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# Genome-wide Nucleosome Occupancy and Organization Modulates the Plasticity of Gene Transcriptional Status in Maize

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## ABSTRACT

**Nucleosomes are fundamental units of chromatin that play critical roles in gene regulation by modulating DNA accessibility. However, their roles in regulating tissue-specific gene transcription are poorly understood. Here, we present genome-wide nucleosome maps of maize shoot and endosperm generated by sequencing the micrococcal nuclease digested nucleosomal DNA. The changes of gene transcriptional status between shoot and endosperm were accompanied by preferential nucleosome loss from the promoters and shifts in the first nucleosome downstream of the transcriptional start sites (+1 nucleosome) and upstream of transcriptional termination sites (−1 nucleosome). Intrinsically DNA-encoded nucleosome organization was largely associated with the capacity of a gene to alter its transcriptional status among different tissues. Compared with tissue-specific genes, constitutively expressed genes showed more pronounced 5′ and 3′ nucleosome-depleted regions as well as further +1 nucleosome to transcriptional start sites and −1 nucleosome to transcriptional termination sites. Moreover, nucleosome organization was more highly correlated with the plasticity of gene transcriptional status than with its expression level when examined using *in vivo* and predicted nucleosome data. In addition, the translational efficiencies of tissue-specific genes appeared to be greater than those of constitutively expressed genes. Taken together, our results indicate that intrinsically DNA-encoded nucleosome organization is important, beyond its role in regulating gene expression levels, in determining the plasticity of gene transcriptional status.**

**Key words:** Nucleosome, Gene transcription, Plasticity, Maize

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## INTRODUCTION

Nucleosomes are the fundamental structural units of chromatin. Each nucleosome consists of 147 bp of DNA wrapped around a core histone octamer (Luger et al., 1997). The genome-wide nucleosome occupancy is determined by a combination of multiple factors (Struhl and Segal, 2013). A basic determinant is the DNA sequence. Because nucleosomal DNA mostly wraps around histone octamers, the properties of the sequence, particularly the bending characteristics, can affect intrinsic histone–DNA interactions and thus influence nucleosome formation (Segal et al., 2006). Studies of *in vitro* reconstructions of nucleosomes in yeast and human demonstrated that certain DNA sequences, notably poly(dA:dT) tracts, intrinsically deter nucleosome formation, whereas non-homopolymeric G/C-rich sequences promote nucleosome formation (Kaplan et al., 2008; Zhang et al., 2009, 2011; Valouev et al., 2011). Recent studies in human cell lines showed that the DNA sequence plays a significant role in the determination of nucleosome position (Sexton et al., 2014, 2016). However, many features of the *in vivo* nucleosome occupancy pattern over genes cannot be reconstituted *in vitro* (Kaplan et al., 2008; Zhang et al., 2009). The nucleosome occupancy can also be affected by some cellular *trans* factors, including ATP-dependent chromatin

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remodelers and site-specific DNA-binding proteins, which can often override the influence of intrinsic DNA sequence preferences to drive nucleosomes occupying intrinsically unfavorable DNA sequences or evict nucleosomes from intrinsically favorable binding sites (Radman-Livaja and Rando, 2010; Bell et al., 2011; Struhl and Segal, 2013). Only ~50% of the *in vivo* nucleosome organization can be explained by intrinsic DNA sequence preferences in *Saccharomyces cerevisiae* (Segal et al., 2006). However, the percentage of nucleosome organization explained by intrinsic DNA sequences is affected by the cell type and analysis method used (Gaffney et al., 2012). Overall, the nucleosome occupancy status *in vivo* is a result of combined effects of the DNA sequence and various cellular factors.

In the past decade, genome-wide nucleosome occupancy maps were generated for many species, including yeast (Yuan et al., 2005; Lee et al., 2007; Brogaard et al., 2012), *Drosophila* (Mavrich et al., 2008), *Arabidopsis* (Chodavarapu et al., 2010; Li et al., 2014; Liu et al., 2015), rice (Wu et al., 2014), and mouse (Teif et al., 2012), as well as human (Schones et al., 2008; Valouev et al., 2011). These studies revealed many fundamental aspects of nucleosome organization, including the relationship between nucleosome positions and gene expression, transcription factor binding sites, DNase I hypersensitive sites, and DNA methylation (Yuan et al., 2005; Lee et al., 2007; Mavrich et al., 2008; Schones et al., 2008; Chodavarapu et al., 2010; Valouev et al., 2011; Brogaard et al., 2012; Teif et al., 2012; Li et al., 2014; Wu et al., 2014; Liu et al., 2015). Although there are differences of nucleosome organization in different species (Mavrich et al., 2008; Wu et al., 2014), many features of nucleosome occupancy patterns are common for a typical gene (Radman-Livaja and Rando, 2010; Struhl and Segal, 2013). For example, nucleosomes are depleted at the promoters and 3′ ends of genes and are strongly positioned downstream of the transcriptional start site (TSS), with the degree of nucleosome positioning gradually decreasing from the 5′–3′ ends of genes (Jiang and Pugh, 2009; Radman-Livaja and Rando, 2010). Nucleosomes can modulate the accessibility of DNA for transcription factors, which often preferentially bind in nucleosome-depleted regions (NDRs), although some, such as Nanog, Sox2, and Oct4, as well as pioneering factors, preferentially bind in regions of high nucleosome occupancy (Jiang and Pugh, 2009; Radman-Livaja and Rando, 2010; Teif et al., 2012). Thus, nucleosome positioning plays a vital role in gene transcriptional regulation (Jiang and Pugh, 2009). Compared with lower-expressing genes, higher-expressing genes tend to have a greater degree of nucleosome depletion at their promoter regions and more well-phased nucleosomes along their gene bodies (Lee et al., 2007; Schones et al., 2008; Li et al., 2014). Moreover, the position of the first nucleosome downstream of the TSS is closely related to polymerase II (Pol II) pausing (Mavrich et al., 2008; Schones et al., 2008). Compared with genes with stalled Pol II, genes with elongating Pol II showed longer distance to the TSS for the first nucleosome downstream of the TSS (Schones et al., 2008). Nucleosome occupancy changes are related to transcriptional changes and are associated with age-dependent alterations, cell differentiation, and reprogramming (Shivaswamy et al., 2008; Bochkis et al., 2014; Hu et al., 2014; West et al., 2014). By measuring nucleosome distributions at a high temporal resolution, nucleosome redistributions were shown to be widespread in genomic responses and likely potentiate regulatory factor binding (Sexton et al., 2014, 2016). In addition, nucleosome organization is associated with the capacity to modulate gene expression upon changing conditions (Tirosh and Barkai, 2008; Choi and Kim, 2009). However, little is known regarding the role of nucleosome organization associated with the plasticity of gene transcriptional status, which refers here to the capacities of genes to alter their transcriptional statuses among different tissues. In contrast to constitutive genes that are transcribed constantly in all tissues, the transcriptional statuses of tissue-specific genes are more "plastic" and thus more variable among different tissues.

Maize (*Zea mays*), an important crop worldwide, is a model species for plant genomic research. In addition to the predicted nucleosome occupancy (Fincher et al., 2013), *in vivo* nucleosome occupancy in maize was first investigated using a differential micrococcal nuclease (MNase) sensitivity assay with microarray quantification (Vera et al., 2014). In a recent study, genome-wide open chromatin regions, which are closely associated with nucleosome organization, were generated by differential MNase sensitivity and high-throughput sequencing of the maize genome (Rodgers-Melnick et al., 2016). MNase hypersensitive regions, consisting of less than 1% of the genome, can explain ~40% of the heritable phenotypic variance in diverse complex traits (Rodgers-Melnick et al., 2016), indicating the importance of nucleosome organization in maize. Here, we present genome-wide nucleosome maps of maize shoot and endosperm generated by the deep sequencing of the nucleosome-protected DNA fragments isolated from MNase-digested chromatin. We determined the general characteristics of the nucleosome occupancy pattern around genes and their correlation with gene transcription. The change of gene transcriptional status between shoot and endosperm is mainly accompanied by the eviction of nucleosomes just upstream of the TSSs, as well as a shift of the first nucleosome downstream of the TSSs and upstream of the transcriptional termination sites (TTSs). In addition to the gene expression level, the nucleosome organization of a gene is also associated with its capacity to alter its transcriptional status among different tissues. Moreover, nucleosome organization is better correlated with the plasticity of gene transcriptional status than gene expression level. Our results expand the understanding of the fundamental roles of nucleosome organization in gene transcriptional regulation.

## RESULTS

### Mapping Nucleosome Occupancy in Maize Shoot and Endosperm

To obtain a genome-wide nucleosome occupancy map in maize and characterize its association with gene transcription, we digested chromatin from 14-day-old shoots and from endosperm 12 days after pollination of the maize inbred B73 (Supplemental Figure 1A), for which the reference genome sequences are available (Schnable et al., 2009), with MNase to generate mononucleosomal DNA fragments, and sequenced the products using the Illumina 100-nt paired-end sequencing strategy (MNase-seq). The two-units and one-unit levels of MNase were chosen for shoot and endosperm samples (Supplemental

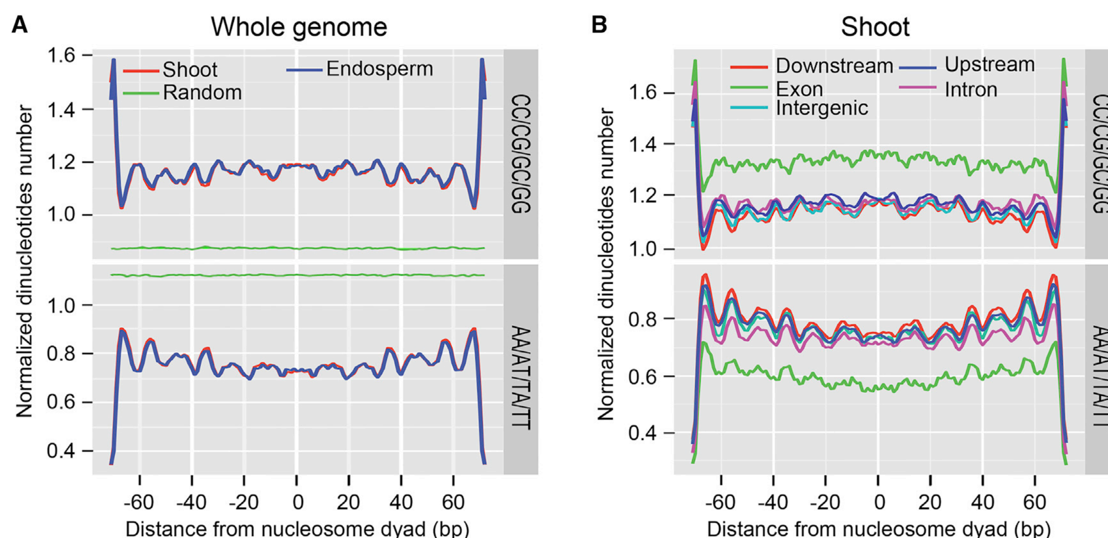


**Figure 1. Sequence Characteristics of Maize Nucleosomal DNA.**

Composite distribution of AA/AT/TA/TT and CC/CG/GC/GG dinucleotides along the 147-bp axis of nucleosomal DNA in the whole genome **(A)** and different genomic regions **(B)**. Random, calculated using the randomly generated 147-bp fragments from the maize genome; Upstream, upstream 2 kb of the transcriptional start sites (TSSs); Downstream, downstream 2 kb of the transcriptional termination sites (TTSs).

Figure 1B), respectively, because ~80% mononucleosomes were generated at these concentrations, which is most suitable for nucleosome occupancy analysis according to a previous report (Weiner et al., 2010). The degree of digestion is also comparable with that performed in other species, such as yeast, *Drosophila*, *Arabidopsis,* rice, mouse, and human (Yuan et al., 2005; Mavrich et al., 2008; Schones et al., 2008; Bochkis et al., 2014; Li et al., 2014; Wu et al., 2014; Liu et al., 2015). Only uniquely aligned paired reads were used to analyze the nucleosome occupancy. Finally, 489 and 522 million effective reads were determined for shoot and endosperm, respectively, which corresponded to an approximately 25-fold coverage of all core nucleosomes assuming that every 200 bp of DNA contains one nucleosome throughout the genome (Supplemental Table 1). As expected, the insert size distributions of the two libraries were highly similar, with the major peaks near the mononucleosomal DNA length being ~150 bp, as determined by the DNA electrophoretogram (Supplemental Figure 1B and 1C), reflecting a comparable degree of digestion for shoot and endosperm. There exists a minor peak at a 20-bp interval from the major peak, which could be caused by the overdigestion of 10 bp from both ends of the nucleosomes. In addition, the genomic distributions (Supplemental Figure 2) and the nucleosome occupancy patterns over genes (see later section) were similar based on the analysis of the paired reads from insert sizes of 120–140 bp and 140–160 bp, indicating that there was no particular bias between the two typical fragments (~130 bp and ~150 bp) in the libraries.

To examine the existence of the 10-bp periodicities of dinucleotides, which is regarded as a general feature of nucleosomal DNA (Struhl and Segal, 2013), we determined the frequency of dinucleotides across nucleosomal DNA. The 147-bp fragments were used to reduce the effect of over- or underdigestion of nucleosomal DNA. As seen in other organisms (Segal et al., 2006; Mavrich et al., 2008; Chodavarapu et al., 2010; Gaffney et al., 2012), clear 10-bp periodicities of AA/AT/TA/TT and CC/CG/GC/GG dinucleotides were found (Figure 1A). Moreover, the 10-bp periodic patterns in shoot and endosperm were almost the same (Figure 1A). We also investigated the nucleotide composition of nucleosomal DNA in different regions of the genome. The AA/AT/TA/TT dinucleotides level for nucleosomes in exons is lower than that in other regions, while the CC/CG/GC/GG dinucleotides level for nucleosomes in exons is higher than that in other regions (Figure 1B and Supplemental Figure 3). Further analysis indicated that this is at least partly because of the higher CG content in exons (Supplemental Figure 4). In addition, the AA/AT/TA/TT dinucleotides' ratio showed a sharp decrease at the borders of nucleosomal DNA fragments (Figure 1), which reflects the sequence preference of MNase digestion at A/T-containing dinucleotides (Dingwall et al., 1981; Hörz and Altenburger, 1981).

## Nucleosome Repeat Length Variation in Different Tissues and Chromosomal Regions

The nucleosome repeat length (NRL), also known as the average distance between two neighboring nucleosomes, is an important parameter for primary chromatin organization and mainly varies with linker DNA lengths (Luger et al., 1997; Fransz and Jong, 2011). We determined NRLs using a previously described method (Valouev et al., 2011), except that the middle positions of paired reads were used to calculate the nucleosome distances instead of the start positions of reads to reduce the effect of non-homogeneous MNase digestion of different nucleosomal DNA fragments. The resulting plot exhibits a wave-like pattern with the periodic appearance of peaks, which reflects the preferred inter-nucleosomal distances (Figure 2A). We then plotted the lines using nucleosome numbers and corresponding peak positions, and obtained phase values of 193.5 bp and 190.7 bp, which represent the NRLs for shoot and endosperm, respectively (Figure 2B). As reported

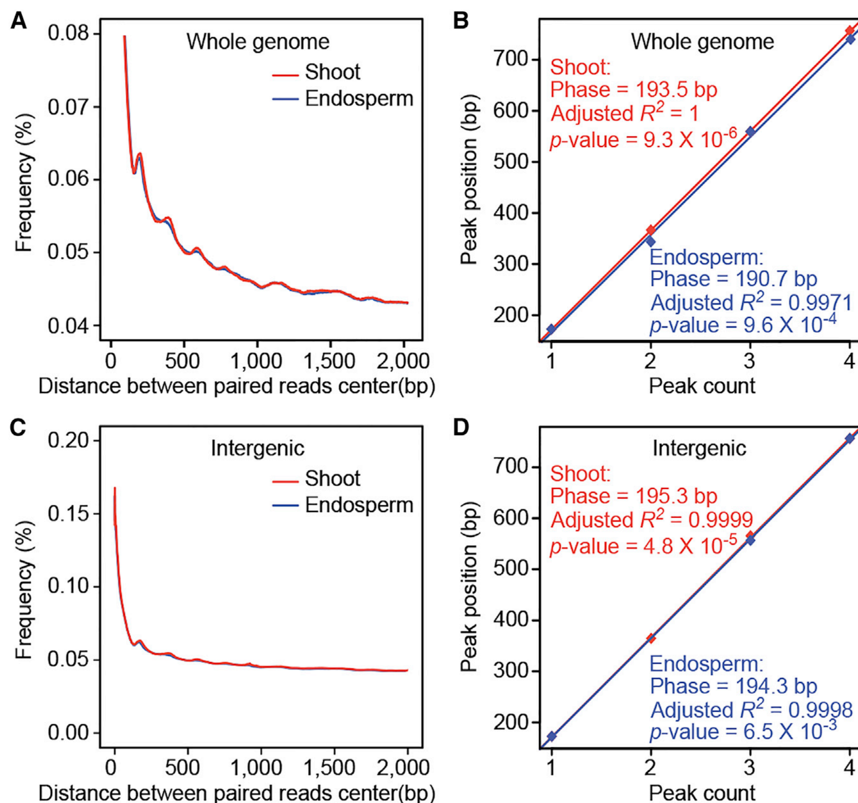


**Figure 2. Nucleosome Repeat Length in Shoot and Endosperm.**

**(A and C)** Distributions of distances between all of the nucleosome middle positions in the whole genome **(A)** and intergenic regions **(C)**.

**(B and D)** The line fitted through the plot of the peak positions versus the corresponding peak counts.

previously, linker histone gene expression, which is tied to linker length differences, tends to be higher in tissues with longer NRLs (Fan et al., 2005; Valouev et al., 2011; Teif et al., 2012). Indeed, we identified six homologous maize genes of *Arabidopsis* histone H1, and the expression levels in shoot are all consistently higher than those in endosperm (Supplemental Table 2). Thus, although the differences in NRLs are small between shoot and endosperm, they might reflect different chromatin statuses in the two tissues. We also compared the NRLs of the intergenic regions in the two tissues and the NRLs of the intergenic regions are 195.3 bp and 194.3 bp for shoot and endosperm (Figure 2C and 2D), respectively, slightly longer than the corresponding genome-wide NRLs. This is consistent with the observation in *in vitro* nucleosome reconstruction that long-linker chromatin is associated with a repressed chromatin state (Routh et al., 2008; Grigoryev et al., 2009). The NRLs of genic regions were not compared because there were not enough peaks in the wave-like pattern for analysis.

## Nucleosome Occupancy Near TSSs and TTSs

To determine the nucleosome occupancy of genes, we used DANPOS (Chen et al., 2013) to analyze the MNase-seq data. We investigated the average nucleosome occupancy patterns over all of the genes to determine the features of nucleosomes near the TSSs and TTSs. The characteristic NDR immediately upstream of the TSS and over the TTS, and a well-positioned nucleosome downstream of the TSS and upstream of the TTS, were observed in both shoot and endosperm (Figure 3A). To further investigate the correlation between nucleosome organization and gene transcription, we performed a transcriptome analysis using the RNA sequencing (RNA-seq) data of shoot and endosperm (Supplemental Table 1). RNA-seq data of both tissues had a high degree of correlation ($R^2 > 0.97$) for expression values between two biological replicates (Supplemental Figure 5). For operability, we defined the unexpressed genes as genes having transcript levels of fragments per kilobase per million (FPKM) lower than 1, although some might still be lowly expressed. Compared with the unexpressed genes, the transcriptionally active genes showed wider and deeper NDRs at both the 5′ and 3′ ends, lower nucleosome occupancies in the gene bodies, and more pronounced phasing of nucleosomes downstream of the TSSs (Figure 3A), which was in agreement with previous reports in yeast, *Arabidopsis*, rice, mouse, and human (Lee et al., 2007; Schones et al., 2008; Teif et al., 2012; Li et al., 2014; West et al., 2014; Wu et al., 2014; Liu et al., 2015). This was further confirmed by comparisons of nucleosome occupancies among genes divided based on a gradient of their expression levels (Supplemental Figure 6A and 6B). These observations are consistent with the positive correlation between gene expression level and signal strength for MNase hypersensitive regions around TSSs in maize (Rodgers-Melnick et al., 2016) because the open chromatin regions largely correspond to NDRs (Furey, 2012). Thus, our results indicated that reduced nucleosome occupancy levels at the promoter region and gene body are associated with increases in gene expression levels.

The degree of MNase digestion and the fragment sizes used for analysis are critical for the determination of nucleosome occupancy (Weiner et al., 2010; Henikoff et al., 2011; Kent et al., 2011; Carone et al., 2014; Vera et al., 2014). To explore whether there are differences in the determination of nucleosome occupancy when using the subnucleosome-sized or the nucleosome-sized DNA fragments generated in our study, we analyzed the nucleosome occupancy using fragments of 120–140 bp and 140–160 bp, respectively, which accounted for most of the fragments used in the analysis (Supplemental Figure 1C). Similar relationships between nucleosome occupancy and gene expression levels were observed based on the two types of reads (Supplemental Figure 7), demonstrating the consistency of the nucleosomal DNA fragments generated in our study. We also analyzed the nucleosome occupancy data of shoot tissue using a 10-fold lower MNase concentration (0.2 U) for digestion (Supplemental Figure 1B). The relationship between nucleosome occupancy and gene transcription was reproduced using the

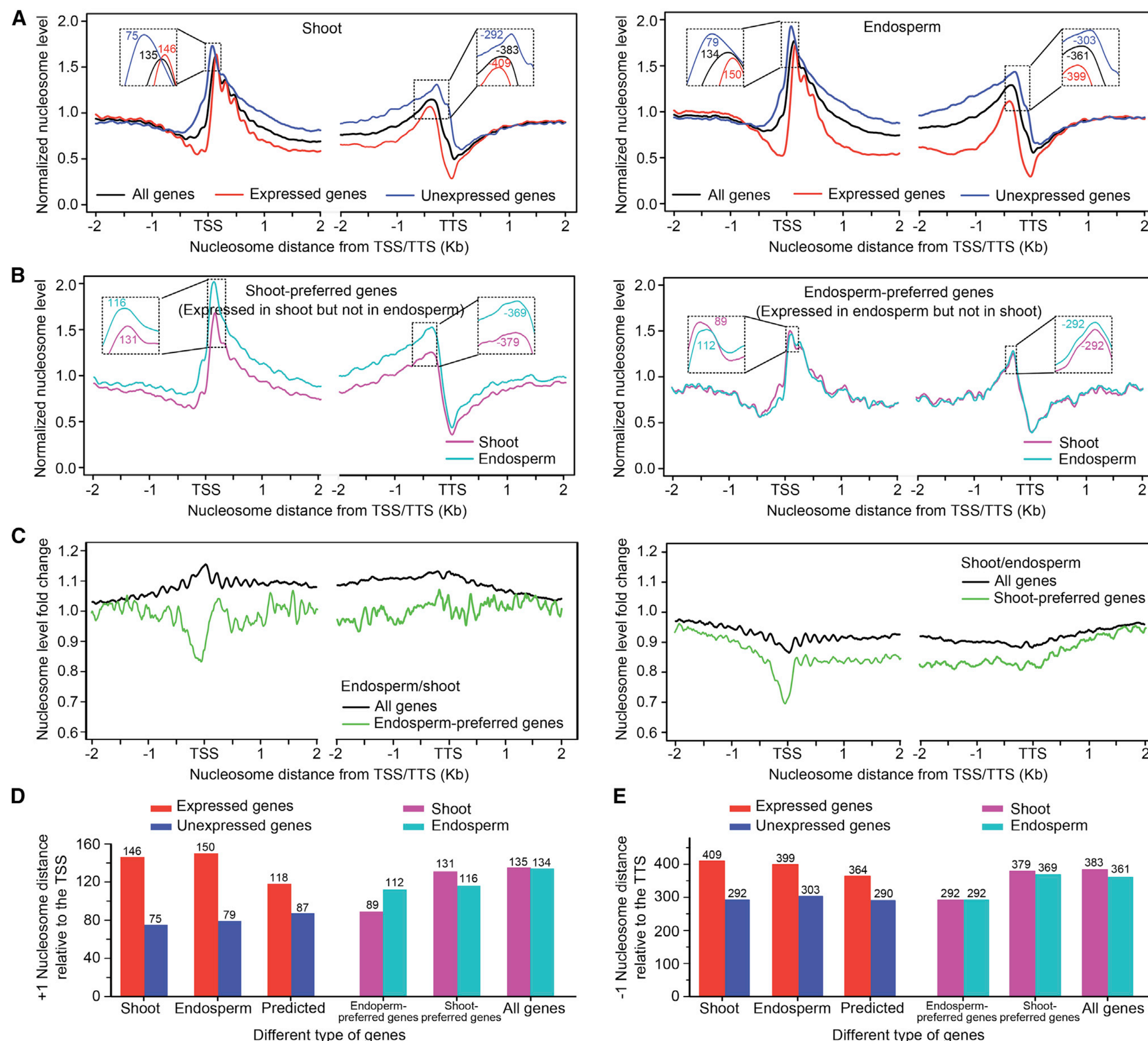


**Figure 3. Nucleosome Organization Near the 5′ and 3′ Ends of Genes in Maize.**

**(A)** Average nucleosome occupancy patterns near the TSSs and TTSs for all genes, and genes classified by their expression levels.

**(B)** Average nucleosome occupancy patterns near the TSSs and TTSs for shoot- and endosperm-preferred genes. The +1 and −1 nucleosomes and their distance relative to the TSSs and TTSs, respectively, are shown in enlarged figures.

**(C)** The fold change of nucleosome occupancy near the TSSs and TTSs for the endosperm- and shoot-preferred genes.

**(D and E)** The +1 **(D)** and −1 **(E)** nucleosome distances relative to the TSSs and TTSs, respectively. Predicted, the distances were obtained based on the nucleosome occupancy data predicted by NuPoP and gene expression levels in shoot; Endosperm-preferred genes, expressed in endosperm but not in shoot; Shoot-preferred genes, expressed in shoot but not in endosperm.

lightly digested nucleosome occupancy data (Supplemental Figure 8). This further confirmed that the NDRs at the 5′ and 3′ ends of genes are existing, rather than the product of over digestion. The nucleosome occupancy level in the transposon region was greater than 1 (the value 1 is the average level of the entire genome), while the nucleosome occupancy level in other regions was less than 1 (Supplemental Figure 9). This perhaps is due to the repressed chromatin state of transposons associated with long-linker chromatin, and thus leads to a greater sensitivity to MNase digestion (Fransz and Jong, 2011; Wu et al., 2014).

The first nucleosome located downstream of the 5′ NDR is the +1 nucleosome. The position of the +1 nucleosome relative to the TSS can be related with Pol II pausing (Mavrich et al., 2008; Schones et al., 2008). The +1 nucleosome in the average nucleosome occupancy pattern of the genes occurred ∼135 bp downstream of the TSS in maize (Figure 3A). Moreover, the position of the +1 nucleosome was associated with gene expression level, with highly expressed genes displaying longer distances of the +1 nucleosome relative to the TSS (Supplemental Figure 6C). The distance of the −1 nucleosome

(the first well-phased nucleosome upstream of the 3′ NDR) relative to the TTS was also positively correlated with the gene expression level (Supplemental Figure 6D). This may be due to the greater distances of the +1 nucleosome relative to the TSS and the −1 nucleosome to the TTS, which could facilitate access for the transcriptional machinery, or serve as a result of transcriptional activity, or both. Thus, there was a remarkable association of the +1 and −1 nucleosome locations with gene transcription.

## Dynamics of Nucleosome Occupancy Related to Transcriptional Status Change

To examine how nucleosome occupancy dynamics are related to gene transcriptional status change among different tissues, we compared the nucleosome occupancy in shoot-preferred genes (referring to genes expressed in shoot but not expressed in endosperm) and endosperm-preferred genes (referring to genes expressed in endosperm but not expressed in shoot). The nucleosome occupancy in shoot was lower than that in endosperm for shoot-preferred genes, consistent with the lower nucleosome occupancy level in shoot for genes expressed or unexpressed in both tissues (Figure 3B and Supplemental Figure 10). The nucleosome occupancy difference between shoot and endosperm for shoot-preferred genes was significantly greater than that of genes expressed or unexpressed in both tissues ($p$ value <2.2e-16, Supplemental Figure 11), suggesting that the expression of shoot-preferred genes was accompanied by an overall reduction of nucleosome occupancy over genes. For endosperm-preferred genes, the nucleosome occupancy patterns were almost the same in shoot and endosperm (Figure 3B), which likely reflected the reduction of nucleosome occupancy in endosperm. Thus, the nucleosome occupancy differences between shoot and endosperm were significantly decreased when compared with genes expressed or unexpressed in both tissues ($p$ value <2.2e-16, Supplemental Figure 11). Overall, these results indicated that the nucleosome occupancy changed along with gene transcriptional status change. A nucleosome occupancy fold-change analysis was performed to better understand the dynamics of nucleosome occupancy related to transcriptional status change. Compared with all genes, the shoot- and endosperm-preferred genes showed an obvious reduction in the nucleosome occupancy in the TSS-proximal region in shoot and endosperm, respectively (Figure 3C). This revealed that the transcriptional change of genes from the silenced to activated status was accompanied by the nucleosome eviction near the TSS, which could promote the accessibility of transcriptional initiation elements. In addition, the nucleosome occupancy level in the gene bodies and 3′ ends of genes was also reduced in the activated status (Figure 3C), reflecting a potential role of transcriptional elongation and termination in nucleosome remodeling.

We also examined the positional variation of the +1 and −1 nucleosomes that were accompanied with transcriptional status change. The +1 nucleosome distance to the TSS for endosperm-preferred genes in endosperm was 23 bp longer than that in shoot, and the +1 nucleosome distance to the TSS for shoot-preferred genes in shoot was 15 bp longer than that in endosperm (Figure 3B and 3D), indicating that the +1 nucleosome shifted toward the downstream direction of the TSS when the transcriptional status of a gene changed from silenced to activated. However, the shifted distance was much less than the difference (~70 bp) in the +1 nucleosome distance to the TSS between expressed and unexpressed genes in shoot or endosperm (Figure 3D). Thus, the variation in the +1 nucleosome position accompanying gene transcriptional status change could account for only part of the difference in the +1 nucleosome location between expressed and unexpressed genes in a tissue, which reflected the effects of intrinsic DNA sequences on nucleosome organization (Kaplan et al., 2008; Zhang et al., 2009, 2011; Valouev et al., 2011). Similarly, the −1 nucleosome position also shifted with gene transcriptional status change, and the distance shifted was far less than the difference in the −1 nucleosome distance to the TTS between expressed and unexpressed genes in shoot or endosperm (Figure 3E).

## Intrinsically DNA-Encoded Nucleosome Organization Is Associated with Gene Transcriptional Status Plasticity in Different Tissues

To examine the contributions of DNA sequences to nucleosome organization, we used NuPoP (Xi et al., 2010) to predict nucleosome organization based on genome sequences. The predicted nucleosome organization is called "intrinsically DNA-encoded nucleosome organization" in our study, which is different from the experimentally measured *in vivo* nucleosome organization that resulted from the combined effects of DNA sequences *per se* and various *trans* factors in the cellular environment. The NuPoP method was built upon a duration hidden Markov model by incorporating the nucleosome and linker DNA information, and can calculate the nucleosome occupancy score for each base pair of the genome (Xi et al., 2010). Overall, the typical patterns of nucleosome occupancy near the TSSs and TTSs, as well as the correlations between nucleosome organization and gene expression level, were reproduced using the predicted nucleosome occupancy data (Figure 4A), which was consistent with the basic roles of DNA sequences in nucleosome organization determination *in vivo*. However, compared with the predicted nucleosome occupancy data, more well-positioned nucleosomes downstream of the TSS and longer distance of the +1 nucleosome to the TSS were observed *in vivo*, especially for expressed genes (Figures 3A and 4A), reflecting the effects of cellular *trans* factors on nucleosome remodeling.

Transcriptional status changes are essential for the development and differentiation of various tissues. The transcriptional status of constitutive genes is not influenced by the change of tissue identities, while the transcriptional status of tissue-specific genes is variable among different tissues. To investigate whether nucleosome organization plays a role in determining the genes being expressed constitutively or tissue-specifically, we first identified 4756 tissue-specific genes that were preferentially expressed in at least one of the given tissues, 2590 constitutive genes expressed in all of the tested tissues, and 22 236 intermediate genes, based on their expression profiles in published RNA-seq data (Jia et al., 2009; Wang et al., 2009; Li et al., 2010; Davidson et al., 2011; Bolduc et al., 2012; Chen et al., 2014), including embryo, endosperm, leaf, root, shoot, shoot apical meristem, cob, tassel, and immature ear tissues. The nucleosome organization patterns of the three types of genes were then analyzed based on the

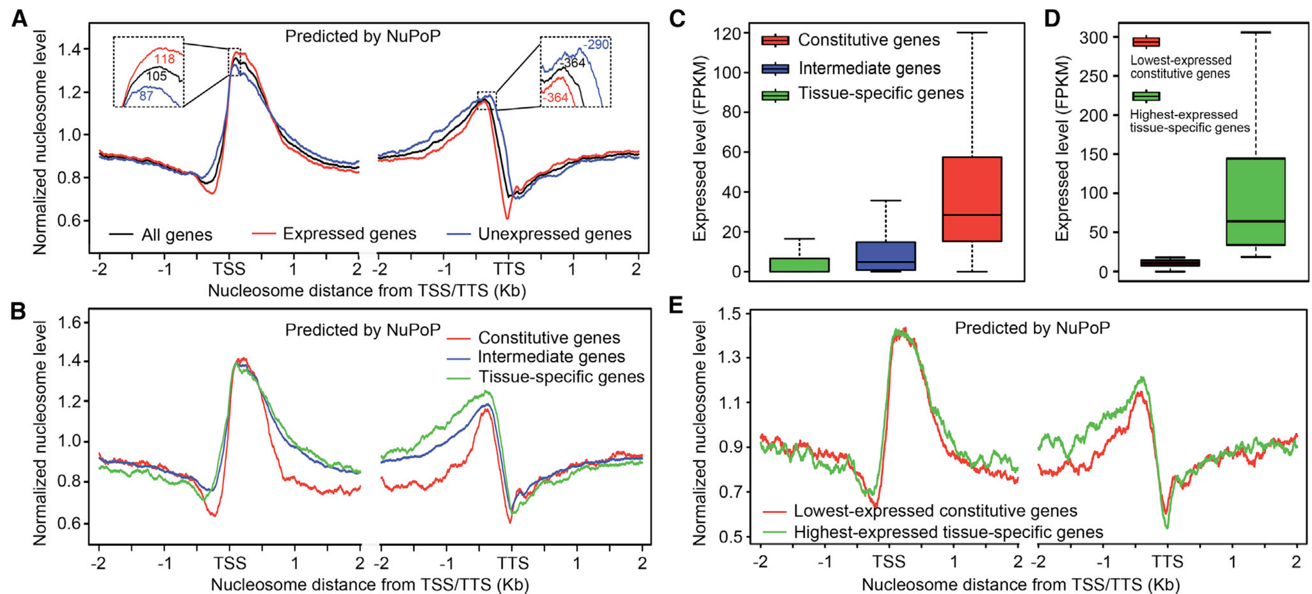


**Figure 4. Correlation between Nucleosome Organization and Gene Transcription.**

**(A and B)** Average nucleosome occupancy patterns near the TSSs and TTSs for genes classified by their expression levels in shoot **(A)** and genes classified based on their transcriptional status in different tissues (see Methods) **(B)** using the nucleosome occupancy data predicted by NuPoP. The +1 and −1 nucleosomes and their distances relative to the TSSs and TTSs, respectively, are shown in enlarged figures.

**(C)** Comparison of expression levels of constitutive, intermediate, and tissue-specific genes in shoot. The box plots display a gradient of expression levels: constitutive > intermediate > tissue-specific genes. All identified tissue-specific genes were used for analysis.

**(D)** Comparison of expression levels of the lowest-expressed constitutive genes and the highest-expressed tissue-specific genes in shoot.

**(E)** Average nucleosome occupancy patterns near the TSSs and TTSs for the lowest-expressed constitutive genes and highest-expressed tissue-specific genes using the nucleosome occupancy data predicted by NuPoP.

predicted nucleosome occupancy data. Constitutive genes had the lowest nucleosome occupancy level, while tissue-specific genes had the highest nucleosome occupancy level in gene bodies (Figure 4B). Moreover, although nucleosome depletion occurred near the TSSs in all three types of genes, the NDRs of the tissue-specific genes were less pronounced than those of constitutive and intermediate genes (Figure 4B). The differences in the nucleosome organization between constitutive and tissue-specific genes were reproduced using the *in vivo* nucleosome occupancy data in both shoot and endosperm (Supplemental Figures 8B and 12). In addition, compared with constitutive genes, tissue-specific genes exhibited closer +1 and −1 nucleosome positions relative to their TSSs and TTSs, respectively (Supplemental Figures 8B and 12). Overall, greater nucleosome occupancy levels near the TSS and TTS, as well as closer +1 and −1 nucleosomes to the TSS and TTS, respectively, were related to the capacities of genes to alter their transcriptional statuses among different tissues. Thus, the expressional pattern, constitutively or tissue-specifically expressed, appears to be associated with the intrinsically DNA-encoded nucleosome organization.

## Relationships among the Intrinsically DNA-Encoded Nucleosome Organization, Expression Level, and Transcriptional Status Plasticity of Genes

The intrinsically DNA-encoded nucleosome organization was associated with both gene expression level and transcriptional status plasticity in different tissues. The expression levels of constitutive genes were typically greater than tissue-specific genes ($p$ value <2.2e-16, Figure 4C). To further investigate the different contributions of intrinsically DNA-encoded nucleosome organization on the expression level and the plasticity of gene transcriptional status, we compared the nucleosome organization of the lowest-expressed constitutive genes and the highest-expressed tissue-specific genes based on their transcript levels in shoot (Figure 4D). This analysis allowed us to distinguish the effects of the intrinsically DNA-encoded nucleosome organization on expression level from its effects on the plasticity of the transcriptional status. Overall, the nucleosome occupancy of the lowest-expressed constitutive genes was lower than that of the highest-expressed tissue-specific genes using predicted nucleosome occupancy data (Figure 4E). Similar results were observed based on an analysis of the *in vivo* data of shoot and endosperm (Supplemental Figures 13 and 14). Moreover, the distances of the +1 nucleosome to the TSS and the −1 nucleosome to the TTS in the lowest-expressed constitutive genes were longer than those of the highest-expressed tissue-specific genes in shoot and endosperm (Supplemental Figures 13 and 14). Thus, nucleosome organization was correlated with the plasticity of the transcriptional status of genes to a greater extent than with their expression levels.

The constitutive genes in general had greater expression levels than the tissue-specifically expressed genes. However, whether the transcriptional products (from both constitutively and tissue-specifically expressed genes) were used equally during the translation process has not been examined. The rate of

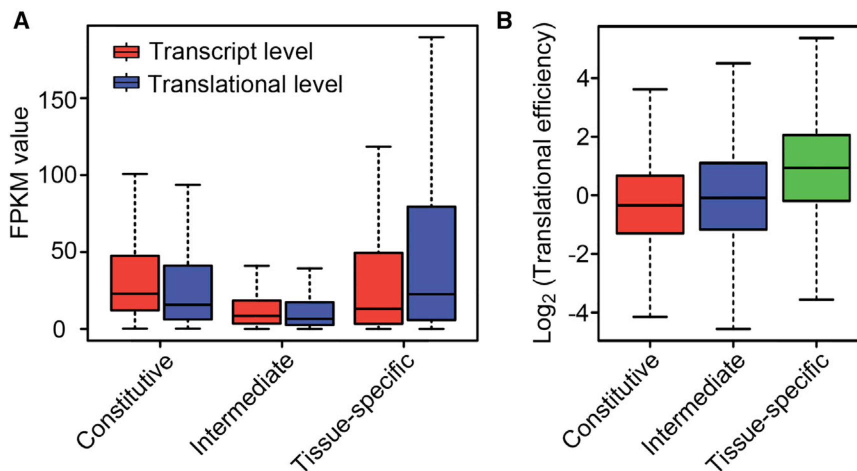


**Figure 5. Analysis of Translational Efficiencies of Constitutive, Intermediate, and Tissue-Specific Genes.**

**(A)** Comparisons of transcript and translational levels of constitutive, intermediate, and tissue-specific genes in shoot.

**(B)** Comparisons of translational efficiencies of constitutive, intermediate, and tissue-specific genes in shoot. Translational efficiency was calculated by $FPKM_{(translational\ level)}/FPKM_{(transcript\ level)}$. Only genes with FPKM > 1 at both transcriptional and translational levels were used.

translation can be measured by analyzing the ribosomal footprints using the recently developed ''ribosome profiling'' method (Ingolia et al., 2011). Using the recently generated ribosome profiling data of maize shoot (Lei et al., 2015), we calculated the translational levels of constitutive, intermediate, and tissue-specific genes. We found that the translational levels of the constitutive genes were relatively lower when compared with their transcript levels, while the translational levels of tissue-specifically expressed genes were relatively higher when compared with their transcript levels (Figure 5A). We further used the translational efficiency (calculated by $FPKM_{translational\ level}/FPKM_{transcript\ level}$) to compare the differences in RNA utilization efficiencies among different types of genes. Constitutive genes had the lowest translational efficiency while tissue-specific genes had the greatest translational efficiency (Figure 5B). The translational efficiencies of intermediate genes fell between those of the constitutive and tissue-specific genes (Figure 5B). Similarly, the translational efficiencies of tissue-specific genes were also greater than those of constitutive genes based on ribosome profiling data from maize endosperm of hybrids between ''B73'' and ''Mo17'' (*p* value <0.01, Supplemental Figure 15). These results are consistent with the hypothesis that intrinsically DNA-encoded nucleosome organization is important, beyond its generally accepted role in the regulation of gene expression levels, for the determination of the plasticity of gene transcriptional status.

## Effects of DNA Sequence Features on Nucleosome Organization of Genes

The roles of DNA sequence properties in determining nucleosome positioning have been observed during nucleosome formation (Kaplan et al., 2008; Zhang et al., 2009; Valouev et al., 2011; Struhl and Segal, 2013). Here, we investigated how DNA sequences affected the nucleosome occupancy of gene bodies in constitutive, intermediate, and tissue-specifically expressed genes as shown in Figure 4B. Using the nucleosome occupancy data predicted by NuPoP (Figure 4B), we analyzed the average nucleosome occupancy levels in exons and introns, respectively, for the three types of genes. The nucleosome occupancy of exons was significantly greater than that of introns (*p* value <2.2e-16, Figure 6A), while the AT content of exons was significantly lower than that of introns for all three types of genes (*p* value <2.2e-16, Figure 6B). Moreover, the constitutive genes had the greatest AT content and the lowest nucleosome occupancy in exons and introns (Figure 6A and 6B). These results were further confirmed by the *in vivo* data of shoot (Supplemental Figure 16) and revealed that nucleosome occupancy variation can be affected by the AT content of the DNA sequences, which was consistent with previous reports that nucleosomes favor positioning in non-homopolymeric G/C-rich regions (Yuan et al., 2005; Kaplan et al., 2008). Next, we investigated how the AT contents of the coding sequences, which are the most important parts of exon regions and are relatively conserved for the maintenance of particular proteins, were adjusted. Constitutive genes had the greatest proportion of codons having two or three A/T nucleotides, while tissue-specific genes had the lowest proportion of codons having two or three A/T nucleotides (Figure 6C), in accordance with the differences in their AT contents (Figure 6B). Thus, the codon degeneracy may have served as a mechanism to modulate the AT content of coding sequences, thereby affecting nucleosome occupancy.

We also investigated how gene structure affects the positions of the +1 and −1 nucleosomes. The constitutive genes had the longest 5′ untranslated region (UTR) and 3′ UTRs, while tissue-specific genes had the shortest 5′ UTRs and 3′ UTRs (Figure 6D and Supplemental Figure 17). Length variations in the 5′ UTRs and 3′ UTRs was correlated with variations in the +1 and −1 nucleosomes distances to the TSS and TTS, respectively (Figure 6D and Supplemental Figure 17). We further plotted the average nucleosome occupancy patterns over the 5′ and 3′ ends of the genes with the start and stop codons, respectively, as data points using the *in vivo* shoot data. Like the nucleosome occupancy patterns generated using TSSs and TTSs as data points (Supplemental Figure 12A), we observed a well-phased nucleosome downstream of the start codon and upstream of the stop codon (Figure 6E), having sharper peaks than those shown in Supplemental Figure 12A. In addition, the major peak near the 5′ ends of genes was located ∼45 bp downstream of the start codon, and the major peak was near the 3′ ends of genes was located ∼75 bp upstream of the stop codon (Figure 6E). The degrees of variation were less than those of the +1 nucleosome distance to the TSS and the −1 nucleosome distance to the TTS among the three types of genes (Supplemental Figure 12A). Thus, the lengths of the 5′ and 3′ UTRs were associated with the distances of the

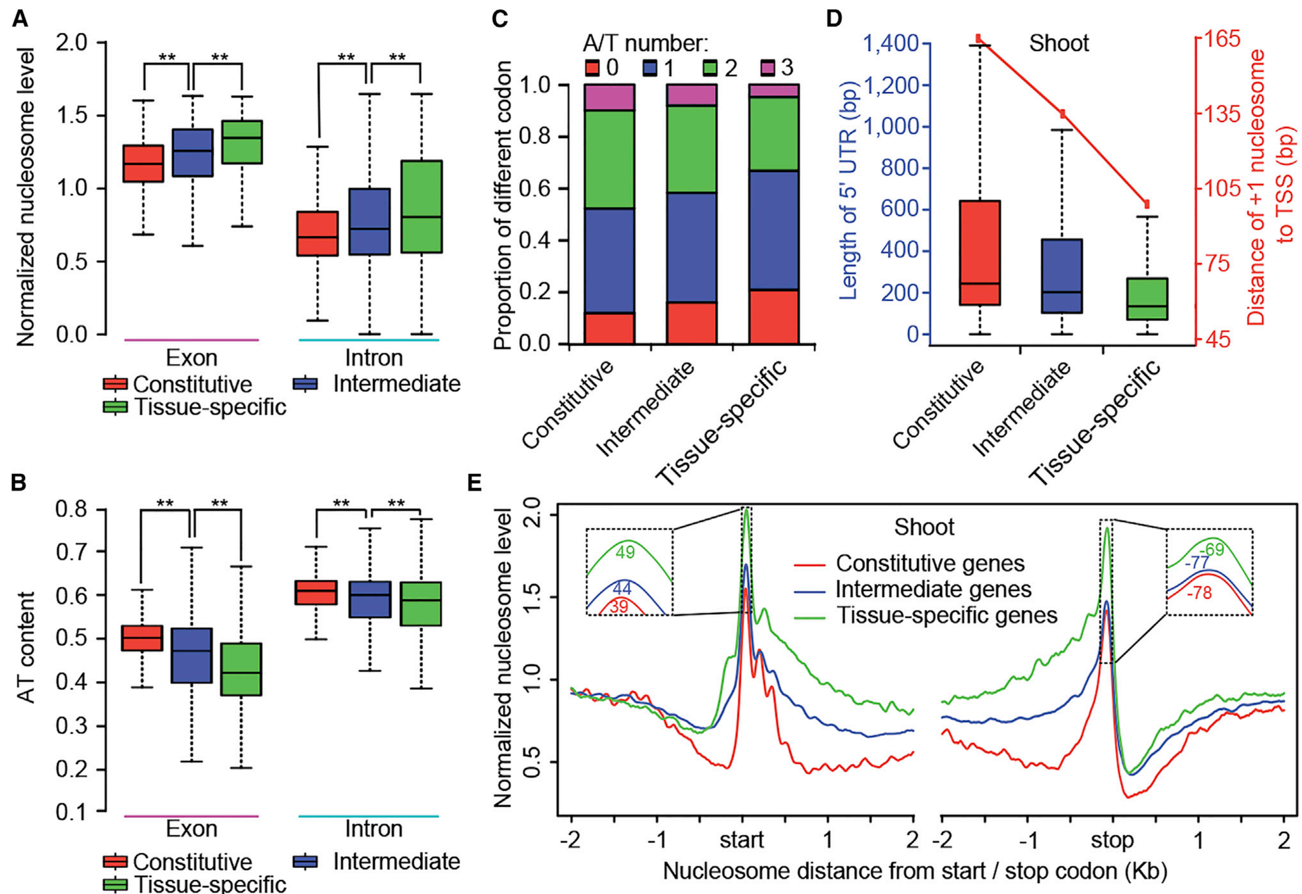


**Figure 6. DNA Sequence Properties that Affect Nucleosome Organization of Genes.**

**(A and B)** Comparisons of nucleosome occupancy **(A)** and AT content **(B)** in exons and introns of constitutive, intermediate, and tissue-specific genes, which were classified based on their expression levels in different tissues (see Methods). The nucleosome occupancy data predicted by NuPoP was used. Asterisks indicate significant difference of nucleosome occupancy or AT content (**$p < 0.01$).

**(C)** Codon components of constitutive, intermediate, and tissue-specific genes. Codons were classified based on the number of As and Ts in the codons.

**(D)** Comparisons of the 5′ UTR lengths with the +1 nucleosome distances relative to the TSSs of constitutive, intermediate, and tissue-specific genes in shoot.

**(E)** Average nucleosome occupancy plotted relative to the start and stop codons of constitutive, intermediate, and tissue-specific genes in shoot. The +1 and −1 nucleosomes and their distances relative to the start and stop codons, respectively, are shown in enlarged figures.

respective +1 and −1 nucleosomes to the TSS and TTS, respectively.

## DISCUSSION

In this study, we presented genome-wide nucleosome occupancy maps of maize shoot and endosperm through the sequencing of mononucleosomal DNA generated by MNase digestion. We confirmed the typical characteristics of nucleosome organization near TSSs and TTSs in maize, including the NDRs in the 5′ and 3′ ends of genes, as well as the locations of the +1 and −1 nucleosomes, which were similar to those reported previously (Radman-Livaja and Rando, 2010; Struhl and Segal, 2013). We also showed the dynamics of nucleosome occupancy that was accompanied with gene transcriptional status change. In addition, we determined that intrinsically DNA-encoded nucleosome organization is associated with plasticity of gene transcriptional status to more of an extent than the correlation with gene expression level reported previously. We found that constitutively and tissue-specifically expressed genes displayed two distinct nucleosome organization patterns.

### Correlation between Nucleosome Organization and Gene Transcription

The nucleosome organization patterns around genes are closely related to gene transcription. The nucleosome occupancy signatures of genes are correlated with gene expression levels in yeast, with genes having significant NDRs near their promoters being highly expressed (Lee et al., 2007). Similar results have also been observed in *Arabidopsis*, rice, and human, in which the expressed genes have greater nucleosome depletion levels surrounding TSSs than the unexpressed genes (Schones et al., 2008; Li et al., 2014; Wu et al., 2014). Moreover, a global nucleosome mapping study in mouse revealed that genes with greater expression levels also have lower nucleosome occupancy levels in the 3′ NDRs (Teif et al., 2012). Consistent with these observations, we found that the 5′ and 3′ NDRs

became more pronounced as the gene expression level increased. In addition, the distances of the +1 nucleosome to the TSS and −1 nucleosome to the TTS were both positively correlated with the gene expression level. The +1 and −1 nucleosomes also shifted away from the TSSs and TTSs, respectively, when the transcriptional status of a gene changed from silenced to activated. However, the shifts were far less than the +1 and −1 nucleosomes' positional differences between expressed and unexpressed genes in the same tissue. This limited impact of the transcription process on nucleosome organization is consistent with the observation that the position of the +1 nucleosome is similar whether the paused Pol II is present in *Drosophila* (Mavrich et al., 2008). Therefore, the differential expressions of genes can only account for part of the variation in nucleosome occupancy among different genes. Because of the important role of the DNA sequence in nucleosome determination (Kaplan et al., 2008; Valouev et al., 2011; Zhang et al., 2011), we speculated that differences in gene sequences also led to variation in the nucleosome occupancy among different genes. Indeed, the nucleosome occupancy in gene bodies was associated with the AT content of the gene, and the positions of the +1 and −1 nucleosomes were associated with the lengths of 5′ and 3′ UTRs, respectively.

### Role of Intrinsically DNA-Encoded Nucleosome Organization in Gene Transcription

The genome-wide nucleosome organization is determined by the DNA sequence and several other cellular factors, including ATP-dependent nucleosome remodeling enzymes and transcription factors (Struhl and Segal, 2013). In yeast, the intrinsically DNA-encoded nucleosome organization can explain ~50% of the *in vivo* nucleosome positions (Segal et al., 2006). By contrast, just over 20% of the human genome is covered by nucleosomes determined by DNA sequence (Valouev et al., 2011). In this study, the NDRs at the 5′ and 3′ ends of genes, and the +1 and −1 nucleosomes, were observed using the predicted intrinsically DNA-encoded nucleosome organization in maize, consistent with recent reports that the DNA sequence plays an important role in regulating nucleosome position (Sexton et al., 2014, 2016). Although different cell/tissue types and methods were used in these studies, these results may reflect the different effects of the DNA sequence on nucleosome organization in different species.

Although previous *in vitro* nucleosome reconstruction studies demonstrated many aspects of the roles of intrinsic DNA sequences in the determination of nucleosome positioning (Kaplan et al., 2008; Zhang et al., 2009, 2011; Valouev et al., 2011), little is known about the roles of the intrinsically DNA-encoded nucleosome organization in gene transcription. In yeast, the canonical nucleosome organization in the gene promoter region is also present in "housekeeping" genes involved in ribosome biogenesis and assembly, as well as organelle organization and biogenesis (Lee et al., 2007). Here, tissue-specific genes displayed nucleosome organizations distinct from those of constitutive genes. Compared with constitutive genes, tissue-specific genes had greater nucleosome occupancy levels surrounding genes, as well as closer +1 and –1 nucleosomes relative to the TSSs and TTSs, respectively. It may be that these characteristics allow for dynamic competition between nucleosome assembly and transcription factor binding, thus influencing the transcriptional statuses of genes, which is consistent with previous reports that genes with relatively high nucleosome occupancy levels in their promoters are more sensitive to chromatin regulation and show more variable expression under both genetic and environmental perturbations (Newman et al., 2006; Choi and Kim, 2008, 2009; Tirosh and Barkai, 2008; Tirosh et al., 2009). In contrast, the relatively open chromatin state of constitutive genes may increase the accessibility of transcription initiation and termination elements, resulting in tissue identities having limited influence on transcriptional status. Thus, a gene's tendency to be constitutively or specifically expressed might be intrinsically encoded by DNA sequences. Notably, different tissue-specific genes possibly being expressed in different tissues does not appear to be associated with intrinsically DNA-encoded nucleosome organization but to be regulated by other tissue-specific elements because the genome sequences are identical in different tissues.

It is generally accepted that intrinsically DNA-encoded nucleosome organization can also affect gene expression levels. Indeed, the expression levels of constitutive genes are significantly greater than those of tissue-specific genes. However, because expression levels can vary drastically among different tissues, the static genome sequence cannot encode a nucleosome organization that copes with different expression levels. We propose that the intrinsically DNA-encoded nucleosome organization of constitutive and tissue-specific genes was not primarily designed to determine gene expression level but to program the corresponding plasticity of the gene transcriptional status. This interpretation is consistent with the observed correlation of intrinsically DNA-encoded nucleosome organization with the plasticity of gene transcriptional status in different tissues, which is greater than its correlation with gene expression level. An analysis of the efficiency of RNA utilization indicated that the translational efficiencies of constitutive genes were lower than those of tissue-specific genes. The effects of intrinsically DNA-encoded nucleosome organization on expression level seem need to be further optimized by translational regulation to obtain appropriate translational levels. This optimization process can be either passive or active. Interestingly, a number of studies have determined potential translational regulating factors, such as the upstream open reading frames in 5′ UTRs and the post-transcriptional $N^6$-methyladenosine modifications of mRNA (Ringnér and Krogh, 2005; Liu et al., 2012, 2013; Lei et al., 2015; Zhou et al., 2015). These potential translational factors can function after transcription to fine-tune the levels of final translational products.

## METHODS

### Plant Materials

The maize (*Z. mays*) inbred line B73 was grown in the field in the summer of 2013 in Beijing, China, to harvest endosperm 12 days after pollination. The seeds of "B73" were planted in the greenhouse in conditions of 30°C for 16 h under light and 25°C for 8 h in the dark. After 14 days, the aerial tissues were harvested. Harvested shoot and endosperm were immediately frozen in liquid nitrogen and stored at −80°C before processing.

### Isolation of Nucleosomal DNA and RNA, Library Construction, and Deep Sequencing

A previously described method (Labonne et al., 2013) was used to isolate mononucleosome core DNA from shoot and endosperm, respectively. In

brief, 4 g of tissue was ground in liquid nitrogen and crosslinked in ice-cold APEL buffer (20 mM Tris–HCl [pH 7.8], 250 mM sucrose, 5 mM $MgCl_2$, 5 mM KCl, 40% glycerol, 0.25% Triton X-100, and 0.1% β-mercaptoethanol) with 1% formaldehyde for 10 min. The suspension was stopped with 125 mM glycine for 5 min and then centrifuged for 10 min at 2000 *g* to precipitate nuclei. Next, isolated nuclei were washed with APEL buffer, resuspended in 1 mL of MNase digestion buffer (50 mM Tris–HCl [pH 7.5], 320 mM sucrose, 4 mM $MgCl_2$, and 1 mM $CaCl_2$), and divided into five portions. The five portions of the shoot sample were independently digested with 0 U, 0.1 U, 0.2 U, 0.5 U, and 2 U of MNase at 37°C for 10 min. The five portions of the endosperm sample were independently digested with 0 U, 0.1 U, 0.2 U, 1 U and 2 U of MNase at 37°C for 10 min. The appropriate levels of 2 U and 1 U of MNase were chosen for shoot and endosperm samples, respectively, because the mononucleosome proportions were most suitable for nucleosome occupancy analysis based on a previous report (Weiner et al., 2010). The mononucleosomes of shoot generated with 0.2 U of MNase (resulting in light digestion) was also used for library construction. EDTA was added to a final concentration of 10 mM to stop the digestion. Crosslinks were then reversed with SDS and proteinase K overnight at 65°C. DNA was purified by phenol–chloroform extraction, precipitated with ethanol, and treated with RNase A. Finally, purified DNA was run on a 2% agarose gel, and the resulting mononucleosomal DNA fragments (~150 bp) were gel extracted. The mononucleosomal DNA fragments were blunt-ended and ligated to adaptors to generate MNase-seq libraries following the Illumina protocol.

Total RNA was extracted using TRIzol reagent. RNA-seq libraries were constructed according the instructions of the Illumina Standard mRNA-seq library preparation kit (Illumina). The MNase-seq and RNA-seq libraries were sequenced on the Illumina HiSeq platform. The MNase-seq library of shoot generated with 0.2 U of MNase was sequenced to generate 150-nt paired-end reads, and all other libraries were sequenced to generate 100-nt paired-end reads.

The ribosome profiling data of endosperm collected 14 days after pollination from hybrids between "B73" and "Mo17" were generated and analyzed together with the published shoot data (Lei et al., 2015). The ribosome profiling libraries were constructed according to the protocol described in our previous study (Lei et al., 2015) and sequenced to generate single-end 50-nt reads on the Illumina HiSeq platform.

### Processing of MNase-Seq Data and Initial Analysis

The raw reads of MNase-seq data were aligned to the "B73" reference genome (RefGen_v2) allowing two mismatches using the Burrows–Wheeler Aligner (BWA) (Li and Durbin, 2010). Only uniquely aligned paired reads were used for further analysis. The 147-bp nucleosomal DNA sequences were used to analyze the distribution of dinucleotides along the 147-bp axis of nucleosomal DNA as described previously (Segal et al., 2006). Dinucleotide counts were normalized with the number of nucleosomal DNA sequences used. A previously described method (Valouev et al., 2011) was used to calculate the NRL in maize. The middle points of paired MNase-seq reads were used to calculate the spacing between two nucleosomes rather than the mapped reads' start positions to reduce the effects of different levels of MNase digestion.

### Defining Nucleosome Occupancy

The *in vivo* nucleosome occupancy levels of shoot and endosperm were calculated using DANPOS (Chen et al., 2013), with the parameters set as -s 1, -a 1, -m 1, -p 1e-5, and -t 1e-5. Only uniquely aligned paired reads of MNase-seq data were used for the nucleosome occupancy analysis. NuPoP software (Xi et al., 2010) was used to predict the intrinsically DNA-encoded nucleosome occupancy of the maize genome with default parameters.

The average nucleosome occupancy patterns near the TSSs, TTSs, start codons, and stop codons were calculated as follows. First, the nucleosome occupancies of each gene in a given gene set were summed over all of the corresponding sites. Then the sum of nucleosome occupancies was normalized using the number of genes and the average nucleosome occupancy of the whole genome. Similarly, the nucleosome occupancies of each site in a given region were summed and then normalized using the sequence length and the average nucleosome occupancy of the whole genome to determine the average nucleosome occupancy of a given region.

### Gene Expression Analysis

The endosperm RNA-seq data generated in this study and shoot RNA-seq data reported in our previous study (Lei et al., 2015) were used to analyze gene expression levels. The row reads were aligned to the "B73" reference genome (RefGen_v2) with TopHat (Trapnell et al., 2009). The uniquely aligned reads were then used to calculate the FPKM values for each gene in the "B73" filtered gene set with Cufflinks (Trapnell et al., 2012). To reduce the influence of transcriptional noise, a given gene was determined to express if its FPKM value was ≥1.

### Identification of Tissue-Specific Expression

The transcriptional statuses of genes in different tissues were determined based on expression data described in our previous study (Chen et al., 2014), which contained the expression levels of genes in 53 different seed and 25 non-seed samples, then classified into different types. We first filtered out the genes that were not identified as expressing in any of the samples using the standard of FPKM <1. We then used the *Z*-score value to select the tissue-specifically expressed genes as the method used previously in maize (Chen et al., 2014). The genes that were expressed in all of the samples were identified as constitutively expressed genes. The remaining genes were regarded as intermediate genes.

## ACCESSION NUMBERS

Sequence data from this article can be found in the National Center for Biotechnology Information Sequence Read Archive (http://www.ncbi.nlm.nih.gov/sra) under accession number SRP078031.

## SUPPLEMENTAL INFORMATION

Supplemental Information is available at *Molecular Plant Online*.

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## AUTHOR CONTRIBUTIONS

J.L. and J.C. designed the experiments. J.C., L.L., W.S., and H.Z. performed the experiments. J.C., E.L., X.Z., and X.D. analyzed the data. J.C. and J.L. wrote the paper.

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