1 Short title: Chromatin dynamics of phosphate-starved rice

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Low-phosphate chromatin dynamics predict a cell wall remodeling network in rice shoots

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One-sentence summary: Combined chromatin structural data reveals specific chromatin-state transitions that correlate with subsets of functionally distinct rice genes differentially expressed under phosphate starvation.

Author contributions: M.F. performed the experiments, analyzed the data, and wrote the article; S.Z provided technical assistance; D-H.O. and G.W. provided bioinformatics assistance; M.D. supervised the data analysis; A.S. conceived the project, supervised the experiments and data analysis, complemented the writing, and agrees to serve as the author responsible for contact and to ensure communication.

Funding information: Funding was provided by the United States Department of Agriculture (USDA-NIFA, 2016-10070); the National Science Foundation (NSF-IOS, 1127051 and NSF-MCB, 1616827); and the Next Generation BioGreen21 Program (PJ01317301) of the Rural Development Administration, Republic of Korea.

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Abstract

Phosphorus (P) is an essential plant macronutrient vital to fundamental metabolic processes. Plant-available P is low in most soils, making it a frequent limiter of growth. Declining P reserves for fertilizer production exacerbates this agricultural challenge. Plants modulate complex responses to fluctuating P levels via global transcriptional regulatory networks. Although chromatin structure plays a substantial role in controlling gene expression, the chromatin dynamics involved in regulating P homeostasis have not been determined. Here we define distinct chromatin states across the rice (Oryza sativa) genome by integrating multiple chromatin marks, including the H2A.Z histone variant, H3K4me3 modification, and nucleosome positioning. In response to P starvation, 40% of all protein-coding genes exhibit a transition from one chromatin state to another at their transcription start site. Several of these transitions are enriched in subsets of genes differentially expressed under P deficiency. The most prominent subset supports the presence of a coordinated signaling network that targets cell wall structure and is regulated in part via a decrease of H3K4me3 at transcription start sites. The P starvation-induced chromatin dynamics and correlated genes identified here will aid in enhancing P use efficiency in crop plants, benefitting global agriculture.
Introduction

Phosphorus (P) is among the most limiting essential nutrients for plants because the primary plant-available form of P, inorganic phosphate (Pi), has poor solubility in most soils (Holford, 1997). As a result, P fertilization of soils is required for crop plants to achieve adequate yields. Unfortunately, P fertilization can result in serious environmental concerns due to nutrient run-off, which will worsen in the future due to the non-renewable nature of P resources (Vance et al., 2003). It is, therefore, necessary to investigate the underlying mechanisms involved in regulating P homeostasis, so as to increase the efficiency of plants to acquire and recycle P. In order to tolerate low-Pi conditions and maintain optimal P levels, plants have evolved a number of physiological, morphological, and biochemical responses, such as reduced growth, altered root system architecture, and secretion of organic acids, phosphatases, and nucleases to acquire more Pi (Secco et al., 2013). These responses are modulated by large transcriptional networks in which the MYB protein PHOSPHATE STARVATION RESPONSE 1 (PHR1) and related transcription factors play key roles (Secco et al., 2013, Sun et al., 2016).

In eukaryotic cells, DNA is complexed with core histones and other chromosomal proteins into chromatin (Luger et al., 1997). Therefore, chromatin structure is a key determinant of gene expression. Despite the fact that a large transcriptional cascade governs responses to low-Pi, relatively little is known regarding the associated chromatin dynamics, although evidence for chromatin-level mechanisms modulating Pi-deficiency responses is emerging. Smith et al. (2010) demonstrated that mutation of the actin-related protein (ARP) gene, ARP6, which encodes a key component of the SWR1 complex that catalyzes H2A.Z deposition (Deal et al., 2007), resulted in decreased H2A.Z localization at a number of Pi deficiency-response genes that were also de-repressed. These changes in H2A.Z and expression also occurred in Pi-deficient wild-type plants (Smith et al., 2010). Recently, we demonstrated similar phenomena in rice in which genome-wide H2A.Z distribution was altered by Pi starvation or RNAi knock-down of ARP6 (Zahraeifard et al., 2018). We also showed that deposition of rice H2A.Z in gene bodies largely resulted in down-regulation, whereas H2A.Z at the TSS was positively or negatively correlated with gene expression, depending on the particular Pi deficiency-response genes affected. In a separate study, we revealed that changes in nucleosome occupancy correlated with genes differentially expressed by Pi starvation, implicating nucleosome remodelers in modulating Pi-deficiency responses in rice (Zhang et al., 2018). Finally, two
chromatin-related components have been shown to play roles in Pi-deficiency induced root hair growth in Arabidopsis. The ALFIN-LIKE 6 (AL6) gene encodes a plant homeodomain (PHD)-containing protein that recognizes H3K4 trimethylation and appears to promote enhanced root hair growth during low-Pi conditions by targeting H3K4me3-marked target genes, such as ETC1, which functions in root hair cell patterning (Chandrika et al., 2013). The second factor necessary for normal induction of root hair growth in response to Pi deficiency is Arabidopsis HDA19, which encodes a histone deacetylase necessary for low-Pi root hair elongation through its role in regulating epidermal cell length (Chen et al., 2015).

Structural components of chromatin, including positioning of nucleosomes, the presence of histone variants, and post-translational modifications of histones, can be altered by a number of mechanisms (Mariño-Ramírez et al., 2005, Venkatesh and Workman, 2015). In contrast to examining individual chromatin modifications, defining the patterns, or states, of chromatin by examining multiple marks simultaneously in their spatial context is more informative to understanding transcriptional changes in response to stress (Ernst and Kellis, 2012, Pan et al., 2017). In rice, two recent studies defined distinct chromatin states by combining multiple histone marks and showed various associations between particular chromatin states and genes differentially expressed by ionizing radiation (Pan et al., 2017) or salinity stress (Zheng et al., 2019). In contrast, no studies have defined chromatin state transitions linked to Pi-deficiency responses in plants. Herein we characterized the impact of Pi starvation on the major histone mark, H3K4me3, as well as on chromatin states generated from the combined occupancy data of H3K4me3, H2A.Z, and nucleosomes. The data reveal several distinct chromatin state transitions that accompany expression changes of key subsets of Pi-starvation response genes.
Results:

H3K4me3 is prominent at the 5′ end of rice protein-coding genes and co-localizes with the H2A.Z histone variant

Previously we demonstrated that dynamics of nucleosome occupancy (Zhang et al., 2018) and H2A.Z deposition (Zahraeifard et al., 2018) were linked to genes differentially expressed in response to Pi starvation in rice shoots. The primary goal herein was to evaluate the combined role of nucleosome occupancy, H2A.Z, and a major histone post-translational modification, H3K4me3, in modulating responses to Pi starvation. We began by determining the genome distribution of H3K4me3 via ChIP-seq on shoots from 36-day-old rice (Oryza sativa ssp. japonica cv. Nipponbare) seedlings (Supplemental Table S1). Genes were categorized into four groups based on the MSU7 genome annotation: protein-coding genes (PCG), ‘pseudogenes’ (PSG, i.e. annotated genes that are neither expressed nor transposable element-related), transposable element-related genes that are expressed (TEG), and transposable element-related genes that are not expressed (TE; Kawahara et al., 2013, Zhang et al., 2018). As shown in Figure 1A, we observed a prominent H3K4me3 peak immediately downstream of the transcription start sites (TSS) of PCG, similar to previous studies (Zhang et al., 2009, Van Dijk et al., 2010, Du et al., 2013, Zong et al., 2013). In contrast to PCG, H3K4me3 abundance was relatively low at PSG, TEG, and TE (Figure 1A). Next we examined whether sub-groups of PCG exhibited different H3K4me3 patterns. Sorting all PCG according to size revealed a strong correlation between H3K4me3 and gene length (Supplemental Figure S1A,B), indicating that the general pattern of H3K4me3 among all PCG is relatively consistent (i.e. a major peak of H3K4me3 at the TSS). Although a TSS-localized peak of H3K4me3 was observed at virtually all PCG, the abundance of the peak varied. Clustering analysis at a 100-bp window across the TSS revealed 4 distinct clusters of H3K4me3 abundance (Supplemental Figure S1C,D). Gene Ontology (GO) enrichment analysis showed that the clusters with high and moderate abundance were enriched (FDR<0.05) with housekeeping genes, whereas the clusters with relatively low H3K4me3 abundance were enriched in stress-responsive genes (Supplemental Dataset 1).

The H3K4me3 localization patterns at different gene types (Figure 1A) are similar to those we recently demonstrated for the H2A.Z histone variant (Zahraeifard et al. 2018; Figure 1B). A key distinction is that the relative difference in abundance of H2A.Z between PCG and
TEG/TE is larger than that of H3K4me3. To further examine the apparent association between H3K4me3 and H2A.Z, we first computed a correlation coefficient using deepTools (Ramírez et al., 2016), which showed that both chromatin marks were correlated across the rice genome ($r = 0.77$; Pearson correlation coefficient; Figure 1C). Next we identified and compared distinct H3K4me3 and H2A.Z peaks using SICER (Zang et al., 2009) and BEDTools (Quinlan and Hall, 2010). This identified 32,886 H3K4me3 peaks and 44,804 H2A.Z peaks, of which 30,813 (93% of H3K4me3 peaks) overlapped (Figure 1D). Finally, we plotted the average abundance of both marks centered at peak summits of H3K4me3 (Supplemental Figure S2A) or H2A.Z (Supplemental Figure S2B). Together, these analyses demonstrate substantial co-occurrence of H3K4me3 and H2A.Z in the rice genome.

**H3K4me3 and H2A.Z abundance have distinct correlations with gene expression in rice**

To compare H3K4me3 abundance with gene expression, we analyzed our previously obtained RNA-seq data (Zahraeifard et al., 2018) from shoot tissues of 36-day-old rice seedlings (Supplemental Table S1). PCG were ranked according to FPKM and divided into five expression quintiles, as well as a sixth group of genes that were not expressed (i.e. FPKM = 0). We found a clear, positive correlation between transcript abundance and H3K4me3 localization around the TSS (Figure 2A,B), consistent with studies from a variety of species (Bernstein et al., 2002, Santos-Rosa et al., 2002, Barski et al., 2007, Zhang et al., 2009, Van Dijk et al., 2010). In contrast, transcript abundance exhibited a general negative correlation with TES- and gene body-localized H3K4me3 (Figure 2A,B). Genes exhibiting no expression were severely depleted in H3K4me3 at the TSS, but had a moderate level of gene-body H3K4me3. Next, we compared the correlation between H3K4me3 abundance and gene expression with that of H2A.Z (Zahraeifard et al., 2018; Figure 2C,D). As with H3K4me3, non-expressed genes were deficient in H2A.Z at the TSS, but contained moderate levels of gene-body H2A.Z. However, for the expression quintiles, H2A.Z exhibited a general negative correlation with expression at both the TSS and, especially, in the gene body. Therefore, although H3K4me3 and H2A.Z are generally co-localized at genic regions, particularly at the TSS of PCG, they have contrasting correlations with gene expression.

**Pi-starvation impacts H3K4me3 localization at protein-coding genes**
To evaluate a potential role for H3K4me3 in modulating Pi-deficiency responses, we carried out H3K4me3 ChIP-seq on shoots from plants subjected to a 24-hour Pi-deficiency treatment (Supplemental Table S1). As shown in Supplemental Figure S3A, Pi-deficiency altered H3K4me3 distribution at PCG, such that the prominent 5′ peak was reduced. Separating PCG into two broad functional categories showed that house-keeping genes exhibited the 5′ peak reduction (Supplemental Figure S3B), whereas stress-responsive genes exhibited an increase in H3K4me3 at the TSS (Supplemental Figure S3C). These data along with our prior studies (Zahraeifard et al., 2018, Zhang et al., 2018) indicate that nucleosome occupancy, H2A.Z, and H3K4me3 each exhibit distinct changes in response to Pi starvation.

**H3K4me3, H2A.Z, and nucleosome occupancy define five chromatin states in the rice genome**

It is becoming increasingly clear that simultaneously examining multiple chromatin marks provides a more robust picture of the dynamic chromatin environment linked to various developmental processes and responses to stimuli (Pan et al., 2017, Yan et al., 2019). Therefore, we integrated our H3K4me3 ChIP-Seq, H2A.Z ChIP-Seq (Zahraeifard et al., 2018), and MNase-Seq (Zhang et al., 2018) datasets to define distinct chromatin states using ChromHMM (Ernst and Kellis, 2012). ChromHMM employs a multivariate Hidden Markov Model that scores the enrichment of each chromatin mark to determine the major recurring combinatorial and spatial patterns of marks, i.e. chromatin states. ChromHMM identified five chromatin states (CS), each distinguishable from the others by differential enrichment of one or more of the marks tested (Figure 3A). Because the enrichment values are relative, a low score for a particular mark does not indicate a lack of the mark. CS1 and CS2 were each deficient in both H2A.Z and H3K4me3, CS3 was enriched in only H2A.Z, CS4 was enriched in both H2A.Z and H3K4me3, and CS5 was enriched in only H3K4me3. Regarding nucleosome density, CS2 and CS3 had moderately higher nucleosome enrichment compared to the other three states. To support the presence of these five particular chromatin states in the rice genome, we repeated the ChromHMM analysis by combining our three datasets from control samples with publically available datasets for two marks recognized as largely repressive, DNA methylation (Secco et al., 2015) and/or H3K27me3 (Zhang et al., 2012) from control, shoot samples. As shown in Supplemental Figure S4, the addition of these marks did not change the combinatorial effect of the three marks we used to
designate the five chromatin states, verifying the relevance of these states in our original ChromHMM analysis. The inclusion of DNA methylation or H3K27me3 alone each yielded a sixth chromatin state, whereas including both repressive marks yielded a sixth and seventh state (Supplemental Figure S4A). In addition, a genome comparison of the five and seven chromatin-state analyses showed similar percentages of the genome represented by each CS except CS1. When the repressive marks were added, some regions of the genome defined as CS1 were changed to CS6 or CS7 (Supplemental Figure S4B,C).

Next we mapped the distribution of the five chromatin states across the genome (divided into 200-bp bins), which revealed several biases with genomic features (Figure 3B,D). CS1 was the major chromatin state, accounting for 63% of the rice genome, and was enriched at TE and TEG. It should be noted that highly repetitive regions of the genome were likely designated CS1 due to low numbers of mappable reads rather than bona fide depletion of the chromatin marks examined. TE and TEG were also enriched in CS2 and CS5. This means that the transposable element-related loci were either deficient in both H2A.Z and H3K4me3 or enriched in H3K4me3 only. In contrast, PSG were enriched in CS2 and CS3, consistent with depletion of both H2A.Z and H3K4me3 or enrichment of only H2A.Z. Finally, PCG were enriched in CS4, consistent with enrichment of both H3K4me3 and H2A.Z. To more specifically characterize PCG, we calculated enrichments at the TSS and TES separately (Figure 3B). Compared to all bins within PCG, the TSS was more enriched in CS4, CS5, and CS3, whereas the TES was more enriched in CS3 and less enriched in CS4. These results indicate for PCGs generally an overall high occupancy of H2A.Z and/or H3K4me3 at the TSS, but an enrichment of only H2A.Z at the TES.

**Pi starvation has a dramatic impact on chromatin signatures**

To characterize the impact of Pi starvation on chromatin signatures we compared the distribution of chromatin states between control and Pi-deficiency conditions. First we analyzed the enrichment of each chromatin state within the four gene types (Figure 3C,D). In response to Pi starvation, the genomic bins within TE, TEG, and PSG increased in CS1, but decreased in CS2, CS4, and CS5, consistent with a loss of H3K4me3. On the other hand, PCG bins decreased in CS1, but increased in CS2, CS3, and CS5. At the TSS of PCG, CS2 and CS4 decreased and CS1 increased, whereas at the TES, CS1 and CS4 decreased and CS2 increased (p-value < 0.01) (Figure 3B,C, Supplemental Figure S5). Because the TSS is an important regulatory region for
gene expression, we investigated the effect of Pi starvation at the TSS of PCG in more detail. First we determined the chromatin state at the TSS of each PCG under control conditions (Supplemental Dataset 2). For each CS sub-group, we generated average plots and heatmaps of the relative enrichment of H3K4me3, H2A.Z, and nucleosome occupancy (Supplemental Figure S6). Next we determined the chromatin state at the TSS of each PCG following Pi starvation (Supplemental Dataset 2). Over 40% of PCG exhibited a chromatin state change, or transition, at their TSS in response to Pi starvation (Supplemental Dataset 2; Figure 4). The largest groups of transitions were CS4 to CS3 (n = 4,088), CS4 to CS5 (n = 2,355), and CS5 to CS1 (n = 2,496) (Figure 4; Supplemental Figure S7). GO enrichment analysis showed significantly enriched GO terms (FDR < 0.05) for eight of the transition groups (Supplemental Dataset 3). We then applied Markov Clustering (MCL) to reduce redundancy in enriched GO processes and generated functional clusters (‘GOMCL’ clusters) to represent the primary functions associated with chromatin state transitions. As shown in Figure 4, the enriched GO terms for CS4-CS3 genes fell into five GOMCL clusters, including transcription factor activity, response to endogenous stimulus, cell wall, oxygen binding, and response to extracellular stimulus. In contrast, CS4-CS5 genes were enriched in GO terms defined by nine GOMCL clusters, which among other functional categories, were related to translation and gene expression, nuclear functions, plastid functions, nucleic acid metabolism, development, and RNA binding. Interestingly, CS5-CS1 genes shared essentially the same enriched GO terms (Supplemental Dataset 3) and GOMCL clusters (Figure 4) as CS4-CS5 genes. One explanation for this is that the CS5-CS1 and CS4-CS5 transitions are frequently found together at the TSS. Indeed, examination of the bins that flank the TSS (Supplemental Figure S8) showed that CS5-CS1 genes were approximately four times more likely than random to exhibit a CS4-CS5 transition in the bin downstream of the TSS (p-value < 0.001). Similarly, the CS4-CS5 genes were 3.5-fold more likely to contain a CS5-CS1 transition upstream of the TSS (p-value < 0.001). In contrast, CS5-CS1 genes with a CS4-CS5 upstream bin, and CS4-CS5 genes with a CS5-CS1 downstream bin were similar to random or under-represented, respectively. Thus, the identification of subgroups of functionally similar genes with CS5-CS1 and CS4-CS5 transitions at their TSS is reflective of these genes containing a specific pair of transitions (CS5-CS1 + CS4-CS5, 5′–3′) at the TSS.

Chromatin state transitions correlate with differential expression of Pi deficiency-responsive genes
We analyzed our recent RNA-seq experiments (Zahraeifard et al., 2018; Supplemental Table S1) to investigate the relationship between gene expression and chromatin state transitions in response to Pi starvation. Differential expression analysis with DESeq2 identified 1385 differentially-expressed genes (DEGs) in response to Pi starvation, 694 up-regulated and 691 down-regulated (adjusted P-value < 0.001; Supplemental Figure S9, Supplemental Dataset 4). GO terms enriched for up-regulated genes included response to stress, lipid metabolic process, and signal transduction, whereas down-regulated genes were enriched in growth, cell-cell signaling, and lipid, carbohydrate, and secondary metabolic processes (Supplemental Table S2, Supplemental Figure S9B,C). Although lipid metabolism was overrepresented in both groups of DEGs, genes linked to carotenoid biosynthesis and alpha-Linolenic acid metabolism were among the up-regulated DEGs, whereas cutin, suberin, and wax biosynthesis were among the down-regulated DEGs. Overall, the functional categories of these DEGs were similar to those from previous transcriptome studies of Pi-deficient plants (Thibaud et al., 2010, Cai et al., 2013, Secco et al., 2013, Zahraeifard et al., 2018).

Next we investigated whether differential expression in response to Pi starvation correlated with distinct CS transitions. We quantified the overlap between the up- or down-regulated DEGs and each CS transition via bootstrapping analyses (1000 iterations) and carried out binomial tests to identify the over- or under-represented DEGs for each CS transition (Figure 5). These analyses revealed a number of significant (p-value < 0.001) biases between DEGs and CS transitions. First, down-regulated DEGs were enriched among CS1-CS3 and CS2-CS3 transitions (i.e. transitions from H2A.Z-deficient chromatin states to an H2A.Z-enriched state), consistent with down-regulation of gene expression being correlated with a gain of H2A.Z. Reciprocally, up-regulation of gene expression correlated with a loss of H2A.Z, as indicated by enrichment of up-regulated genes among CS3-CS1 transition genes. These observations support a role for H2A.Z as a repressive chromatin mark during Pi starvation, in which some genes are repressed by the deposition of H2A.Z, whereas other genes are induced (de-repressed) by loss of H2A.Z. Second, genes containing H2A.Z and H3K4me3 that exhibited decreases in both marks in response to Pi deficiency (i.e. CS4 to CS1 transition) were enriched among up-regulated genes. This suggests a negative role for not only H2A.Z, but also H3K4me3, in which the loss of both marks from this group of genes results in their de-repression. Third, up- and down-regulated DEGs were both enriched among CS4-CS3 transition genes (i.e. those with a decrease in
H3K4me3 but maintenance of H2A.Z). Interestingly, this suggests a possible dual role of
H3K4me3 in Pi-responsive gene modulation. Finally, the other two prominent groups of
transitions, CS5-CS1 and CS4-CS5, which contain many translation-related genes, were under-
represented among down-regulated DEGs. This indicates that genes exhibiting these transitions,
or pair of transitions (Supplemental Figure S8), at the TSS are unlikely to be differentially
expressed after 24-hour Pi deficiency. Because a number of translation-related genes were
previously shown to be down-regulated by long-term (21-day) Pi deficiency in rice shoots
(Secco et al., 2013), we carried out a bootstrapping analysis to test whether our CS5-CS1 and
CS4-CS5 genes were enriched among those DEGs. Indeed, both CS5-CS1 and CS4-CS5 groups
were enriched (p-value < 0.01) among genes down-regulated by long-term Pi deficiency
(Supplemental Figure S10). This suggests that the chromatin dynamics observed at these genes
after 24 hours of Pi starvation is a prelude to decreased transcript abundance not observable until
after a longer duration of Pi deficiency.

In addition to the correlations between DEGs and distinct chromatin state transitions,
there were also correlations between DEGs and groups of genes that did not transition (Figure 5).
Both up- and down-regulated DEGs were significantly enriched among CS3 genes that did not
transition (i.e. CS3-CS3), and were under-represented among CS1-CS1 and CS5-CS5 genes.
Furthermore, up-regulated DEGs were enriched among CS4-CS4 genes and under-represented
among CS2-CS2 genes. These results show that responsive genes are likely to contain H2A.Z,
which is consistent with previous reports (Coleman-Derr and Zilberman, 2012, Zahraeifard et al.,
2018). Taken together, these biases demonstrate that specific chromatin dynamics at the TSS are
linked to subsets of genes differentially expressed by Pi starvation.

**Differentially-expressed genes exhibiting a CS4 to CS3 chromatin transition suggest a
coordinated Pi-deficiency regulatory network targeting the apoplast**

As shown above, the largest group of genes exhibiting a chromatin state shift in response
to Pi deficiency was the CS4-CS3 transition group (Figure 4, Supplemental Dataset 3). These
genes were also significantly enriched among both up- and down-regulated DEGs (Figure 5). To
gain insight into the predicted functions of the DEGs that exhibited a CS4-CS3 transition, we
carried out GO term enrichment analysis, which identified four significantly enriched (FDR <
0.05) terms: cell wall, external encapsulating structure, response to biotic stress, and catalytic
activity (Supplemental Figure S11). Due to the relatively limited GO term assignments for rice loci, we carried out extensive data mining on the CS4-CS3 DEGs, which allowed us to assign putative functional and subcellular localization information to over 90% (178 of 196) of the DEGs (Supplemental Dataset 5). These DEGs encode components with putative functions in signal transduction (37%), cell wall structure (23%), lipid composition (13%), transcription regulation (10%), secondary metabolism (9%), primary metabolism, or cell growth (3%), which are mostly targeted to the apoplast (31%), plasma membrane (28%), nucleus (18%), cytosol (10%), or plastid (6%; Figure 6A). Strikingly, more than half (53%) of the CS4-CS3 DEGs are predicted to encode proteins targeted to the apoplast or plasma membrane, and have functions in signaling or cell wall and lipid composition. Among this group are a number of pectinases, arabinogalactan proteins (AGPs), and expansins that mostly are down-regulated by the 24-hour Pi-deficiency treatment (Figure 6B, Supplemental Dataset 5). A previous study in Arabidopsis identified a similar response of cell wall hydrolytic enzyme-encoding loci in roots subjected to Pi-deficiency treatments of 1, 6, and/or 24 hours (Lin et al., 2011). Together this suggests that modification of the cell wall is an early and prominent response to Pi starvation in roots and shoots across species. In addition to the down-regulation of cell wall-related components was a large group of signaling components, including many receptor-like kinases (RLKs) that were predominantly up-regulated (Figure 6B, Supplemental Dataset 5). One of the RLKs is a Catharanthus roseus RLK1-like kinase orthologous to Arabidopsis FERONIA (FER), which has been shown to regulate cell expansion in response to diverse developmental and environmental cues (Liao et al., 2017). For example, during salinity stress, FER maintains cell wall integrity, and is necessary for root growth recovery (Feng et al., 2018). Recently it was demonstrated that FER is one component of a signaling module that transduces cell-wall signals during salt stress (Zhao et al., 2018). In the absence of salt stress, a group of apoplastic leucine-rich repeat extensins (LRX) bind to RAPID ALKALINIZATION FACTOR (RALF) peptides. In response to salt stress, LRX and RALF dissociate, and RALF peptides bind FER. This results in FER internalization and, subsequently, inhibition of growth and initiation of stress responses. Calcium transients and SITE-1 PROTEASE (S1P) activity also play roles in RALF/FER signaling (Stegmann et al., 2017, Feng et al., 2018). Notably, our CS4-CS3 DEG list also contains six genes encoding RALF peptides (out of 14 total in the rice genome; (Campbell and Turner, 2017) an LRX, several Ca2+ transport-related components (e.g. Ca2+ ATPase and calmodulin), and
two S1P proteases (Supplemental Dataset 5). In addition to the signaling and cell wall components among the CS4-CS3 DEGs were a number of transcription factors, including five AP2 superfamily factors, two HLH factors, and two WRKY transcription factors. These represent families of transcription factors known to be responsive to a number of biotic and abiotic stressors. Interestingly, examining the CS4-CS3 DEGs as a whole suggests an integrated network whereby Pi-deficiency initiates reduced cellular growth and low-Pi tolerance mechanisms. A proposed model of this network is shown in Figure 7. In this working model, low-Pi signals entering the apoplast interact with signaling components, including receptor-like and wall-associated kinases. Also, Pi starvation may lead to a transfer of RALF peptides from LRX to the rice FER ortholog and subsequent internalization of FER, which is likely regulated in part by Ca^{2+}. Next, the transduced low-Pi signals lead to differential expression of many regulatory and structural genes including a number of stress-responsive transcription factors. These changes in gene expression, which coincide with chromatin remodeling (i.e. a transition from CS4 to CS3), drive robust metabolic and physiological adaptations, such as cell wall modification, lipid and wax remodeling, and altered secondary metabolite biosynthesis. These modifications, in turn, result in slowed cellular growth and tolerance to sustained Pi starvation. This hypothetical model provides a strong framework for future functional studies aimed at investigating the roles of the specific components in modulating cellular responses to Pi starvation in rice.
**Discussion**

**H3K4me3 and H2A.Z exhibit overlapping and divergent localization patterns**

Despite being widely recognized as marks of active transcription, assigning specific roles for H3K4me3 and H2A.Z in regulating transcription has been challenging. For instance, H3K4me3 is often assumed to promote transcription, but loss or severe depletion of H3K4me3 levels results in relatively few gene expression changes (Clouaïre et al., 2012, Margaritis et al., 2012). Also, whereas loss of H3K4me3 at most genes has no impact on expression, H3K4me3 has been linked to both activation and repression of subsets of genes (Weiner et al., 2015, Cano-Rodriguez et al., 2016). Like H3K4me3, H2A.Z is often associated with gene activity, but plays a complex role in modulating gene expression. Evidence indicates that H2A.Z acts to both promote and repress gene expression, depending on the environmental or developmental context, genic location, and relevant loci (Deal et al., 2007, Zilberman et al., 2008, March-Díaz and Reyes, 2009, Kumar and Wigge, 2010, Smith et al., 2010, Berriri et al., 2016, Sura et al., 2017, Zahraeifard et al., 2018). Interactions among multiple chromatin modifications add complexity to identifying specific chromatin-level mechanisms that modulate gene expression, particularly in light of contradictory findings. For example, Arabidopsis H2A.Z has been proposed to facilitate H3K4 trimethylation at miR156 loci (Xu et al., 2018) but antagonize H3K4me3 abundance at anthocyanin biosynthetic genes (Cai et al., 2019). Thus, there is a need to investigate multiple aspects of the chromatin environment in order to gain insight into chromatin-level mechanisms that impact gene expression.

Herein we used ChromHMM to combine our H3K4me3 ChIP-Seq data from this study with our previous H2A.Z ChIP-Seq (Zahraeifard et al., 2018) and MNase-Seq (Zhang et al., 2018) data to define five chromatin states (CS1-CS5) in rice shoots. Genic regions were enriched in CS4, which is characterized by moderate nucleosome occupancy and relatively high levels of H2A.Z and H3K4me3. The TSSs of protein-coding genes were also enriched in CS4, as well as CS3 and CS5, which contain only H2A.Z or H3K4me3, respectively. In contrast, the TES of protein-coding genes was only enriched in CS3. This suggests that H3K4me3 functions mostly at the TSS, whereas H2A.Z functions across the gene. This is generally consistent with previous reports on the functions of H3K4me3 and H2A.Z. Studies in a number of organisms have shown that H3K4me3 localizes near the TSS of active protein-coding genes (Santos-Rosa et al., 2002,
Liu et al., 2005, Bernstein et al., 2006, Barski et al., 2007, Zhang et al., 2009, Van Dijk et al., 2010, Du et al., 2013, Zong et al., 2013). Our data further support this by showing a prominent peak of H3K4me3 at the TSS of rice PCG (Figure 1A) that is positively correlated with basal gene expression (Figure 2). H2A.Z is also abundant at the TSS of PCG, but appears to play roles in gene expression by localizing to gene bodies and the TES as well (Coleman-Derr and Zilberman, 2012, Sura et al., 2017, Zahraeifard et al., 2018). In contrast to H3K4me3, TSS-localized H2A.Z is negatively correlated with basal expression (Figure 2; Zilberman et al., 2008, Coleman-Derr and Zilberman, 2012, Yelagandula et al., 2014, Dai et al., 2017, Zhang et al., 2017, Zahraeifard et al., 2018). Interestingly, our data show that abundance of H2A.Z and H3K4me3 downstream of the TSS region is negatively correlated with expression. Previous studies have reported this phenomenon for H2A.Z (Zilberman et al., 2008, Coleman-Derr and Zilberman, 2012, Sura et al., 2017), but Arabidopsis H3K4me3 was shown to be positively regulated with expression (Van Dijk et al., 2010). This may reflect a difference in the role of H3K4me3 at the 3′ genic region in different plant species. On the other hand, a H3K4me3 profile of genes from an allotetraploid cotton genotype generally showed a negative correlation with expression, whereas a diploid cotton genotype in the same study exhibited a positive correlation (You et al., 2017). H3K4me3 at the TES was reported to play a role in modulating antisense transcription, thereby repressing sense transcription (Ponting et al., 2009, Cui et al., 2012). Therefore, genotypic or cell type-dependent differences in antisense transcription may contribute to the correlation of TES-localized H3K4me3 with sense transcription. Further investigation is required to understand the nature of the differences in 3′ H3K4me3-dependent regulation of gene expression across samples and species.

Pi-starvation induced chromatin dynamics correlate with gene repression and induction

Often, the disruption of histone modifiers, such as H3K4 methyltransferases, through mutagenesis do not have substantial impacts on global steady-state transcription (Guo et al., 2010, Chen et al., 2017, Howe et al., 2017). On the other hand, a number of studies have identified significant roles for particular histone or chromatin modifiers in differential expression in response to environmental stimuli (Ding et al., 2011, Ding et al., 2012, Weiner et al., 2015). Our data support this by revealing that more than 40% of all rice PCG in shoots exhibit a chromatin state transition at their TSS in response to a 24-hour Pi-deficiency treatment, and that
several specific transitions correlate with subgroups of genes differentially expressed by Pi starvation. It is noteworthy that although we found significant correlations between chromatin dynamics and subsets of differentially expressed genes, there were many genes exhibiting a chromatin state transition that were not differentially expressed (Figure 4). This lack of a strong global correlation between chromatin dynamics and changes in gene expression has been reported previously (Zong et al., 2013, Fiziev et al., 2017), and likely reflects regulation of steady-state transcript levels by other, unobserved, molecular processes, such as the presence of appropriate transcription factors and other chromatin modifications. Also, the duration of our Pi-starvation treatment was likely not adequate to detect changes in transcript abundance of some genes despite the detection of chromatin state changes (Secco et al., 2013; Supplemental Figure S10). Nevertheless, the correlations between changes in chromatin signatures and gene expression observed herein highlight the importance of chromatin dynamics during differential gene expression in response to Pi starvation.

Genes with CS1-CS3 or CS2-CS3 transitions exhibit increases in nucleosome occupancy and H2A.Z deposition in response to Pi starvation, and are enriched in down-regulated genes, whereas CS3-CS1 genes, which exhibit decreases in nucleosome occupancy and H2A.Z, are enriched in up-regulated genes. These correlations are consistent with a repressive role for H2A.Z at the TSS in modulating Pi deficiency-response genes. This is consistent with our recent work in rice (Zahraeifard et al., 2018) and previous reports in Arabidopsis (Dai et al., 2017, Sura et al., 2017), which all provide evidence for H2A.Z acting as a repressor of expression when localized at gene bodies or the TSS. Work in Arabidopsis also showed general co-localization of H2A.Z and H3K4me3 in promoter regions, but a negative correlation of the two marks at the TSS of genes exhibiting relatively high H2A.Z (Dai et al., 2017). The same study also showed a positive correlation between nucleosome occupancy and H2A.Z at the +1 nucleosome, suggesting that H2A.Z deposition at the +1 nucleosome is linked to high nucleosome occupancy, low H3K4me3 abundance, and low gene accessibility (Dai et al., 2017). Our data bolster support for a model where H2A.Z at the TSS, likely the +1 nucleosome, regulates a subset of Pi deficiency-response genes that contain low levels of H3K4me3 and relatively low basal expression. In response to Pi starvation, H2A.Z is either removed or deposited, resulting in de-repression (CS3-CS1) or repression (CS1-CS3/CS2-CS3), respectively. Similar to CS3-CS1, genes with a CS4-CS1 transition, which exhibit a loss of both H2A.Z and H3K4me3, are
enriched in up-regulated genes (Figure 6). These genes tend to be more highly expressed during control conditions than CS3 genes, and therefore have a stronger requirement for H3K4me3 for basal expression. In response to Pi starvation, the combined loss of H2A.Z and H3K4me3 may reflect some dependence of H3K4me3 on H2A.Z at these genes, similar to how H2A.Z was suggested to facilitate H3K4me3 deposition at two miR156-encoding genes in Arabidopsis (Xu et al., 2018).

Among the gene groups that exhibit chromatin state transitions, the CS4-CS3 group contains the largest number of genes, and is characterized by a loss of H3K4me3, but maintenance of H2A.Z, during Pi starvation. Interestingly, these genes are enriched among both up- and down-regulated genes, indicating that loss of H3K4me3 is linked to gene activation and repression during Pi deficiency. In contrast to H2A.Z, H3K4me3 is generally not recognized as playing a negative role in gene expression. Studies in a variety of plant species and tissues have examined the change in genic levels of H3K4me3 in response to environmental stressors (Tsuji et al., 2006, Sokol et al., 2007, Kim et al., 2008, Van Dijk et al., 2010, Jaskiewicz et al., 2011, Zeng et al., 2019). These studies generally reported increases in H3K4me3 at genes up-regulated by stress. However, most of the studies examined relatively small numbers of genes, and the genome-level studies that compared average H3K4me3 genic profiles between control and stressed samples found substantial decreases in 5’ localization of H3K4me3 in response to stress (Zong et al., 2013, Zeng et al., 2019). We observed a similar effect when comparing the H3K4me3 profiles for all PCG between control and Pi-deficiency conditions (Figure S3A). One explanation for our CS4-CS3 genes being linked to both induction and repression is that the TSSs of the corresponding genes contain bivalent histone modifications. Bivalent domains are characterized by containing both active and repressive histone modifications. First described in mouse embryonic stem cells were bivalent domains containing H3K4me3 and H3K27me3, in which H3K4me3 is proposed to poise genes for activation, whereas H3K27me3 maintains the genes in a repressed state (Bernstein et al., 2006). A recent study in potato tuber found an association between genes containing the bivalent H3K4me3 and H3K27me3 marks and differential expression in response to cold stress (Zeng et al., 2019). Interestingly, the bivalent mark was enriched among up-regulated genes linked to stress responses, as well as down-regulated genes linked to developmental processes. The authors proposed that the bivalent H3K4me3-H3K27me3 domain confers greater accessibility to regulatory proteins that can induce
or repress genes in response to cold stress. A similar phenomenon might explain our observed
correlations between the CS4-CS3 transition and both up- or down-regulated genes in response
to Pi starvation. A decrease in H3K4me3 at the TSS may reflect a switch from nucleosomes
modified with only H3K4me3 to nucleosomes containing both H3K4me3 and H3K27me3. This
would favor enhanced DNA accessibility, which could facilitate the targeting of transcriptional
machinery for induction or repression. Recently, an interaction between H2A.Z deposition and
H3K27 tri-methylation was reported in Arabidopsis, in which H2A.Z deposition promotes
H3K27 tri-methylation (Carter et al., 2018). It is possible that the maintenance of H2A.Z at the
CS4-CS3 genes is required for proper H3K27me3 deposition at the bivalent marks. Future
experiments that examine H3K27me3 localization would shed light on this hypothesis.

**Differential expression of cell wall-related genes correlates with decreased H3K4me3 and
maintenance of H2A.Z**

Cell walls provide rigidity to plant cells but are also restrictive to cell expansion. Thus,
cells must simultaneously weaken cell wall structure and maintain turgor and cell integrity to
achieve growth (Voxeur et al., 2016). Correspondingly, plants must employ signaling
mechanisms aimed at regulating cell wall structure in response to developmental and
environmental cues. Several plasma-membrane localized receptor-like kinases, such as FER,
have been implicated in cell-wall integrity sensing in response to a variety of environmental
stressors (Liao et al., 2017). The majority of our CS4-CS3 DEGs encode putative apoplastic or
plasma membrane proteins with predicted roles in signaling and cell wall composition (Figure
6A). The signaling genes were mostly up-regulated, whereas the cell wall-related genes were
largely down-regulated (Figure 6B). Comparing the transcriptomic profile of the CS4-CS3 DEGs
to public transcriptome studies using Genevestigator (Hruz et al., 2008) revealed substantial
overlap with several pairwise comparisons from a previous study on rice lamina joint
development (Zhou et al., 2017). Comparisons between older stages of development (maturation
or post-maturation) with a younger stage showed similar expression profiles as our CS4-CS3 Pi-
deficiency DEGs (not shown). Interestingly, cell wall thickening is a prominent feature during
younger stages of lamina joint development, and this declines over time. This suggests that Pi
starvation results in decreased cell wall thickening, or more generally, a decrease in cell
elongation. Transcriptomic profiles of several biotic and abiotic (e.g. salinity and heat) stressors
also showed high similarity to our CS4-CS3 DEG profile, suggesting the apparent apoplastic signaling network overlaps with multiple stressors. Our CS4-CS3 DEG list contains many orthologs of Arabidopsis components involved in salinity stress responses, including FER, LRX, and RALF peptides (Zhao et al., 2018). It is of interest to evaluate whether the rice orthologs exhibit similar functions in response to stressors including salinity and Pi starvation.

A distinct pair of chromatin state transitions may poise translation-related genes for repression

Following the CS4-CS3 gene group, the transitions with the most genes were the CS5-CS1 and CS4-CS5 groups, which were enriched with similar functional categories of genes (Figure 4) including those related to translation, particularly a number of ribosomal protein genes. Examination of the two bins adjacent to the TSS revealed that a number of these genes contained both transitions with the CS5-CS1 transition immediately upstream of the CS4-CS5 transition. Our bootstrapping results showed that these genes are not enriched among our DEGs. On the contrary, members of the CS4-CS5 subgroup are under-represented among down-regulated DEGs (Figure 6). Interestingly, a group of genes shown in a previous study (Secco et al., 2013) to be down-regulated after 21 days of Pi deficiency were enriched among our CS5-CS1/CS4-CS5 genes (Supplemental Figure S10). This might indicate that 24 hours of Pi deficiency is sufficient to observe chromatin dynamics at these genes without observing a corresponding detectable decline in transcript abundance. We propose that the sequential CS5-CS1 and CS4-CS5 transitions observed at the TSS reflect genes under control conditions that contain low H2A.Z and high H3K4me3 in the -1 nucleosome and high levels of both marks in the +1 nucleosome. Pi starvation, then, results in a moderate loss of nucleosome occupancy at both the +1 and -1 nucleosomes, and specific removal of H3K4me3 from the -1 nucleosome and H2A.Z from the +1 nucleosome. In yeast, Spp1 promotes the H3K4 trimethylase activity of the Set1 complex (Morillon et al., 2005). As a result, deletion of Spp1 results in substantial loss of global H3K4me3 levels, but the remaining H3K4me3 (approximately 20%) is not evenly distributed among genes. Genes that retain the highest levels of H3K4me3 in Δspp1 mutants are enriched in ribosomal protein genes and other translation-related genes, whereas genes exhibiting the most severe H3K4me3 depletion are enriched in stress-related genes (Howe et al., 2014). Also, the Spp1-independent genes tend to be more highly expressed during control conditions,
and repressed during environmental stress, whereas the Spp1-dependent genes generally exhibit low expression during control conditions and induced expression during stress. Finally, in response to diamide stress, many yeast ribosomal protein genes are down-regulated and exhibit a decrease in H3K4me3 (Weiner et al., 2015). Our data suggest that rice employs different mechanisms to modulate H3K4me3 levels at distinct gene groups, similar to yeast. This is consistent with our CS4-CS3 and CS5-CS1 gene groups undergoing decreases in H3K4me3 via different chromatin remodeling complexes. Future studies on the roles of H3K4me3 and H2A.Z, in conjunction with additional marks such as H3K27me3, in the Pi deficiency-dependent regulation of gene expression will provide valuable information on the chromatin dynamics that impact low-Pi adaptation mechanisms.

Materials and methods:

Plant material and growth conditions

Sterilization and pre-germination (1 day at 37°C followed by 2 days at 28°C) were carried out on rice cultivar Nipponbare (Oryza sativa ssp. japonica) seeds. Seeds were transferred to 12-h light/12-h dark at 30°C/22°C conditions to germinate for 14 days. Seedlings were grown hydroponically in modified Yoshida Rice culture media as described (Yoshida et al., 1971, Secco et al., 2013). The solution was replaced every 7 days. After 21 days, seedlings were used for a 24-hour Pi-deficiency treatment (modified Yoshida Rice solution without NaH2PO4).

ChIP-seq

Four grams of frozen shoot tissue from 24-hour Pi-deficiency or control treatment was used to perform chromatin immunoprecipitation (ChIP) as previously described (Zahraeifard et al., 2018) using an antibody (Millipore; lot number 2648189) against H3K4me3, a custom polyclonal antibody against rice H2A.Z (Zahraeifard et al., 2018), and input genomic DNA as a control. Three biological replicates were used for both input and antibody treatments. Purification of ChIP DNA was carried out with the Clean and Concentrator kit (Zymo Research). Libraries were constructed using 1:20 diluted adaptor from Kapa Biosystems Hyper Library Construction Kit and 10 cycles of DNA amplification. Libraries were quantitated (qPCR) and multiplexed, and single-end sequencing was completed with a HiSeq2500 (Illumina) using a
HiSeq SBS sequencing kit (version 4) for 101 cycles at the University of Illinois Roy J. Carver Biotechnology Center. Approximately 147 million ChIP-seq reads were quality-checked and cleaned using FastQC and Trimmomatic-0.33 (Andrews, 2010, Bolger et al., 2014). Using Bowtie, the reads were aligned to MSU Rice Genome Annotation Release 7.1 (MSU7.1) with one mismatch allowed to retain uniquely mapped reads. The SICER software package (Zang et al., 2009) was used to define the H3K4me3 enrichment regions with the following parameters (W = 200, G = 200, FDR < 1.00E-02). The input genomic DNA was used as a background control. Differential H3K4me3 enrichment peaks between control and Pi-deficiency samples were determined using SICER-df.sh shell script (W = 200, G = 200, FDR < 1.00E-02). We defined the existence of peaks with protein-coding genes (PCG) if 50% of peaks overlapped with PCG (including 250 bp upstream and downstream) using BEDTools intersect (Quinlan and Hall, 2010). The genome-wide distribution pattern of H3K4me3 and the published profile of H2A.Z (Zahraeifard et al., 2018) were visualized using ngs.plot (Shen et al., 2014). K-means clustering within ngs.plot was used to find different patterns of H3K4me3. Gene ontology (GO) terms enriched among clusters were analyzed with BiNGO and visualized with Cytoscape (Maere, Heymans et al. 2005).

**RNA-seq analysis**

RNA-sequencing reads were generated previously (Zahraeifard et al., 2018). A minimum of 58 million high-quality RNA-seq reads (100-bp single end) per sample were mapped to the MSU Rice Genome Annotation Release 7.1 (MSU7.1) using Bowtie2 tools (Langmead and Salzberg, 2012). Fragments per kilobase of transcript per million mapped reads (FPKM) were calculated with the Cuffdiff tool (Trapnell et al., 2012). DESeq2 was applied to identify differentially expressed genes (DEGs) (Love et al., 2014). The cutoff (adjusted P-value < 0.001) recommended for a small-sample RNA-seq experiment was used (Soneson and Delorenzi, 2013). Gene ontology (GO) terms enriched among DEGs were analyzed with BiNGO and visualized with Cytoscape (Maere et al., 2005).

**Chromatin States Analysis**

We used ChromHMM (Ernst and Kellis, 2012) with default parameters to characterize the chromatin state maps for control and Pi-deficiency samples. We used the published profiles of H2A.Z ChIP-seq (Zahraeifard et al., 2018) and nucleosome occupancy (Zhang et al., 2018)
(MNase-seq), as well as the H3K4me3 profile generated in this study. All input data were binarized with BinarizedBam, included in ChromHMM (Ernst and Kellis, 2012), and input genomic DNA was used to adjust binarization thresholds locally. The common model of chromatin states in both control and Pi-deficiency samples was developed by concatenating the marks using a hidden Markov model. Five chromatin states were generated based on the learned model parameters as described in ChromHMM (Ernst and Kellis, 2012). Chromatin state changes were analyzed using a previously described method (Fiziev et al., 2017). Briefly, the control and –Pi genomes were divided into 200-bp bins, each occupied by one chromatin state, and the chromatin state annotations of control and Pi-deficiency genomes were overlapped. The number of bins in each possible chromatin state were counted and called as the observed number. The expected number was calculated by multiplying the number of bins in the two chromatin states involved in each transition (a change in transition from control to Pi-deficiency sample) and divided by total bins in the genome to calculate enrichment scores. Similarity between each pair of chromatin states was controlled by dividing the enrichment scores of each state transition to the enrichment scores of the reverse state transition. The distribution of chromatin states were identified using CEAS software (Shin et al., 2009). Each protein-coding gene was assigned to one chromatin state based on the state of the 200-bp bin encompassing the transcription start site. For bootstrapping analysis, we used a custom FORTRAN script (Zahraeifard et al., 2018) to obtain the same number of randomly selected genes and estimate the percentage of overlap between these genes and each group of state transitions (1000 iterations). Binomial distribution tests were carried out with R (pbinom, P-value < 1.00E-03). For the chromatin state transition plot (Figure 4), chromatin states in control samples were differentially color-coded. Genes in each control chromatin state were sorted based on their positions within each chromosome. Chromatin transitions for each gene were connected with lines of colors matching those used for control chromatin states. Genes in each chromatin transition were positioned according to their expression changes upon Pi-deficiency treatment, with up-regulated genes on the top and down-regulated on the bottom. These transition connections were plotted with ggplot2 (Wickham, 2016). For the circos plot (Figure 3), each rice chromosome was partitioned into bins of 5 kbp. Chromatin states were merged from 200-bp bins to 5-kbp bins in both control and Pi-deficiency samples. The most dominant chromatin state in each merged bin, or the chromatin state of the previous bin if most dominant chromatin state could not be determined, was selected as the
chromatin state for that bin. For gene type partitioning, the most dominant gene type, in base pairs, was used as the bin type for each bin. Chromatin states, differential expression status, and bin types for the merged bins were determined using customized scripts and visualized with the R package circlize (Gu et al., 2014).

**Gene Ontology (GO) clustering**

To identify enriched gene functions associated with the selected chromatin state transitions we followed a two-step process. First, we searched for enriched GO terms in gene clusters assigned to each chromatin state using the enrichment tool BINGO (Maere et al., 2005). However, a single gene can be assigned to multiple GO terms and there is inherent redundancy in processes reported on functional lists generated by GO enrichment analysis tools that cannot be manually assessed, especially when large lists representing thousands of genes are used. Therefore, we used the Markov Clustering Algorithm (Van Dongen and Abreu-Goodger, 2012) on these primary lists of enriched functions to identify non-redundant functional clusters. Briefly, each GO term was represented by a node in a network, and an edge connecting two nodes in the network represent how many genes are shared between each node. We computed a similarity value for each edge based on how many genes were shared between the nodes for each edge to cluster closely related groups. We used an Overlap Coefficient set to a 0.5 cutoff and a granularity value set to 2 for Markov Clustering. The source code written in python for clustering is available at https://github.com/Guannan-Wang/GOMCL. We identified the clusters with the largest number of genes, the hub nodes with the largest number of connections to other nodes, and the most significant clusters with high enrichment for further assessment of these representative functions associated with each chromatin state.

**Accession numbers**

H3K4me3 ChIP-seq and RNA-seq datasets from this article were submitted to the NCBI Sequence Read Archive (SRA) Database under the accession, SRP102668.

**Supplemental Data**
Supplemental Figure S1. H3K4me3 abundance is strongly correlated with gene length but varies in abundance at the transcription start site (TSS).

Supplemental Figure S2. Peaks of H3K4me3 and H2A.Z are correlated in the rice genome.

Supplemental Figure S3. 24-hour Pi deficiency alters the H3K4me3 enrichment pattern across rice protein coding genes (PCG).

Supplemental Figure S4. Comparison of defined chromatin state (CS) predictions.

Supplemental Figure S5. Fold changes of chromatin state (CS) enrichments between the Pi-deficiency and control (-Pi/ctrl) samples.

Supplemental Figure S6. The H3K4me3, H2AZ, and MNase densities of each chromatin state.

Supplemental Figure S7. The average H3K4me3, H2AZ, and MNase density plots of the genes in the three largest chromatin state transitions in control (Ctrl) and Pi-deficiency (–Pi) samples.

Supplemental Figure S8. CS5-CS1 and CS4-CS5 transitions occur in sequence.

Supplemental Figure S9. Identification of differentially expressed genes (DEGs) in response to 24 hours of Pi deficiency.

Supplemental Figure S10. Bootstrapping analysis showing the overlap between genes exhibiting chromatin state transitions (CS5-CS1, CS4-CS5) and differentially expressed genes (DEG) that are down-regulated in shoots following a 21-day Pi-deficiency treatment.

Supplemental Figure S11. Gene Ontology (GO) terms enriched in differentially expressed genes (DEGs) that have a chromatin state (CS) transition of CS4 to CS3.

Supplemental Table S1. Summary of ChIP-seq and RNA-seq libraries (short reads).

Supplemental Table S2. Summary of gene ontology (GO) analysis of genes differentially expressed under phosphate deficiency (-Pi).

Supplementary Dataset S1. Significantly enriched GO terms for four sub-groups of protein-coding genes displaying different H3K4me3 abundance levels at the TSS.

Supplementary Dataset S2. Chromatin states of all PCG at their TSS in control and phosphate deficiency samples.

Supplementary Dataset S3. Significantly enriched GO terms for eight gene groups exhibiting specific chromatin transitions.

Supplementary Dataset S4. Up-regulated and down-regulated genes in response to Pi starvation.
Supplementary Dataset S5. Differentially expressed genes showing a CS4-CS3 chromatin transition.

Acknowledgements

The authors thank High Performance Computing at Louisiana State University (HPC@LSU) for providing computer resources. We also thank Aliasghar Sepehri for sharing the custom FORTRAN script to perform bootstrapping analyses.

Figure legends

Figure 1. H3K4me3 abundance is predominantly associated with the transcription start site (TSS) and co-localizes with H2A.Z. Distribution of H3K4me3 (A) and H2A.Z (B) among four gene types in shoots from rice seedlings grown under control conditions. Control input reads were used for ChIP-Seq read normalization. PCG, protein coding genes; PG, pseudogenes; TE, non-expressed transposable element-related genes; TEG, expressed transposable element-related genes. (C) Scatter plot of read counts from H3K4me3 and H2A.Z samples (Pearson correlation = 0.77). (D) Venn diagram showing the number of H3K4me3- and H2A.Z-enrichment peaks and the overlap.

Figure 2. Correlations of H3K4me3 and H2A.Z distribution with gene expression for rice protein-coding genes. (A) Heat map of H3K4me3 distribution from 500 bp upstream of the transcription start site (TSS) to 500 bp downstream of the transcription end site (TES) in control shoot samples for six gene groups ordered based on transcript abundance level (FPKM), defined as 1st (highest) to 5th (lowest) or no expression (zero). (B,C, and D) Distribution of H3K4me3 (B) or H2AZ (C) or both (D) at the same gene groups as in (A). Control input reads were used for ChIP-Seq read normalization.

Figure 3. Chromatin state predictions for control (Ctrl) and Pi-deficiency (–Pi) samples defined by H3K4me3, H2A.Z, and nucleosome occupancy. (A) Emission parameters for 5 chromatin
states (CS1–5). The darker blue color corresponds to a greater probability of observing the mark in the state. Overlap fold enrichment of various genomic regions with 5 chromatin states in Ctrl (B) and –Pi (C) samples. PCG, protein coding genes; PSG, pseudogenes; TE, non-expressed transposable element-related genes; TEG, expressed transposable element-related genes; TSS, transcription start site of PCG; TES, transcription termination site of PCG. (D) Circos plot showing the chromatin states (in 5-kbp bins) of the whole genome. The first and second rings show the chromatin state in phosphate starved (CS –Pi) and control (CS ctrl) samples, respectively. The third ring shows the log2 fold change (FC) of differentially expressed genes, and the last ring represents four gene types (GT). Inter, intergenic region. Roman numerals represent chromosome numbers.

**Figure 4.** Chromatin state (CS) transitions of protein-coding genes from control (Ctrl) to Pi-deficiency (-Pi) conditions. (Left) The size of each segment represents the number of genes in each CS and the width of each ribbon represents the number of genes with a transition to another CS. (Right) Networks representing Gene Ontology Markov Clustering terms enriched in CS5-CS1, CS4-CS3 and CS4-CS5 groups. Cytoscape was used to visualize enriched GO terms. Node (circle) size represents the number of genes in each node.

**Figure 5.** Chromatin state (CS) transitions are associated with differentially expressed genes (DEGs) under phosphate (Pi) deficiency. (A) Bootstrapping analysis showing the overlap between genes exhibiting CS transitions and down-regulated or up-regulated genes in response to Pi deficiency. Randomly selected genes with the same number of DEGs per CS transition were used as a control, and values are means (±SD) for 1000 iterations. (B) Values are the average probability of each chromatin mark at the CS shown. The category of DEG (up or down) that is biased to the CS is shown at right.

**Figure 6.** Predicted functions and subcellular locations of differentially-expressed genes (DEGs) having a chromatin state (CS) transition of CS4 to CS3. DEGs are grouped based on the putative functional categories shown. (A) The number of DEGs predicted to localize to distinct subcellular locations. (B) The number of DEGs that are up- or down-regulated in each functional category.
Figure 7. Predicted interactions and functions of differentially expressed genes having a chromatin state (CS) transition of CS4 to CS3. Text color indicates up-regulation (red), down-regulation (blue), or a combination of up- and down-regulation (purple). Abbreviations: 2OG, 2OG-Fe oxygenase; a/b bar, A/B barrel; a/b fold, alpha/beta fold hydrolase; ACO, 1-aminocyclopropane-1-carboxylate oxidase; a-glu, heparan-alpha-glucosaminide N-acetyltransferase; AGP, arabinogalactan protein; AIR, auxin response protein; AOS, allene oxide synthase; AP2; B-gal, beta-galactosidase; B-glu, Beta glucan synthase; B-gluc, beta-glucuronidase; BTB, Bric-a-Brac, Tramtrack, Broad Complex protein; bZIP; Ca ATPase; CaM, Calmodulin-related calcium sensor; CHASE; CLP, ATP-dependent caseinolytic protease/crotonase; COBRA, AtCOBRA-like; E3 lig, ubiquitin ligase; EXP, expansin; FA, fatty acid hydroxylase; FER, AtFERONIA ortholog; FORK, FORKED1-like; G3P, glycerol-3-phosphate acyltransferase; GASR, GASA/GAST/ Snakin; GDSL, GDSL-like lipase/acylhydrodase; GDG, gibberellin receptor; GlyHy, glycosyl hydrolase; HAD, HAD phosphoethanolamine/phosphocholine phosphatase; HLH; HLH helix-loop-helix transcription factor; HSF, heat shock factor; HXXXD, HXXXD-type acyl-transferase; ILR, IAA-amino acid hydrolase; Integ, cell wall integrity protein; IQ CaM, IQ calmodulin-binding motif protein; JAZ, ZIM domain-containing JAZ protein; kCoA, 3-ketoacyl-CoA synthase; kinase; KNOT, knotted-1-like homeobox protein; LIG, lignin dirigent; lipase; LRX, leucine-rich repeat extensin; LTPL, Protease inhibitor/seed storage/LTP protein; LYM, lysM domain-containing GPI-anchored protein; MEE, maternal effect embryo arrest; MYB; N-Gly, shiga/ricin-like N-glycosidase; NPC, non-specific phospholipase; OXI, oxidoreductase; P450, cytochrome P450; PDD, PD-(D/E)XK nuclease superfamily protein; PEC, pectinase; PLA, phospholipase A; PLATZ; PNM, phosphoethanolamine N-methyltransferase; POX, peroxidase; PPR, pentatricopeptide repeat protein; RALF, Rapid ALkalinization Factor; RLK, receptor-like kinase; S1P, Subtilisin Site-1 Protease; SAM, S-adenosyl-L-methionine-dependent methyltransferases; SRO, OsSRO1c; SULF, sulfotransferase; UDP, UDP-glucuronosyl/UDP-glucosyltransferase; UVB, ultraviolet-B-repressible protein; VQ, VQ domain containing protein; WAK, wall-associated kinase; WAX, WAX2-like; WRKY; ZGT, ZGT circadian clock coupling factor; ZR1, FYVE zinc finger domain protein.
Figure 1. H3K4me3 abundance is predominantly associated with the transcription start site (TSS) and co-localizes with H2A.Z. Distribution of H3K4me3 (A) and H2A.Z (B) among four gene types in shoots from rice seedlings grown under control conditions. Control input reads were used for ChIP-Seq read normalization. PCG, protein coding genes; PG, pseudogenes; TE, non-expressed transposable element-related genes; TEG, expressed transposable element-related genes. (C) Scatter plot of log2 (ChIP-Seq/input) read counts from H3K4me3 and H2A.Z samples (Pearson correlation = 0.77). (D) Venn diagram showing the number of H3K4me3- and H2A.Z-enrichment peaks and the overlap.
Figure 2. Correlations of H3K4me3 and H2A.Z distribution with gene expression for rice protein-coding genes. (A) Heat map of H3K4me3 distribution from 500 bp upstream of the transcription start site (TSS) to 500 bp downstream of the transcription end site (TES) in control shoot samples for six gene groups ordered based on transcript abundance level (FPKM), defined as 1<sup>st</sup> (highest) to 5<sup>th</sup> (lowest) or no expression (zero). (B, C, and D) Distribution of H3K4me3 (B) or H2AZ (C) or both (D) at the same gene groups as in (A). Control input reads were used for ChIP-Seq read normalization.
Figure 3. Chromatin state predictions for control (Ctrl) and Pi-deficiency (–Pi) samples defined by H3K4me3, H2A.Z, and nucleosome occupancy. (A) Emission parameters for 5 chromatin states (CS1–5). The darker blue color corresponds to a greater probability of observing the mark in the state. Overlap fold enrichment of various genomic regions with 5 chromatin states in Ctrl (B) and –Pi (C) samples. PCG, protein coding genes; PSG, pseudogenes; TE, non-expressed transposable element-related genes; TEG, expressed transposable element-related genes; TSS, transcription start site of PCG; TES, transcription termination site of PCG. (D) Circos plot showing the chromatin states (in 5-kbp bins) of the whole genome. The first and second rings show the chromatin state in phosphate starved (CS –Pi) and control (CS ctrl) samples, respectively. The third ring shows the log2 fold change (FC) of differentially expressed genes, and the last ring represents four gene types (GT). Inter, intergenic region; Roman numerals represent chromosome numbers.
Figure 4. Chromatin state (CS) transitions of protein-coding genes from control (Ctrl) to Pi deficiency (-Pi) conditions. (Left) The size of each segment represents the number of genes in each CS and the width of each ribbon represents the number of genes with a transition to another CS. (Right) Networks representing Gene Ontology Markov Clustering terms enriched in CS5-CS1, CS4-CS3 and CS4-CS5 groups. Cytoscape was used to visualize enriched GO terms. Node (circle) size represents the number of genes in each node.
Figure 5. Chromatin state (CS) transitions are associated with differentially-expressed genes (DEGs) under phosphate deficiency. (A) Bootstrapping analysis showing the overlap between genes exhibiting CS transitions and down-regulated or up-regulated genes in response to Pi deficiency. Randomly-selected genes with the same number of DEGs per CS transition were used as a control, and values are means (±SD) for 1000 iterations. (B) Values are the average probability of each chromatin mark at the CS shown. The category of DEG (up or down) that is biased to the CS is shown at right.
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