Differential Chromosomal Localization of Centromeric Histone CENP-A Contributes to Nematode Programmed DNA Elimination

Graphical Abstract

Highlights
- CENP-A genome distribution suggests that *Ascaris* has holocentric chromosomes
- Genomic regions lost during somatic DNA elimination have reduced CENP-A level
- CENP-A is present in these same genomic regions in the mitotic germline
- Changes in CENP-A localization contribute to programmed DNA elimination

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In Brief
Kang et al. show that nematodes with programmed DNA elimination have holocentric chromosomes in the mitotic germline. DNA sequences lost in early embryos during programmed DNA elimination have reduced levels of CENP-A and kinetochore components. Thus, developmental changes in CENP-A localization contribute to sequences lost in programmed DNA elimination.

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Differential Chromosomal Localization of Centromeric Histone CENP-A Contributes to Nematode Programmed DNA Elimination

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SUMMARY

The stability of the genome is paramount to organisms. However, diverse eukaryotes carry out programmed DNA elimination in which portions or entire chromosomes are lost in early development or during sex determination. During early development of the parasitic nematode, Ascaris suum, 13% of the genome is eliminated. How different genomic segments are reproducibly retained or discarded is unknown. Here, we show that centromeric histone CENP-A localization plays a key role in this process. We show that Ascaris chromosomes are holocentric during germline mitoses, with CENP-A distributed along their length. Prior to DNA elimination in the four-cell embryo, CENP-A is significantly diminished in chromosome regions that will be lost. This leads to the absence of kinetochores and microtubule attachment sites necessary for chromosome segregation, resulting in loss of these regions upon mitosis. Our data suggest that changes in CENP-A localization specify which portions of chromosomes will be lost during programmed DNA elimination.

INTRODUCTION

Maintenance of genome stability is an important process in all organisms. Genome instability can lead to cell death and disease. However, examples are known where genome instability through developmentally regulated DNA loss or rearrangement is integral in the biology of the organism. A well-known example is vertebrate immunoglobulin gene rearrangement that enables antibody and T cell receptor diversification (Jung et al., 2006; Nishana and Raghavan, 2012). DNA rearrangement and elimination are also involved in the extensive remodeling of the somatic genome that occurs during development of the macronucleus in ciliates (Chalker and Yao, 2011). In addition, programmed DNA elimination also occurs during the development of diverse metazoans, including some nematodes, copepod crustaceans, insects, lampreys, hagfish, zebra finches, and marsupials (Wang and Davis, 2014).

Programmed DNA elimination was first described in 1887 by Boveri (1887) in the intestinal nematode of horses, Parascaris. In the related pig and human parasite Ascaris suum, DNA elimination occurs during the third through fifth cleavages (four- to 16-cell stage) of early development in five distinct somatic precursor cells that give rise to different cell lineages (Bonnieve, 1902; Meyer, 1895; Tobler et al., 1992). The genome in germline cells remains intact. During DNA elimination mitoses in the precursor somatic cells, chromosomes are broken and the fragments that undergo DNA elimination remain at the metaphase plate while the retained DNA is segregated into daughter cells (Niedermaier and Moritz, 2000). Using high-throughput sequencing, we previously compared the germline and somatic genomes of a single male Ascaris and found that 13% of the germline genome (43 Mb) is lost in forming the somatic genome, including single-copy DNA for at least 685 germline-expressed genes (Wang et al., 2012). This suggests that DNA elimination is an irreversible mechanism for germline gene silencing in Ascaris somatic cells (Wang et al., 2012). A key question is what determines which chromosomes fragments will be kept and which will be eliminated.

Caenorhabditis elegans has holocentric chromosomes, and other nematode chromosomes may be holocentric (Albertson and Thomson, 1982; Goday et al., 1985; Maddox et al., 2004; Pimpinelli and Goday, 1989). A prevailing model of holocentric chromosomes is that multiple centromeric regions, kinetochores, and microtubule attachment sites are punctuated along the length of the chromosome (Drinnenberg et al., 2016; Maddox et al., 2004; McKinley and Cheeseman, 2016; Melters et al., 2012; Steiner and Henikoff, 2015). During nematode programmed DNA elimination, chromosomes break and some chromosome fragments are retained while others are lost. This raises a key question for what leads to the lack of segregation of those portions of Ascaris chromosomes that are eliminated.

We hypothesized that, during DNA elimination, either the centromere-kinetochore function of eliminated genomic regions or microtubule attachment to the centromere-kinetochore is compromised or inhibited, leading to the lack of segregation.
and consequent loss of the DNA from these chromosome regions. To test whether the centromeres/kinetochores or microtubule attachment play a regulatory role during Ascaris DNA elimination, we generated antibodies to the histone H3 variant CENP-A (also known as CenH3), the epigenetic mark of centromeres (Black and Bassett, 2008; Chen and Mellone, 2016; DeRop et al., 2012; Drinnenberg et al., 2016; Earnshaw, 2015; Fukagawa and Earnshaw, 2014; McKinley and Cheeseman, 2016), and antibodies to components of the kinetochore. Our immunofluorescence staining data showed that CENP-A is reduced in chromosome regions that will be lost during DNA elimination, indicating centromere-kinetochore function is compromised in regions that will be eliminated. Chromatin immunoprecipitation sequencing (ChIP-seq) data indicated that during Ascaris germline mitoses, CENP-A is deposited diffusely along Ascaris holocentric chromosomes and present within the chromosome regions that will be lost. However, CENP-A is later significantly reduced in these regions prior to DNA elimination. This CENP-A reduction leads to the loss of kinetochores and microtubule attachment sites necessary for chromosome segregation, thus leading to the DNA loss during elimination. Overall, our data suggest that Ascaris CENP-A localization contributes to the identification of regions to be retained and lost during DNA elimination. Thus, CENP-A localization plays a regulatory role and contributes to the mechanism of DNA elimination.

RESULTS

CENP-A Is Reduced on Chromosome Regions that Are Eliminated

To examine the contribution of centromeres/kinetochores in Ascaris DNA elimination, we identified several key protein components of the centromere and kinetochore in Ascaris (see Figures S1) (Cheerambathur and Desai, 2014; Chen and Mellone, 2016; Drinnenberg et al., 2016; Earnshaw, 2015; Fukagawa and Earnshaw, 2014; Hori and Fukagawa, 2012; Lampert and Westermarck, 2011; McKinley and Cheeseman, 2016; Müller and Almouzni, 2014; Westtorpe and Straight, 2013). We generated polyclonal antibodies to the Ascaris histone H3 variant CENP-A, the inner kinetochore protein CENP-C, and the outer kinetochore protein NDC80 (see the Supplemental Information). These three proteins represent the key regions of the centromere and kinetochore organization and are required for chromosome segregation in the model nematode C. elegans (Buchwitz et al., 1999; Cheeseman et al., 2004, 2006; Desai et al., 2003; Kitagawa, 2009; Maddox et al., 2004; Monen et al., 2005; Moore and Roth, 2001; Oegema et al., 2001).

Western blot analyses with these antibodies demonstrated that the antibodies recognize nuclear proteins of the expected molecular weight (see Figure S1D). We then used these antibodies to examine mitoses and chromosome segregation in early Ascaris embryos. All three antibodies stained interphase nuclei and chromosomes during mitosis, consistent with their known functions (Figure S1E). Consistent with holocentric chromosome organization, CENP-A localized all along the outer surface of metaphase chromosomes facing the spindle poles (Buchwitz et al., 1999) (Figure 1E). We next asked if these centromere-kinetochore components were present or absent at anaphase on chromosome regions destined for loss during DNA elimination where retained and eliminated chromosomes are easily identified. Two distinct CENP-A antibodies stain the chromosomes that will be retained during DNA elimination mitoses, but CENP-A staining is greatly reduced on chromosomes that will be eliminated (Figures 1A and 1B, red arrows) (CENP-A intensity is ~13% in the eliminated DNA compared to the retained DNA). Immunohistochemistry with CENP-C and NDC80 antibodies demonstrated that like CENP-A, these kinetochore components were present on chromosomes that will be retained during DNA elimination mitoses, but were greatly reduced on chromosomes that will be eliminated (Figures 1C and 1D, red arrows). Overall, these data suggest that during Ascaris programmed DNA elimination, limited or no functional centromeres/kinetochores are assembled on the eliminated chromosome regions due to the lack of CENP-A localization.

Next, we asked whether chromosome regions that will be eliminated lack CENP-A in the cell cycle preceding the DNA elimination mitosis using a closely related nematode, Parascaris univalens. Parascaris undergoes DNA elimination in early embryos, has very high sequence similarity with Ascaris, and has been used in conjunction with Ascaris as a model for nematode DNA elimination. Parascaris germline has a single pair of large chromosomes that consist of long condensed and heterochromatic arms that are eliminated and a central region that is fragmented into smaller retained chromosomes (Niedermaier and Moritz, 2000) (Figures 1F and 1G). This chromosomal organization makes it easier to distinguish retained versus eliminated chromosomal regions prior to DNA elimination. Consistent with their loss in elimination, the long arms have greatly reduced CENP-A at the one-cell stage compared to the central region that will form new retained chromosomes (Figures 1F and 1G, yellow arrow) that exhibit significant CENP-A staining (Figure 1G, white arrows). These data suggest that CENP-A marks regions for retention prior to the DNA elimination mitosis in Parascaris. Overall these data indicate that CENP-A localization on particular regions of chromosomes immediately prior to and during DNA elimination likely determines which portions of chromosomes will be retained and eliminated in the somatic cells.

High-Resolution CENP-A Mapping Reveals Reduced CENP-A Localization in the Genomic Regions that Will Be Eliminated

We next carried out native CENP-A ChIP-seq to obtain whole-genome, base-pair resolution maps of CENP-A. CENP-A is organized into clusters, dispersed throughout the Ascaris genome, that extend along the length of the chromosomes (Figure 2A). If the location of CENP-A in the genome represents functional centromeres/kinetochores, we would expect the inner kinetochore component CENP-C to co-localize with CENP-A. ChIP-seq analyses demonstrate that CENP-C localization shows a strong correlation with CENP-A (Figures 2A, 2B, and S2A–S2C). These data strongly suggest that (1) our CENP-A and CENP-C ChIP-seq data represent centromeric regions with kinetochores; (2) Ascaris has holocentric chromosomes; and (3)
the holocentromeres typically extend along the length of the *Ascaris* chromosome.

We next characterized *Ascaris* CENP-A nucleosomes. We compared the size of the DNA from immunoprecipitated *Ascaris* CENP-A nucleosomes to all input nucleosomes (mostly H3 based nucleosomes) (Figures 2C and S2D). Based on the observed DNA length for *Ascaris* CENP-A compared to the core H3 nucleosomes (Figures 2C and S2D), the DNA size of an *Ascaris* CENP-A mono-nucleosome was ~6 bp smaller than for a core H3 nucleosome. This smaller DNA size for CENP-A nucleosomes is consistent with previous CENP-A nucleosome observations in other organisms (Hasson et al., 2013), further suggesting that our analyses are mapping octameric *Ascaris* CENP-A nucleosomes. *Ascaris* CENP-A is present primarily in mono- or di-nucleosomes flanked by H3 nucleosomes (Figure 2C). Overall, these data suggest that our genome mapping of CENP-A and its localization likely corresponds to centromere-kinetochore sites in *Ascaris*.

We next carried out CENP-A ChIP-seq at the four-cell stage (60 hr) to further examine the CENP-A reduction we observed by immunohistochemistry (Figure 1) in regions that undergo DNA elimination. We found that compared to retained regions, the number of CENP-A peaks, the genomic area covered by CENP-A, and the level of CENP-A reads are all greatly reduced in DNA regions that will be eliminated (Figures 2D and 2E). These data are consistent with a DNA elimination mechanism where CENP-A reduction leads to the absence of kinetochores and microtubule attachment sites necessary for chromosome elimination.
As the consequence of this loss of DNA regions during segregation, thus leading to the loss of these DNA regions during *Ascaris* programmed DNA elimination.

**Changes in CENP-A Localization Define DNA that Will Be Retained and Eliminated**

To examine whether the reduced CENP-A in regions that undergo DNA elimination at the four-cell stage is inherited from germ cells or re-organized during development, we carried out CENP-A ChIP-seq on the *Ascaris* mitotic germline and compared the CENP-A coverage in DNA regions destined for retention or elimination in the mitotic germline to that present in the four-cell embryos. In contrast to the four-cell stages where there is little CENP-A localization in regions that undergo DNA elimination, we found that CENP-A is abundant in the mitotic germline regions that will undergo DNA elimination later in embryos (Figure 3A). We next compared CENP-A peaks numbers, the genomic regions covered by CENP-A peaks, and the CENP-A reads numbers in regions that will be eliminated (Figures 3B and 3C; Tables S1 and S2) in different stages. All these features of CENP-A are reduced in four-cell stages prior to DNA elimination compared to the germline tissues. The extensive and dynamic changes in CENP-A distribution in *Ascaris* suggest that this key epigenetic mark contributes to the specific loss of DNA sequences.

**CENP-A Deposition, Epigenetic Marks, and Transcription**

To explore what factors might contribute to *Ascaris* CENP-A localization or its removal during DNA elimination, we examined the relationship between CENP-A, RNA expression (RNA sequencing [RNA-seq]), and several active (H3K4me3, H3K36me3, and H4K20me1) or repressive histone marks (H3K9me2 and H3K27me3) (Figures 4A and S4A–S4C; Table S3). CENP-A is primarily concentrated in lowly or non-transcribed genomic regions (including intergenic regions) (correlation coefficient, r = −0.546) (Figures 4A and S4A), and it is not associated with repetitive sequence or enriched on promoter regions. In addition, CENP-A showed no significant correlation with either the active or repressive histone marks examined (Figures 4A, S4B, and S4C). Overall, these data indicate that CENP-A is in general inversely correlated with transcription and is not correlated with any of the specific histone marks examined.

In *C. elegans*, germline transcription plays a role in defining CENP-A deposition. Genomic regions that are transcribed in the *C. elegans* germline lack CENP-A deposition in the early embryo (Gassmann et al., 2012). To examine whether transcription plays a role in the *Ascaris* CENP-A deposition changes
Figure 3. Dynamic CENP-A Localization Defines DNA to Be Retained and Eliminated

(A) CENP-A localization in the mitotic regions of germline (testis and ovary) and four-cell embryos. CENP-A enriched regions (positive values) are in green, and CENP-A depleted regions (negative values) are in red (Z scores; see the Supplemental Experimental Procedures). The purple arc and light blue shading represent the genomic region that will be eliminated. Circles from outer to inner represent (1) genes (red, + strand; blue, − strand); (2) testis CENP-A; (3) ovary CENP-A; and (4) four-cell embryo CENP-A.

(B) CENP-A peaks are reduced prior to DNA elimination in regions of the genome that will undergo elimination. Changes in CENP-A peaks between the germline and four-cell embryos in a region eliminated (scaffold ag84) (see Figure 3C for the overall changes).

(C) CENP-A is reduced before DNA elimination (see also Table S1). CENP-A is present at high levels in the mitotic germline (Testis-1 and Ovary-1) within regions that will become eliminated in embryos and then is greatly reduced before DNA elimination (four-cell).
associated with DNA elimination, we compared RNA-seq and CENP-A data (Figures 4B, S3A, and S3B). CENP-A enriched regions and transcriptionally active regions are mutually exclusive throughout Ascaris development (Figure 4B). Notably, transcriptionally active regions have low levels of CENP-A (Figures 4B [left and middle] and S4D), and developmental transcription changes have limited impact on the level of CENP-A deposition unless very large transcriptional changes occur (Figures 4B, S4D, and S4E). Overall, our data suggest that (1) CENP-A enrichment and transcriptional changes are generally differentially organized in the genome, (2) only very large changes in RNA expression can impact the levels of CENP-A deposition, and (3) germline or early embryo transcription does not appear to play a direct role in defining CENP-A deposition or removal for DNA elimination.

**DISCUSSION**

**CENP-A Localization in the Ascaris Genome Defines Sequences for Retention or Elimination**

During programmed DNA elimination in nematodes, chromosomes break, and portions of chromosomes are segregated and retained in daughter cells while other parts of chromosomes remain at the metaphase plate, are not segregated, and are eventually degraded and lost (Figure 4C, part 3). A key question is what determines which portions of chromosomes are retained
and which will be lost during DNA elimination when all regions of holocentric chromosomes (by definition) should be functional for segregation. We assessed whether centromere/kinetochore component assembly or microtubule attachment is compromised in the genomic regions lost during nematode DNA elimination by using antibodies we generated to CENP-A, CENP-C (inner kinetochore), and NDC80 (outer kinetochore) proteins. CENP-A is in general uniformly localized along chromosomes in the mitotic germline, consistent with a holocentric chromosome organization (Figure 3). Immediately preceding and during DNA elimination, chromosome regions that will be lost have significantly reduced CENP-A, CENP-C, and NDC80 immunohistochemical staining (Figure 1). Early embryo ChIP-seq data also demonstrate that the regions that will be lost have significantly reduced levels of CENP-A. However, in contrast, genome regions in the mitotic germline chromosomes that will be lost in early embryos have significant CENP-A. During development, CENP-A localization is subsequently reduced or CENP-A is removed in specific chromosome regions, thereby compromising centromere-kinetochore assembly and facilitating the loss of these chromosome regions during DNA elimination (Figure 4C).

In the closely related parasitic nematode *Parascaris*, one pair of very large chromosomes undergoes DNA elimination in the second and third division of embryogenesis. The highly heterochromatic and condensed arms of these large chromosomes are lost during DNA elimination (Figures 1F and 1G) (Niedermaier and Moritz, 2000; Pimpinelli and Goday, 1989). Pioneering cytological studies described a long kinetochore extending along the length of the mitotic germline chromosomes, suggesting their holocentric nature (Goday et al., 1985). Just prior to DNA elimination, the heterochromatic arms were proposed to lose kinetochore activity and microtubule attachment, leading to their loss in DNA elimination (Figures 1F and 1G) (Goday et al., 1992). Our data provide a mechanistic explanation for the proposed change in the kinetochore distribution.

The reduction of CENP-A from *Ascaris* germline tissues to the four-cell embryos could be due to a combination of defective CENP-A deposition (including during DNA replication) and active removal of CENP-A. CENP-A may not be completely lost from eliminated regions. We note that the minimal amount of CENP-A nucleosome(s) (and its organization) that is required to establish microtubule attachment on a holocentric chromosome, and how many of these functional centromere sites are needed to segregate chromosomes to the daughter cells during mitosis, remains unknown.

**Determinants of CENP-A Deposition and CENP-A Nucleosomes**

As the presence of CENP-A appears to be a key event in defining the retention or elimination of portions of chromosomes during DNA elimination, we sought to understand where and how CENP-A is localized in the genome. Unlike in many organisms, *Ascaris* CENP-A deposition is not associated with repetitive sequences or with heterochromatin. In particular, there is no apparent CENP-A enrichment with the major 121-bp repetitive sequences (with >99% lost during DNA elimination) in the germline tissues and early embryos. In addition, no DNA sequence motifs or other attributes appear to be associated with CENP-A localization. These are also attributes observed for CENP-A deposition in the model nematode *C. elegans* (Gassmann et al., 2012; Steiner and Henikoff, 2014), and they are consistent with the observation that CENP-A deposition can occur and facilitate segregation of any extrachromosomal array in *C. elegans* (Stinchcomb et al., 1985; Yuen et al., 2011). The majority of *Ascaris* CENP-A is organized into peaks of 1–15 kb (median of ~3.5 kb) that appear uniformly distributed across *Ascaris* chromosomes. In general, *Ascaris* CENP-A deposition is inversely correlated with regions of the genome that are actively transcribed. However, this is not an exclusive relationship as there are regions of the genome with actively expressed genes where CENP-A can be deposited. These genes are expressed at relatively low levels, whereas genes that are expressed at very high levels in any stage generally preclude CENP-A deposition (Figure 4B).

We used ChIP-seq to analyze the relationship between active and repressive histone marks (H3K4me3, H3K36me3, H4K20me1, and H3K27me3) and CENP-A deposition. Despite the inverse correlation between CENP-A and transcription, we observed no correlations with any of these histone marks and CENP-A deposition. It remains to be determined in *Ascaris* whether there are specific histone marks that are strongly associated with CENP-A that may help establish or preclude CENP-A deposition and centromere-kinetochore assembly.

**Nematode CENP-A Deposition, Centromeres/Kinetochores, and Holocentric Chromosomes**

Cytological data indicate that the model nematode *C. elegans* chromosomes are holocentric with centromere-kinetochore regions distributed along the length of the chromosomes in early embryos (Albertson and Thomson, 1982). By using CENP-A ChIP-DNA microarray (ChIP-chip), Gassmann et al. demonstrated that CENP-A diffusely occupies ~2,900 broad, low-density domains of ~10–12 kb that cover about half of the genome (Gassmann et al., 2012). In contrast, in a more recent study using *C. elegans* CENP-A ChIP-seq, Steiner and Henikoff suggested that CENP-A is present in high-density and discrete point-like peaks that are coincident with transcription factor hotspots (Steiner and Henikoff, 2014). It remains to be determined whether these observed differences are due to the different experimental conditions and methods used or data interpretation. In our *Ascaris* analyses, we used MNase digestion conditions and a native ChIP-seq protocol similar to those described by Steiner and Henikoff (2014). However, we did not observe any strong, discrete point-like CENP-A peaks in *Ascaris*. We also found that the length of DNA wrapped around *Ascaris* CENP-A nucleosome is ~6 bp smaller than a canonical H3 nucleosome, consistent with the octameric nucleosome model that suggests CENP-A nucleosomes have loose DNA termini (Hasson et al., 2013). In addition, our CENP-C ChIP-seq data also showed a strong correlation with CENP-A data, in agreement with a diffuse, low-density CENP-A model (Gassmann et al., 2012). In *Parascaris*, electron microscopy analysis of the mitotic germline and early embryo chromosomes did not demonstrate regular interruptions along the length of the kinetochore regions, suggesting that the ascarid holocentric organization is more diffusely...
distributed, as classically described, rather than polycentric (Goday et al., 1985, 1992). Our CENP-A data in Ascaris appear consistent with these electron microscopy (EM) studies and thus favor a broad, diffusively distributed holocentric chromosome organization model.

CENP-A and Ciliate DNA Rearrangement

A recent study described the deletion of the CENP-A gene during DNA rearrangement in ciliates (Lhuillier-Akakpo et al., 2016). However, the loss of CENP-A in the formation of the ciliate macronucleus does not appear to lead to the direct loss of macronuclear chromosomes. In contrast, the nematode CENP-A gene is not lost, but CENP-A localization is regulated and reduced on chromosome regions that will undergo DNA elimination, contributing to the loss of particular DNA regions.

Conclusions

Programmed DNA elimination in ascarid nematodes occurs during early embryo development. Chromosomes break, new chromosomes form, and regions of chromosomes are lost. Our data demonstrate that CENP-A, the key centromere component, extends along the length of the mitotic germline chromosomes, consistent with a holocentric organization of the germline chromosomes. CENP-A is then reduced in specific chromosome regions that will be eliminated in early development. Thus, regulation of CENP-A localization or its removal prior to DNA elimination defines and specifies which portions of somatic chromosomes will be lost during programmed DNA elimination. Our studies provide key insights into what determines which sequences are retained and which are eliminated in programmed DNA elimination, a phenomenon that occurs in a breadth of organisms including ciliates, nematodes, arthropods, crustaceans, and vertebrates.

EXPERIMENTAL PROCEDURES

Ascaris, Antibodies, and Immunohistochemistry

Collection of Ascaris tissues, zygotes, and zygote embryonation were as previously described (Wang et al., 2011, 2014). Preparation of polyclonal antibodies and monoclonal histone antibodies used are described in the Supplemental Experimental Procedures. Ascaris embryo immunohistochemistry was carried out as described (Wang et al., 2014), using a modified freeze-crack method to permeabilize and fix embryos.

Nuclei Isolation and ChIP-Seq

Nuclei were isolated from de-coated embryos or Ascaris germline tissues as described in the Supplemental Experimental Procedures. For native ChIP, a protocol modified from Steiner and Henikoff (2014) was used. For CENP-A ChIP, 5–10 million nuclei were isolated from germline tissues or different stages of embryos. CENP-A was immunoprecipitated from the extract by incubation with 15 μg of affinity purified antibody and pre-cleared protein A beads. For CENP-C ChIP, a cross-linked method modified from Patel et al. (2014) was used with the 20 μg of affinity purified antibody. The details are described in the Supplemental Experimental Procedures.

Sequencing and Data Analysis

Sequencing libraries and data analysis are described in the Supplemental Experimental Procedures. Data are made available in the UCSC Genome Browser track data hubs (https://genome.ucsc.edu/cgi-bin/hgHubConnect) using the “My Hubs” tab with the following link: http://amc-sandbox.ucdenver.edu/User14/hub.txt.

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ACCESSION NUMBERS

The accession number for the ChIP-seq data reported in this paper is NCBI GEO: GSE76914.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.07.079.

REFERENCES


