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H2A.Z contributes to trithorax activity at the *AGAMOUS* locus

Dear Editor,

In multicellular eukaryotes, Polycomb repression heritably silences gene expression programs not needed or detrimental for a given developmental stage or tissue (Schuettengruber et al., 2017). During cell fate reprogramming, Polycomb silencing can be overcome by the combined activity of multiple Trithorax group (TrxG) proteins (Wu et al., 2012; Liang et al., 2015; Schuettengruber et al., 2017). TrxG proteins are genetically defined as suppressors of homeotic defects caused by loss of Polycomb function and have diverse enzymatic activities (Schuettengruber et al., 2017). We used a genetic enhancer screen to identify candidate TrxG proteins and uncovered TrxG activity for components of the SWR1 chromatin remodeling complex, which deposits the histone variant H2A.Z (Deal et al., 2007; March-Diaz et al., 2008).

In *Arabidopsis*, floral homeotic genes are repressed by Polycomb Repressive Complex 2 (PRC2) in vegetative tissues (Goodrich et al., 1997) and several different TrxG proteins contribute to reversal of PRC2-mediated silencing of floral homeotic genes during flower development (Wu et al., 2012; Liang et al., 2015). Among these, double mutants in the SWI/SNF chromatin remodeling ATPases BRAHMA (BRM) and SPLAYED (SYD) fail to pattern floral organs (Wu et al., 2012). BRM and SYD are recruited to the floral homeotic *AGAMOUS* (*AG*) locus by the plant-specific pioneer transcription factor LEAFY (LFY), which promotes floral fate and directs flower patterning (Wu et al., 2012; Jin et al., 2021; Lai et al., 2021). Indeed, mutations in several TrxG proteins enhance floral homeotic defects of the weak *lfy-5* mutant (Wu et al., 2012; Liang et al., 2015).

In this study, we set out to identify additional TrxG factors using a genetic enhancer screen in the *lfy-5* mutant background. After fast neutron or chemical (ethyl methanesulfonate [EMS]) mutagenesis, we identified 59 and 53 mutants with enhanced floral homeotic defects, respectively. Since chromatin regulators act in multiple genetic pathways (Goodrich et al., 1997; Wagner and Meyerowitz, 2002; Schuettengruber et al., 2017), we implemented a secondary screen for pleiotropic phenotypes. The additional phenotypes of the remaining 49 fast neutron and 39 EMS mutants include dwarfism, stem fasciation, terminal flowers, and compressed inflorescences (Supplemental Figure 1). A large complementation group of 22 pleiotropic fast neutron *lfy-5* enhancers was early flowering and had a bushy growth habit (Supplemental Figure 1). Map-based cloning of two allelic members of this complementation group placed the lesion near the centromere on chromosome three (Supplemental Figure 2A). Large deletions (>12 kb) in both mutants encompassed ACTIN RELATED PROTEIN 6 (ARP6) (Supplemental Figure 2B), consistent with the observed pleiotropic phenotypes (Deal et al., 2007). Moreover, *ARP6* expression was abolished in both mutants (Supplemental Figure 2C). ARP6 is a component of the *Arabidopsis* SWR1 complex, which deposits the histone variant H2A.Z on chromatin (Deal et al., 2007; March-Diaz et al., 2008). We therefore named these mutants *arp6-12* and *arp6-13*.

To assess the contribution of the SWR1 complex to flower patterning, we generated *lfy-2 arp6-3* and *lfy-2 pie1-5* double mutants in the Columbia ecotype. *arp6-3* and *pie1-5* are known mutants in ARP6 and in the catalytic subunit of the *Arabidopsis* SWR1 complex, respectively (Deal et al., 2007; March-Diaz et al., 2008). Like the *lfy-5 arp6-12* and *lfy-5 arp6-13* double mutants in the Landsberg *erecta* (L*er*) ecotype, *lfy-2 arp6-3* and *lfy-2 pie1-5* lacked petals, stamens, and fused carpels, displaying floral homeotic defects similar to those of the *lfy-1* null mutant (Figure 1A). Analysis of gene expression in *lfy-5 arp6-12* and *lfy-5 arp6-13* revealed strongly reduced accumulation of the floral homeotic gene *AG* relative to *lfy-5*, to a level similar to that observed in the *lfy-6* null mutant (Figure 1B). We confirmed that the *ARP6* deletion underlies the *lfy-5 arp6-12* mutant phenotype by genetic rescue. Independent rescued *lfy-5 arp6-12* lines phenocopied *lfy-5* mutant flowers, as expected (Supplemental Figure 2D and S2E). In addition, the rescue lines restored *AG* expression (Supplemental Figure 2F). The combined data implicate the H2A.Z depositing SWR1 complex in upregulation of the floral homeotic gene *AG*.

To test whether ARP6 acts as a *bona fide* TrxG protein, we crossed *arp6-12* to a mutant in *CURLY LEAF* (*CLF*), the catalytic subunit of *Arabidopsis* PRC2 (Goodrich et al., 1997). *clf-2* forms small, upward-curled leaves that misexpress *AG* and other Polycomb targets (Goodrich et al., 1997). As previously observed for other TrxG proteins in *Arabidopsis* (Wu et al., 2012; Liang et al., 2015), *arp6-12* rescued the upward leaf curling of *clf-2* (Figures 1C and Supplemental Figure 3A). In addition, *arp6-12 clf-2* mutants also partially rescued the small leaf size typical of *clf-2* (Figures 1C and Supplemental Figure 3B). Finally, loss of *ARP6* function reduced the ectopic accumulation of *AG* in *clf-2* (Figure 1D). The combined data indicate that ARP6 acts as a TrxG protein at the *AG* locus.

To further explore the role of ARP6 and H2A.Z in activation of floral homeotic gene expression, we employed *ap1 cal* pAP1:AP1-GR inflorescences. In the absence of steroid treatment, *ap1 cal* pAP1:AP1-GR mutants arrest after forming stage 2 flowers, prior to activation of *AG* expression (Yan et al., 2019). At this stage, the *AG* locus is covered by histone H3 lysine 27 trimethylation (H3K27me3) deposited by PRC2 while activating methylation at lysine 4 (H3K4me3) is absent (Supplemental Figure 4A and 4B). We performed H2A.Z and ARP6 chromatin immunoprecipitation (ChIP)-qPCR, which revealed strong association of ARP6 and of H2A.Z with the *AG* locus at this stage (Figure 1E), indicating

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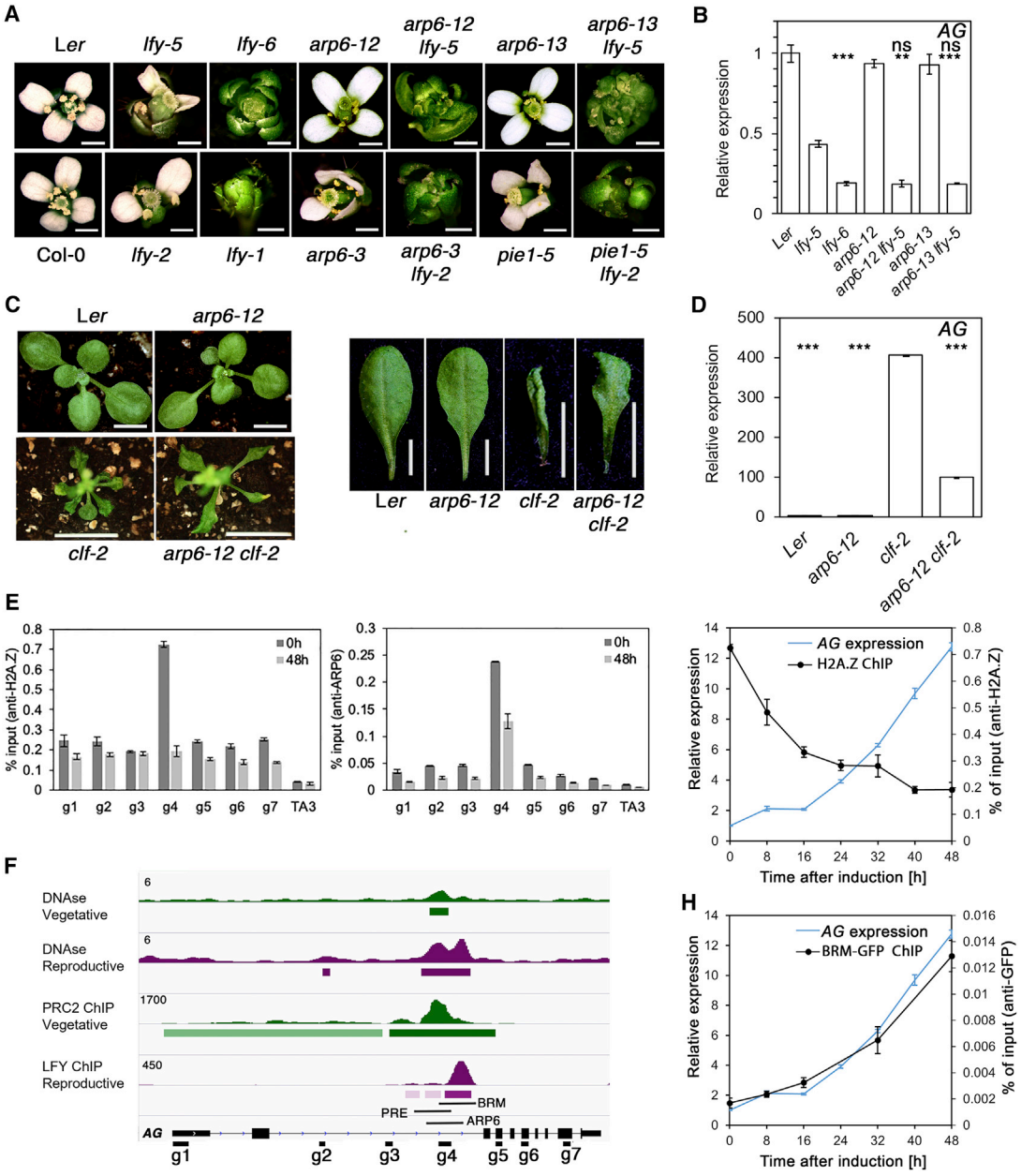

Figure 1. ARP6/SWR1 acts as a Trithorax group protein and binds a PRE/TRE element at the *AG* locus.

(A) Floral homeotic defects of mutants in SWR1 complex components *ARP6* or *PIE* in the weak *LFY* mutant (*lfy-5* or *lfy-2*) background relative to the single mutants, the *LFY* null mutant (*lfy-6* or *lfy-1*) and wild type (L*er* or Col). Scale bars, 1 mm.

(B) *AG* floral homeotic gene expression in the genotypes shown in **(A)**. Mean ± SEM of three biological replicates. Top: *P* values relative to relative to *lfy-6*: 0.84 (*arp6-12 lfy-5*), 0.78 (*arp6-13 lfy-5*), or *lfy-5* (below): 4E-04 (*lfy-6*), 0.001 (*arp6-12 lfy-5*), 2E-04 (*arp6-13 lfy-5*), two-tailed Student's *t*-test.

(C) Rosette leaves in the wild-type (L*er*), *arp6-12*, PRC2 mutant *clf-2*, and *clf-2 arp6-12* in 16-day-old seedlings (left) or individual leaves (right). Scale bars: seedlings, 0.5 cm (L*er*, *arp6-12*), 1 cm (*clf-2*, *arp6-12 clf-2*); leaves, 1 cm.

(D) *AG* expression in the genotypes shown in **(C)**. Mean ± SEM of three biological replicates. *P* values (two-tailed Students *t*-test) relative to *clf-2*: 1E-07 (L*er*), 1E-07(*arp6-12*), 3E-07 (*arp6-12 clf-2*).

(E) ChIP-qPCR for H2A.Z (left) and ARP6 (right) at genomic regions (g1-g7) of *AG* and at a negative control locus (TA3 retrotransposon). *ap1 cal* pAP1:AP1-GR inflorescences were mock (0 h) or dexamethasone treated (48 h). Mean ± SEM of one representative of two biological replicates.

(F) Browser view of published DNase hypersensitivity, PRC2 (FIE), and LFY occupancy in intron two of *AG*. Assays were conducted during vegetative development (green) or in inflorescences (purple). Significant peaks (summit qval $\leq 10^{-10}$) according to MACS2 are marked by horizontal bars, with the color saturation proportional to the negative log10 q value (as for the narrowPeak file format in ENCODE). Black lines: PRE, the H2A.Z/ARP6 occupied ChIP-qPCR amplicon (g4), and the SWI/SNF (SYD and BRM) bound region. Below: *AG* locus diagram with genomic regions tested.

(G and H) Time-course of *AG* expression and H2A.Z **(G)** or BRM-GFP **(H)** occupancy at the *AG* locus genomic region 4 (g4 amplicon) in mock-treated (0 h) *ap1cal* pAP1:AP1-GR inflorescences and in 8-h intervals after dexamethasone treatment. Mean ± SEM of one representative of two biological replicates.

that the observed effect of SWR1/H2A.Z on *AG* upregulation is direct. A small H2A.Z ChIP sequencing (ChIP-seq) peak in the *AG* intron was also observed in leaves collected at the reproductive stage (Supplemental Figure 4A).

On the basis of ChIP-seq and chromatin accessibility analyses, the site at the *AG* intron two occupied by H2A.Z and ARP6 is near a region bound by PRC2 in vegetative tissues (Figure 1E and 1F) that functions as a Polycomb response element (PRE) (Xiao et al., 2017). Moreover, LFY and the SWI/SNF chromatin remodelers SPLAYED (SYD) and BRAHMA (BRM), which LFY recruits to the *AG* locus to overcome Polycomb repression, also bind near the H2A.Z marked region (Figure 1F) (Wu et al., 2012). A low DNase hypersensitive peak overlaps with the PRE during vegetative development, while, in inflorescences, a much larger DNase peak encompasses both the PRE and the LFY/H2A.Z marked region (Figure 1F). The combined findings suggest that ARP6 and H2A.Z occupy a PRE and Trithorax response element (TRE) at the *AG* locus. PRE/TREs are not well characterized in plants but are known to act as epigenetic switch elements in *Drosophila* (Ringrose and Paro, 2007).

Next, we assessed ARP6 and H2A.Z occupancy after steroid activation of *ap1 cal* pAP1:AP1-GR. *AG* upregulation is first detectable 48 h after steroid activation (Yan et al., 2019) (Supplemental Figure 4B). At this time point, ARP6 binding to the *AG* locus was reduced, while H2A.Z occupancy was already at background levels (Figure 1F). This observation is reminiscent of the reported H2A.Z loss from poised enhancers prior to gene activation in animals (Giaimo et al., 2019) and may suggest an active mechanism for H2A.Z removal. No change in H3K27me3, H3K4me3, or H3 acetylation (H3K27ac) was detected at *AG* 48 h after steroid activation compared with mock treatment (Supplemental Figure 4A and 4B).

To further dissect the temporal series of events during reversal of Polycomb repression at the *AG* locus, we examined the *AG* locus chromatin and gene activation during synchronous resumption of flower development after steroid activation of *ap1 cal* pAP1:AP1-GR. *AG* expression increased gradually, starting circa 24 h post steroid application (Figure 1G). By contrast, H2A.Z occupancy at the *AG* PRE/TRE declined rapidly and was already reduced to 40% 16 h post treatment (Figure 1G). Recruitment dynamics of the SWI/SNF chromatin remodeling ATPase BRM, on the other hand, closely paralleled the temporal upregulation of *AG* expression (Figure 1H). Thus, during flower patterning, reduction in H2A.Z occupancy at the PRE/TRE occurs prior to, and thus is unlikely to be a consequence of, SWI/SNF recruitment and *AG* transcription. Our combined data indicate that H2A.Z facilitates conversion from Polycomb repressed to poised chromatin at the *AG* PRE/TRE epigenetic switch element prior to locus activation.

In summary, our study establishes a link that connects ARP6/SWR1-directed H2A.Z deposition to reversal of Polycomb repression at a floral homeotic gene, which is likely executed in concert with other TrxG factors (Wu et al., 2012; Liang et al., 2015). We further show that ARP6 is a *bona fide* TrxG protein. SWR1 deposits H2A.Z, a histone variant implicated in both gene activation and gene repression in the context of chromatin in multicellular eukaryotes (Deal et al., 2007; March-Diaz et al., 2008; Giaimo et al., 2019; Gomez-Zambrano et al., 2019). While gene repression by H2A.Z has been linked to its ubiquitylation by Polycomb Repressive Complex 1 (PRC1) in *Arabidopsis* (Gomez-Zambrano et al., 2019), the *AG* locus does not display H2A ubiquitylation prior to locus activation but is marked by both H2A.Z and HK27me3 (Gomez-Zambrano et al., 2019). Moreover, H2A.Z was reported to colocalize with H3K27me3 genome wide in *Arabidopsis* and is present at bivalent/bistable chromatin domains in animals (Carter et al., 2018; Giaimo et al., 2019; Gomez-Zambrano et al., 2019). These data are consistent with a role for H2A.Z in epigenetic state switching at the PRE/TRE of the *AG* locus. It is possible that H2A.Z poises the *AG* locus PRE/TRE for activation by facilitating binding of the LFY pioneer transcription factor (Jin et al., 2021; Lai et al., 2021); both H2A.Z and LFY act early in the transcriptional activation of *AG* and both bind a similar region at this locus. Indeed, preferential association of pioneer transcription factors with H2A.Z-containing nucleosomes has been reported in animals (Giaimo et al., 2019). How H2A.Z is evicted is unclear; this may be aided by H2A.Z acetylation or by histone chaperones (Giaimo et al., 2019).

SUPPLEMENTAL INFORMATION

Supplemental information is available at *Molecular Plant Online*.

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AUTHOR CONTRIBUTIONS

U.-S.L., T.B., A.Y., J.X., and D.W. designed the experiments. U.-S.L. identified and characterized the *arp6* deletion mutants. T.B. conduced phenotypic analyses. A.Y. performed fast neutron and EMS mutagenesis and screens. U.-S.L. and J.X. performed the molecular experiments. U.-S.L., T.B., A.Y., and D.W. analyzed the data. D.W. wrote the manuscript with contributions from all authors.

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