A Chromodomain-Helicase-DNA-Binding Factor Functions in Chromatin Modification and Gene Regulation[^1]

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Proteins in the Chromodomain-Helicase/ATPase-DNA-binding domain (CHD) family are divided into three groups. The function of group I CHD proteins in nucleosome positioning is well established, while that of group II members (represented by CHD3/Mi2) remains unclear. Using high-throughput approaches, we investigated the function of the group II rice (*Oryza sativa*) CHD protein CHR729 in nucleosome positioning, gene expression, histone methylation, and binding. Our data revealed that the *chr729* mutation led to increased nucleosome occupancy in the rice genome and altered the expression and histone H3K4me3 modification of many, mainly underexpressed, genes. Further analysis showed that the mutation affected both the deposition and depletion of H3K4me3 in distinct chromatin regions, concomitant changes in H3K27me3 modification.

Genetic and genomic analyses revealed that CHR729 and JMJ703, an H3K4 demethylase, had agonistic, antagonistic, and independent functions in modulating H3K4me3 and the expression of subsets of genes. In addition, CHR729 binding was enriched in H3K4me3-marked genic and H3K27me3-marked intergenic regions. The results indicate that CHR729 has distinct functions in regulating H3K4me3 and H3K27me3 modifications and gene expression at different chromatin domains and provide insight into chromatin regulation of bivalent genes marked by both H3K4me3 and H3K27me3.

Chromatin structure is regulated by DNA methylation, histone modifications, and chromatin remodeling. Chromatin-remodeling factors are molecular motors that use the energy from ATP hydrolysis to slide nucleosomes along or off DNA, thereby regulating the accessibility of the underlying DNA to various nuclear factors [Narlikar et al., 2013]. In multicellular eukaryotes, there are several well-conserved but functionally diverse families of ATP-dependent chromatin-remodeling enzymes [Flaus et al., 2006], among which are the Chromodomain-Helicase/ATPase-DNA-binding domain (CHD) proteins [Ho and Crabtree, 2010; Murawska and Brehm, 2011]. Besides yeast (*Saccharomyces cerevisiae*) that has only one CHD protein (Chd1), other higher eukaryotes have several CHD members. In human (*Homo sapiens*), this family has nine members, classified into three groups according to their domain similarity: group I (CHD1 and CHD2), group II (CHD3/Mi2, CHD4, and CHD5), and group III (CHD6, CHD7, CHD8, and CHD9; Murawska and Brehm, 2011).

The yeast Chd1 was shown to be important for maintaining nucleosome structure over transcription units, and its mutants exhibit a high degree of aberrant nucleosomal structures [Gkikopoulos et al., 2011; Hennig et al., 2012; Pointner et al., 2012; Zentner et al., 2013]. Chd1 binds to highly transcribed genes marked by H3K36me3 [Park et al., 2014; Skene et al., 2014]. Moreover, human CHD1 specifically recognizes H3K4me3 and is considered as an effector of this active histone modification [Sims et al., 2007; Lin et al., 2011]. A recent study suggests that CHD1 plays a role to maintain the boundaries between H3K4me3 and H3K36me3 in highly transcribed genes [Lee et al., 2017].

Group II CHD proteins are characterized by the presence of one or two plant homeodomain fingers at the N terminus. Group II proteins CHD3/Mi-2, CHD4, and CHD5 in fruit fly (*Drosophila melanogaster*) and mammalian cells are interchangeable components of the nucleosome remodeling and histone deacetylase complexes regulating transcriptional repression [Zhang et al., 1998; Millard et al., 2016; Hoffmeister et al., 2017]. The gene activation function of CHD3/Mi2 was also reported [Murawska et al., 2008; Aichinger et al., 2009]. The mechanisms by which group II proteins remodel nucleosome in vitro have been studied in human and fruit fly. CHD3 and CHD4 in human display...
differential preference for center-positioned and end-positioned nucleosomes (Hoffmeister et al., 2017), whereas dMi2 only slide end-positioned nucleosomes to a center position, which is analogous to yeast Chd1 activity in spacing nucleosomes in the gene body in vivo (Brehm et al., 2000; Stockdale et al., 2006; Ocampo et al., 2016). However, in vivo remodeling activity of group II proteins has not been demonstrated yet. Group III proteins were reported to act both as transcriptional coactivators and corepressors in humans (Nishiyama et al., 2009; Bajpai et al., 2010).

In plants, several CHD genes were identified. They belong only to groups I and II, and their polygenetic relationship has been analyzed (Hu et al., 2012). The Arabidopsis (Arabidopsis thaliana) group I protein CHD-RELATED5 (CHR5) was shown to enhance the expression of genes required for embryogenesis by reducing nucleosome levels near the transcriptional start site (TSS; Shen et al., 2015). The Arabidopsis group II CHD3/Mi2-like protein PICKLE (PKL) was initially found to repress embryonic traits after seed germination (Ogas et al., 1997). In contrast to animal CHD3/Mi2 proteins that mediate histone deacetylation, PKL was found to promote H3K27me3 over target genes (Zhang et al., 2008a; Carter et al., 2018) and was not found to be in a protein complex (Ho et al., 2013). CHR4 is phylogenetically distinct from PKL and has divergent domain architecture (Hu et al., 2012; Ho et al., 2013). Besides, the SANT domain, which is possessed by all CHD3 proteins and presumably is responsible for DNA binding, is absent in CHR4 (Ho et al., 2013). The depletion of CHR4 does not affect either plant normal development or the pkl phenotype (Aichinger et al., 2009). However, recent data revealed that CHR4 mediates the response to endogenous flowering pathways in the inflorescence meristem to promote floral identity (Sang et al., 2020). The rice (Oryza sativa) group II CHD3/Mi2-like protein CHR729 (closely related to CHR4) was shown to bind to H3K4me2 and H3K27me3 respectively through the chromodomains and the plant homeodomain finger of the protein (Hu et al., 2012). In contrast to the mild phenotypes of pkl (embryonic traits in seedling) and chr4 (delayed flowering time in a quintuple mutant) in Arabidopsis, the CHR729 loss-of-function mutation affects many aspects of plant development, including seedling growth, leaf morphology, cuticle wax biosynthesis, root development, and chloroplast function (Hu et al., 2012; Zhao et al., 2012; Ma et al., 2015; Wang et al., 2016; Guo et al., 2019). A recent study showed that CHR729 is required for H3K4me3 and the expression of reactive oxygen species-related genes (Cho et al., 2018). Thus, CHR729 appears as an important chromatin factor required for rice gene expression and plant growth. However, the precise mechanism by which CHR729 regulates chromatin modification and gene expression is currently unclear.

In this work, we studied the function of CHR729 in nucleosome occupancy, histone methylation, and gene expression by using several genome-wide high-throughput approaches. Our results indicate that CHR729 represses nucleosome accumulation but has a distinct function in H3K4m3 and gene expression at different chromatin domains.

**RESULTS**

CHR729 Represses Nucleosome Occupancy in the Genic Region

To study the chromatin function of CHR729 in gene expression and nucleosome positioning, we performed RNA sequencing (RNA-seq) and nucleosome profiling of wild-type and chr729 12-d-old seedling tissues. For RNA-seq, three biological replicates were performed, and more than 26 million reads per sample were obtained. The mapping rate was more than 85%, and the three biological replicates were highly correlated (Supplemental Fig. S1; Supplemental Table S1). More than 20,000 expressed genes (fragments per million per kilobase [FPKM] > 1) were detected in the wild type and chr729 (Supplemental Table S1). For nucleosome profiling, chromatin isolated from rice seedling tissues was digested with micrococcal nuclease (MNase) to create nuclease-resistant DNA ladders of about 200 bp and was analyzed by Illumina paired-end DNA sequencing. Two biological replicates were performed. More than 40 million micrococcal nuclease sequencing (MNase-seq) reads per sample were obtained, and the overall alignment rates were more than 98% (Supplemental Table S2). About 95,000 nucleosomes per sample were identified using iNPS software (Chen et al., 2014).
To examine the nucleosome profile at the genome-wide level, we first aligned all rice protein-coding genes (n = 39,049) relative to the TSS and obtained identical nucleosome distribution profiles from independent biological replicates (Fig. 1A; Supplemental Fig. S2A). A prominent +1 nucleosome was found downstream of the TSS, which was followed by four clearly phased nucleosomes. A clear nucleosome-free region (NFR) was observed upstream of the TSS. Nucleosome phasing at the promoters was less clear. Comparison with the RNA-seq data revealed a positive association of gene expression with the depth of NFR and the level of nucleosome phasing in the coding region but a negative association of gene expression with nucleosome enrichment at the −1 position in rice (Fig. 1B). In TEGs, the overall nucleosome level was low (Fig. 1A), which was likely due to a low MNase digestion efficiency of heterochromatin regions (Zhang et al., 2012b). The nucleosome profiles and features were consistent with previously reported nucleosome organization in Arabidopsis (Li et al., 2014; Pass et al., 2017) and rice (Zhang et al., 2018).

The chr729 mutation resulted in a general elevation of nucleosome occupancy in the genic region (Fig. 1A; Supplemental Fig. S2A), while it did not clearly affect nucleosome phasing relative to the TSS or the distance between nucleosomes. In TEGs, the chr729 mutation also increased the nucleosome levels (Fig. 1A; Supplemental Fig. S2A). This suggests that, unlike CHD1 (group I) that is required for nucleosome enrichment at +1 and subsequent positions (Gkikopoulos et al., 2011), CHR729 functions to repress nucleosome occupancy.

RNA-seq analysis revealed 1,000 up-regulated and 435 down-regulated differentially expressed genes (DEGs) in chr729 compared with the wild type (Fig. 1C), which corresponded mainly to otherwise lowly expressed genes (see below). Among the DEGs, there were 303 TEGs (193 up and 110 down), 18 of which were close (within 5 kb distance) to misregulated genes. We then analyzed the nucleosome profile of the DEGs, which revealed no clear nucleosome phasing as observed with all non-TE genes (Fig. 1, A and D). This may be because a regular nucleosome pattern can only be obtained from a large number of genes. However, both up- and down-regulated DEGs showed higher nucleosome enrichment in the gene body in the mutant compared with the wild type (Fig. 1D; Supplemental...
Fig. S2B), suggesting that the differential gene expression was not directly related to changes of nucleosome occupancy in the mutant.

CHR729 Regulates Gene Expression by Modulating H3K4me3

To investigate whether chr729-affected gene expression was connected to histone methylation changes in the mutant, we performed chromatin immunoprecipitation sequencing (ChIP-seq) analysis of chr729 and wild-type 12-d-old seedlings using H3K4me3 and H3K27me3 antibodies. Two biological replicates were performed and more than 20 million reads per sample were obtained. The overall mapping rate of ChIP-seq reads was about 80%, and the two biological replicates are highly correlated (Supplemental Table S3). The ChIP-seq analysis revealed 24,626 H3K4me3-marked genes and 7,482 H3K27me3-marked genes in wild-type plants, consistent with previous data (Liu et al., 2015). We first examined the modification levels in all non-TE genes and TEGs and found that the overall level of H3K4me3 was not altered, but that of H3K27me3 in the gene body was slightly reduced in the mutant (Fig. 2A; Supplemental Fig. S3A). Then we investigated H3K4me3 and H3K27me3 levels in chr729 DEGs. Metaplot analysis revealed some increase of H3K4me3 in the up-regulated DEGs but a drastic decrease of the mark in the down-regulated DEGs in chr729 compared with the wild type (Fig. 2B; Supplemental Fig. S3B). By contrast, the H3K27me3 level in the chr729 DEGs was not clearly affected by the mutation (Fig. 2B; Supplemental Fig. S3B). Quantitative analysis of the histone methylation level in each DEG revealed a positive correlation ($r = 0.585$) between H3K4me3 and gene expression changes in chr729, while no significant correlation was found between H3K27me3 and gene expression changes in the mutant (Fig. 2C). These results suggest that CHR729-regulated gene expression involves H3K4me3.

Next, we performed quantitative analysis to identify differential H3K4me3 peaks (TPM fold change $>2$, $P < 0.01$) in chr729 compared with the wild type. In total, 759 hyper-H3K4me3 and 1,283 hypo-H3K4me3 peaks were identified in the mutant (Fig. 3A). In the wild-type background, the H3K4me3 level of the hyper-peaks was low, while that of the hypo-peaks was high (Fig. 3B), suggesting that CHR729 may promote either the deposition or depletion of H3K4me3 in different

**Figure 2.** chr729-affected gene expression is associated with H3K4me3. A, Metaplots of H3K4me3 and H3K27me3 profiles near non-TE genes and TEGs in the wild type (wt) and chr729. B, Metaplots of H3K4me3 and H3K27me3 profiles near chr729 DEGs. C, Scatterplot showing the relationship between gene expression change and histone modification change in chr729 DEGs. The x axis represents gene expression level (FPKM fold change (chr729/wild type)), and the y axis represents H3K4me3 or H3K27me3 level (tags per million mapped tags [TPM] value in the gene body) fold change (chr729/wild type).
chromatin regions. However, it is not excluded that some of the changes are outcomes of gene expression changes. To study whether CHR729-dependent H3K4me3 change was connected with H3K27me3, we analyzed H3K27me3 level in the chr729 differential H3K4me3 peaks in both the wild-type and chr729 backgrounds. In the wild-type background, the H3K27me3 level was much lower in the hyper-H3K4me3 peak than in the hypo-peak regions (Fig. 3B). In the chr729 background, a substantial increase of H3K27me3 was observed in the hyper-H3K4me3 peaks while a decrease of H3K27me3 was detected in the hypo-H3K4me3 peaks (Fig. 3B; Supplemental Fig. S4). The analysis suggested that CHR727 represses H3K4me3 in chromatin regions with low levels of H3K4me3 and H3K27me3 but promotes H3K4me3 in regions modified by both marks at relatively higher levels.

**Relationship between CHR729 and JMJ703 in Regulating H3K4me3**

JMJ703 is a previously characterized H3K4me3 demethylase and is required for rice development (Chen et al., 2013; Liu et al., 2015). CHR729 and JMJ703 were shown to antagonistically regulate gene expression by controlling H3K4me3 and H3K27me3 at an epiallele produced during tissue culture (Chen et al., 2015). JMJ703 was shown to also regulate H3K27me3 (Liu et al., 2015). To study the functional relationship between JMJ703 and CHR729 in genome-wide histone methylation, we performed H3K4me3 and H3K27me3 ChIP-seq analysis of the jmj703 mutant (Chen et al., 2013) using the same tissues and antibodies as for chr729. Two biological replicates were performed with high reproducibility (Supplemental Table S3). Genome-wide metaplot analysis revealed no overall alteration of H3K4me3 or H3K27me3 in jmj703 (Supplemental Fig. S5). Quantitative study using the same method as for chr729 uncovered 465 hyper-H3K4me3 peaks but only 14 hypo-H3K4me3 peaks in jmj703 (Fig. 3A), consistent with the H3K4me3 demethylase activity of the protein. Among the 465 jmj703 hyper-H3K4me3 peaks, 123 corresponded to the chr729 hyper-H3K4me3 peaks (Fig. 3C), indicating a partial overlap between CHR729 and JMJ703-repressed H3K4me3 regions.

Then, we made the chr729/jmj703 double mutant by genetic crosses. The double mutant showed a more severe phenotype than the single mutants at the mature stage (Supplemental Fig. S6), suggesting that the developmental functions of the two genes are not identical. We performed H3K4me3 and H3K27me3 ChIP-seq analysis of the double mutant using the same tissues...
and conditions as for the single mutants, two biological replicates were performed, and the data were qualified (Supplemental Table S3). The double mutant showed 816 hyper-H3K4me3 and 905 hypo-H3K4me3 peaks relative to the wild type (Supplemental Fig. S7). Among the 123 overlapping hyper-H3K4me3 peaks detected in the single mutants, 111 remained to be hypermethylated in the double mutant (Fig. 3C), confirming that CHR729 and JMJ703 are both required to repress H3K4me3 in these genomic regions. Besides, 355 hyper-H3K4me3 peaks specific to chr729 and 198 hyper-H3K4me3 peaks specific to jmj703 also remained to be hypermethylated in the double mutant (Fig. 3, C and D), indicating that CHR729 and JMJ703 independently repress H3K4me3 in these regions. However, 281 hyper-H3K4me3 peaks specific to chr729 and 144 hyper-H3K4me3 peaks specific to jmj703 were no longer identified in the double mutant (Fig. 3, C and D), suggesting that CHR729 and JMJ703 play antagonistic roles in regulating H3K4me3 in these regions. As expected, most (787 out of 1,283) of the chr729 regions. As expected, most (787 out of 1,283) of the chr729 peaks still were not detected in the double mutant. However, 489 of the chr729 hypo-H3K4me3 peaks still were not detected in the double mutant (Fig. 3C). One hypothesis would be that CHR729 promotes H3K4me3 in these regions by preventing JMJ703 access or activity. H3K4me3 levels in some of the peaks were lower in the double mutant than in the wild type (Fig. 3D), suggesting that other histone demethylases might be also involved. Collectively, the analysis suggests that, depending on genomic regions, CHR729 and JMJ703 may have agonistic, antagonistic, or independent functions in H3K4me3 regulation.

CHR729 and JMJ703 Oppositely Regulate the Expression of a Subset of Genes

To study the functional relationship between CHR729 and JMJ703 in gene expression, we performed RNA-seq analysis of jmj703 and chr729/jmj703 plants using the same tissues and conditions as for chr729. We obtained more than 20 million reads per sample with a mapping rate of about 90% (Supplemental Table S1). The three biological replicates of RNA-seq data showed high reproducibility (Supplemental Fig. S8). The analysis revealed that the jmj703 mutation affected relatively few (146 up-regulated and 53 down-regulated) genes (Supplemental Fig. S9A), consistent with previous results (Cui et al., 2013). The jmj703 up-regulated DEGs showed increased H3K4me3, but the down-regulated DEGs displayed no obvious change of the mark (Fig. 4A; Supplemental Fig. S9B). The double mutations resulted in up-regulation of 2,212 genes and down-regulation of 481 genes compared with the wild type (Supplemental Fig. S10A). Most (more than two-thirds) of the chr729 or jmj703 DEGs overlapped with that of the double mutant (Supplemental Fig. S10B). Scatterplot analysis of the chr729 DEGs against their expression changes in the double mutant revealed that about one-third (146 out of 434) of the chr729 down-regulated DEGs were up-regulated and 187 of the chr729 up-regulated DEGs were down-regulated in the double mutant relative to chr729 (Fig. 4B). These (333 in total) genes are likely to be oppositely regulated by CHR729 and JMJ703 and are defined hereafter as reversely regulated DEGs. As expected, the expression level of the reversely regulated DEGs in the double mutant was similar (or restored back) to the wild-type level, but the other double mutant DEGs showed a similar expression level to that in chr729 (Fig. 4C). In addition, we noticed that the expression level of the chr729 up-regulated DEGs in the wild type was much lower than in the down-regulated DEGs (Fig. 4C), suggesting that CHR729 contributes to the repression of lowly expressed or silent genes but is required for the expression of more active genes.

The overall change of H3K4me3 levels in the reversely regulated DEGs in chr729 versus the wild type was reversely correlated (r = −0.957) with that in the double mutant versus chr729 (Fig. 4D), suggesting that H3K4me3 is a key factor in the expression of genes oppositely regulated by CHR729 and JMJ703. Especially, the H3K4me3 level of the down-regulated group of the reversely regulated DEGs was clearly reduced in chr729, but it was restored back to the wild-type level in the double mutant (Fig. 4E; Supplemental Fig. S11A). Other down-regulated genes also showed lower H3K4me3 in chr729 than in the wild type and jmj703. This was likely a consequence of their reduced expression in chr729, as their H3K4me3 level remained lower in the double mutant (Fig. 4E). These data indicate that the CHR729-promoted expression of the reversely regulated genes is dependent on H3K4me3 deposition and is opposed by JMJ703-mediated demethylation. Increase of H3K4me3 was detected in the up-regulated group of the reversely regulated DEGs in chr729, which was reduced back to the wild-type level in the chr729/jmj703 double mutant (Supplemental Fig. S11B). By contrast, neither chr729 nor the double mutation had a clear effect on H3K27me3 in the chr729 DEGs (Supplemental Fig. S11, C and D). Together, these data indicate that CHR729 and JMJ703 antagonistically regulate the expression of a subset of genes by modulating H3K4me3.

CHR729 Up- and Down-Regulated H3K4me3 Regions Display Distinct Chromatin Features

Next, we asked whether CHR729-regulated H3K4me3 was present in a specific chromatin context. We collected published rice chromatin modification data in wild-type plants, along with the nucleosome and RNA-seq data of the wild type obtained in this study, to plot against the chr729 differential H3K4me3 peaks. We found that compared with the genome average levels, the chr729 hyper-H3K4me3 peaks displayed much lower levels of H3K4me3 and H3K27me3 and DNase accessibility at the peak shoulders (Fig. 5A). A small nucleosome peak was detected in the hyper-H3K4me3 peak regions, but the overall nucleosome level was slightly lower than the average. By contrast, chr729 hypo-H3K4me3 peaks
showed the average level of H3K4me3 but a much higher than average level of H3K27me3 in the peaks and the flanking regions. In addition, nucleosome density showed a clear decrease in the hypo-H3K4me3 peaks. Thus, CHR729 up- and down-regulated H3K4me3 regions displayed distinct chromatin modification features. It is worth noting that the levels of H3K4me2 and H3K4me1 in the chr729 hyper-H3K4me3 and hypo-H3K4me3 peaks were both lower than the averages, but H3K4me1 showed a sharp decrease in the hypo-H3K4me3 peaks. No clear difference of H2A.Z and histone acetylation but slightly higher H3K9me2 levels were detected in the chr729 hyper-H3K4me3 and hypo-H3K4me3 peaks compared with genome averages (Fig. 5A). The transcription (mRNA) level was lower in both the hyper-H3K4me3 and hypo-H3K4me3 peaks compared with the average, and that in hyper-peaks was extremely low (Fig. 5A), confirming that CHR729 mainly regulates underexpressed genes (Hu et al., 2012).

**CHR729 Binding Is Detected in Both Genic and Intergenic Regions**

To study CHR729-binding profiles in the genome, we transformed wild-type rice plants with pCHR729:CHR729-sGFP (Supplemental Fig. S12). We used the transgenic seedlings and anti-GFP for ChIP-seq analysis to detect CHR729-binding sites. More than 28 million reads were obtained, and 3,427 CHR729-binding peaks were identified by MACS (Zhang et al., 2008b). The relatively low number of binding peaks might be partly due to competitive binding of the endogenous CHR729.

Among the 3,427 CHR729-binding peaks, 1,899 were located in the gene body or promoter (within the TSS upstream 2 kb) regions and 1,428 in intergenic (greater than 2 kb from the TSS) regions (Fig. 5B). The genic CHR729 peaks showed a high H3K4me3 level, while the intergenic CHR729-binding peaks showed high levels of H3K27me3 (Fig. 5C), which is consist with our previous finding that CHR729 binds to H3K27me3 in vitro (Hu et al., 2012). About one-third (1,345 out of 3,913) of H3K27me peaks in the wild type are found in the intergenic regions, which likely correspond to regulatory elements (Lu et al., 2019; Ricci et al., 2019). H3K27me3 enrichment in CHR729 intergenic peaks suggests that CHR729 may be involved in the activity of regulatory elements in the rice genome. We observed strong CHR729-binding signals in regions corresponding to the hyper-H3K4me3 peaks (Fig. 5A). By contrast, the enrichment of CHR729 binding was weak in the hypo-H3K4me3 peak regions (Fig. 5A). We hypothesize that CHR729 may directly bind to and repress H3K4me3 in chr729 hyper-peak regions but may indirectly promote H3K4me3 in chr729 hypo-peak regions, which showed the genome average level of
H3K4me3, higher than the average level of H3K27me3 and depletion of H3K4me1 (Fig. 5A).

**DISCUSSION**

CHD1 (group I) has been shown to play an important role for maintaining nucleosome structure over transcription units (Gkikopoulos et al., 2011; Hennig et al., 2012; Pointner et al., 2012; Zentner et al., 2013). A recent study indicated that loss of function of a group III CHD protein causes an increase of intragenic nucleosome spacing and deregulation of gene expression in the genus Dictyostelium (Platt et al., 2017). Our results here revealed that the rice group II protein CHR729 had a function to reduce nucleosome levels over the genic regions without clearly affecting nucleosome spacing or phasing, indicating that group II CHD proteins may...
play a distinct role in regulating nucleosome occupancy. However, the effect of chr729 mutation on nucleosome occupancy was relatively subtle. We speculate that this may be due to functional redundancy, as rice has three CHD3 proteins: CHR729, CHR702, and CHR703 (Hu et al., 2012, 2014). CHR729 mutation caused a general increase of nucleosome occupancy on both up- and down-regulated DEGs, suggesting that nucleosome occupancy change alone may be insufficient to lead to a clear change of gene expression. Similarly, it was shown that mutation of Chd1 alters genome-wide nucleosome arrangement but has less effect on gene expression. The function of CHR729 in gene expression may result from a combination of its actions in chromatin, such as histone modification. Indeed, an Arabidopsis CHD3 protein, namely CHR4, was shown to interact with histone modification enzymes (Sang et al., 2020).

It was shown that the Arabidopsis CHD3 protein PKL promotes H3K27me3 for gene repression (Zhang et al., 2008a, 2012a; Carter et al., 2018). However, PKL also reduces H3K27me3 at specific target genes in particular tissues and environments (Jing et al., 2013). In this work, we showed that CHR729 promoted the deposition and depletion of H3K4me3 in distinct chromatin regions in rice, which associated with gene expression. Thus, it appears that CHR729 has a divergent function compared with PKL. CHR729-modulated depletion of H5K4me3, as well as H3K27me3, was mainly detected in lowly expressed regions, and CHR729-promoted H3K4me3 and H3K27me3 were detected in moderately expressed regions (Figs. 3A and B, and 5A; Supplemental Fig. S4), suggesting that CHR729 has distinct functions in different chromatin regions. A recent study showed that the Arabidopsis chr4 mutation also results in large numbers of hyper-methylated and hypomethylated regions of H3K27me3 and H3K4me3 in the genome. In addition, a number of genes including the flowering-time regulators are differentially marked by both H3K27me3 and H3K4me3 in the Arabidopsis chr4 mutant. The wheat (Triticum aestivum) CHR729, TaCHR729, was shown to be recruited by a basic helix-loop-helix-type transcription factor to the promoter of TaKC56 to promote H3K4me3 (Wang et al., 2019). The rice and wheat CHR729 proteins are closely related to Arabidopsis CHR4 (Mansfield et al., 2011; Hu et al., 2012), supporting the hypothesis that plant CHR4 homologs play a role in modulating both H3K4me3 and H3K27me3.

The data presented here indicate that CHR729-mediated H3K4me3 deposition and depletion regions display distinct chromatin features. The chr729 hyper-H3K4me3 peaks displayed background levels of H3K4me3 (as well as H3K27me3) and gene expression (Fig. 5A), while the chr729 hypo-H3K4me3 peak regions showed high levels of H3K4me3 and H3K27me3 marks and moderate gene expression levels. The observation that CHR729 binding was enriched in chr729 hyper-H3K4me3 regions indicates that CHR729 is directly involved in the inhibition of H3K4me3 in these regions. Whether CHR729 binding alters chromatin structure and affects the access of histone methylation writers or erasers to inhibit H3K4me3 remains to be investigated. The interaction of CHR4 and SDC29, an H3K4 methyltransferase, has been demonstrated in Arabidopsis, suggesting that the remodeler may recruit writers to promote H3K4me3 at targeted loci (Sang et al., 2020). The observation that hyper-H3K4me3 peaks detected in jmj703 single and chr729/jmj703 double mutants partly overlapped with that detected in chr729 (Fig. 3, C and D) indicates that the histone demethylase JM1703 is one of the H3K4me3 inhibitors in those regions. H3K4me3 and H3K27me3 respectively mark active and silent genes but jointly mark so-called bivalent genes. The concomitant change of H3K27me3 and H3K4me3 in chr729 indicates that CHR729 plays a role in the establishment of bivalent genes, suggesting that CHR729-regulated chromatin structure might allow access of methyltransferases or prevent that of demethylases of the two marks in rice. One possibility is that CHR729-mediated repression of nucleosome is involved in marking and/or the expression of bivalent genes, as the genomic regions corresponding to chr729 hypo-H3K4me3 peaks showed a low nucleosome level (Fig. 5A), which is augmented in bivalent genes in the chr729 mutant (Supplemental Fig. S13). Indeed, our data indicate that CHR729 promotes H3K4me3 (as well as H3K27me3) and the expression of bivalent genes (Fig. 5A), part of which is achieved by opposing JMJ703 (Fig. 3). Similarly, in the Arabidopsis chr4 mutant, a substantial number of genes including the flowering-time regulators are differentially marked by both H3K27me3 and H3K4me3 (Sang et al., 2020). As bivalent genes are in many cases poised for activation by cellular stimuli, one possible mechanism would be that CHR729 binding may promote the expression of these genes by reducing intragenic nucleosome levels during plant responses to internal and external stimuli. The results showing that Arabidopsis CHR4 mediates the response to endogenous flowering pathways in the inflorescence meristem to promote floral identity (Sang et al., 2020) are in favor of this hypothesis.

MATERIALS AND METHODS

Plant Material and Treatments

Rice (Oryza sativa spp. japonica) plants were germinated and grown on hormone-free, one-half-strength Murashige and Skoog medium under 16/8 h of light/dark at 30°C/25°C. The previously characterized mutant lines chr729 (Hu et al., 2012) and jmj703 (Chen et al., 2013) were used in this study. Pchr729::CHR729::sGFP plasmid (Zhao et al., 2012) was transformed into the Hwayoung variety of rice by Agrobacterium tumefaciens-mediated rice transformation. Transgenic plants were confirmed by detecting DNA fragments and transcripts of GFP. PCR was performed using sGFP primers (forward primer, 5′-AGGAGAACCGCcatAACGGGT-3′; reverse primer, 5′-GAACACCGCA GACACTG-3′). Actin (forward primer, 5′-GGCCACTCCACCTGATCCAT-3′; reverse primer, 5′-GCCACCCCATGATCTTCAT-3′) was detected as a control. Seedling leaves of all samples were harvested at 12 d after germination for chromatin and total RNA extraction. For each RNA-seq sample, three biological replicates were performed with six plants in each replicate. For H3K4me3 and H3K27me3 ChIP-seq samples, two biological replicates were
performed with 25 to 30 plants in each replicate. For MNase-seq, two biological replicates were performed with 12 plants in each replicate.

RNA-Seq and Data Analysis

RNA samples were isolated using TRIzol reagent (Invitrogen). The RNA-seq libraries were prepared using the Illumina TruSeq RNA Sample Preparation Kit and sequenced on Illumina HiSeq 2000 with the PE150 (paired-end 150 nucleotide) method. RNA-seq data were filtered by Trimmomatic (version 0.33) to remove contaminations and low-quality reads. Hisat2 (version 2.1.0; Kim et al., 2015) was used to map clean reads to the rice genome (MSU7.0; http://rice.plantbiology.msu.edu/), featureCounts (v2.0.0; Liao et al., 2014) and DESeq2 (v1.26.0; Love et al., 2014) were used to calculate DEGs with adjusted P < 0.05 and fold change > 2.

MNase-Seq and Data Analysis

The nuclear extraction and MNase treatment were performed according to previously described methods (Zhang et al., 2012b; Li et al., 2014) with modifications. For nuclear extraction, 12-old seedling leaves grown on one-half-strength Murashige and Skoog medium were ground into powder in liquid nitrogen, suspended in nuclear isolation buffer (20 mm Tris-HCl, 50 mm EDTA, 5 mm spermine, 0.15 mm spermidine, 0.1% [v/v] mercaptoethanol, and 40% [v/v] glycerol at pH 7.5), and used for nuclei isolation by following standard protocols. The prepared nuclei pellet was suspended in MNase buffer with 2 units mL⁻¹ MNase for 5 min at 37°C. Mononucleosome-sized DNA was collected for sequencing as described previously (Zhang et al., 2013). A 100- to 200-bp DNA fragment was excised from a 2% (w/v) agarose gel, purified for Illumina library construction, and sequenced in the paired-end mode using the Illumina platform.

Trimmomatic (version 0.32) was used to filter out low-quality reads. Clean reads were mapped to the rice genome (MSU7.0; http://rice.plantbiology.msu.edu/) by Bowtie2 (version 2.2.5), allowing up to two mismatches. SAMtools (version 0.1.19) was used to remove potential PCR duplicates, and reads with low mapping quality (less than 20) were also discarded. Nucleosome positions were identified using iNPS software (Chen et al., 2014) with default parameters. Nucleosome occupancy was determined as averaged reads per million mapped reads. In drawing nucleosome distribution curves, the TSS/transcription termination site ± 1-kb regions were divided into 20-bp bins, and the average tag density in each bin was plotted.

Chromatin Immunoprecipitation

Two grams of rice seedling leaves was cross-linked by 1% (v/v) formaldehyde and used for chromatin extraction. Chromatin was fragmented to 500 bp by sonication and then incubated with antibody-coated beads (Invitrogen/Life Technologies; 10001D) overnight. After extensive washing, immunoprecipitated chromatin was de-cross-linked and retrieved for sequencing, and unimmunoprecipitated chromatin was used as input. Anti-H3K4me3 (Abcam; ab8980), H3K27me3 (Abclonal; A16199), and GFP (Abcam; ab2900) antibodies were used.

ChIP-Seq and Data Analysis

DNA from chromatin immunoprecipitation was used to construct sequencing libraries following the protocol provided by the Illumina TruSeq ChIP Sample Prep Set A and sequenced on Illumina HiSeq 2000 or HiSeq 2500. Trimmomatic (version 0.32) was used to filter out low-quality reads. Clean reads were mapped to the rice genome (MSU7.0; http://rice.plantbiology.msu.edu/) by Bowtie2 (version 2.2.5), allowing up to two mismatches. SAMtools (version 0.1.19) was used to remove potential PCR duplicates. MACS software (version 1.4.2; Zhang et al., 2008b) was used to call histone modification peaks by default parameters (bandwidth, 300 bp; model fold, 10, 30; P = 1.00e-5), and the input sample was used as a control. Differential peaks were calculated using the HOMER (v4.11) software package (Heinz et al., 2010), by fold change > 2 and P < 0.01. Heat maps were generated by the R package heatmap and deepTools2.0 (Ramírez et al., 2016).

H3K4me3 marked genes were defined as genes that have the H3K4me3 peak overlapped with the gene body. For H3K27me3 marked genes, both genes with H3K27me3 peaks overlapped with the gene body and genes within a broad local enrichment were counted. The locations of genes within a broad local enrichment were analyzed as previously described (Pauer et al., 2009). The modification level in each gene was calculated by TPM in the gene body. The significance of correlation was tested by F test (P < 0.01). For metaplots of modification over genes or peaks, the gene body (or peak) and 2-kb flanking regions were divided into 50 equal intervals, and the average TPM in each interval was plotted.

Accession Numbers

The high-throughput sequencing data generated in this study are deposited in Sequence Read Archive under accession number PRJNA548416. Publicly available data sets used in this study are as follows: GSE81436 (H3K9me2), GSE111308 (H3K9ac), GSE26734 (H3K4me2), GSE128434 (H3K4me1), PRJNA142219 (DHS), and PRJNA380652 (H2A.Z). The MSU7.0 locus identifiers for the genes mentioned in this study are as follows: CHR729 (Os07g31450) and JM703 (Os05g10770).

Supplemental Data

The following supplemental materials are available.

Supplemental Figure S1. Correlation matrix of wild-type and chr729 RNA-seq data.
Supplemental Figure S2. Nucleosome occupancy change in chr729.
Supplemental Figure S3. Metaplots of H3K4me3 profiles in chr729 DEGs.
Supplemental Figure S4. Heat map showing the H3K4me3 and H3K27me3 levels in chr729 hyper-H3K4me3 and hypo-H3K4me3 peaks.
Supplemental Figure S5. Metaplots of H3K4me3 and H3K27me3 profiles near non-TE genes and TE-G in the wild type and jm703.
Supplemental Figure S6. Phenotypes of chr729, jm703, and chr729jm703.
Supplemental Figure S7. MA-plot presenting H3K4me3 changes in chr729/jm703 compared with the wild type.
Supplemental Figure S8. Correlation matrix of jm703 and chr729jm703 RNA-seq data.
Supplemental Figure S9. Gene expression and H3K4me3 change in jm703.
Supplemental Figure S10. Gene expression analysis of chr729jm703.
Supplemental Figure S11. H3K4me3 and H3K27me3 levels in chr729 DEGs that are reversed regulated in jm703.
Supplemental Figure S12. Reverse transcription-PCR test of Pchr729c:CHR729:nGFP transgenic plants.
Supplemental Figure S13. Average nucleosome levels of H3K4me3 marked genes, H3K27me3 marked genes, and bivalent genes in the wild type and chr729.
Supplemental Table S1. RNA-seq data alignment summary.
Supplemental Table S2. MNase-seq data alignment summary.
Supplemental Table S3. ChIP-seq data alignment summary.

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