Gene Expression and Chromatin Modifications Associated with Maize Centromeres

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ABSTRACT  Centromeres are defined by the presence of CENH3, a variant of histone H3. Centromeres in most plant species contain exclusively highly repetitive DNA sequences, which has hindered research on structure and function of centromeric chromatin. Several maize centromeres have been nearly completely sequenced, providing a sequence-based platform for genomic and epigenomic research of plant centromeres. Here we report a high resolution map of CENH3 nucleosomes in the maize genome. Although CENH3 nucleosomes are spaced ~190 bp on average, CENH3 nucleosomes that occupied CentC, a 156-bp centromeric satellite repeat, showed clear positioning aligning with CentC monomers. Maize centromeres contain alternating CENH3-enriched and CENH3-depleted subdomains, which account for 87% and 13% of the centromeres, respectively. A number of annotated genes were identified in the centromeres, including 11 active genes that were located exclusively in CENH3-depleted subdomains. The euchromatic histone modification marks, including H3K4me3, H3K36me3 and H3K9ac, detected in maize centromeres were associated mainly with the active genes. Interestingly, maize centromeres also have lower levels of the heterochromatin histone modification mark H3K27me2 relative to pericentromeric regions. We conclude that neither H3K27me2 nor the three euchromatic histone modifications are likely to serve as functionally important epigenetic marks of centromere identity in maize.
of micrococcal nuclease (MNase; Sigma-Aldrich, N5386_200U), respectively. ChIP experiments, including nuclei purification, followed published protocols (Zhang et al. 2012). Both ChIPed DNA and input DNA corresponding to 0.2 U and 5 U MNase trimmed chromatin input were used for ChIP-seq library preparation following published methods (Henikoff et al. 2011) to recover and sequence small DNA fragments. The DNA libraries were sequenced using the Illumina HiSeqation 2000 platform. For MNase digestion of naked DNA, DNA was extracted from a B73 developing ear using a DNase Plant Mini Kit (Qiagen), and digested with MNase (New England Biolabs, #M0247S) as follows: a master mix consisting of 140 ng/μl DNA, 82 gel units/μl MNase, 150 ng/μl BSA in 1X micrococcal nuclease buffer was prepared then divided into five 150-μl aliquots on ice. All aliquots were simultaneously transferred to a 37°C water bath then removed one at a time after 1, 4, and 8 min (at which time the bulk of DNA had been digested to fragments of less than 200 bp in length). The digested was purified and prepared for Illumina sequencing as described (Gent et al. 2014).

Gene expression analysis using quantitative real-time PCR

RNA was extracted from 10-day-old maize (B73) seedlings using an RNeasy Plant Mini Kit (Qiagen, 74904) following the manufacturer’s instructions. RNA samples were treated with TURBO DNase (Ambion, AM1907), and reverse transcribed with SuperScript III (Invitrogen, 18080-051). The expression levels of a set of seven centromeric genes (Supporting Information, Table S1) relative to the Actin reference gene were validated by both semiquantitative real-time PCR (semiqRT-PCR) and quantitative real-time PCR (qRT-PCR) carried out on a Thermal Cycler (Bio-Rad, C1000) using Taq DNA polymerase (Takara, R001A), and on MJ Research Opticon 2 (Bio-Rad Laboratories) using the SYBR Advantage qPCR Premix (Clontech, 639676), respectively. Annealing temperature was set to 60°C. SemiqRT-PCR and qRT-PCR primers are provided in Table S1.

Sequence mapping and identification of CENH3-binding domains

Raw sequencing reads were trimmed by Cutadapt (Martin 2011) to remove low quality nucleotides (with quality score less than 30) and adapters. Pairs with reads of less than 26 bp in length for either read after quality and adapter trimming were discarded. Reads were mapped to the B73 reference genome (ReffGen_v3, http://plants.ensembl.org/Zea_mays) by BWA-MEM using default parameters (Li 2013). Only

MATERIALS AND METHODS

MNase digestion, ChIP, and ChIP-seq

Ten-day-old maize (B73) seedlings grown in the greenhouse were collected and ground into fine powder in liquid nitrogen for nuclei extraction. The input chromatin for anti-CENH3 ChIP experiments was prepared from purified nuclei digested with 0.2 unit (U) and 5 U of micrococcal nuclease (MNase; Sigma-Aldrich, N5386_200U), respectively. ChIP experiments, including nuclei purification, followed published protocols (Zhang et al. 2012). Both ChIPed DNA and input DNA corresponding to 0.2 U and 5 U MNase trimmed chromatin input were used for ChIP-seq library preparation following published methods (Henikoff et al. 2011) to recover and sequence small DNA fragments. The DNA libraries were sequenced using the Illumina HiSeqation 2000 platform. For MNase digestion of naked DNA, DNA was extracted from a B73 developing ear using a DNeasy Plant Mini Kit (Qiagen), and digested with MNase (New England Biolabs, #M0247S) as follows: a master mix consisting of 140 ng/μl DNA, 82 gel units/μl MNase, 150 ng/μl BSA in 1X micrococcal nuclease buffer was prepared then divided into five 150-μl aliquots on ice. All aliquots were simultaneously transferred to a 37°C water bath then removed one at a time after 1, 4, and 8 min (at which time the bulk of DNA had been digested to fragments of less than 200 bp in length). The digested was purified and prepared for Illumina sequencing as described (Gent et al. 2014).

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Some regions may have fewer reads due to inefficient mapping at highly repetitive regions. Small CENH3-binding subdomains of less than 30 kb were discarded.

Transcriptome, histone modification, and CENH3-depleted subdomains

Transcriptome (SRR445245 and SRR445382) and ChIP data of H3K27me3, H3K36me3, H3K4me3 and H3K9ac (GSE1528) (Wang et al. 2009), and H3K27me2 (Gent et al. 2014) (SRR1584368 and SRR1584369) were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/). Transcriptome reads were mapped using TopHat (Trapnell et al. 2009), and ChIP reads were mapped using BWA-MEM with default parameters (Li 2013). Only uniquely mapped reads were retained. Gene expression in centromeres was analyzed by Cufflinks (Trapnell et al. 2010) using gene annotation AGPv3.21 (http://plants.ensembl.org/Zea_mays) with default parameters. We used MACS2 (-B --broad --g 740000000 -tradeline) (Zhang et al. 2008) and SICER (window size 200 bp, gap size 400 bp, effective genome fraction 0.4, redundancy threshold 1, fragment size 150 bp, and FDR 0.05) to identify histone modification peaks in centromeres, and retained peaks that were consistently discovered by both algorithms for further analysis.

To analyze the relationship between histone modification and maize centromere expansion in oat, we divided the expanded regions of Cen2, Cen5, Cen9, and Cen10 into 1-kb windows and calculated read density of H3K4me2, H3K36me3, H3K9ac, H3K27me3, and H3K27me2 in each window. Read densities were then normalized by the mappability of each window. Read densities of nonexpanded regions were calculated in the same manner. The significant differences of each histone modification between expanded regions and nonexpanded regions were determined by bootstrap resampling of read densities from expanded regions and nonexpanded regions.

Nucleosome positioning and phasing on CentC

We analyzed the nucleosome positioning using nucleR (Flores and Orozco 2011) with slight modification. Reads mapped to centromeres were extracted and trimmed to 40 bp around the center of each pair. Read depth of each position in centromeres was then determined by BEDTools (Quinlan and Hall 2010). Read depth was analyzed by nucleR with parameter “pcKeepComp” set to 0.01. “pcKeepComp” was set to 0.02 to identify closely located peaks on CentC arrays. We annotated the maize satellite repeat CentC using CENSOR (“-nofilter -s -bprn -filter = none”) (Kohany et al. 2006). The consensus CentC sequence was generated by CAP3 (-j 200 -s 1000 -o 100 -p 80) (Huang and Madan 1999) using ~9850 (82% of total CentC monomers) CentC monomers. Paired-end reads were merged by FLASH (-M 100 -z -t 1) (Magoc and Salzberg 2011), and then mapped to the B73 genome and a CentC tetramer using BWA-MEM (Zhang et al. 2013). Phasing of CENH3 nucleosomes was analyzed using previously described methods (Zhang et al. 2013).

Data availability

The CENH3 ChIP-seq sequencing data are available from NCBI sequence read archive (SRA) under accession SRP057754, the MNase-digested maize genomic DNA sequencing data are under accession SRR049952 (http://www.ncbi.nlm.nih.gov/sra).

RESULTS

High-resolution mapping of CENH3-binding domains of maize centromeres

We conducted two independent ChIP experiments using anti-CENH3 antibody (Zhong et al. 2002). Chromatin isolated from B73 seedling tissue was digested by either 0.2 U or 5 U of MNase. The sample treated with 5U MNase was nearly fully digested (Figure 1A). Mononucleosomal DNA fragments of approximately 100–200 bp were gel-purified from both samples for library construction. A total of four libraries, two from ChIPed DNA and two from input DNA, were sequenced using the Illumina HiSequation 2000 platform, producing
a total of 283 million 100-bp paired-end reads from the four libraries (Table S2). Sequence reads were mapped to maize B73 genome (version 3) (Schnable et al. 2009). Only uniquely mapped reads with perfect matches to the B73 genome were retained for further analysis.

Approximately 23% of the uniquely-mapped reads from the 0.2 U and 11% from the 5 U libraries were mapped to the centromeres (Table S2), which represented 45-fold and 27-fold CENH3 ChIP-seq sequence enrichments in centromeres compared to input. The ChIP-seq read density across the maize genome was highly correlated between the two libraries ($R = 0.90, p < 2.2 \times 10^{-16}$, Spearman’s rank correlation). The lengths of the uniquely-mapped sequence reads from the 0.2 U ChIP and input libraries peaked at 158 bp and 159 bp, respectively, i.e., longer than the 147 bp that would wrap the canonical nucleosome core particle. These results indicate that the 0.2 U chromatin sample was underdigested and most reads contained linker sequences. The lengths of the sequence reads from the 5 U ChIP and input libraries peaked at 147 bp, with two minor peaks at 123 bp and 133 bp, respectively (Figure 1B). The minor peaks were also clearly visible in the input, of which only a small percentage of the reads is visible in the input, of which only a small percentage of the reads is expected to be derived from CENH3-containing nucleosomes. Thus, these smaller fragments are abundant independently of CENH3, but are particularly enriched by CENH3 ChIP.

We divided each chromosome into 10-kb nonoverlapping windows and calculated ChIP-seq read enrichment within each window. Unambiguous enrichment peaks were observed in centromeric regions of chromosomes 2, 3, 4, 5, 8, 9, and 10 (Figure S1), consistent with previous centromere mapping results (Wolfguber et al. 2009; Wang et al. 2014; Gent et al. 2015). Several minor peaks were also observed in chromosomal arms (Figure S1), which may result from misassembly or misplacement of centromere-associated repetitive DNA sequences. We observed very narrow peaks in centromeres 6 and 7, and no unambiguous peak in centromere 1, suggesting that these three centromeres contain mainly highly repetitive DNA sequences. Indeed, fiber-FISH mapping revealed that both Cen1 and Cen7 contain megabase-sized arrays of the CentC satellite repeats (Jin et al. 2004). We excluded Cen1, Cen6, and Cen7 in further analysis.

We used SICER (Zang et al. 2009) to identify CENH3-binding domains using the 0.2 U and 5 U ChIP-seq data, independently. The CENH3-binding regions identified by the two datasets were highly correlated (Table S3). The consensus CENH3-binding regions identified in both datasets were then considered as the functional centromeres. We considered the SICER-defined peaks of CENH3 enrichment as the centromere of each chromosome (Table 1). The CENH3-binding domains ranged from 0.93 Mb of Cen4 to 1.88 Mb for both Cen5 and Cen8. This range, however, may not accurately reflect the true variation of centromere size because highly repetitive sequences in the centromeres are either missing in the reference genome or the relevant ChIP-seq reads were not mappable. Cen2 and Cen5, the two best assembled maize centromeres, spanned 1.82 and 1.88 Mb, respectively.

**CENH3-depleted subdomains in maize centromeres**

The CENH3 ChIP-seq reads were not evenly enriched in maize centromeres, which could be related to the lack of unique sequence in some regions in centromeres, and/or could indicate the existence of intermingled CENH3-enriched and CENH3-depleted subdomains as reported in several animals and plant species (Blower et al. 2002; Yan et al. 2008; Gong et al. 2012). To profile CENH3 binding in maize centromeres, we first determined each 10-kb window of each centromere as mappable or unmappable based on mapping 150-bp sequence reads that were generated computationally from the maize genome. Unmappable 10-kb windows in the centromeres were excluded because the CENH3-binding in these regions cannot be determined. A total of 1350 Mb mappable regions were identified in maize genome, including 6.5 Mb in centromeres, which comprised 53% of the identified centromeric sequences. We then divided the centromeres into 10-kb windows, and determined the CENH3 read enrichment of each window by comparing CENH3 ChIP-seq data to the input data (see Materials and Methods). We identified a total of 846 kb of CENH3-depleted subdomains (see Materials and Methods for definition of a CENH3-depleted subdomain) within the seven maize centromeres, which consisted of 13% of mappable centromeric regions (Table 2). The CENH3-depleted subdomains were interspersed among CENH3-enriched regions in centromeres (Figure 2). The percentages of CENH3-depleted subdomains were different among centromeres, ranging from 9.9% (Cen5) to 26.2% (Cen3) of the mappable sequences (Table 2). For this and all subsequent analyses, we combined both the 0.2 U and 5 U ChIP datasets into a single set.

**Nucleosome positioning in maize centromeres**

Although CENH3 ChIP-seq datasets were developed previously (Wolfguber et al. 2009; Wang et al. 2014; Gent et al. 2015), we produced the highest volume of ChIP-seq reads from a specific maize genotype. This high volume of paired-end reads provided an opportunity to investigate the positioning of individual maize CENH3 nucleosomes. Each pair of sequence reads was first merged into a single DNA fragment, which provided information on the size and boundaries of individual DNA fragment that wrap single CENH3 nucleosomes. Approximately 93–95% of the paired reads were merged to a single fragment from the two libraries. Mapping merged fragments to the B73 genome revealed many sharp peaks in centromeres, which represent well-positioned CENH3 nucleosomes (Figure 3).
We observed sharp peaks associated with individual CentC monomers in Cen2 (Figure 3A), indicating that CENH3 nucleosomes preferentially bind to CentC repeats such that the same histone-DNA contacts are formed; this phenomenon is termed nucleosome phasing and is related to innate sequence preferences of histones. Phasing of CENH3 on CentC arrays was further evidenced by phasograms of CentC arrays with a periodicity of approximately the same length as the CentC monomer (~156 bp) (Figure 3C). In contrast, we did not detect a similar nucleosome phasing associated with non-CentC sequences in Cen2.

We next examined if the cutting site pattern associated with the CentC repeats was caused by sequence preference of MNase using a large sequence dataset derived from MNase-digested B73 genomic DNA. The distribution patterns of the cutting sites on the CentC repeat were clearly different between naked DNA and ChIPed DNA (Figure 3B). The cutting sites on naked CentC biased to A/T sites, consistent with the sequence preference of MNase (Horz and Altenburger 1981; Drew 1984; Telford and Stewart 1989). By contrast, the cutting sites of ChIPed DNA did not show A/T preferences (Figure 3B). Among the top 20% most frequently cut sites on the CentC repeat, 92% were A or T on naked DNA, but only 45% were A or T on ChIPed DNA.

Transcription of genes in maize centromeres

CENH3-depleted subdomains in rice centromeres are occupied by canonical nucleosomes and contain active genes (Wu et al. 2011). We next investigated the transcriptional activity associated with maize centromeres. A total of 34 protein-coding genes were annotated in seven maize centromeres, of which 21 were located in CENH3-depleted subdomains, and 13 located in CENH3-enriched regions (Table S4). Eleven of the 21 genes (52%) in CENH3-depleted subdomains were expressed in shoot and/or root tissues based on RNA-seq datasets (Wang et al. 2009). In contrast, transcription was not detected for any of the 13 genes located in CENH3-enriched regions (Table S4). Six of 13 inactive genes located in the CENH3-enriched regions were shorter than 100 amino acids, and lack homologs (>70% identity) in other plant species, suggesting that these are likely nonfunctional genes. Maize centromeres also included a total of nine genes annotated as "low confidence genes" or "novel transcript" that lack homologs in other plant species, or lack evidence of transcription and/or intact coding capacity (Table S4). Analysis of RNA-seq datasets revealed potential transcription associated with eight of the low confidence genes as well as one "novel transcript" in Cen2 (Table S4). Only two of these genes, which showed low levels of transcription (FPKM of 1.17 and 1.23, respectively) in roots (but not in leaf), were located in CENH3-enriched regions.
We selected seven centromeric genes to validate their transcription. These genes have different expression levels based on RNA-seq data. Transcription of all seven genes in seedlings was detected by both qRT-PCR and semi-qRT-PCR, including genes GRMZM2G175425 and GRMZM2G137715 with FPKM 1.1 and 2.3, respectively (Figure 4). The expression of a low confidence gene GRMZM5G820434 (FPKM 5.6) was also confirmed.

Histone modifications associated with CENH3-depleted subdomains

We next investigated the histone modification patterns associated with maize centromeres. Genome-wide datasets of active histone marks H3K4me3, H3K36me3, and H3K9ac, and repressive histone mark H3K27me3 were developed using maize shoot tissue (Wang et al. 2009). We identified 64 peaks in seven centromeres (see Materials and Methods), which were associated with one to four of these histone marks (Table S5). Most of these peaks (62 of 64) were associated with the three active marks, and only two peaks associated with the repressive mark H3K27me3. In addition, most peaks (54 of 64) were found in CENH3-depleted subdomains. Interestingly, 47 of the 54 peaks located in CENH3-depleted subdomains were associated with annotated genes (Table S5). H3K36me3 was the most prevalent modification found in the CENH3-depleted subdomains (Table 2). Of the 20 transcribed centromeric genes, 17 (85%) were associated with active histone modifications (Figure 2 and Table S5). H3K4me3 and H3K9ac are generally enriched around transcription start sites of most maize genes. By contrast, H3K36me3 is more broadly associated with gene bodies (Figure S2).

Thus, the centromeric genes showed similar histone modification patterns as most maize genes (Figure S2). These results indicate that CENH3-depleted subdomains in maize centromeres are occupied by canonical nucleosomes and share similar chromatin properties with euchromatic regions in the maize genome.

H3K27me2 is a heterochromatic histone mark and is enriched with repetitive elements in the maize genome (Shi and Dawe 2006; Gent et al. 2014). Interestingly, H3K27me2 was generally depleted in centromeres compared to the flanking pericentromeric regions (Figure 5 and Figure S3). The reduced H3K27me2 in centromeres relative to pericentromeres was not due to mappability of the sequences in centromeric regions because there was no mapping bias of random reads generated from the B73 genome (see Materials and Methods). These results were also in agreement with previous cytological studies, in which H3K27me2 signals were detectable in centromeres, but were relatively weaker than in pericentromeric regions (Shi and Dawe 2006; Jin et al. 2008). In addition, H3K27me2 was also significantly reduced in the CENH3-enriched subdomains relative to the CENH3-depleted subdomains (Kolmogorov-Smirnov test, one-tailed) (Figure S4).

Chromatin features associated with expanded maize centromeres in the genetic background of oat

The CENH3-binding domains of maize centromeres expand from ~1.8 Mb to 3.6 Mb in the genetic background of oat to adapt to a similar size as oat centromeres (Wang et al. 2014). Interestingly, several centromeres, including Cen2, Cen5, Cen9, and Cen10, expand exclusively toward a single direction, either in the short or in the long arm, suggesting...
Expression of centromeric genes in maize. (A) qRT-PCR validation of a set of seven centromeric genes. RNAs were isolated from 10-day-old seedlings of B73. The y-axis represents the relative expression level normalized by setting the expression of the Actin gene to 100. (B) Semi-qRT-PCR analysis of the same set of centromeric genes. Validated genes include: Lane 2: GRMZM2G040843 (FPKM 22.8); Lane 3: GRMZM2G175425 (FPKM 1.1); Lane 4: GRMZM2G137715 (FPKM 2.3); Lane 5: GRMZM2G820434 (FPKM 5.6); Lane 6: GRMZM2G071042 (FPKM 13.7); Lane 7: GRMZM2G409893 (FPKM 5.7); and Lane 8: GRMZM2G083935 (FPKM 3.0). Note: the levels of expression of each gene correlated in qRT-PCR and semi-qRT-PCR experiments.

that the expansion direction is not random. The expanded regions appear to be relatively deficient for active genes (Wang et al. 2014).

To further explore the potential barriers that prohibit maize centromere expansion in oat background, we compared the histone modification patterns associated with the expanded region flanking one side of the original centromere (Figure 6, A–D, regions marked by red bars) with the patterns associated with nonexpanded region flanking the other side of the centromere (Figure 6, A–D, region marked by blue bars). We analyzed the average numbers of ChIP-seq reads, using in 1-kb windows, of H3K4me3, H3K36me3, and H3K9ac and H3K27me3 associated with expanded and nonexpanded regions in four centromeres (Cen2, Cen5, Cen9, and Cen10). The expanded regions contained significantly lower numbers of reads (p-value < 1 × 10⁻⁵, two-tailed) of H3K4me3, H3K36me3, and H3K9ac than nonexpanded regions (Figure 6, E–G). Thus, the expanded regions were less euchromatic than the nonexpanded region. A similar read density of H3K27me3 was observed in expanded and nonexpanded region (p-value = 0.69, two-tailed), probably due to the lack of H3K27me3 in the pericentromeric regions (Figure 6H). We also found similar read density of a heterochromatin mark H3K27me2 in expanded and nonexpanded regions (p-value = 0.50, two-tailed) (Figure 6I), suggesting that the heterochromatin marked by H3K27me2 is not likely to play a role in the one-directional expansion of the centromeres.

**DISCUSSION**

We determined patterns of maize CENH3 nucleosome occupancy across centromeres by combining CENH3 ChIP and deep sequencing. Consistent with previous studies in rice (Yan et al. 2008; Zhang et al. 2013), we found that regions with high CENH3 enrichment were interspersed with regions of low CENH3 enrichment, at least some of which contain expressed genes. Although the chromatin environment of centromeres differs from the rest of the genome (Verdaasdonk and Bloom 2011), we found that expressed genes located in centromeres exhibit histone modification associated with typical active genes, including H3K36me3, H3K4me3, and H3K9ac (Figure S2). The fact that expressed genes in centromeres have lower CENH3 enrichment than other parts of centromeres suggests that transcription may inhibit CENH3 accumulation. We also speculate that very high levels of CENH3 within genes would be incompatible with transcription because of a different N-terminal tail structure that cannot be modified with the H3 modifications associated with transcription regulation (Gassmann et al. 2012; Wang et al. 2014). Regardless of how CENH3 affects or is affected by transcription, it is clear that the expressed genes can exist inside of functional centromeres in maize.

Comparison of CENH3 nucleosome distribution in maize and rice reveals several interesting differences. First, maize centromeres are much larger than rice centromeres (~2 Mb vs. ~750 kb). Second, maize centromeres appear to have greater CENH3 nucleosome occupancy across their centromeres: 87% of maize centromeres were made of regions (~90%) with high CENH3 enrichment, compared with 38–49% of rice centromeres (Yan et al. 2008). These numbers should be interpreted with caution, however, as the rice study measured enrichment using a
Figure 6  Centromere expansion and histone modifications. (A–D) Diagrammatic illustrations of expansion of maize Cen2, Cen5, Cen9, and Cen10, respectively, in the genetic background of oat (Wang et al. 2014). Green lines represent CENH3 ChIP-seq read density in maize, red lines represent CENH3 ChIP-seq read density in oat. Green horizontal bars show the locations of the four centromeres in maize. Red horizontal bars show the locations of expanded regions in oat. Each blue horizontal bar represents a nonexpanded region of the same size as the red bar of the same centromere. (E–I) Quantification of H3K4me3, H3K36me3, H3K9ac, H3K27me3, and H3K27me2, respectively, in original maize centromeres (green lines), expanded centromeric regions in oat (red lines) and nonexpanded regions (blue lines). The histone modification data are presented as the average of four centromeres (Cen2, Cen5, Cen9, and Cen10). The y-axis represents the number of 1-kb windows. The x-axis represents the average number of reads in each 1-kb window.
normalization-free method that does not allow precise measurements of CENH3 enrichment. Further supporting evidence for greater CENH3 nucleosome occupancy in maize centromeres than in rice comes from the different numbers of genes in centromeres in each species: while maize centromeres contain from one to eight genes in each centromere, rice centromeres contain from 17 to 21 genes (Yan et al. 2008).

High occupancy of CENH3 nucleosomes in maize is not limited to regions containing the CentC repeats, nor is it an artifact from mapping reads to areas of low sequence complexity. First, Cent2 and Cent5 contain the lowest amounts of CentC repeats among B73 centromeres (Albert et al. 2010), but regions of high CENH3 enrichment make up nearly 90% of these two centromeres. Second, CentC and the two dominant centromeric retrotransposons CRM1 and CRM2 of maize are highly enriched for CENH3 nucleosomes (Zhong et al. 2002; Jin et al. 2004; Wolfrubger et al. 2009; Gent et al. 2011). Differences in centromere size and CENH3 abundance between maize and rice may be a consequence of maize having much a larger genome and chromosome size. An elegant immunofluorescence-based comparison of centromere and genome size revealed that centromere size correlates with genome size among the genetically related grass species, suggesting large amounts of cenH3 benefit the stabilization of the spindle and separation of large chromosomes (Zhang and Dawe 2012). Based on these data, we propose that abundant CENH3 nucleosomes are important for centromere function in species with large chromosomes, and we predict that characterization of additional large-chromosome species will reveal maize-like centromeres that are either large or have a high percentage of CENH3-enriched subdomain within the centromeres.

Consistent with prior studies of nucleosome organization for the maize centromeric repetitive elements CentC, CRM1, and CRM2 (Gent et al. 2011), we found that the dominant pattern of nucleosome spacing across centromeres revealed by micrococcal nuclease digestion was approximately 190 bp between nucleosome midpoints (Figure 3C). However, we also found evidence for reproducible positioning, or phasing, of nucleosomes relative to the CentC repeat (Figure 3A). Phasing of CENH3 nucleosomes on the 156-bp CentC repeat mirrors the recently reported phasing of cenH3 nucleosomes along the 155-bp CentO repeats in rice (Zhang et al. 2013). Thus, nucleosome phasing mediated by centromeric tandem repeats may be a common mechanism that contributes toward centromere/kinetochore function. The existence of distinct fragment sizes following MNase digestion in both input and ChIP (Figure 1) could reflect flexibility of DNA wrapping around cenH3 nucleosomes (Hasson et al. 2013). Another potential explanation for smaller DNA fragments is that nucleosomes are not limited to the classical eight-histone and 147-bp wrapping DNA structure, and such alternative histone complexes are particularly abundant among cenH3 containing nucleosomes. Indeed, multiple experimental approaches in diverse species have indicated that cenH3 nucleosomes can exist in a complex of histone tetramers rather than the canonical histone octamer (Dalal et al. 2007; Dimitriadis et al. 2010; Shivaraju et al. 2012).

Centromeric chromatin is associated with distinct histone modification patterns as compared to classical euchromatin and heterochromatin in several model eukaryotes. In humans and Drosophila melanogaster, CENP-A nucleosomes are interspersed with H3 nucleosomes marked by H3K4me2. These H3 nucleosomes are associated neither with the euchromatic mark H3K4me3 nor with the heterochromatic marks H3K9me2 and H3K9me3 (Sullivan and Karpen 2004). H3 nucleosomes marked with H3K4me were also detected in S. pombe centromeres (Cam et al. 2005). However, this distinct association of H3K4me2 with centromeres was not found in rice centromeres. Transcribed genes in rice centromeres, however, were associated with classical euchromatic histone modifications marks, including H3K4me2, H3K4me3, H3K36me3, and H3K4ac (Wu et al. 2011). We detected a similar association of euchromatic histone modifications with active genes in maize centromeres (Figure 2). Maize centromeres had reduced levels of the heterochromatic mark H3K27me2 relative to pericentromeric regions (Figure 5 and Figure S3), which agrees with cytological observations that maize centromeres were weakly stained with immunofluorescence from heterochromatic histone modification marks (Shi and Dawe 2006; Jin et al. 2008). Thus, maize centromeres appear to be dominated by subdomains enriched with CENH3 and have a relative depletion of histone modifications associated with H3 histone. We find no evidence that histone H3 modifications studied in this work serve as a major epigenetic mark for centromere identity.

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