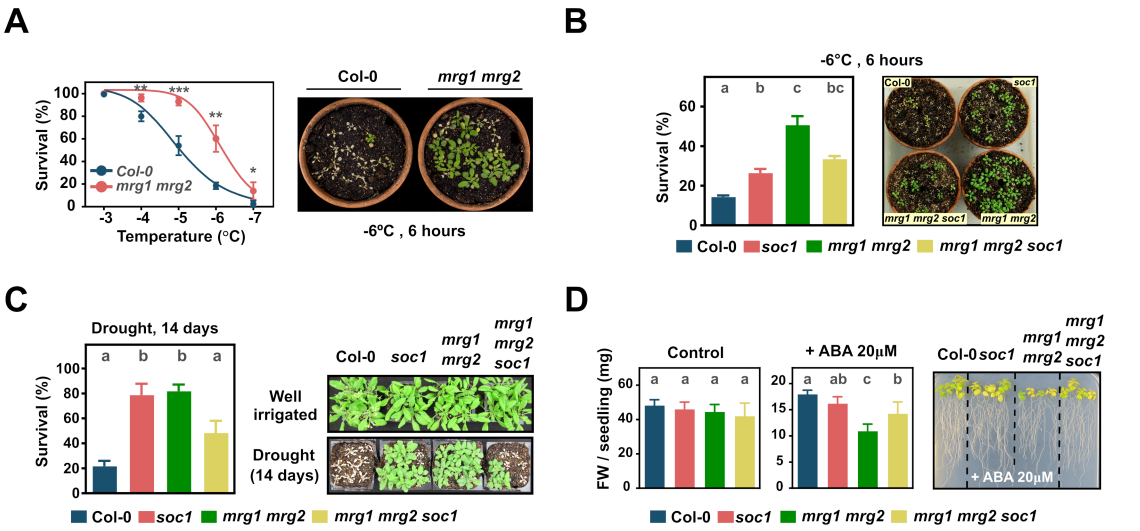

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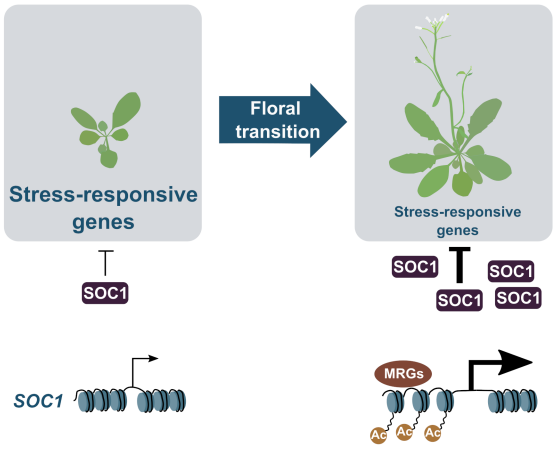

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