DNA Methylation, H2A.Z, and the Regulation of Constitutive Expression

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The most well-studied function of DNA methylation in eukaryotic cells is the transcriptional silencing of genes and transposons. More recent results showed that many eukaryotes methylate the bodies of genes as well and that this methylation correlates with transcriptional activity rather than repression. The purpose of gene body methylation remains mysterious, but is potentially related to the histone variant H2A.Z. Studies in plants and animals have shown that the genome-wide distributions of H2A.Z and DNA methylation are strikingly anticorrelated. Furthermore, we and other investigators have shown that this relationship is likely to be the result of an ancient but unknown mechanism by which DNA methylation prevents the incorporation of H2A.Z. Recently, we discovered strong correlations between the presence of H2A.Z within gene bodies, the degree to which a gene's expression varies across tissue types or environmental conditions, and transcriptional misregulation in an *h2a.z* mutant. We propose that one basal function of gene body methylation is the establishment of constitutive expression patterns within housekeeping genes by excluding H2A.Z from their bodies.

Eukaryotic gene expression is heavily influenced by the local chromatin environment, which is defined in part by the incorporation of histone variants, posttranslational modification of histones, and DNA methylation in the form of 5-methylcytosine. DNA methylation is a conserved feature of eukaryotic chromatin that is present in all vertebrates examined to date as well as in many invertebrates, fungi, and plants (Cokus et al. 2008; Feng et al. 2010; Zemach et al. 2010b; Glastad et al. 2011). Studies of numerous species have demonstrated that the silencing of transposable elements (TEs) and repetitive sequences is a common function of DNA methylation (Suzuki and Bird 2008). In this role, DNA methylation acts as a genomic immune system, suppressing the transcription and transposition of invading DNA elements and thereby maintaining genome integrity (Zemach and Zilberman 2010). However, the discovery of gene body methylation in many plant and animal species (Simmen et al. 1999; Tran et al. 2005; Zhang et al. 2006; Zilberman et al. 2007; Lister et al. 2008; Wang et al. 2009; Feng et al. 2010; Xiang et al. 2010; Zemach et al. 2010b; Sarda et al. 2012), some of which do not methylate TEs, suggests an ancient function for DNA methylation in the regulation of euchromatin.

DIFFERENTIAL TARGETING OF DNA METHYLATION TO GENES AND TRANSPOSONS

Plant genes and TEs are methylated differently. Plants heavily methylate TEs in three sequence contexts (CG, C*H*G, and C*HH*, where H = A, T, or C), whereas methylation of genes is at lower levels, generally limited to CG sites, and is usually excluded from the 5′ and 3′ ends (Zhang et al. 2006; Zilberman et al. 2007; Cokus et al. 2008; Lister et al. 2008; Law and Jacobsen 2010; Zemach et al. 2010b). The exclusion of DNA methylation from the ends of genes has also been found in invertebrate genomes and to a lesser extent in mammals (Zemach and Zilberman 2010). Considering that TEs are interspersed among genes in many eukaryotes, these results imply that the mechanisms that target and maintain DNA methylation must be both accurate and precise.

In plants, the initial establishment of DNA methylation at TEs is thought to be mediated primarily by the RNA-directed DNA methylation pathway (RdDM), which targets repeats and TEs for C*HH* methylation through regions of small interfering RNA (siRNA)–DNA sequence homology (Furner and Matzke 2011). These siRNAs are produced by DICER-LIKE nucleases from longer double-stranded RNAs (dsRNAs) thought to be derived from plant-specific RNA polymerase IV transcripts of repetitive elements that are copied into dsRNA by RNA-dependent RNA polymerase 2 (Meyer 2010). Each siRNA strand is then incorporated into ARGONAUTE complexes that are likely to target homologous nascent RNA polymerase V transcripts to mediate DNA methylation catalyzed by members of the domains rearranged methyltransferase (DRM) family (Law and Jacobsen 2010; Haag and Pikaard 2011). Although precise and accurate, such de novo methylation requires active targeting in each new cell.

In the absence of an active signal, plant and animal DNA methylation patterns are maintained by the DNMT1 family of methyltransferases (Law and Jacobsen 2010). To achieve this epigenetic memory, the DNMT1

enzymes target symmetrical CG sites that are left hemimethylated following DNA replication (Goll and Bestor 2005). Virtually all CG methylation within the *Arabidopsis thaliana* genome is catalyzed by MET1, a DNMT1 homolog (Cokus et al. 2008; Lister et al. 2008). Other factors act as positive regulators of CG DNA methylation (Law and Jacobsen 2010). One such factor, the *Arabidopsis* nucleosome remodeling ATPase DDM1 (Brzeski and Jerzmanowski 2003), is required for DNA methylation of TEs (Lippman et al. 2004), as is its mammalian homolog Lsh (Huang et al. 2004; Tao et al. 2011).

Distinct patterns of DNA methylation in genes and TEs are also maintained by the active removal of methylation and associated histone modifications from genes. In most eukaryotes, transposons are marked by histone methylation of lysine 9 of histone H3 (H3K9me), and the loss of this modification leads to hypomethylation of TE sequences in plants and fungi (Saze and Kakutani 2011). Recently, the histone demethylase IBM1, a member of the JHDM2/KDM3 family, was shown to remove H3K9me from *Arabidopsis* genic sequences (Inagaki and Kakutani 2010). Loss-of-function *ibm1* mutants cause an increase in both H3K9me and non-CG methylation in genes (Saze et al. 2008; Miura et al. 2009; Inagaki and Kakutani 2010). This *ibm1*-induced genic non-CG hypermethylation is attributed primarily to the activity of CMT3, a third type of methyltransferase found in plants, which is responsible for DNA methylation in the C*H*G context (Lindroth et al. 2001; Law and Jacobsen 2010). CMT3 contains a chromodomain that binds H3K9me, potentially offering a distinct method from RdDM for the targeting of new DNA methylation (Lindroth et al. 2004; Du et al. 2012). Additionally, *Arabidopsis* enzymes that catalyze H3K9me, such as KRYPTONITE (KYP), contain SRA domains that are capable of binding to methylated cytosines (Johnson et al. 2007). This mutual binding by KYP and CMT3 of their reciprocal enzymatic products creates a positive feedback loop that allows for the propagation and reinforcement of both C*H*G methylation and H3K9me across targeted sequences that normally consist primarily of TEs (Furner and Matzke 2011).

GENIC METHYLATION IS ASSOCIATED WITH CONSTITUTIVE EXPRESSION

Genome-wide analyses in a variety of eukaryotic species have revealed that gene body methylation most likely existed in the ancestors of most or all extant eukaryotes and remains well conserved among plants and animals today (Lister et al. 2009; Feng et al. 2010; Xiang et al. 2010; Zemach et al. 2010a,b). Despite its ancient origins, the purpose of genic methylation remains a mystery (Zemach and Zilberman 2010). Genic methylation was proposed to prevent cryptic promoters from firing within the bodies of actively transcribed genes (Zilberman et al. 2007), and the preferential methylation of plant and animal exons has led to the suggestion that gene body methylation plays a role in exon definition and splicing (Lorincz et al. 2004; Chodavarapu et al. 2010; Shukla et al. 2012). The evaluation of these and other potential biological functions of genic methylation may be aided by the now well-established relationship between DNA methylation and transcription.

Unlike DNA methylation at promoters, gene body methylation does not appear to antagonize transcription; on the contrary, targets of genic methylation tend to be expressed (Suzuki and Bird 2008). Gene body methylation is highest in moderately transcribed plant and animal genes, with the lowest levels of genic methylation in either transcriptional extreme (Zhang et al. 2006; Zilberman et al. 2007; Zemach et al. 2010b). In *Arabidopsis*, we found that genic methylation is positively correlated with gene expression up to the 70th transcriptional percentile, after which the relationship becomes strongly negative (Zilberman et al. 2007). We found similar results in pufferfish as well as in several invertebrates, including honeybee, silkworm, sea anemone, and sea squirt (Zemach et al. 2010b).

Curiously, genic methylation also appears to correlate with constitutive gene expression. This relationship was first discovered in *Arabidopsis*, where body methylated genes were shown to be more constitutively expressed (as measured by Shannon's entropy) than other genes (Zhang et al. 2006). Further analysis of *Arabidopsis* methylation data has shown that genes with body methylation are enriched for housekeeping functions, whereas those with no gene body methylation are more likely to exhibit high tissue and environmental condition specificity (Aceituno et al. 2008). Functional analysis of body methylated genes from another plant species (*Populus trichocarpa*) revealed significant enrichment for gene categories that are typically constitutively expressed, including translation/protein metabolism, nucleic acid binding, and RNA metabolism (Vining et al. 2012). Similar results were found in four invertebrates species, with body methylated genes showing enrichment for housekeeping functions, such as translation, ribosome biogenesis, RNA splicing, and protein localization (Zeng and Yi 2010; Sarda et al. 2012). These data suggest that in addition to the ancestral relationship between gene body methylation and transcription level, the correlation of genic methylation with constitutive expression is an invention that predates the divergence of plants and animals. Enrichment of methylation in constitutively expressed housekeeping genes argues against a major role for gene body methylation in the control of developmentally regulated alternative splicing. The biological significance of gene body methylation may instead be explained by the discovery that DNA methylation and the histone variant H2A.Z, another well-conserved feature of eukaryotic chromatin, are anticorrelated on a genome-wide basis in plants and animals (Zilberman et al. 2008; Conerly et al. 2010; Edwards et al. 2010; Zemach et al. 2010b).

THE HISTONE VARIANT H2A.Z

In addition to the canonical histones, a variety of histone variants play important roles in diverse cellular pro-

cesses, including meiotic recombination, chromosome segregation, DNA repair, transcription initiation, and sex chromosome condensation (Talbert and Henikoff 2010). H2A.Z, which replaces canonical H2A, is perhaps the most well conserved of all histone variants, and considerable progress has been made toward understanding the role of H2A.Z in eukaryotic biology (Zlatanova and Thakar 2008). Unlike some other histone variants, which are lineage specific (Talbert and Henikoff 2010), H2A.Z appears to have had a single origin at the root of the eukaryotic tree and shares high amino acid sequence identity among species as diverse as *Saccharomyces cerevisiae, Drosophila melanogaster, Homo sapiens*, and *Arabidopsis thaliana* (Redon et al. 2002; Zlatanova and Thakar 2008). Mutant analyses have revealed that the unicellular yeasts *S. cerevisiae* and *Schizosaccharomyces pombe* can tolerate a loss of H2A.Z, although they exhibit slower growth and environmental sensitivities (Jackson and Gorovsky 2000; Redon et al. 2002). In contrast, H2A.Z is strictly required for animal viability, with mutants exhibiting early developmental arrest and death (Liu et al. 1996; Clarkson et al. 1999; Faast et al. 2001; Ridgway et al. 2004). Unlike many of these species, which have only a single copy of *H2A.Z*, the *Arabidopsis* genome contains three. Loss of two of the three *Arabidopsis H2A.Z* genes leads to a variety of developmental phenotypes, including a loss of apical dominance, smaller and fewer rosette leaves, decreased fertility, and early flowering (March-Diaz et al. 2008). A strong loss-of-function *h2a.z* mutant with insertions in all three *H2A.Z* genes exhibits a similar, but considerably stronger, phenotype (Coleman-Derr and Zilberman 2012), yet remains viable, which potentially makes *Arabidopsis* an unusual and useful model system among multicellular species for understanding H2A.Z biology.

The high degree of H2A.Z conservation among eukaryotes, in conjunction with the phenotypes of *h2a.z* mutants, suggests the existence of one or more ancient and important functions for this variant. A considerable proportion of H2A.Z research has focused on analyzing differences between the protein structures of H2A.Z and H2A in order to reveal these core, H2A.Z-specific functions (Billon and Cote 2011). The crystal structure of an H2A.Z-containing nucleosome revealed key amino acid residues that affect how H2A.Z molecules interact with one another and with the H3/H4 core particle, potentially altering nucleosome stability (Suto et al. 2000). However, similarly to many aspects of H2A.Z biology, how H2A.Z influences nucleosome stability remains controversial, and experiments have found evidence both for stabilizing (Fan et al. 2002; Park et al. 2004; Thambirajah et al. 2006; Ishibashi et al. 2009) and destabilizing (Suto et al. 2000; Placek et al. 2005; Zhang et al. 2005) effects.

Other H2A.Z amino acid residues are required for interaction with a specific chromatin remodeler (SWR1) and the subsequent deposition of H2A.Z into chromatin (Wu et al. 2005; Jensen et al. 2011; Wang et al. 2011). This multisubunit, ATP-dependent chromatin remodeling complex was first discovered in yeast, where it was shown to be capable of replacing H2A/H2B dimers with H2A.Z/H2B in vitro and in vivo (Kobor et al. 2004; Krogan et al. 2004; Mizuguchi et al. 2004; Li et al. 2005). More recently, homologous remodelers with similar functional capabilities have been discovered in animals (Kusch et al. 2004; Zhang et al. 2005; Ruhl et al. 2006; Gevry et al. 2007; Wong et al. 2007; Cuadrado et al. 2011). In *Arabidopsis*, the SWR1 homolog PIE1 has been demonstrated to interact with H2A.Z and to be required for the deposition of H2A.Z (Choi et al. 2007; Deal et al. 2007; March-Diaz et al. 2008). Mutations in the catalytic subunit *PIE1*, as well as in other members of the PIE1 complex, produce phenotypes that are broadly similar to those found in the *Arabidopsis h2a.z* mutants, although the presence of several phenotypic differences suggests that mutations in *PIE1* and *H2A.Z* are not completely functionally redundant (Noh and Amasino 2003; Deal et al. 2005; Choi et al. 2007; March-Diaz et al. 2007, 2008). Whether PIE1 has H2A.Z-independent functions, that have been shown for SWR1 homologs in animals, remains unclear (Kobor et al. 2004; Auger et al. 2008; Bowman et al. 2011). Possibly indicative of such nonredundant roles, we recently demonstrated that double mutants of *pie1* and *h2a.z* are not viable, exhibiting developmental arrest shortly after germination (Coleman-Derr and Zilberman 2012). This is quite different from results reported in yeast, where simultaneous mutation of *SWR1* and *H2A.Z* (*HTZ1*) leads to amelioration of the phenotypes found in *htz1* mutants alone (Morillo-Huesca et al. 2010)—a result hypothesized to be caused by SWR1-mediated remodeling in the absence of its substrate. Although the cause of the more severe phenotype in *h2a.z;pie1 Arabidopsis* plants remains unclear, our results suggest that there may be substantial differences in H2A.Z-related function among species.

Recent work has shown that yeast INO80, a chromatin remodeler belonging to the same subfamily as SWR1 (Morrison and Shen 2009), can regulate the genome-wide distribution of H2A.Z by promoting the eviction of H2A.Z from promoters during transcriptional induction (Papamichos-Chronakis et al. 2011). This discovery, alongside the finding that H2A.Z is incorporated into nucleosomes at low levels in both *pie1* and *swr1* mutants (Kobor et al. 2004; Wu et al. 2005; Deal et al. 2007), suggests that similar remodelers may also be capable of depositing H2A.Z in *Arabidopsis*. Homologs of *INO80* exist in *Arabidopsis* (Fritsch et al. 2004), and an important unanswered question is whether any of these remodelers possess similar H2A.Z remodeling capabilities and assist in shaping the characteristic genomic distribution pattern of H2A.Z.

H2A.Z HAS DIFFERENT EFFECTS ON TRANSCRIPTION AT THE TRANSCRIPTION START SITES AND IN GENE BODIES

Genome-wide localization experiments in fungi, animals, and plants have all shown that H2A.Z is preferentially found within the few nucleosomes surrounding the transcription start sites (TSS) of genes (Guillemette and

Gaudreau 2006; Creyghton et al. 2008; Zilberman et al. 2008), although which genes show enrichment seems to vary depending on the species. In yeast, the majority of genes have a peak of H2A.Z at their 5′ end, regardless of whether they are active or inactive (Guillemette et al. 2005; Li et al. 2005). In contrast, the 5′ enrichment of H2A.Z is more frequent in active animal genes (Whittle et al. 2008; Hardy et al. 2009; Jin et al. 2009). Interestingly, the genes enriched in H2A.Z change during mammalian embryonic stem (ES) cell differentiation (Creyghton et al. 2008). Contrary to the general conclusion that active genes are enriched for H2A.Z in animals, genes showing enrichment in the ES cell lineage are overrepresented for silent developmental genes required in later cell types (Creyghton et al. 2008). Consistent with this finding, *Caenorhabditis elegans* genes that exhibit high H2A.Z are enriched for gene ontology terms related to embryonic development, larval development, and gamete generation (Whittle et al. 2008). The somewhat contradictory data regarding H2A.Z localization are likely to reflect differences among species but might also be attributable to differences in how genic H2A.Z enrichment is measured.

Most research on H2A.Z has focused on the 5′ peak of H2A.Z at the TSS of genes, and only a few studies have reported the presence of H2A.Z in other parts of the gene. However, a recent reexamination of the available genome-wide mapping data from animals and fungi showed substantial presence of H2A.Z within gene bodies (Fujimoto et al. 2012). Another study suggests that as much as 40% of all H2A.Z-containing nucleosomes may reside downstream from the promoter in human genes (Tolstorukov et al. 2009). In *Arabidopsis*, we have shown that, in addition to H2A.Z enrichment at the TSS, many genes exhibit considerable levels of H2A.Z across their coding regions (Zilberman et al. 2008; Coleman-Derr and Zilberman 2012). Substantial evidence suggests that the H2A.Z-mediated functions in gene bodies and promoters are different (Fujimoto et al. 2012).

Many studies have focused on dissecting the relationship between H2A.Z and transcription. Counterintuitively, H2A.Z enrichment at yeast promoters is simultaneously required for, and inversely correlated with, gene activity (Guillemette et al. 2005; Zhang et al. 2005; Millar et al. 2006). *PHO5*, a gene that is repressed on phosphate-rich media and active under low-phosphate conditions, shows a loss of H2A.Z upon transcriptional induction under phosphate deprivation (Millar et al. 2006). A return to phosphate-rich conditions resulted in transcriptional repression and increased H2A.Z occupancy (Millar et al. 2006). Similar results were found for the *GAL1* and *GAL10* loci, which require H2A.Z for transcriptional activation by galactose (Adam et al. 2001). Overall, a number of yeast genes required for growth under specific conditions show defects in transcriptional activation in an *h2a.z* mutant (Santisteban et al. 2000; Larochelle and Gaudreau 2003; Dhillon et al. 2006; Wan et al. 2009).

In contrast to this pattern in yeast, most studies in animals report that H2A.Z exhibits a positive correlation with transcription. In humans and *Drosophila*, H2A.Z enrichment at promoters correlates with the level of gene expression (Barski et al. 2007; Mavrich et al. 2008), and in humans H2A.Z has been shown to associate with gene promoters upon their induction and to aid in the recruitment of the transcriptional machinery (Hardy et al. 2009). However, some studies suggest a more complex relationship. H2A.Z is present at silent retinoic acid response (RAR) genes in mouse ES cells and is removed upon the transcriptional activation of these genes by retinoic acid (Amat and Gudas 2011). In *C. elegans* and the pufferfish *Tetraodon nigroviridis*, H2A.Z enrichment at promoters is positively correlated with transcription up to a point, after which the correlation becomes negative (Whittle et al. 2008; Zemach et al. 2010b).

Similar to the results from *C. elegans* and pufferfish, we have found that promoter H2A.Z enrichment and transcription display a roughly parabolic relationship in *Arabidopsis*, with H2A.Z at its highest in moderately transcribed genes (Zilberman et al. 2008). The presence of this correlation between H2A.Z and transcription level in plants and at least some animals suggests that it may be the ancestral relationship for eukaryotes. Interestingly, the relationship between transcription and H2A.Z enrichment within gene bodies in *Arabidopsis* shows a negative correlation, with the lowest expressed genes showing the greatest gene body enrichment of H2A.Z (Zilberman et al. 2008; Coleman-Derr and Zilberman 2012). Several studies in animals have noted that, unlike promoter enrichment of H2A.Z, the presence of H2A.Z within gene bodies is negatively correlated with transcription (Barski et al. 2007; Hardy et al. 2009; Zemach et al. 2010b). Given the distinct correlations between transcription and H2A.Z enrichment at promoters and gene bodies, it is likely that some of the apparent discrepancies among published transcriptional studies are attributable to differences in how H2A.Z enrichment was measured within genes.

DNA METHYLATION AFFECTS THE PATTERNS OF H2A.Z ENRICHMENT

The distribution patterns of DNA methylation and H2A.Z are quite different; whereas H2A.Z is primarily found at the 5′ ends of genes, DNA methylation is typically associated with TEs and other repetitive sequences and is generally confined to gene bodies. Not surprisingly, the global distribution patterns of DNA methylation and H2A.Z are strongly anticorrelated (Zilberman et al. 2008; Conerly et al. 2010). However, this anticorrelation exists at the local level as well, irrespective of the type of sequence. For example, the relatively few TEs that have escaped DNA methylation in *Arabidopsis* are enriched for H2A.Z. Similarly, genes with significant levels of DNA methylation are depleted of H2A.Z (Zilberman et al. 2008). The opposing distributions of these two chromatin marks suggest that one or both may be acting to prevent the incorporation of the other.

We have shown that changes in DNA methylation patterning in *Arabidopsis*, induced by a loss of the primary maintenance methyltransferase MET1, led to opposite changes in H2A.Z deposition (Zilberman et al. 2008). Although all TEs show a strong loss of CG DNA methylation in the *met1* background, only about half show transcriptional derepression; yet TEs are equally H2A.Z-enriched in *met1* plants regardless of expression, which strongly argues that changes in DNA methylation rather than changes in transcription cause the redistribution of H2A.Z in *met1* (Zilberman et al. 2008). In support of these results, experiments with human tumor cells showed that changes in DNA methylation associated with cancer progression are linked with opposite changes in H2A.Z levels (Conerly et al. 2010). A more recent study, also with human cancer cells, found that treatment with the demethylating agent 5-Aza-CDR leads to loss of DNA methylation and subsequent increases in H2A.Z at many loci (Yang et al. 2012).

Mounting evidence suggests that this relationship is unidirectional. Recently, we conducted whole-genome shotgun bisulfite sequencing in an *h2a.z* loss-of-function line in *Arabidopsis* and found that a loss of H2A.Z has only a minimal effect on genic methylation profiles (Coleman-Derr and Zilberman 2012). In support of this, Conerly et al. (2010) found almost no change in methylation at active gene promoters in the transition from wild-type to Em-MYC cells, despite a loss of H2A.Z from the promoters of these genes. Similarly, a study using siRNA knockdown of the human SWR1 homolog SRCAP found that reduction in H2A.Z levels had no effect on DNA methylation (Yang et al. 2012). Taken together, these results strongly suggest that the genome-wide anticorrelation between H2A.Z and DNA methylation is largely the result of DNA methylation acting to prevent the incorporation of H2A.Z into chromatin and that this is an ancient feature of eukaryotes.

H2A.Z REGULATES RESPONSIVE GENES

In contrast to the enrichment of DNA methylation in constitutively expressed genes, studies in many species have revealed an association between H2A.Z and various classes of inducible genes. Genes that are misregulated upon loss of H2A.Z in yeast or animals tend to be required only in specific environmental conditions or tissue types, respectively (Adam et al. 2001; Millar et al. 2006; Updike and Mango 2006; Creyghton et al. 2008; Whittle et al. 2008; Amat and Gudas 2011; Petter et al. 2011; Sadeghi et al. 2011). In *Arabidopsis*, plants lacking PIE1 exhibit misregulation of many genes involved in the innate immune response (March-Diaz et al. 2008). Recent work has shown that *Arabidopsis* plants with a mutated copy of *ARP6*, a component of the PIE1 complex, inappropriately express temperature response genes, which leads to the proposal that H2A.Z acts specifically as a thermosensor in plants (Kumar and Wigge 2010). Other experiments with *arp6* mutants have shown that H2A.Z is deposited at a number of phosphate starvation response genes and that a loss of H2A.Z leads to their derepression (Smith et al. 2009).

Our functional analysis of the genes up-regulated in an *Arabidopsis h2a.z* loss-of-function mutant revealed a significant overrepresentation for response genes in general (Coleman-Derr and Zilberman 2012). In fact, all of the top overrepresented gene ontology terms corresponded to "response" categories (Coleman-Derr and Zilberman 2012). Importantly, although all three previously reported response-related categories (innate immune response, response to temperature stimulus, and response to phosphate starvation) shown to be misregulated in *arp6* and *pie1* mutants are overrepresented in our data set, there are also many examples of unrelated response categories, including response to wounding, water, carbohydrate stimulus, endogenous stimulus, salt stress, and hormone stimulus (Coleman-Derr and Zilberman 2012). This result demonstrates that a loss of H2A.Z in plants is likely to lead to misregulation of response genes in general, rather than a specific category of response genes.

In a similar vein, we found that the degree of transcriptional misregulation in loss-of-function *h2a.z* plants correlated with levels of gene responsiveness, with the most misregulated genes tending to be the least constitutively expressed (Coleman-Derr and Zilberman 2012). Additionally, we found that the degree of misregulation in *h2a.z* directly correlates with the level of H2A.Z body enrichment and that the presence of H2A.Z within gene bodies is positively associated with measures of gene responsiveness (Coleman-Derr and Zilberman 2012). In agreement with our work, analyses of H2A.Z levels in yeast genes demonstrated significant enrichment in genes that are differentially expressed after environmental stresses (Sadeghi et al. 2011). Taken together, these findings suggest that the presence of H2A.Z within gene bodies promotes variability of levels and patterns of expression and may antagonize constitutive gene expression. Although the mechanism by which H2A.Z exerts this effect remains unclear, we hypothesize that the presence of H2A.Z in gene bodies leads to a more open chromatin state, altering the accessibility of the coding sequence to binding factors that influence the transcriptional state and rate.

CONCLUSIONS

The discovery of gene body methylation in many diverse groups of eukaryotes has challenged the hypothesis that DNA methylation functions primarily to silence parasitic DNA sequences and has opened the door to a broader evolutionary understanding of epigenetics in the context of gene regulation. Mounting evidence from plants and animals suggests that the anticorrelation between DNA methylation and H2A.Z is caused by the exclusion of H2A.Z from methylated DNA. Therefore, we propose that one basal function of genic DNA methylation is the stabilization of housekeeping gene expression by antagonizing H2A.Z deposition. Because H2A.Z has been linked to the expression of inducible genes in

many species (Adam et al. 2001; Millar et al. 2006; Zanton and Pugh 2006; Creyghton et al. 2008; Whittle et al. 2008; Smith et al. 2009; Kumar and Wigge 2010; Amat and Gudas 2011; Petter et al. 2011; Sadeghi et al. 2011), including species such as *S. cerevisiae* and *C. elegans* that lack DNA methylation, H2A.Z is a more plausible candidate for a direct regulator of gene responsiveness than DNA methylation.

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