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The histone variant H2A.Z and chromatin remodeler BRAHMA act coordinately and antagonistically to regulate transcription and nucleosome dynamics in *Arabidopsis*

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Summary:

Plants adapt to environmental changes by regulating transcription and chromatin organization. The histone H2A variant H2A.Z and the SWI2/SNF2 ATPase BRAHMA (BRM) have overlapping roles in positively and negatively regulating environmentally responsive genes in *Arabidopsis*, but the extent of this overlap was uncharacterized. Both factors have been associated with various changes in nucleosome positioning and stability in different contexts, but their specific roles in transcriptional regulation and chromatin organization need further characterization. We show that H2A.Z and BRM colocalize at thousands of sites, where they interact both cooperatively and antagonistically in transcriptional repression and activation of genes involved in development and responses to environmental stimuli. We identified 8 classes of genes that show distinct relationships between H2A.Z and BRM with respect to their roles in transcription. These include activating and silencing transcription both redundantly and antagonistically. We found that H2A.Z contributes to a range of different nucleosome properties, while BRM stabilizes nucleosomes where it binds and destabilizes or repositions flanking nucleosomes. We also found that at many genes regulated by both BRM and H2A.Z, both factors overlap with binding sites of the light-regulated transcription factor FAR1-Related Sequence 9 (FRS9) and that a subset of these FRS9 binding sites are dependent on H2A.Z and BRM for accessibility. Collectively, we comprehensively characterized the antagonistic and cooperative contributions of H2A.Z and BRM to transcriptional regulation, and illuminated several interrelated roles in chromatin organization. The variability observed in their individual functions implies that both BRM and H2A.Z have more context-dependent roles than previously assumed.

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Author contribution: RD and ET conceived the research plan. ET performed the experiments and analyzed the data with guidance from RD. ET wrote the article with direction and contributions from RD.

Accession numbers

All high throughput sequencing data described in this paper have been deposited to the NCBI GEO database under record number GSE108450. The BRM ChIP-seq data used in our analysis is available under GEO number SRX1184288. The PIF4 and PIF ChIP-seq data used in our analyses are available under GEO numbers SRX1005830 (PIF4) and SRX1495297 (PIF5).

Keywords

histone; H2A.Z; nucleosome; BRAHMA; chromatin remodel; transcription; transcription factor; *Arabidopsis thaliana*

INTRODUCTION

As sessile organisms, plants have evolved a plethora of physiological responses to endure adverse environmental conditions. External signals are often transmitted to the nucleus, triggering a transcriptional response network that facilitates a multidimensional response to external stimuli (Rymen and Sugimoto 2012; Barah et al., 2016, Urano et al., 2010). In eukaryotes, DNA associates with histones and other nuclear proteins to form a highly condensed chromatin structure. The arrangement of these proteins can either facilitate or obstruct transcription factor (TF) binding to target regulatory sequences, and therefore impact the ability of transcriptional machinery to modulate transcriptional responses (Weber et al., 2014; Lai and Pugh 2017).

At its most basic level, chromatin is made up of DNA wrapped around an octamer of histones to form nucleosomes (Luger et al., 1997). Chromatin-binding proteins such as histone post-translational modifying enzymes and chromatin remodelers interact with nucleosomes and influence their positioning, stability, and their ability to interact with other proteins, thus regulating DNA accessibility (Vergara and Gutierrez 2017). Chromatin remodeling complexes (CRCs) use the energy of ATP-hydrolysis to disrupt the interaction between DNA and histones in order to evict nucleosomes, eject histone dimers, slide nucleosomes, or exchange canonical histones for variant forms (Narlikar et al., 2013; Clapier et al., 2017). Chromatin remodeling is a key part of regulating genome stability, DNA replication, DNA damage repair, and transcription, which in turn affects development, homeostasis, and how an organism responds to changes in its environment (Probst and Mittelsten Scheid 2015; Vergara and Gutierrez 2017).

The combined effects of different chromatin-regulating proteins at a locus can create opposing and redundant forces that maintain proper transcription level and integrate a multitude of endogenous and exogenous signals (Vergara and Gutierrez 2017). One way to define the individual contributions of chromatin regulating factors *in vivo* is to evaluate how such proteins coordinately or antagonistically contribute to chromatin organization and transcription. Studies in *Arabidopsis thaliana* evaluating the histone H2A variant H2A.Z (deposited by the SWR1 CRC) and the SWI2/SNF2 CRC separately have revealed that they both individually modulate chromatin organization and transcription to regulate developmental processes and responses to the environment (Wu et al., 2015; Lee and Seo 2017). More directly, Farrona et al. (2011) proposed that the SWI2/SNF2 ATPase BRAHMA (BRM) and the incorporation of H2A.Z into chromatin by the SWR1 CRC have antagonistic roles in modulating *Flowering Locus C* (*FLC*) transcript levels and the developmental timing of flowering. In yeast, mutations in H2A.Z increase dependence on the SWI2/SNF2 complex for transcriptional activation of several genes, implying that the histone variant and chromatin remodeler have cooperative functions (Santisteban et al.,

2000). Furthermore, in mammals, SWI2/SNF2 subunits interact with H2A.Z, although the implications of this interaction have not been explored (Goldman et al., 2010; Li et al., 2012; Zhang et al., 2017b). While the SWR1 CRC and the SWI2/SNF2 complex have parallel roles in development and environmental responses in plants, there is a dearth of studies that focus on the direct intersection of these two complexes in chromatin and transcriptional regulation. Since an antagonistic relationship was already established between H2A.Z and BRM at one *Arabidopsis* gene (Farrona et al. 2011), we decided to characterize the extent to which these two factors genetically interact in transcriptional regulation and chromatin organization genome-wide.

In this study, we demonstrate that H2A.Z and BRM interact to regulate transcription across the *Arabidopsis* genome and found that the types of nucleosomal changes found at BRM binding sites are impacted by whether they contain H2A.Z nucleosomes or not. We assessed the overall and direct transcriptional contributions of H2A.Z and BRM by performing transcriptional profiling in combination with BRM and H2A.Z localization information in wild type, single mutants, and double mutants. For these experiments, we used mutants lacking BRM, mutants for the ARP6 subunit of the SWR1 CRC that are defective in H2A.Z incorporation into chromatin, or double mutants depleted for both. We identified 8 different classes of BRM-H2A.Z co-targeted genes where transcription is coordinately or antagonistically regulated by these factors, including genes that are up- or down- regulated only in double mutants. By experimentally verifying that these genes are direct targets of H2A.Z or BRM, the regulatory relationships we identified allude to cooperative and antagonistic functions between BRM and H2A.Z in chromatin regulation and transcription.

We further explored whether BRM and H2A.Z influence nucleosome organization to facilitate these transcriptional changes by measuring nucleosome occupancy and positioning. We found that BRM is involved in nucleosome stabilization at nucleosome-depleted regions (NDR), both distal and proximal to transcription start sites (TSS), and contributes to destabilization and/or repositioning of flanking nucleosomes. On the other hand, H2A.Z-containing nucleosomes show highly variable changes in nucleosome properties upon H2A.Z depletion. At loci where both H2A.Z and BRM are found together in the genome, BRM usually destabilizes nucleosomes, especially the +1 nucleosome, while H2A.Z alone can also destabilize +1 nucleosome at some loci. In addition, we identified binding sites of light-responsive TFs that are enriched at BRM and H2A.Z co-targeted genes, and show that nucleosome occupancy is dependent on BRM and H2A.Z at the binding sites for the FAR1-Related Sequence 9 transcription factor. These findings point to a role for both H2A.Z and BRM in regulating nucleosome positioning and stability in coordinately and antagonistically regulated genes involved in light response and responses to other stimuli. Collectively, our findings indicate that the relationship between BRM and H2A.Z is more complex than solely antagonizing or complementing the chromatin organizing function of the other, and our datasets will be useful for future studies to explore the contexts in which BRM and H2A.Z contribute to chromatin organization and transcriptional regulation.

RESULTS

Analysis of transcriptional changes in *arp6* and *brm* mutants

Both H2A.Z and BRM contribute to transcriptional repression and transcriptional activation, but it is unknown how the role of one factor at a gene might affect the role of the other in transcriptional regulation. (Marques et al., 2010; Archacki et al., 2016). To identify the genes in *Arabidopsis* that are transcriptionally regulated by BRM and H2A.Z, we performed RNA sequencing (RNA-seq) experiments. We used plants homozygous for the *brm-1* allele, since it was previously characterized as a null (Hurtado et al., 2006). To study plants with H2A.Z-depleted nucleosomes, we used null mutants for the ARP6 component of the SWR1 chromatin remodeling complex (CRC) which is primarily responsible for incorporating H2A.Z into nucleosomes (Deal et al., 2007). In *Arabidopsis*, three genes encode the pool of H2A.Z proteins and there are no completely null triple H2A.Z mutants available, which complicates genetic work with H2A.Z (Coleman-Derr and Zilberman 2012). *arp6* mutants provide a logical proxy for H2A.Z mutants in our genetic study, since other studies have verified that ARP6 is required for proper H2A.Z incorporation into nucleosomes and *arp6* mutants phenocopy H2A.Z mutants (Sura et al., 2017; March-Diaz et al., 2008; Berriri et al., 2016). Therefore, we identified genes that are differentially expressed (DE) in *brm-1* mutants, *arp6-1* mutants, and/or *arp6-1;brm-1* double mutants compared to wild type (WT) plants. RNA was isolated from shoot tissue of developmentally staged plants with 4-5 leaves. We collected tissue based on developmental stage instead of plant age because *brm* and *arp6;brm* double mutants have delayed developmental progression relative to WT (Figure S1A)(Boyes et al., 2001;Hurtado et al., 2006). After performing RNA-seq, we focused our analyses on genes that had >1.5x fold change and a false discovery rate of <0.2. We identified 2,109 genes that were DE in *arp6* (1,036 genes up-regulated and 1,073 genes down-regulated), 4,250 genes DE in *brm* (2,317 genes up and 1,933 genes down), and 3,203 genes DE in *arp6;brm* mutants (1,517 genes up and 1,686 genes down) (Fig. S1B, Summarized in Table S1). The gene sets we defined as DE in *arp6-1* and *brm-1* overlap significantly with those defined in previous studies (Sura et al., 2017;Archacki et al., 2016). Gene Ontology terms that were overrepresented among DE genes in each mutant were largely associated with responses to biotic and abiotic stress (Table S2).

Assessing H2A.Z and BRM localization

Although many genes are DE in *arp6* and *brm* mutants, these are not necessarily direct targets of H2A.Z or BRM. Since ‘regulation of transcription’ is a Gene Ontology term enriched in DE gene sets from each mutant (Table S2), the genes that are mis-expressed in the mutants may go on to cause secondary changes in transcription that are not directly related to BRM or H2A.Z function. To identify the genes directly targeted by H2A.Z and BRM, we analyzed our RNA-seq data in combination with data from BRM and H2A.Z chromatin immunoprecipitation experiments followed by sequencing (ChIP-seq). To assess BRM localization, we used previously published BRM-GFP ChIP-seq peaks generated from 14 day-old seedlings grown on plates (Li et al., 2016). Archacki et al. (2016) compared these ChIP-seq enriched sites to their BRM ChIP-chip enriched sites generated from 3 week-old plants grown on soil. The authors saw that even in different growth conditions and a different developmental stage, which consequently included different tissues, BRM was

stably associated with similar sites with significant overlap between the two datasets (Archacki et al., 2016). Since our WT plants are at the same developmental stage as the published BRM ChIP-seq data we selected, we are confident that they sufficiently represent BRM localization for our experiments.

To assess H2A.Z localization relative to DE genes, we performed ChIP-seq for H2A.Z on shoot tissue from developmentally staged plants with 4-5 leaves from *arp6* mutants, *brm* mutants, and WT plants. Once sequence reads were mapped to the genome, Homer software (Heinz et al., 2010) was used to determine significant peaks in ChIP-seq read signal indicative of H2A.Z localization for each genotype. Since we are using *arp6* mutants as a proxy for H2A.Z mutants, we plotted H2A.Z ChIP-seq read signal from *arp6* mutants at H2A.Z peaks and confirmed that nucleosomes that normally contain H2A.Z are depleted of H2A.Z in *arp6* mutants (Fig. S2A and C). To focus our analysis on sites where H2A.Z localization is dependent on ARP6, we removed the few H2A.Z peaks called in WT that overlapped with peaks called in *arp6* mutants (n=801). This left us with 11,877 ARP6-dependent H2A.Z peaks to assess how H2A.Z localization relates to transcriptional changes observed in the *arp6* mutants.

Combining the BRM and H2A.Z ChIP-seq data sets, we also tested whether the presence of H2A.Z-containing nucleosomes is dependent on BRM function. Evaluating H2A.Z ChIP-seq signals in WT plants and *brm* mutants revealed a minor subset of H2A.Z sites with slightly altered H2A.Z content in *brm* mutants (Fig. S2A-B). However, BRM does not appear to play a general role in regulating the levels of H2A.Z-containing nucleosomes in chromatin. There is still the possibility that H2A.Z could impact BRM localization to chromatin, but we are not able to test this with our current data.

Identifying differentially expressed H2A.Z and BRM target genes

To determine which of the transcriptional changes detected in our RNA-seq data are directly associated with H2A.Z and BRM localization, we identified DE direct target genes for either factor by integrating transcriptome data with H2A.Z and BRM ChIP-seq data. If an H2A.Z or BRM ChIP-seq peak fell within the body of a DE gene or if the closest TSS to a binding site was a DE gene, the gene was considered a direct target of that factor.

Only 471 (45%) of up- and 449 (42%) of down-regulated *arp6* DE genes are directly associated with an ARP6-dependent H2A.Z peak (Fig. S1C, D and Table S1). In *brm* mutants, 1,552 (67%) of up- and 786 (41%) of down-regulated genes are direct targets of BRM (Fig. S1C, D and Table S1). In *arp6;brm* double mutants, the changes in gene expression were considered direct effects of the mutations if the DE gene was a target of H2A.Z, BRM, or both. Therefore, 1,082 (71.3%) of the up- and 1,058 (62.8%) down-regulated genes were direct targets of either factor in the *arp6;brm* mutants (Fig. S1C, D and Table S1). Thus, we defined H2A.Z and BRM DE target genes 1) by RNA-seq data showing that the genes are DE in the *arp6*, *brm*, or *arp6;brm* mutants respectively, as well as 2) using ChIP-seq data to confirm that H2A.Z or BRM are normally found at these genes in WT plants. From this point on in the study, we focused our analysis on the DE BRM and H2A.Z target genes, and considered their transcription to be directly regulated by H2A.Z and/or BRM. Collectively, the defined DE target genes support the notion that both BRM and

H2A.Z contribute to gene repression and activation in different contexts (Marques et al., 2010; Archacki et al., 2016).

To better understand what types of genes are directly targeted by H2A.Z and BRM, we performed a Gene Ontology (GO) enrichment analysis on DE genes in each mutant that are direct targets of either factor. The DE genes in *arp6* mutants or in *brm* mutants that are direct targets of either H2A.Z or BRM are individually enriched for similar GO terms compared to the list of GO terms that describe the total list of DE genes in either mutant (Table S2). We also identified many GO terms for genes targeted by both H2A.Z and BRM that include terms that relate to development/growth and responses to environmental stimuli (e.g. fungal response, osmotic stress, cold, and light) and hormones (auxin, ethylene, jasmonic acid, salicylic acid) (Table S2).

H2A.Z and BRM coordinately and antagonistically regulate transcription

Since previous studies indicated that H2A.Z and BRM antagonistically regulate transcription of the *FLC* gene in *Arabidopsis*, we tested whether this antagonistic relationship extends to other genes across the genome (Farrona et al., 2011). We also tested the hypothesis that H2A.Z and BRM could work together to regulate gene transcription as suggested by their roles in yeast (Santisteban et al., 2000). After verifying which differentially expressed genes are direct targets of H2A.Z and BRM using ChIP-seq data, we used the transcriptional changes in the *arp6;brm* double mutants relative to their changes in single mutants to identify 8 gene classes that are either coordinately or antagonistically regulated by H2A.Z and BRM (Fig. 1A, Fig. S3A, B and Table S1). To describe these gene classes, we will refer to genotypes and transcriptional changes with the following abbreviations: A=*arp6-1*, B=*brm-1*, D=*arp6-1;brm-1* double mutant, “+” = genes up-regulated in the specified mutant, “-” = genes down-regulated in the specified mutant, “=” = genes not DE in the specified mutant, n = number of genes in the class. We first identified genes that are coordinately regulated by H2A.Z and BRM. This category includes target genes that are up-regulated in both *arp6-1* and *brm-1* mutants compared to WT plants (Class 1: A+, B+, n=70), targets down-regulated in both *arp6* and *brm* mutants relative to WT (Class 2: A-, B-, n=51), and target genes with no change in transcript level in the individual mutants, but that are up- (Class 3: A=, B=, D+, n=159) or down-regulated (Class 4: A=, B=, D-, n=88) in the *arp6;brm* double mutants relative to WT (Fig. 1A, S3A). Classes 1 and 2 indicate that both H2A.Z and BRM are independently required for the proper regulation of these genes (Fig. 1, S3A). Classes 3 and 4 are DE target genes in the double mutants but not the single mutants, which are particularly interesting because these are genes where BRM and H2A.Z functions appear to be redundant and compensate for the other with respect to transcription (Fig. 1A, Fig. S3A).

We also identified different classes of target genes where H2A.Z and BRM act antagonistically. This category of genes includes those either up- or down-regulated in a single mutant but that are neither DE in the other mutant nor the double mutant (Class 5: A+, B=, D=, n=91; Class 6: A-, B=, D=, n=99; Class 7: A=, B+, D=, n=324; Class 8: A=, B-, D=, n=305) (Fig. 1A, Fig. S3B). Since the loss of the second factor in double mutants suppresses the change in transcript levels observed in the single mutant, H2A.Z and BRM

seem to have opposing functions at these genes that become evident by comparison of single and double mutants (Fig. S3B). Using class 5 as an example, these are H2A.Z- and BRM-targeted genes that have increased transcript levels in the *arp6* mutants when H2A.Z is depleted from nucleosomes. These same genes, however, are no longer significantly differentially expressed relative to WT when BRM is also depleted in the double mutants. Therefore, it seems that H2A.Z does not merely play a repressive role at these genes but does so by opposing the positive regulatory contribution of BRM at genes in class 5 (Fig. S3B). Alternatively, at genes in class 6, H2A.Z opposes the repressive role of BRM (Fig. S3B). Reciprocally, BRM also opposes the positive and negative contributions of H2A.Z to transcriptional regulation in classes 7 and 8, respectively (Fig. S3B).

Interestingly, we did not observe a significant class of genes that displayed the antagonistic relationship between H2A.Z and BRM previously observed for the *FLC* gene (Farrona et al. 2011). Based on the reported behavior of *FLC*, we anticipated identifying this putative class as being reduced in *arp6*, increased in *brm*, and also increased in *arp6/brm* plants. However, we did observe this behavior for *FLC* by quantitative RT-PCR (Figure S1E), and the same trends were apparent by RNA-seq, although these differences were not statistically significant.

To determine the processes that may be influenced by these genetic interactions between H2A.Z and BRM, we evaluated GO terms for biological processes significantly enriched in our 8 coordinately or antagonistically regulated gene sets (Table S2). The GO terms collectively enriched for genes in the 8 classes of H2A.Z and BRM DE co-targets represent terms relating to transcriptional regulation, development, and responses to the environment similar to target genes DE in both mutants (Fig. 1B and Table S2).

BRM contributes to nucleosome stability and positioning at nucleosome-depleted regions.

The chromatin remodeling roles of BRM as a SWI2/SNF2 ATPase and H2A.Z incorporation into nucleosomes by the SWR1 CRC both can affect nucleosome stability and positioning at individual loci (Han 2012; Wu et al 2012; Brzezinka et al., 2016; Rudnizky et al., 2016). Identifying sites where both H2A.Z and BRM influence chromatin organization allows us to determine whether the presence of one could antagonize or enhance the chromatin modulating function of the other.

To assess the genome wide contributions of H2A.Z and BRM to nucleosome organization, we performed Micrococcal Nuclease (MNase) digestion followed by sequencing (MNase-seq) on shoot tissue from 4-5 leaf developmentally-staged *arp6*, *brm*, and *arp6;brm* and WT plants. The nuclease activity of MNase specifically digests nucleosome-free DNA and leaves behind nucleosome-protected DNA, which provides a measure of where and how often a nucleosome is associated with a locus (Allan et al., 2012). Thus, MNase-seq experiments allow us to evaluate how H2A.Z and BRM influence nucleosome occupancy and positioning (Allan et al., 2012; Zhang et al., 2015). Using H2A.Z and BRM ChIP-seq data in combination with MNase-seq data, we evaluated nucleosomal changes that occur in our mutants at sites enriched for either H2A.Z, BRM, or both in order to focus our analysis and describe how H2A.Z and BRM influence nucleosome stability and positioning.

To first survey the extent to which BRM contributes to nucleosome occupancy and positioning across the genome, we evaluated nucleosome patterns in *brm* mutants compared to WT plants using MNase-seq data. By plotting nucleosome read signals across sites where BRM localizes, we found that BRM is enriched at nucleosome-depleted regions (NDRs) that are generally flanked on either side by well-positioned nucleosomes (Fig. 2, Fig. S4A). The nucleosome patterns surrounding BRM peaks at DE genes support the idea that BRM binds both to the NDR adjacent to TSSs and also to upstream sites with open chromatin structure, such as potential regulatory regions within promoters or enhancers (Fig. S4B). However, when we compare between WT and *brm* mutant nucleosome levels, we do not observe notable changes in nucleosome occupancy in the mutants across all sites or at specific DE gene sets where BRM localizes (Fig. 2, Fig. S4). Therefore, it seems that BRM is not required to produce these NDRs where it binds, but may perform other functions once targeted there.

Since locus-specific studies have described roles for BRM and other SWI2/SNF2 subunits in nucleosome positioning and destabilization, we decided to quantify how often genomic BRM sites are associated with different types of nucleosome dynamics (Han et al 2012; Wu et al., 2015; Brzezinka et al., 2016; Sacharowski et al., 2015). Using DANPOS2 software, nucleosomes were defined as dynamic if they had significant changes in nucleosome positioning (different position of nucleosome read summits), occupancy (different height of nucleosome read summits), fuzziness (difference in the standard deviation of nucleosome read positions), or any combination of the three in mutants relative to nucleosomes in WT tissue (Chen et al., 2013). We found that 25% of BRM peaks have significant nucleosome changes between the two genotypes (based on an FDR cutoff of <0.05) (Fig. S5).

To identify how often nucleosome occupancy, positioning, or fuzziness depends on BRM, we quantified the proportion of specific types of nucleosome changes that are observed among all nucleosomes considered dynamic between WT plants and *brm* mutants specifically where BRM localizes. We observed enrichment for decreased nucleosome occupancy in combination with enrichment for increased nucleosome fuzziness at BRM peaks in *brm* mutants (Fig. S6B-C). The chromatin landscape flanking BRM binding sites appears to have different nucleosome occupancy levels in *brm* mutants than nucleosomes within BRM bindings sites, based on the previous nucleosome read plots (Fig. 2). Therefore, we evaluated whether the types of nucleosome changes at the bordering regions of BRM ChIP-seq peaks (nucleosomes that fall within 200 bp of either end of a defined BRM ChIP-seq peak) are enriched for different types of changes than those observed for nucleosomes found where BRM directly associates within peak centers (any nucleosome that is contained within the defined BRM binding sites) (illustrated in Fig. S6A). We observe enrichment for changes in positioning and increases in occupancy of nucleosomes flanking BRM sites in *brm* mutants (Fig. S6D-E). This suggests that BRM contributes to the stability of any nucleosomes that are found where BRM binds and to the destabilization of bordering nucleosomes (Fig. S6A). Therefore, our MNase-seq data in combination with BRM ChIP-seq data demonstrate that BRM localizes to NDRs and contributes more to nucleosome stability where it directly associates with chromatin, while contributing more to destabilization or the positioning of flanking nucleosomes (Fig. S6A).

H2A.Z has a variable influence on the surrounding nucleosome landscape

When H2A.Z is incorporated into nucleosomes, it can change both intra- and inter-nucleosomal interactions as well as the interactions between nucleosomes and other nuclear proteins (Bonisch and Hake 2012). Consequently, H2A.Z-containing nucleosomes have been associated with a range of nucleosome dynamics including changes in nucleosome stability and positioning (Bonisch and Hake 2012; Rudnizky et al., 2016). Before assessing whether specific types of nucleosomal changes are enriched at sites where H2A.Z is found in relation to BRM function, we used MNase-seq experiments to survey whether H2A.Z-containing nucleosomes are enriched for specific types of nucleosomal changes in *arp6* mutants compared to WT plants.

When we evaluated nucleosome occupancy in the *arp6* mutants, we noticed that there were large gaps in nucleosome read signals across the genome (Fig. S7). We therefore compared our *arp6* MNase-seq nucleosome signals to *arp6* genomic DNA and found that the gaps in nucleosome read signals correspond to large genomic deletions in the *arp6* mutants (Fig. S7). These deletions are in line with the roles of the SWR1 complex and H2A.Z in maintaining genome stability and previous reports that specifically show that *arp6* mutants have a greater crossover density, are more susceptible to DNA damage, and have meiotic defects (Choi et al., 2013; Rosa et al., 2013). These deletions would skew our MNase-seq results, making them appear as a loss of a nucleosome in the mutant compared to WT when instead there was a loss of genomic DNA in this mutant line. We therefore mapped the deletions using CNVnator software which reported 1,545 deletions (>200 bp) compared to the TAIR10 reference genome (Abyzov et al., 2011). Although some of these deletions are strain differences, since they were also missing in our WT plants compared to the reference genome, the total deleted portion collectively covers 5.88 megabases of DNA, which is a large portion of the ~145 megabase Arabidopsis genome (Bevan and Walsh 2005). To ensure that we are analyzing nucleosome dynamics at regions of the genome that are present in *arp6* and *arp6;brm* mutants, we removed nucleosomes from our analysis if they were called as dynamic by DANPOS2 but also overlapped with deleted regions. We also required a minimum of 1 read per 10 bp area visualized as a cutoff when analyzing nucleosome plot profiles to exclude deleted regions from our analyses.

After accounting for the deleted regions, we analyzed our MNase-seq data from WT plants and *arp6* mutants in combination with our H2A.Z ChIP-seq data. We specifically evaluated whether different types of nucleosomal changes (changes in positioning, occupancy, or fuzziness) were enriched at nucleosomes that normally contain H2A.Z, but that are depleted of H2A.Z in *arp6* mutants. Only a fraction of nucleosomes that normally contain H2A.Z in WT plants (14.6%, Fig. S8A) had significant changes when comparing them to nucleosomes in *arp6* plants using the DANPOS2 software. We quantified the proportion of nucleosomes that changed in *arp6* mutants that had changes in occupancy, fuzziness, or positioning, using the same definitions we used to analyze nucleosomal changes in *brm* mutants. We found that the collection of nucleosomes that normally contain H2A.Z in WT but lose H2A.Z in *arp6* mutants experience both increases and decreases in fuzziness, increases and decreases in occupancy, and changes in positioning (these categories are not mutually exclusive) (Fig. S8B). There was a slight preference for changes in nucleosome occupancy (both up and

down), but no enrichment for either increases or decreases in fuzziness or changes in positioning at sites where H2A.Z localizes. This further supports the idea that additional factors interacting with H2A.Z help to determine how H2A.Z contributes to nucleosome occupancy, fuzziness, or positioning. Contrary to our observations of nucleosome occupancy increases and decreases, another group has reported a general decrease in nucleosome occupancy in *arp6* mutants (Dai et al., 2017). Considering the presence of deletions in *arp6* mutants, such a decrease in nucleosome occupancy could also be explained by changes in the genome rather than changes to chromatin organization if it is not properly accounted for.

BRM contributes to nucleosome destabilization more often when it is in proximity to H2A.Z

Since both BRM and H2A.Z contribute to nucleosome stability and positioning individually, we wanted to evaluate whether they work coordinately or antagonistically on nucleosomes where they overlap in the genome. We defined 2,963 regions of overlap between BRM ChIP-seq peaks and H2A.Z ChIP-seq peaks (significant by Fisher's exact test, p -value $< 2.2 \times 10^{-16}$) (Fig. 3A). By plotting the ChIP-seq read signals for BRM and H2A.Z at these regions of overlap and dividing them into 4 K-means sub-clusters, we determined that these are primarily regions of peripheral overlap between H2A.Z and BRM instead of sites with strong co-localization (Fig. 3B).

To determine whether the presence of one factor modifies how the other contributes to nucleosome dynamics, we divided BRM peaks into those with and without overlap with H2A.Z. Then we used DANPOS2 to classify the types of nucleosome changes (occupancy, fuzziness, or position changes) that are found at BRM peaks with and without H2A.Z. Next, we measured the proportion of BRM peaks that do or do not overlap with H2A.Z in WT plants and that show specific nucleosomal changes between *brm* mutants and WT plants. This analysis suggested that BRM peaks with H2A.Z experience more decreases in fuzziness, more increases in occupancy, and more nucleosome position changes in *brm* mutants compared to nucleosomes at BRM sites that do not have H2A.Z (Fig. 3C). These changes were deemed significant by comparing the changes observed at BRM peaks with and without H2A.Z to sets of BRM peaks with and without overlaps with a random control set of H2A.Z size-matched peaks using Fisher's exact test. This control set showed no enrichment for any particular type of nucleosome change (Fig. S9B). This suggests that at sites where H2A.Z is found, BRM has a greater role in decreasing nucleosome stability.

Reciprocally, we also evaluated whether nucleosomes at H2A.Z peaks exhibit a particular kind of nucleosomal change with and without BRM. We did not observe any particular difference in the types of nucleosomal changes observed in *arp6* or *brm* mutants between H2A.Z peaks that do or do not overlap with BRM in WT plants (Fig. S9A). This suggests that the presence of BRM does not consistently impact the role of H2A.Z in regulating nucleosome occupancy, fuzziness, or positioning.

We further identified 88 regions of H2A.Z-BRM overlap that also contained significant nucleosome changes in both respective mutants compared to WT. These regions allow a more direct comparison between the roles that BRM and H2A.Z play in nucleosome dynamics. At these regions of shared overlap, H2A.Z and BRM both contribute to significant changes in nucleosome positioning at 42% (37/88) of nucleosomes, however only

21% (19/88) of these nucleosomes show changes in positioning in both genotypes. Based on nucleosome changes observed in *arp6* mutants, H2A.Z contributes evenly to increases and decreases in the degree of nucleosome occupancy changes (Fig. 3D) and fuzziness changes (Fig. 3E) in these regions of BRM/H2A.Z overlap. These observations demonstrate that H2A.Z has a range of contributions to nucleosome stability at these sites, consistent with what is observed at H2A.Z sites alone (Fig. S8B).

Alternatively, *brm* mutants have a greater proportion of nucleosomes with an increase in occupancy and decrease in fuzziness compared to WT at regions of H2A.Z/BRM overlap where both are required for specific nucleosome patterns (Fig. 3D and E). These data indicate that BRM plays a greater role in nucleosome destabilization at sites where it overlaps with H2A.Z (Fig. 3C-E). This is consistent with the fact that there are more increases in nucleosome occupancy at BRM peak borders than at the centers (Fig. S6A, E) and that H2A.Z and BRM have more peripheral overlaps (Fig. 3B). Collectively, these observations indicate that H2A.Z and BRM do not solely antagonize the function of the other with respect to nucleosome properties, but can also cause similar changes in nucleosome organization.

BRM contributes to nucleosome destabilization of +1 nucleosomes at genes coordinately regulated with H2A.Z.

To assess the roles of H2A.Z and BRM in nucleosome stability as they relate to transcriptional regulation, we plotted the average profiles of nucleosome read signals from WT, *arp6*, *brm*, and *arp6;brm* plants surrounding the transcription start sites (TSS) of DE target genes (Fig. 4). We focused our analysis on TSSs from the 8 antagonistically or coordinately regulated DE H2A.Z/BRM target gene classes we identified earlier in the study based on their transcriptional changes in the mutants (Fig. 1A). While there are some nucleosome occupancy changes detected within gene bodies in mutants, we primarily focused our analysis on changes observed for the +1 nucleosome because it acts as a first physical barrier for transcriptional regulation (Weber et al., 2014).

At these DE gene classes, *brm*, *arp6* and *arp6;brm* mutants showed an increase in +1 nucleosome occupancy, with the most dramatic changes seen in the coordinately regulated gene classes 1 and 2 (Fig. 4A and Fig. S10). The *brm* and *arp6;brm* mutants also show +1 nucleosome occupancy increases at gene classes 3 and 4 (Fig. 4A and Fig. S10). It is interesting to note that the role of BRM in +1 nucleosome stabilization is unaffected by the direction of transcriptional change (Fig. 4A, D and Fig. S10). In *arp6* mutants, +1 nucleosome occupancy is mostly unchanged at genes DE in *arp6* or *arp6;brm* mutants (Classes 3-6) but show slight increases in nucleosome stability where H2A.Z opposes the regulatory functions of BRM at DE genes in *brm* mutants (Classes 7 and 8; Fig. 4A, D and Fig. S10). Although the loss of BRM results in +1 nucleosome occupancy increases at genes, especially in classes 3 and 4, significant changes in transcription do not happen in these gene classes until there is a loss of both H2A.Z and BRM in the *arp6;brm* mutants (Fig. 4A and Fig. S10). This means that at these gene classes (3 and 4), the increase in nucleosome stability in the *brm* mutants is not sufficient to cause significant changes in transcription until the loss of H2A.Z also occurs.

BRM is enriched at the NDR just upstream of the +1 nucleosome at our 8 gene classes (Fig. 4B), so it may be influencing +1 nucleosome stability by interacting with the +1 nucleosome either peripherally or through recruiting other chromatin modifying factors to interact with the +1 nucleosome. Having more stable +1 nucleosomes at BRM targets in *brm* mutants is consistent with our observations that BRM contributes to nucleosome destabilization at the borders/flanking regions where it localizes and particularly when it co-localizes with H2A.Z (Fig. 3C and Fig. S6). Further work to determine whether BRM destabilizes the +1 nucleosomes or whether it recruits or blocks other factors which indirectly contribute to +1 nucleosome stabilization will help us better understand the role of BRM in transcriptional regulation.

BRM and H2A.Z may interact with TFs to facilitate transcriptional regulation and influence nucleosome occupancy

Our work presenting variable nucleosome changes where the two factors overlap as well as at DE co-targeted genes (Fig. 3D-E, S6, S8) suggests that the BRM-H2A.Z relationship is much more complex than a simple antagonism. However, H2A.Z and/or BRM may have more consistent roles in chromatin regulation through interactions with specific transcription factors (TFs). A number of previously reported interactions between either H2A.Z or BRM and TFs prompted us to evaluate how H2A.Z and BRM might contribute to nucleosome organization surrounding TF binding sites where they co-localize (Wu 2012; Efroni et al., 2013; Vercruyssen et al., 2014; Zhao et al., 2015; Buszewicz et al., 2016; Zhang et al., 2016; Cortijo et al., 2017; John et al., 2008; Sacharowski et al., 2015; Jegu et al., 2017).

To identify TFs that may be associated with specific regulatory relationships between H2A.Z and BRM, we identified significantly enriched sequence motifs found in accessible chromatin regions associated with each of the 8 DE gene classes we identified as targets of H2A.Z and BRM (Fig. 1A). Accessible chromatin sites were defined in a previous study using an ATAC-seq data set from leaf mesophyll cells (Sijacic et al. 2018), which is the predominant cell type in our tissue. The motifs enriched at accessible regions across 7 of our DE gene classes are enriched for the target motifs of 78 different TFs (none were enriched for gene class 1) (Table S3). Of the factors identified, 15 have been previously reported to associate with the SWI2/SNF2 complex in Arabidopsis (Table S3; Efroni et al., 2013; Jegu et al., 2017; Zhang et al., 2017a). Several of the TFs identified are involved in responses to light (SOC1, FRS9, HY5, MYC2, CIB2, BZR1, BIM1/2/3, and PIF1/3/4/5/7). These factors are intriguing because they are consistent with the multiple GO terms relating to responses to light stimuli that are enriched individually in our 8 classes of DE H2A.Z-BRM target genes and for genes regulated individually by H2A.Z and BRM (Table S2).

Experimentally determined genomic binding sites for several factors identified by our motif discovery analysis are publicly available (O'malley et al., 2016; Pedmale et al., 2016) and many of these sites overlap with H2A.Z and BRM sites in the genome (Fig. S11A). Therefore, we further evaluated whether nucleosome patterns at the binding sites for several of the light-responsive transcription factors are impacted in our mutants where they overlap with both BRM and H2A.Z. For this analysis, we plotted MNase-seq nucleosome signals from *arp6*, *brm*, *arp6,brm* and WT plants across sized-scaled TF binding sites. For most

factors, we did not detect consistent changes in nucleosome patterns at their binding sites from the MNase-seq average profile plots in our mutants relative to WT. However, we did detect changes in nucleosome occupancy in *brm* and *arp6* mutants for H2A.Z-BRM sites that were also targeted by the FAR1-Related Sequence 9 (FRS9) transcription factor. FRS9, a member of the FRS far-red light responsive TF family, was predicted to regulate genes in 6 of our classes of DE H2A.Z-BRM targeted genes based on our motif discovery analysis (Lin et al. 2004). Little is known about FRS9 function, but it is expressed in young rosette tissue and regulates the inhibition of hypocotyl elongation by red light (Lin et al. 2004).

Since relationships between nucleosomes and TF binding sites do not follow a simple presence-absence pattern, we evaluated how 4 K-means clustered nucleosome patterns associated with FRS9 binding sites are affected in *arp6*, *brm*, or *arp6;brm* double mutants compared to those in WT plants. At FRS9 sites overlapping with both BRM and H2A.Z peaks, there was an increase in nucleosome occupancy in the *brm* and *arp6;brm* mutants with a notable subset of nucleosomes that also have increased occupancy in *arp6* mutants (Fig. 5). These occupancy changes demonstrate that both factors can contribute to nucleosome destabilization and DNA accessibility at FRS9 binding sites (Fig. 5B).

These changes in occupancy were not detected at FRS9 sites that have neither H2A.Z nor BRM present (Fig. S11D), demonstrating that changes in nucleosome stability observed in the mutants can be attributed to losing H2A.Z and BRM. To better understand the individual contributions of BRM and H2A.Z, we plotted the average nucleosome signal in the *arp6*, *brm* and *arp6;brm* mutants and WT plants at FRS9 sites that overlapped with BRM and not H2A.Z and, conversely, those that overlap H2A.Z and not BRM. In the *brm* and *arp6;brm* mutants, FRS9 sites overlapping with BRM but not H2A.Z had increased occupancy for bordering nucleosomes, but a decrease in occupancy within FRS9 binding site regions that already had the most accessibility (Fig. S11B, especially clusters 2 and 3). Since we see an increase and decrease in nucleosome occupancy so close together, BRM may be responsible for moving nucleosomes from the bordering regions into the FRS9 binding sites to maintain more specific control of FRS9 or other TFs binding there. FRS9 sites that are associated with H2A.Z but not BRM and those that have H2A.Z and BRM display more nucleosome phasing than those observed in the sites that do not have H2A.Z (Fig. S11B-D and Fig. 5B). Since this phasing is not disrupted in the *arp6* mutants, but rather we observed an increase in the occupancy of the already well-positioned nucleosomes (Fig. S11C), H2A.Z may be needed at FRS9 sites to modulate chromatin accessibility in response to these already well-positioned nucleosomes.

DISCUSSION

H2A.Z and BRM have both redundant and antagonistic roles in regulating transcription

We initially set out to test the hypothesis that BRM antagonizes the activating function of H2A.Z by stabilizing or repositioning nucleosomes. This hypothesis was proposed in response to their antagonistic relationship in regulating *FLC* transcription (Farrona et al., 2011). Through the work reported here, we learned that the relationship between BRM and H2A.Z in transcriptional regulation is not a simple antagonism, but includes several different relationships. We identified gene sets where BRM antagonizes the repressive function and

the activating function of H2A.Z (Classes 7 and 8), and reciprocally, where H2A.Z antagonizes the activating and repressive functions of BRM (Classes 5 and 6). We also identified genes that depend on either H2A.Z or BRM to modulate transcript level (Classes 1 and 2) and genes where the additive function of both factors contributes to transcriptional repression or activation (Classes 3 and 4) (summarized in Fig. 6). By identifying genes that are direct targets of BRM and H2A.Z, we provide an additional layer of genomic information about regulatory factors acting at the targeted genes and regulating the associated processes. These gene sets can now serve as a robust starting point for understanding their individual roles in transcriptional regulation in even greater detail.

H2A.Z levels in chromatin are independent of BRM

One hypothesis that would explain how BRM and H2A.Z coordinately or antagonistically regulate transcription is that one factor may regulate the ability of the other factor to associate with the loci that they both target. Others have observed increased H2A.Z protein levels in nuclear fractions in RNAi knock down plants for the BAF60 SWI2/SNF2 subunit. However, these were measurements of total nuclear H2A.Z levels and not necessarily H2A.Z levels in chromatin (Jegu et al., 2014). Since *brm* mutants do not have a consistent increase or decrease in H2A.Z-containing nucleosome levels in our ChIP-seq experiments (Fig. S2A), our results indicate that BRM does not generally affect H2A.Z levels in chromatin. An alternative hypothesis to explain interactions between H2A.Z and BRM would be that H2A.Z may recruit BRM, because H2A.Z plays a role in recruiting the SWI2/SNF2 complex to at least one locus in human cells (Gevry et al., 2009). However, this seems unlikely since BRM and H2A.Z have a relatively small, yet significant, overlap in the genome (Fig. 3A).

BRM and H2A.Z destabilize +1 nucleosomes

At DE BRM-H2A.Z co-targeted genes, BRM localizes just upstream of the TSS and H2A.Z is enriched at the +1 nucleosome (Fig. 4B). When H2A.Z and BRM coordinately regulate gene transcription, they both contribute to +1 nucleosome destabilization (Fig. 4A, Classes 1 and 2). BRM and H2A.Z have been associated with +1 nucleosome stability in combination with other factors as well. Mutants for the FORGETTER1 TF that interacts with BRM perturb +1 nucleosome occupancy of genes involved in heat stress memory (Brzezinka et al., 2016). Our data showing an increase in +1 nucleosome occupancy in *brm* mutants support a role for BRM in contributing to how FORGETTER1 destabilizes +1 nucleosomes after heat exposure. At the +1 nucleosomes of some heat responsive genes, H2A.Z eviction contributes to nucleosome destabilization, emphasizing a role for H2A.Z in +1 nucleosome stability (Cortijo et al., 2017). We found, however, that when H2A.Z is found proximal to BRM at co-targeted genes, H2A.Z tends to destabilize +1 nucleosomes of DE genes (Fig. 4A, D and Fig. 6).

While the +1 nucleosome presents a barrier to transcription (Weber et al., 2014), the direction of transcriptional changes observed in BRM mutants is not inherently coupled to the change in nucleosome stability caused by BRM. For example, in classes 3 and 4 of co-regulated genes, +1 nucleosome occupancy increases in BRM mutants at genes transcriptionally regulated by BRM and H2A.Z, but there are no significant transcription

changes until the genome is also depleted of H2A.Z-containing nucleosomes in *arp6;brm* double mutants (Fig. 4A). Changes in transcriptional regulation can also correspond with changes in the accessibility of the DNA that is associated with the +1 nucleosome without changing the nucleosome occupancy (Huebert et al., 2012). Additionally, changes in occupancy can be uncoupled from transcription changes (Mueller et al., 2017). This means that although +1 nucleosomes appear to have an increase in nucleosome occupancy in BRM mutants, and H2A.Z does not show a consistent change in nucleosome occupancy at these gene classes (Fig. 4A), the actual DNA that associates with them may have different degrees of accessibility depending on other factors such as histone modifications or interactions with other chromatin interacting proteins.

It is also important to keep in mind that the data we generated come from a pool of different types of cells, so that nucleosomal changes in a subpopulation of cells could be masked by signals from other cell types. In addition to contributing to transcriptional initiation, H2A.Z can help facilitate transcriptional elongation in *Arabidopsis* (Rudnizky et al., 2016; Weber et al 2014). The overall destabilization role of both factors in some co-regulated genes may also allude to both H2A.Z and BRM contributing to transcriptional elongation rather than strictly transcriptional initiation at co-targeted genes.

BRM destabilizes nucleosomes flanking NDRs

In this paper, we focus on describing where BRM and H2A.Z intersect in the genome to understand how they each impact the role of the other in transcription and nucleosome organization. However, H2A.Z and BRM each function independently in general, as we see in the large number of non-overlapping regions from BRM and H2A.Z ChIP-seq data (Figure 3A). The specific role of BRM in chromatin regulation to date has been evaluated locus by locus, and we report how BRM contributes to global nucleosome organization in *Arabidopsis* (Wu et al., 2015; Han 2015; Brzezinka et al., 2016). We demonstrate that BRM localizes to NDRs across the genome and is flanked by well-positioned nucleosomes whose stability can depend on BRM (Fig. S6, and Fig. 6). These results expand on previous locus-specific studies by both finding that well-positioned nucleosomes surrounding BRM binding sites is a general genomic trend and that the stability of many of these flanking nucleosomes depends on BRM (Sacharowski et al., 2015; Wu et al., 2015). Since the BAF60 subunit of the SWI2/SNF2 complex has been observed localizing to open chromatin, we provide further evidence that the SWI2/SNF2 complex binds to NDRs by finding that the BRM SWI2/SNF2 ATPase also binds to NDRs (Jegu et al., 2017). The fact that we see an increase in +1 nucleosome occupancy in the absence of BRM and H2A.Z at genes where both are needed for proper transcriptional regulation (especially Classes 1 and 2) is consistent with studies showing that both factors disrupt interactions between DNA and nucleosomes (Schnitzler et al., 2001; Rudnizky et al., 2016). It is interesting to note that although BRM localizes to NDRs it appears that other factors establish the open conformation of these regions, as these sites remain depleted of nucleosomes in *brm* mutants (Fig. 2).

BRM sites found near H2A.Z-containing nucleosomes show more nucleosome occupancy increases in *brm* mutants, more changes in nucleosome positions, as well as decreases in nucleosome fuzziness score compared to those BRM sites without H2A.Z (Fig. 3C). This

suggests that BRM opposes the nucleosome stabilizing functions of H2A.Z at some BRM-bound sites. Other studies using recombinant human proteins suggest that modifications to the nucleosome acidic patch can enhance chromatin remodeling activity (Dann et al., 2017). One of the differences between H2A and H2A.Z is that H2A.Z has an extended acidic patch that has been proposed to help facilitate remodeling activity of the ISWI complex (Goldman et al., 2010). Therefore, the extended acid patch of H2A.Z-containing nucleosomes may promote nucleosome remodeling by BRM.

BRM and H2A.Z interact with binding sites for light-responsive TFs

Based on our GO analyses, H2A.Z and BRM regulate the transcription of genes that are involved in defense, temperature and light responses, as well as growth (Fig. 1B and Table S2). This supports the idea that both H2A.Z and BRM contribute to the balance between normal growth and responses to stimuli. More specifically, overlapping DE target genes suggest that H2A.Z and BRM are important to integrate signals and regulate transcription in response to light stimuli. Since we show that BRM and H2A.Z often overlap with binding sites for FRS9 and other light responsive transcription factors (Figure S11A), our findings indicate that interacting with light responsive TFs may be one way that H2A.Z and BRM could mediate responses to light stimuli. We show that FRS9 binding sites are present in each of the 8 classes of DE co-targets of H2A.Z and BRM. We further find that many of these FRS9 binding sites are dependent on H2A.Z and/or BRM for full accessibility, suggesting that FRS9 binding may be affected in the mutants (Fig 5B).

Additional factors may contribute to the roles of BRM and H2A.Z in chromatin organization and transcriptional regulation

Some of the transcriptional and nucleosomal changes that we observed in *brm* or *arp6* mutants may not be due to the specific catalytic functions of BRM or inherent properties of H2A.Z incorporation into chromatin by the SWR1 complex, but could rather be contributed by other chromatin regulating factors that interact with them. The SWI2/SNF2 complex is known to interact with a histone acetyltransferase (HD2C), a H3K27me3 histone demethylase (REF6), and potentially the ISWI CRC (Brzezinka et al., 2016; Buszewicz et al., 2016; Li et al., 2016). BRM also antagonizes the silencing function of the Polycomb Repressive Complex 2 (Li et al., 2015). Additionally, interchanging subunits of the SWI2/SNF2 complex can confer unique functions to modulate specific developmental processes (Vercruyssen et al., 2014; Sacharowski et al., 2015). This could mean that some of the variability in BRM's role in chromatin regulation could correspond with which SWI2/SNF2 subunits co-localize with it. BRM and the paralogous SWI2/SNF2 ATPase SPLAYED have both unique and redundant roles in *Arabidopsis*, so some contributions from BRM that are redundant with SPLAYED will be obscured from our analyses (Bezhani et al., 2007).

In other organisms, post-translational modifications to H2A.Z, such as ubiquitination and acetylation, have been shown to correlate with the role of H2A.Z in transcriptional repression and activation, respectively (Marques et al., 2010; Dalvai et al., 2012; Valdes-Mora et al., 2012). Assuming that similar post-translational modifications to H2A.Z exist in *Arabidopsis*, they likely contribute to some of the variability in nucleosome positioning and stability that we describe for H2A.Z. However, more work is needed to create a

comprehensive description of how histone-modifying enzymes interact with H2A.Z to affect chromatin organization and regulate transcription.

Conclusions

Within the nucleus, combinatorial effects from a range of factors regulate chromatin organization in different contexts. This can make it difficult to understand the extent to which any one factor contributes to chromatin organization as a whole. *In vitro* studies simplify the system to understand individual chromatin-modulating components, but they are far removed from the constant flux of regulatory pressures that a locus experiences *in vivo*. In our study, we attempted to parse the contributions of H2A.Z and BRM *in vivo* and chose to simplify our approach by specifically evaluating direct target loci of H2A.Z and BRM. We found that not only do H2A.Z and BRM cooperate at co-targeted genes to positively and negatively regulate transcription, but some of their roles are functionally redundant (Fig. 6). In addition, we identified genes where H2A.Z and BRM act either negatively or positively to affect transcript level in ways that are opposed by the other factor. We discovered that BRM contributes to nucleosome stabilization within its binding sites, while it has more destabilizing effects on flanking nucleosomes and in combination with H2A.Z. At co-targeted DE genes, BRM and H2A.Z contribute to nucleosome stability to varying degrees, but they appear to both regulate +1 nucleosome occupancy where either is required for transcriptional regulation (Fig. 4). Some of the different regulatory relationships between H2A.Z and BRM may be explained by their interactions with TF binding sites, such as those of FRS9 (Fig. 5). For example, H2A.Z and BRM may modulate the accessibility of sites for TF binding, which could affect the activity of individual TFs or combinations thereof and result in a variety of different transcriptional outcomes.

Both H2A.Z and the SWI2/SNF2 complex have also been implicated in regulating larger scale nuclear organization in other organisms, contributing to chromatin looping and chromosome localization within the nucleus (Yoshida et al., 2010; Light et al., 2010; Maruyama et al., 2012; Kitamura et al., 2015; Imbalzano et al., 2013). The fact that H2A.Z and the SWR1 complex associate with nuclear scaffold/matrix attachment regions in *Arabidopsis* suggests that similar functions for both are yet to be described in plants (Lee and Seo 2017). Likewise, the SWI2/SNF2 complex has been implicated in chromatin looping in *Arabidopsis* and other organisms, as well as *in vitro* (Jegu et al., 2014, Kim et al., 2009, Bazett-Jones et al., 1999). Therefore, the DE genes that are direct targets of H2A.Z and BRM will provide useful information for assessing the roles of H2A.Z or BRM in larger scale chromatin organization in future studies. Finally, the 34 genomic data sets that were generated in this study will provide valuable reference resources in further studies of H2A.Z, SWR1, BRM, SWI2/SNF2, as well as determinants of nucleosome positioning and properties.

EXPERIMENTAL PROCEDURES

Plant material

We used previously characterized *Arabidopsis* T-DNA insertion lines *arp6-1* (GARLIC_599_G03; Deal et al., 2005) and *brm-1* (SALK_030046, Hurtado et al., 2006)

and genotyped the strains using primers described previously (Deal et al., 2005; Hurtado et al., 2006). The *arp6-1;brm-1* mutant was generated from genetic crosses of *arp6-1* homozygous and *brm-1* heterozygous lines. Plants were sown on soil, stratified at 4 °C for two days, and then moved to grow at 20 °C in long day light conditions (16 hr light/8 hr dark). Above ground plant tissue for all genomic experiments was collected at 10 hrs after dawn from 4-5 leaf developmentally staged plants (Boyes et al., 2001) from the following genetic backgrounds: WT (collected 12-13 days post stratification (dps)); *arp6-1* (12-14 dps); *brm-1* (13-16 dps); and *arp6;brm* (16-24 dps, delayed collection due to delayed germination). One cotyledon was removed from each plant to use for genotyping with the Phire™ Plant Direct PCR Kit (Thermo Scientific).

RNA-seq material

Three plants each for three biological replicates of 4-5 leaf developmentally staged above soil seedling material were collected and pooled for WT, *arp6*, *brm*, and *arp6;brm* plants. RNA was isolated using the Spectrum Plant Total RNA Kit (Sigma) and incubated at 37 °C for 30 min with DNase to remove DNA using the Turbo DNA-free kit (Ambion). The integrity of the RNA was confirmed on a 2% agarose gel in 1x TAE visualized with GELRED nucleic acid stain (Sigma), and the samples were quantified with a spectrophotometer. Libraries were prepared from 100 ng of RNA from each sample using the Ovation RNA-seq for Model Organisms kit (NuGEN), which is a strand specific library preparation kit that depletes the transcripts of rRNA. Libraries were quantified with qPCR (NEB), pooled, and sequenced with the Illumina NextSeq500 to generate paired-end 36-nt sequence reads.

RNA-seq data analysis

Sequencing reads were mapped to the TAIR10 *Arabidopsis thaliana* reference genome using Tophat2 (using the second strand option and default parameters), generating an average of 75.5M mapped reads per library. The accepted hits file was name-sorted (option -n) rather than position sorted and indexed using SAMtools (Trapnell et al., 2012; Li et al., 2009). Read counts were quantified for each exon using the htseq-counts program, with name order and strict intersection options (Anders et al., 2015). Differential expression was calculated using edgeR software (Robinson et al., 2010; McCarthy et al., 2012). Differentially expressed genes were determined with a false discovery rate (FDR) cutoff of <0.2 and a log₂ fold change of ± 0.6 (~1.5 x fold change). GO terms were generated using AgriGO for the total set of genes that were DE in the mutants relative to WT plants. GeneCodis was used to analyze GO terms for DE direct target genes of H2A.Z and BRM (Carmona-Saez et al., 2007; Nogales-Cadenas et al., 2009; Tabas-Madrid et al., 2012; Tian et al., 2017). These two separate programs were used for GO analyses based on how generally (AgriGO) or specifically (GeneCodis) they summarized the overlap between gene lists.

ChIP-seq material

For ChIP-seq experiments, we collected at least 0.5 g of tissue from two biological replicates each of WT, *arp6-1*, and *brm-1* plants. (WT, 12-13 dps; *arp6*, 12-14 dps; *brm*, 13-16 dps; *arp6;brm* 16-24 dps). Above ground developmentally staged 4-5 leaf plant tissue was collected at 10 hrs after dawn, cross-linked as described previously (Gendrel et al., 2005),

frozen, and ground in liquid nitrogen. Nuclei were isolated as previously described (Gendrel et al., 2005). Chromatin was sonicated using a Bioruptor® (Diagenode) (40 min on high (45 sec on/ 15 sec off)). Each sample was diluted in 1.1 mL of ChIP dilution buffer (described in Gendrel et al., 2005) and 50 µl was saved as the input sample. Then H2A.Z-containing chromatin was immunoprecipitated from the 1.1 mL of chromatin solution using 2 µg of H2A.Z antibody purified to specifically recognize unmodified H2A.Z peptides (Deal et al., 2007). The chromatin solution was incubated with the H2A.Z antibody for 2 hr then for 1 more hour in combination with 60 µl of Dynabeads™ Protein-A magnetic beads (Invitrogen). DNA collected from the immunoprecipitation and from the inputs was purified using 1.8x volume of SPRI beads (Beckman Coulter) then quantified with Quant-IT™ Picogreen® dsDNA Assay Kit (Invitrogen). Sequencing libraries were prepared from 1 ng of DNA per sample with the Accel-NGS® 2S Plus DNA Library kit (Swift Biosciences) and sequenced on an Illumina NextSeq500 using 76 nt single-end reads.

ChIP-seq data analysis

ChIP-seq reads were mapped to the TAIR10 *A. thaliana* reference genome with Bowtie2, using default parameters (Langmead and Salzberg 2012). An average of 13.9 M reads were converted to binary files, sorted, indexed and quality filtered (with the -q 2 option) using SAMtools software (Li et al., 2009). H2A.Z peaks were called with Homer findpeaks software, using options “style histone” and “-region” (Heinz et al., 2010). H2A.Z peaks from two biological replicates were intersected to find the regions that were called in both replicates for each genotype using Bedtools software (Quinlan 2014). H2A.Z peaks in WT that overlapped with H2A.Z peaks called in *arp6* mutants with less than a 2-fold difference in enrichment between the two genotypes were removed from the analysis to ensure that the datasets analyzed represent ARP6-dependent H2A.Z peaks. These were the H2A.Z peaks we used throughout the study. We integrated BRM-GFP ChIP-seq peaks into our analysis from a previously published data set (Li et al., 2016). Also, we used DAP-seq peaks for FRS9 (O'malley et al., 2016). H2A.Z and BRM ChIP-seq peaks were annotated based on the genes that they overlapped (-u ODS option) or were assigned to the nearest TSS (-u TSS option) using PeakAnnotator software (Salmon-Divon et al., 2010). Before preparing bigwig files, we first used the SAMtools view command (with option -s) to scale data sets so that all samples had same number of reads. We also combined the two biological replicates with SAMtools merge. Using the deepTools software suite, we then prepared bigwig files using default parameters for the bamCoverage program, then we subtracted the input signal from the ChIP signals by 10 bp bins with the bamCompare command for each genotype (Li et al., 2009; Li 2011; Ramirez et al., 2014). Heatmaps and average profile plots were generated from these bigwigs using deepTools computeMatrix, plotHeatmap, and plotProfile programs (Ramirez et al., 2014).

MNase-seq material

Tissue from two biological replicates of 100 mg of pooled above ground 4-5 leaf stage plants was collected from WT, *arp6-1*, *brm-1*, and *arp6-1;brm-1* plants grown on soil in long day light conditions (16 hr light/ 8 hr dark). Nuclei were isolated as described previously (Gendrel et al., 2005). After purification, nuclei were resuspended in 500 µl of TM2 solution (10 mM Tris (pH 7.5), 2 mM MgCl₂, and 1X Roche Complete protease inhibitor tablet). We

spun nuclei down at 3,000 x g for 10 min then removed the supernatant and re-suspended the pellet in 500 µl of MNase reaction buffer (16 mM Tris-Cl (pH 8.0), 50 mM NaCl, 2.5 mM CaCl₂, 1 mM EDTA, Protease inhibitor tablet). Samples consisting of 500 µl of nuclei were incubated with 7.5 U MNase for 7.5 min at 37 °C, and then the reaction was stopped by adding EDTA to a final concentration of 10 mM. Nuclei were lysed by adding SDS (to 1% of the final sample volume). The solution was mixed and spun down at 1,300 x g for 3 min to remove insoluble debris. After moving the supernatant to a new tube, samples were treated with RNase A (1 mg/mL, Ambion) and then with Proteinase K (Invitrogen) to remove RNA and proteins, respectively. DNA fragments were purified with MinElute PCR purification kit (Qiagen). To purify nucleosome associated DNA fragments that were <400 bp, we used a 0.6x bead-to-sample ratio of SPRI beads prepared as described previously (Faircloth and Glenn 2014). Sequencing libraries were prepared with the ThruPLEX® DNA-Seq Kit (Rubicon Genomics), using 25 ng of MNase-digested DNA as the input. Purified, indexed libraries were pooled and sequenced on an Illumina NextSeq500 generating 76 bp paired-end reads.

MNase-seq data analysis

Sequence reads were mapped to the TAIR10 *Arabidopsis thaliana* reference genome using Bowtie2 (using default parameters except for -p 6) and were then further sorted and indexed using SAMtools (Li et al., 2009; Langmead and Salzberg 2012). We filtered reads using the SAMtools view command, with the -q 2 option to filter for quality and option -f 0x02 to filter for properly paired reads. Libraries were subsampled using the SAMtools -s parameter to normalize all samples to the same number of reads (30.46 M reads). We analyzed the mapped reads from each biological replicate for each of the four genotypes to generate nucleosome peak files and nucleosome occupancy wiggle files using the DANPOS2 dpos program (Chen et al., 2013). Values in the *.allPeaks.xls file output from the dpos program were used to determine dynamic nucleosomes. These dynamic nucleosomes are defined as those with a FDR <0.05 for the difference between the occupancy value at the summit position of a point of difference in control and treatment samples (point_diff_FDR <0.05) and then individual types of dynamic nucleosome changes were described with the following additional criteria. Fuzziness scores were defined as the standard deviation of read positions in each peak. Significant nucleosome fuzziness changes were defined as those with a FDR of <0.05 for the difference between WT and mutant fuzziness scores (fuzziness_diff_FDR <0.05). Significant occupancy changes were defined as those with a FDR of <0.05 of the difference between the occupancy value at the peak summit position in the WT and the mutant (smt_diff_FDR <0.05). Position shifts were defined as a 20-95 bp difference in peak summit position between WT and mutant nucleosomes (treat2control_dis 20-95 bp). To measure nucleosome occupancy across the genome, we converted the DANPOS generated wiggle files to bigwig files using the wigToBigWig software (UCSC). Heatmaps were generated from these bigwig files using deepTools software: computeMatrix, plotHeatmap, and plotProfile programs (Ramirez et al., 2014).

Identifying deletions in arp6 mutants

We isolated genomic DNA from mature rosette leaf material (~5 mg per plant) from 50 pooled *arp6* mutants using a standard phenol:chloroform extraction followed by ethanol

precipitation. We used 1 µg of sonicated DNA to prepare a sequencing library (NEXTflex Rapid DNA-seq (option 2), Bioo Scientific), and then sequenced on an Illumina HiSeq2000, generating 125 bp paired-end reads. Sequenced reads were mapped using BWA mem software, indexed and quality sorted (-s option) using SAMtools, and randomly subsetted using a python script, leaving 61.5 M mapped reads (Li and Durbin 2009; Li et al., 2009; Li 2011). Deletions were called in the arp6 mutant using CNVnator (v0.3.3) software using bin sizes of 100 bp (Abyzov et al., 2011).

Motif enrichment analysis

ATAC-seq transposase hypersensitivity sites (THSs) from mesophyll cells were identified and annotated previously (Sijacic et al., 2017). We used python scripts to pull out the annotated mesophyll THSs that were associated with genes from our 8 differentially expressed BRM and H2A.Z target gene classes (defined in Fig.1D). We scaled all THSs to be 150 bp in width and then used the Regulatory Sequence Analysis Tool for plants (RSAT plants) to obtain the corresponding DNA sequences and mask any repeated sequences (Medina-Rivera et al., 2015). Motifs that were enriched in our lists of THSs were discovered using DREME and MEME programs from the MEME-ChIP suite then paired with TFs predicted to bind to the motifs using the Tomtom program (Bailey et al., 2009) THS sequences were compared to both the CIS-BP and DAP-seq TF binding databases for these analyses (Weirauch et al., 2014; O'malley et al., 2016). Motifs and their associated TF were considered significant they had an E-value of < 0.05.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance Statement: The histone variant H2A.Z and the chromatin remodeler BRM have overlapping roles in gene activation and repression, but the extent of their physical and regulatory interactions was unknown. We used genome-wide profiling methods to identify sites where both factors overlap, and found that H2A.Z and BRM have both cooperative and antagonistic roles with respect to transcription and chromatin organization.

Figure 1. H2A.Z and BRM regulate transcription through various cooperative and antagonistic relationships.

(A) Heatmap showing the average log₂ fold change of genes in 8 classes of genes regulated antagonistically and coordinately by BRM and H2A.Z. These classes were assigned based on transcriptional changes observed between *arp6*, *brm-1*, or *arp6-1;brm-1* mutants compared to transcript levels in WT plants. Genes up-regulated are indicated in yellow and down-regulated are indicated in blue with a gradient to black representing no change in transcript level relative to WT. The 8 different classes are indicated by various colors to the left and are divided into gene classes based on their pattern of transcription change in the different genotypes. Coordinately regulated genes include those that are up- (1-red) or down-regulated (2-orange) in both *arp6* and *brm* mutants relative to WT, genes that are up- (3-yellow) or down-regulated (4-light green) in the *arp6;brm* double mutant, but not the single mutants. Antagonistically regulated genes are divided into those genes that are up- (5-dark green) or down-regulated (6-light blue) in the *arp6* mutants but not *brm* or *arp6;brm* double mutants relative to WT, or genes that are up- (7-dark blue) or down-regulated (8-pink) in the *brm* mutants but not *arp6* or *arp6;brm* double mutants relative to WT. White boxes around gene sets highlight the significantly DE genes used to define the corresponding gene class, and the number of genes in each class (n) is shown to the right of the heatmap. (B) Select Gene Ontology Terms enriched for genes that are targeted by both H2A.Z and BRM and make up the combined set of 8 gene classes defined in the heatmap (A).

Figure 2. BRM localizes to nucleosome depleted regions flanked by two well-positioned nucleosomes.

Profile plot showing nucleosome read signals ± 1 kb around all BRM peaks size-scaled to be 1 kb wide. Nucleosome reads are from an MNase-seq experiment from WT plants (blue line) and *brm* mutants (red line).

Figure 3. BRM destabilizes nucleosomes where BRM and H2A.Z overlap.

(A) Venn diagram shows the number of BRM peaks, H2A.Z peaks, and regions of overlap between the two. (B) Average profile plots and heatmaps show four K-means clustered H2A.Z and BRM ChIP-seq read signal patterns (normalized to input) at regions of overlap between BRM and H2A.Z peaks. (C) Histogram summarizing the percent of BRM peaks that exhibit different types of nucleosomal changes in *brm* mutants subdivided into whether the peaks overlap with H2A.Z (black) or do not overlap with H2A.Z (grey). Asterisks indicate that the proportion of nucleosomes with the observed changes are greater than expected from a randomly selected subset of BRM peaks (Fisher's exact test, p-value < 0.05). Nucleosomal changes described in mutants (grey dotted line) compared to wild type nucleosome patterns (black line) are depicted below the graph. Regions of overlap with dynamic nucleosomes identified in both mutants relative to wild type nucleosomes (n=88) were further evaluated. Scatter plots display the log₂ fold change in nucleosome occupancy (C) and fuzziness (D) in regions of H2A.Z/BRM overlap that contain dynamic nucleosomes in both *brm* mutants (y-axis) and *arp6* mutants (x-axis) compared to WT.

Figure 4. BRM and H2A.Z destabilize the +1 nucleosome at DE targets.

(A) Profile plots showing the average nucleosome read signal from WT plants (black), *arp6* (orange), *arp6;brm* (blue), and *brm* mutants (red) ± 500 bp around the TSSs of the 8 classes of DE H2A.Z and BRM target genes. Black triangles on the x-axis indicate the position of the +1 nucleosome. The diagrams above the plots describe the genetic relationships between BRM and H2A.Z/ARP6 for each gene class and are the same as those described in Fig. 1. (B) Profile plot showing the read signal for WT nucleosomes (black), H2A.Z ChIP-seq (blue), and BRM ChIP-seq (orange), averaged across ± 500 bp up- and downstream of the TSSs of the DE BRM and H2A.Z target genes. (C) Diagram representing how we used ChIP-seq, MNase-seq, and RNA-seq data sets in the previous figures, to evaluated the relationship between BRM localization in WT (orange), H2A.Z localization in WT (light blue) and nucleosomes (blue circles) around the TSSs of DE BRM and H2A.Z target genes. (D) Table summarizes the extent to which the +1 nucleosomes become stabilized in *brm*, *arp6;brm* and *arp6* mutants at the 8 DE BRM-H2A.Z target gene classes defined in Fig 1. The level of nucleosome stabilization in the mutant was defined based on the overlaps between different measures of variance at the +1 nucleosome read signals plotted in Fig. S6.

The degree to which the +1 nucleosome was stabilized in the mutants compared to WT is defined as no change (– = mean of one falls within the 95% confidence interval of the other); a small change (+ = the mean of one sample does not overlap with the 95% confidence interval of the other); a medium change (++ = the standard error of one sample does not overlap with the 95% confidence interval of the other); or a large change in nucleosome occupancy (+++ = there is no overlap between 95% confidence intervals for the two samples).

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Figure 5. H2A.Z and BRM contribute to nucleosome stability at FRS9 binding sites.

(A) Average K-means clustered profile plots showing H2A.Z ChIP-seq (blue), BRM ChIP-seq (orange) and WT nucleosome read signals from MNase-seq experiments (black) across FRS9 ChIP-seq binding sites scaled to 500 bp regions. Signals are subdivided into 4 K-means clusters based on nucleosome patterns (depicted below graphs). (B) Average profile plots show nucleosome profiles from WT (black), *arp6* (blue), *arp6;brm* (red), and *brm* (orange) plants across K-means clustered FRS9 binding sites where H2A.Z and BRM localize. The arrows indicate sites that experience nucleosomal changes in *arp6*, *brm*, and/or *arp6;brm* mutants.

Figure 6. Model for BRM and H2A.Z interactions.

We evaluated localization of the BRM SWI2/SNF2 subunit and H2A.Z with chromatin immunoprecipitation followed by sequencing of isolated DNA (ChIP-seq, green), changes in steady state transcript levels with RNA-sequencing (RNA-seq, purple), and measured changes in nucleosome occupancy and positioning by sequencing nucleosomal DNA after digestion of chromatin with Micrococcal nuclease (MNase-seq, red) in genetic mutants disrupting function of one or both factors compared to WT. The BRM-containing SWI2/SNF2 complex (orange) and H2A.Z incorporated into nucleosomes by the SWR1 complex (light blue) can act antagonistically or coordinately to repress or activate for transcription directly. With the MNase-seq experiment combined with the ChIP-seq experiment, we found that BRM stabilized or repositioned nucleosomes that H2A.Z was responsible for stabilizing or destabilizing (shown as red and green dashed lines). Both H2A.Z and H2A.Z overlap at sites enriched for additional transcription factors (Turquoise).