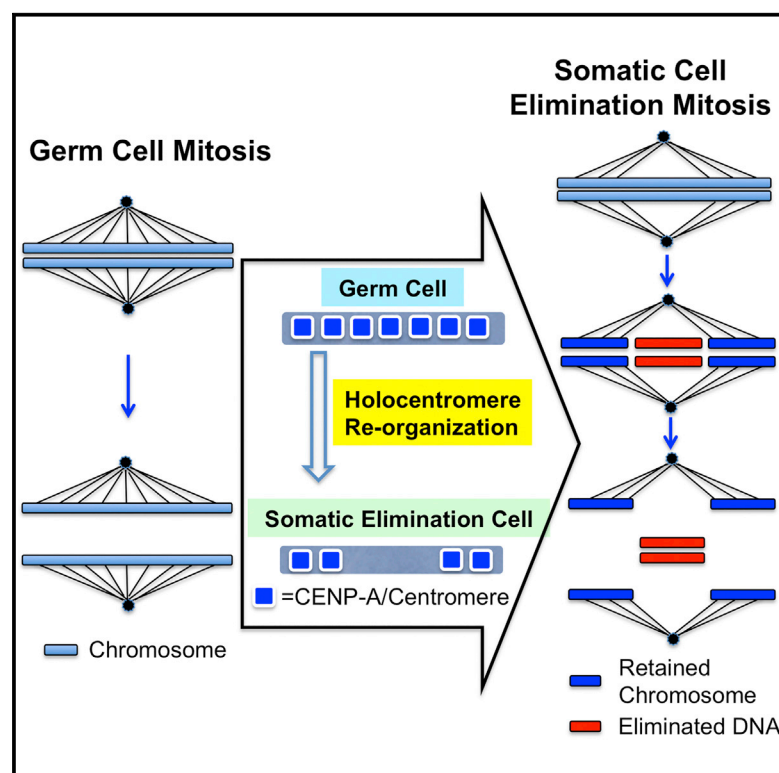


# Cell Reports

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

### Graphical Abstract



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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.

### 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

### Accession Numbers

GSE76914



Kang et al., 2016, Cell Reports 16, 2308–2316  
August 30, 2016 © 2016 The Author(s).  
<http://dx.doi.org/10.1016/j.celrep.2016.07.079>

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

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<http://dx.doi.org/10.1016/j.celrep.2016.07.079>

## 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 metazoa, 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 (Bonnievie, 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 chromosome 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; De Rop 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 Westermann, 2011; McKinley and Cheeseman, 2016; Müller and Almouzni, 2014; Westhorpe 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).

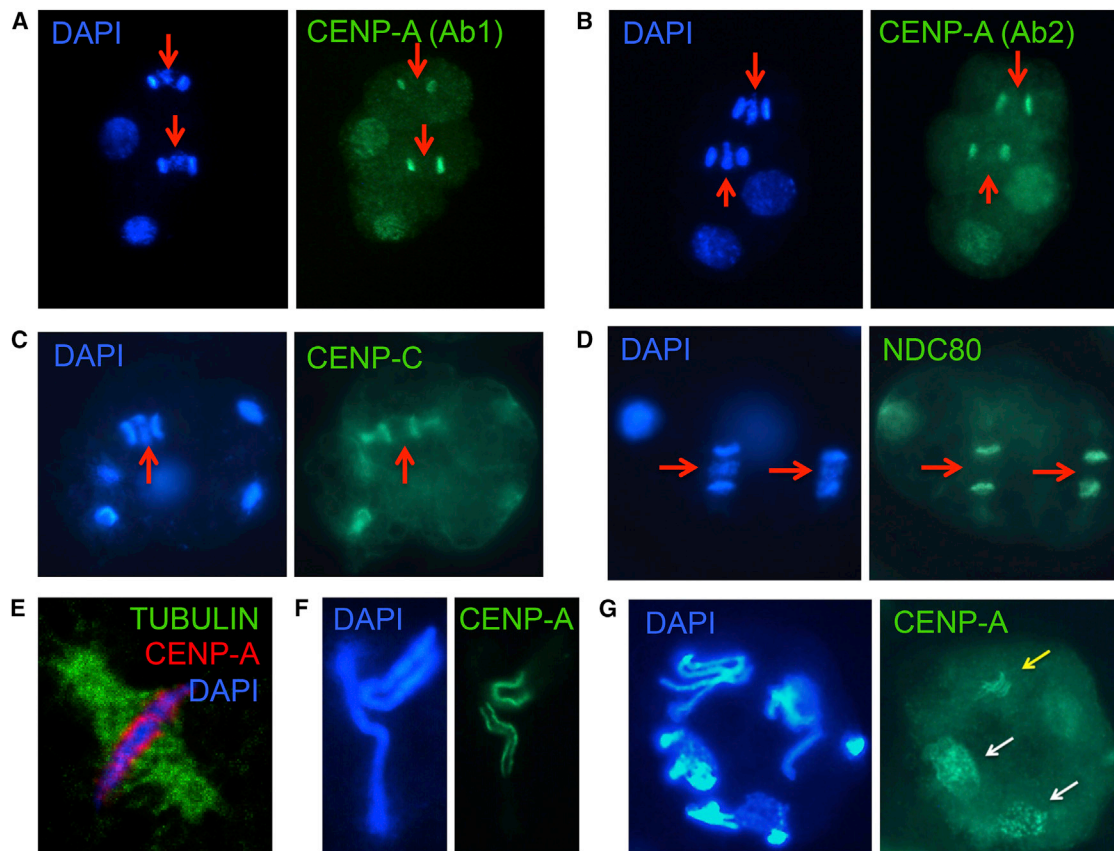
Western blot analyses with these antibodies demonstrated that the antibodies recognize nuclear proteins of the expected molecular weight (see Figure S1D). We then used these antibodies to examine mitoses and chromosome segregation in early *Ascaris* embryos. All three antibodies stained interphase nuclei 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)



**Figure 1. CENP-A Is Reduced in Genomic Regions Lost during Nematode Programmed DNA Elimination**

(A and B) CENP-A is reduced in genomic regions that remain at the metaphase plate and will be lost during *Ascaris* DNA elimination. Immunohistochemical (IHC) staining of CENP-A on a four-cell *Ascaris* embryo with two cells undergoing DNA elimination mitoses (anaphase) indicates the DNA that will be lost (red arrows) has much less CENP-A than the DNA that will be segregated and retained. The staining observed is the same using two independently prepared antibodies (Ab1 and Ab2) made against a CENP-A peptide (A) or a fusion protein (B) (see Figure S1).

(C and D) CENP-C (C) and NDC80 (D) are also reduced on *Ascaris* chromosome regions that remain at the metaphase plate and will be eliminated.

(E) IHC staining of microtubules and CENP-A in a two-cell *Ascaris* embryo at metaphase illustrates that CENP-A extends along the length and is localized asymmetrically to the centrosome side of the chromosomes.

(F) IHC staining of CENP-A in a one-cell *Parascaris univalens* embryo during prometaphase. Note that CENP-A is reduced on the long arms of *Parascaris* chromosomes just before DNA elimination. These regions will be lost in subsequent cell divisions during DNA elimination, whereas the central chromosome region with CENP-A will form many new retained chromosomes (see G).

(G) IHC staining of CENP-A in a four-cell embryo in *Parascaris* undergoing DNA elimination. Note that CENP-A only stains euchromatin regions (yellow arrows) in chromosomes prior to DNA elimination and that the many new and retained chromosomes (white arrows) formed from this region during elimination.

The size of *Ascaris* and *Parascaris* embryos are  $\sim 70 \times 45 \mu\text{m}$  and  $\sim 50 \times 50 \mu\text{m}$ , respectively.

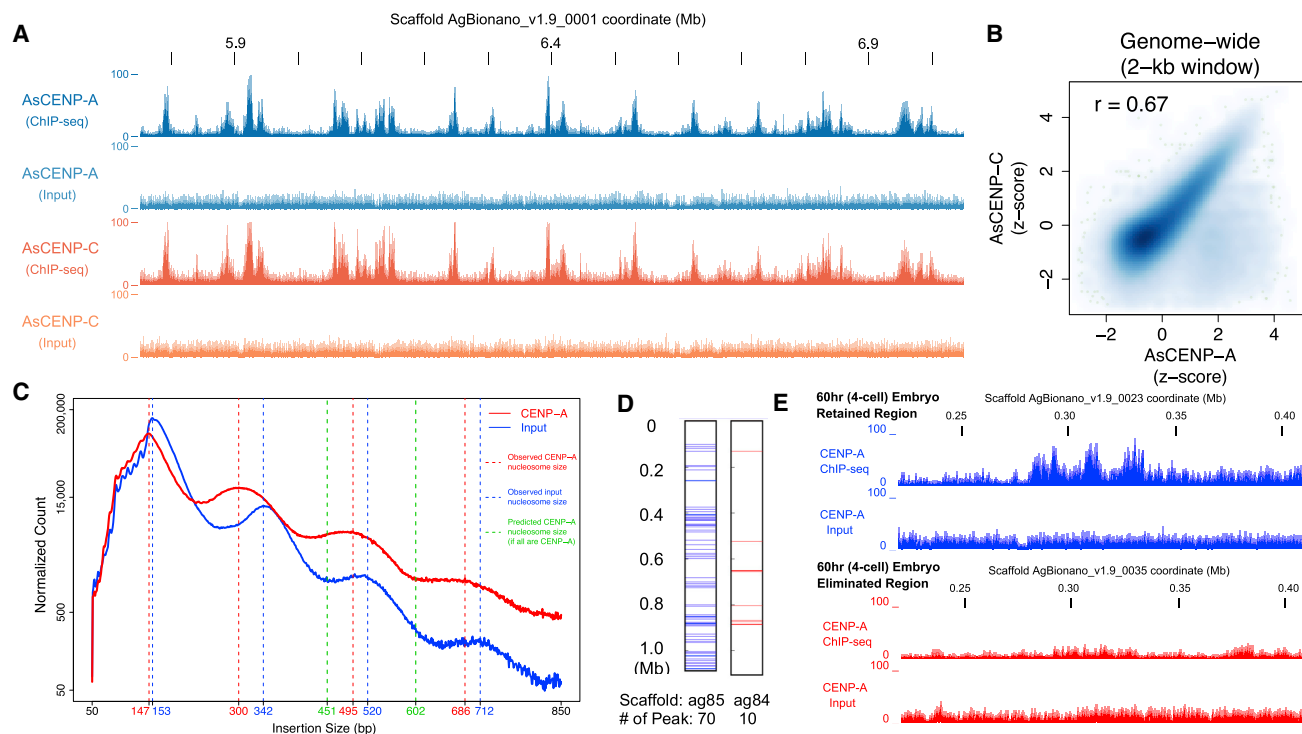
the holocentromeres typically extend along the length of the *Ascaris* chromosome.

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  $\sim 6$  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





**Figure 2. Genome-wide Mapping Demonstrates *Ascaris* CENP-A Is Reduced in Eliminated Regions**

(A and B) CENP-A and CENP-C genome-wide ChIP-seq co-localization. (A) Representative region of an *Ascaris* chromosome (data from 32 to 64-cell embryos). (B) Strong genome-wide correlation between CENP-A and CENP-C localization. (C) *Ascaris* CENP-A nucleosomes are smaller than H3 nucleosomes (see the [Supplemental Experimental Procedures](#)). (D) CENP-A peaks in retained (ag85, blue) and eliminated (ag84, red) scaffolds just prior to DNA elimination (four-cell stage). Overall, there is at least a 3-fold reduction of CENP-A peaks in eliminated regions (see [Table S1](#)). (E) CENP-A is greatly reduced in genomic regions that will be eliminated (red) compared to retained (blue) regions. Representative scaffolds with data from four-cell embryos are shown.

segregation, thus leading to the loss of these DNA regions during *Ascaris* programmed DNA elimination.

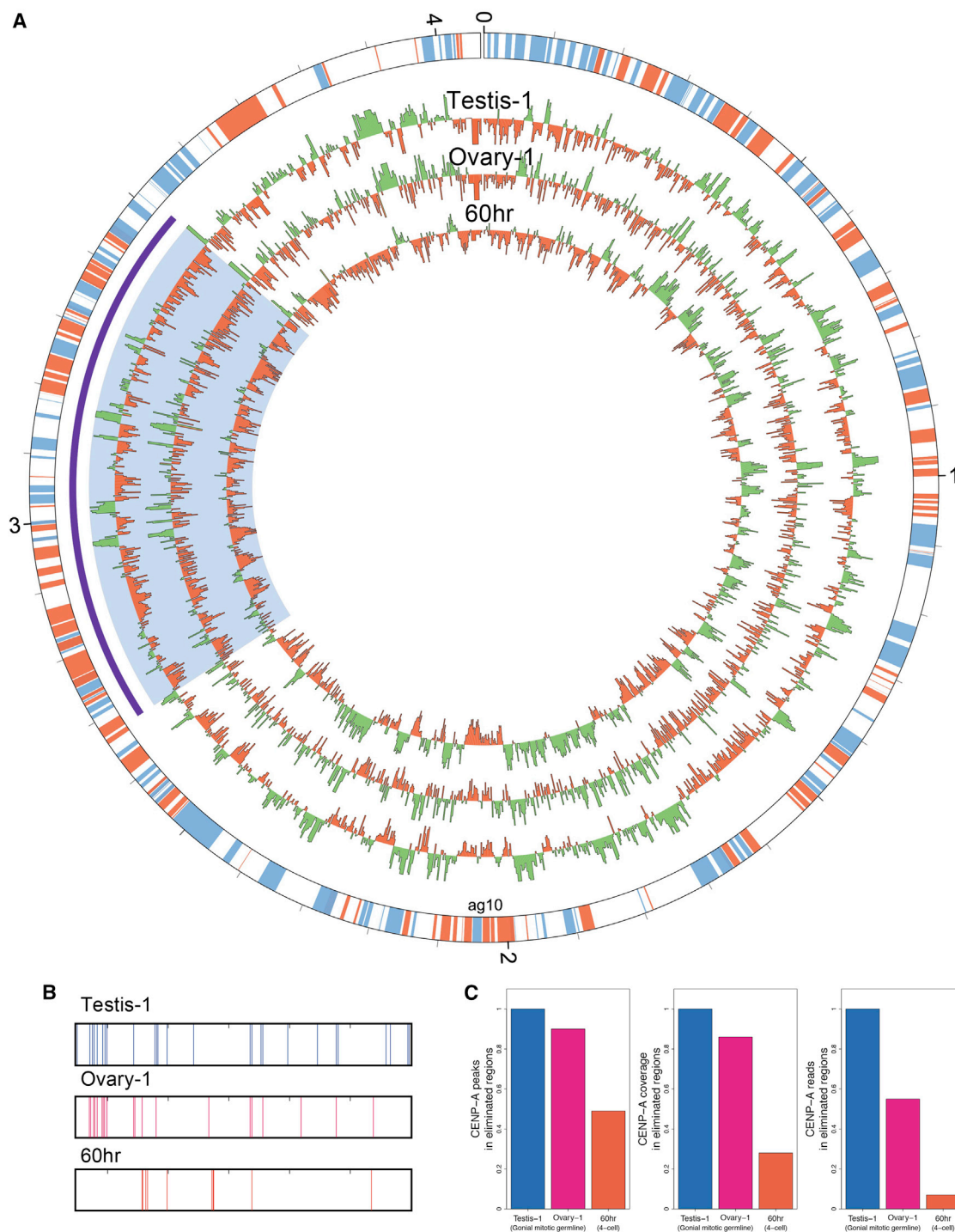
### Changes in CENP-A Localization Define DNA that Will Be Retained and Eliminated

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

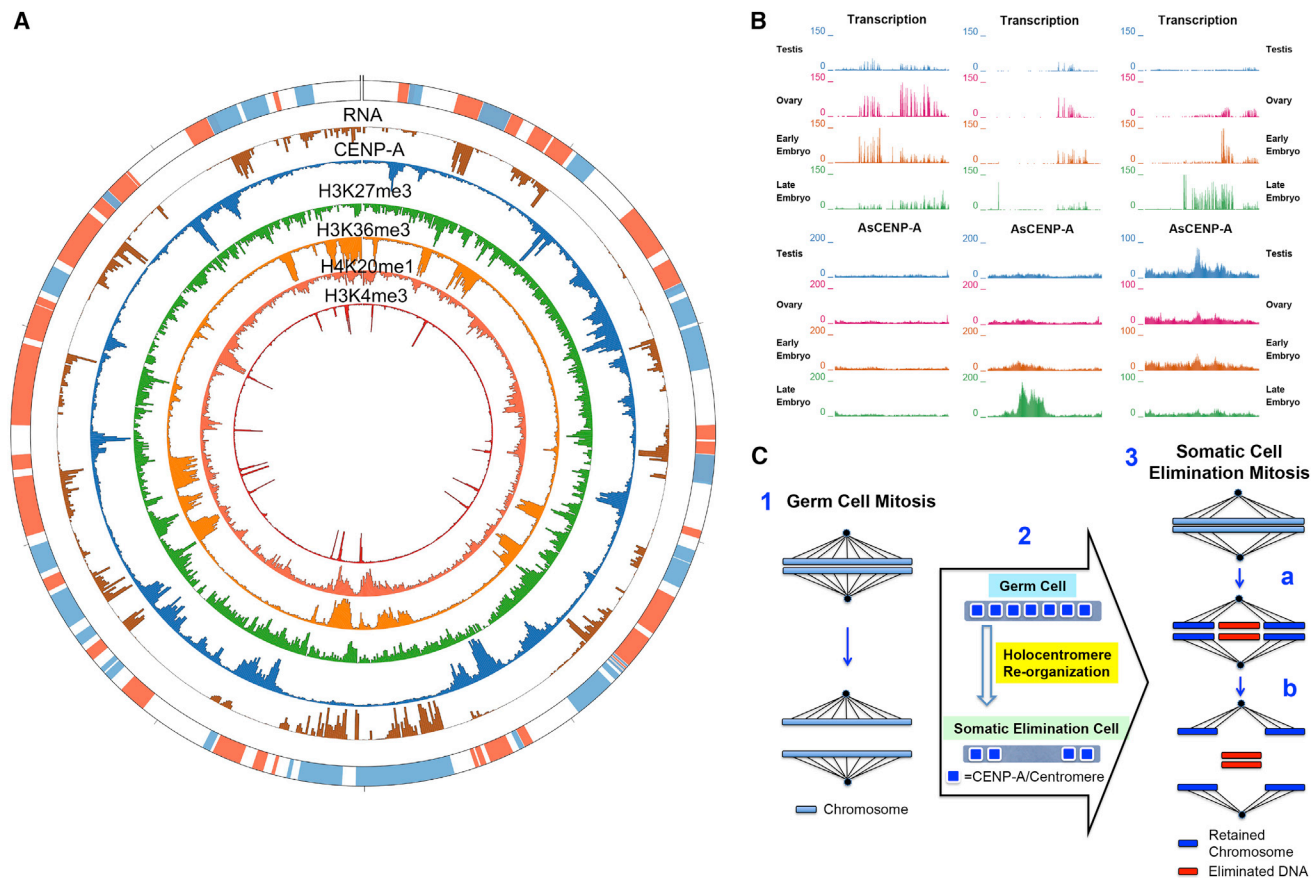


**Figure 3. Dynamic CENP-A Localization Defines DNA to Be Retained and Eliminated**

(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).



**Figure 4. *Ascaris* CENP-A Deposition, Histone Marks, and Transcription**

(A) CENP-A, histone marks, and RNA (32- to 64-cell embryos) associated with a 1-Mb region of an *Ascaris* chromosome (ag1 5.5–6.5 Mb). Circles from outer to inner represent (1) genes (red, + strand; blue, – strand); (2) RNA (brown); (3) CENP-A (blue); (4) H3K27me3 (green); (5) H3K36me3 (light orange); (6) H4K20me1 (dark orange); and (7) H3K4me3 (red).

(B) mRNA expression (RNA-seq) and CENP-A (ChIP-seq) in different stages and tissues. Three representative 50-kb regions of the *Ascaris* chromosome (ag17) are shown. Data were averaged from three replicates with normalization (see the [Supplemental Experimental Procedures](#)). Shown are transcriptionally active regions where there is lack of CENP-A (left and middle); CENP-A enriched regions where there is little transcription (middle); and a genomic region that illustrates both CENP-A localization and RNA transcription (right). CENP-A localization is in general not associated with transcription changes (left and middle), but is in some cases where transcription is highly dynamic, CENP-A localization can be affected (right) (see also [Figure S4E](#)).

(C) Model of programmed DNA elimination in nematodes. (1) Holocentric mitotic germline chromosomes. (2) Re-organization of CENP-A and centromeres/kinetochores during development in regions that will be eliminated. (3) DNA elimination mitosis. Chromosomes in early embryo somatic cells undergo chromosome breaks at metaphase (a). CENP-A is reduced in the regions to be eliminated prior to chromosome breakage. Retained chromosome fragments (blue) have centromere-kinetochore sites for microtubule attachment to facilitate chromosome segregation, whereas chromosomal fragments that will be eliminated (red) remain at the metaphase plate, are not segregated, and are lost (b).

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 in all developmental stages examined 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 transcribed regions are in general 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 *Ascaris* 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>.

### 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>.

### AUTHOR CONTRIBUTIONS

Y.K., J.W., and R.E.D. designed the experiments. Y.K., J.W., S.K., A.N., and R.E.D. conducted the experiments. Y.K., J.W., and R.E.D. analyzed and interpreted the data. J.W. carried out bioinformatics analyses, and Y.K., J.W., and R.E.D. wrote the manuscript. H.K. provided monoclonal histone antibodies.

### ACKNOWLEDGMENTS

We thank Paul Megee and Chad Pearson for their input; Florian Steiner for sharing detailed protocols for nematode CENP-A ChIP-seq; Arshad Desai for help identifying *Ascaris* CENP-A and CENP-C; Martin Nielsen for *Parascaris* material; Richard Komuniecki, Amanda Ortega, Jeff Myers, and Routh Packing Co. for their support and hospitality in collecting *Ascaris* material; and Lee Niswander, David Bentley, Chad Pearson, and Mark Johnston for comments on the manuscript. This work was supported in part by NIH grants to R.E.D. (AI0149558 and AI114054).

Received: February 11, 2016

Revised: June 13, 2016

Accepted: July 27, 2016

Published: August 18, 2016

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**Cell Reports, Volume 16**

**Supplemental Information**

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

**Yuanyuan Kang, Jianbin Wang, Ashley Neff, Stella Kratzer, Hiroshi Kimura, and Richard E. Davis**

## Supplemental Text

**CENP-A.** We identified two potential orthologs of CENP-A in *Ascaris* (Fig. S1A). One ortholog was highly expressed at the time of DNA elimination (ASU\_14124, CENP-A). Two isoforms of *Ascaris* CENP-A (ASU\_14124) were identified in RNA-seq from diverse adult tissues, larvae, and embryo stages, but only one is predominantly present in early embryos during DNA elimination and was used to make an N-terminal fusion protein (amino acids 1-73) with GST for antibody generation. Another CENP-A ortholog (ASU\_11276, CENP-A-like) is primarily expressed in the testes, present at very low levels during DNA elimination, and is then gone later in early development (Fig. S1F). We synthesized peptides to the N-terminal regions of both of the CENP-A proteins (ASU\_14124, CENP-A = GAPRFGKKAIEGHIRVDC and ASU\_11276, CENP-A-like = SDFAKYEPKKGIACK), fused them to KLH, and used them as immunogens to prepare rabbit polyclonal antibodies (Covance). Both the peptide and fusion protein antibodies to CENP-A (ASU\_14124) identified a nuclear protein of the predicted molecular weight and stained interphase nuclei and mitotic chromosomes in early embryos (Fig. S1F, S1E, and Fig. 1A-B). In contrast, the antibodies to the CENP-A-like protein (ASU\_11276) did not recognize a protein of the predicted molecular weight or stain nuclei or chromosomes in early embryos. Thus, the antibodies to the peptide and fusion protein of CENP-A (ASU\_14124) were used in all experiments described in the paper.

**CENP-C.** We identified three potential orthologs of *Ascaris* CENP-C based on similarity with known CENP-C proteins in *C. elegans* (Fig. S1B). RNAs (Fig. S1F) for two of the orthologs (CENP-C1, ASU\_05506, and CENP-C2, ASU\_05420) were expressed during DNA elimination, whereas one (CENP-C3, ASU\_08074) was testis specific and expressed at very low levels in early embryos. We generated GST fusion proteins to portions of CENP-C1 (amino acids 885-1092) and CENP-C2 (amino acids 720-870) (see Fig. S1B) and used them as immunogens to prepare rabbit polyclonal antibodies (Covance). Antibodies were not made to CENP-C3 as it is expressed at such low levels in early embryos where DNA elimination occurs. Both CENP-C1 and CENP-C2 identified nuclear proteins of the expected molecular weight and stained interphase nuclei, mitotic chromosomes, are absent on DNA that will be eliminated



during elimination mitoses in early embryos, and co-localize with CENP-A ChIP-seq (Figs. 1-2). Antibody data shown in the paper are for CENP-C2.

## Supplemental Experimental Procedures

**Antibody generation.** We generated polyclonal antibodies to an *Ascaris* CENP-A peptide and a fusion protein, CENP-C fusion protein, and NDC80 fusion protein. CENP-A peptide (GAPRFGKKAIEGHIRVDC from gene ASU\_14124) was fused to KLH and used as immunogens to generate polyclonal rabbit antibodies (Covance). The CENP-A (ASU\_14124) amino acids 1-73, CENP-C1 amino acids 885-1092, CENP-C2 amino acids 720-870, and NDC80 amino acids 22-127 were fused to GST using the Bam H1 site of pGEX-6P-1 (GE Healthcare); the proteins were expressed in *E. coli*, purified using Glutathione Sepharose 4B columns, and used as the immunogens for the initial boost to generate polyclonal rabbit antibodies (Covance). For additional boosts, we used the *Ascaris* proteins cleaved from GST-fusions bound to Glutathione Sepharose 4B by treatment with PreScission protease (GE Healthcare). Antibodies were affinity purified using either the peptides linked to SulfoLink beads (Pierce) or the *Ascaris* proteins (without GST) linked to a mix of Affigel-10/15 (BioRad).

**Histone antibodies.** Monoclonal histone antibodies (H3K9me2, H3K9me3, H3K36me3, H3K27me3, H3K4me3, and H4K20me1) were from Hiroshi Kimura (Chandra et al., 2012; Kimura et al., 2008) and have been previously validated for use in the modENCODE project for *C. elegans* and other organisms (see antibody validation database: <http://compbio.med.harvard.edu/antibodies/>).

**Immunohistochemistry.** *Ascaris* embryo immunohistochemistry was carried as described (Wang et al., 2014) using a modified freeze-crack method to permeabilize and fix embryos. Briefly, decoated embryos were suspended in 50% methanol and 2% formaldehyde solution and were frozen and thawed 3 times using a dry ice/ethanol bath. The embryos were re-hydrated with 25% methanol in PBS pH 7.4 for 1 min. After washing twice with PBS pH 7.4, the embryos were incubated in signal enhancer solution (Invitrogen I36933) for 30 min at RT. The embryos were then re-suspended in blocking solution (0.5% BSA in PBS pH7.4) for 30 min at RT, followed by overnight incubation in primary antibodies at 4°C, and then a 2 hr incubation in secondary antibodies (Invitrogen) at room temperature. Nuclei were stained with DAPI.

## Nuclei Isolation

De-coated embryos were washed with Nuclei Extraction Buffer A (20 mM Tris-HCl pH 7.8, 1.5 mM  $MgCl_2$ , 1 mM EGTA, 0.5 M Sucrose, 10 mM KCl, 1 mM DTT, 1 mM Spermine, 1 mM Spermidine, 0.5 mM PMSF). Nuclei were released using a metal Dounce homogenizer with 10 strokes each of the loose- and then tight-fitting pestles in Nuclei Extraction Buffer B (Nuclei extraction Buffer A supplemented with 0.1% Triton X-100, 0.15% NP-40, 0.5mM PMSF and protease inhibitor cocktail [Roche]). The nuclei were pelleted at 750×g for 10 min. Nuclei were washed once with Nuclei Extraction Buffer A and then Nuclei Extraction Buffer C (1.5mM  $MgCl_2$ , 1mM EGTA, 1 M Sucrose, 10mM KCl, 20mM Tris-HCl, pH 7.8, 1mM DTT, 1mM Spermine, 1mM Spermidine, 0.5mM PMSF) at 750×g for 10 min. For nuclei isolation from the *Ascaris* germline, dissected and frozen regions of the germline were ground to a fine powder in liquid nitrogen and nuclei isolated as described above.

### **Native ChIP**

For CENP-A ChIP, 5-10 million nuclei for germline tissues and different stages of embryos were washed with TE buffer (10 mM Tris-HCl, pH 8.0, 0.5 mM EDTA) 3 times and then suspended in 3 ml of Digestion Buffer (10 mM Tris pH 7.5, 2 mM  $MgCl_2$ , 0.5 mM PMSF, and 1x Complete Protease Inhibitor Cocktail [Roche]) pre-warmed for 5 min at 37°C.  $CaCl_2$  was added to a final concentration of 2 mM and then 30 units of micrococcal nuclease (MNase; Affymetrix) added to a final concentration of 10 units/ml. After 2.5 min, the nuclease reaction was stopped by the addition of EDTA to a final concentration of 30 mM. Chromatin was solubilized by cavitation using needle extraction (4 times through a 20 1/2 gauge needle and then 4 times through a 26 1/2 gauge needle), a protocol modified from Steiner and Henikoff (Steiner and Henikoff, 2014). Soluble chromatin was collected by centrifugation at 1000×g for 5 min, the pellet further solubilized by incubating the pellet in Native ChIP washing buffer 1 (10 mM Tris pH7.5, 10 mM EDTA, 0.1%Triton X-100, 100 mM NaCl, 0.5 mM PMSF and 1x Complete Protease Inhibitor Cocktail (Roche)) for 4 hr at 4°C, and the supernatants pooled. The pooled soluble chromatin fractions were adjusted to 100 mM NaCl, debris removed by centrifugation 4 times at 13,000g for 5 min, and then pre-cleared with protein A (Invitrogen) for 2 hr. Protein A beads were pre-blocked by incubation with 0.1 mM yeast tRNA and 0.03% BSA for 2 hr. CENP-A was immunoprecipitated overnight from the extract by incubation with 15 µg affinity purified anti-CENP-A antibody and pre-cleared protein A beads. Beads

were then collected and washed three times in Native ChIP Washing Buffer 1 and then two times with Native ChIP Washing Buffer 2 (10 mM Tris-HCl pH 7.5, 10 mM EDTA, 100 mM NaCl, 0.5 mM PMSF). Chromatin was treated with RNase and Proteinase K at 100 µg/ml, the DNA isolated with phenol:chloroform extraction, and DNA precipitated with ethanol in the presence of 10 µg glycogen. For Native ChIP on histone modifications, the reaction with MNase was extended to 20 min and the amount of antibody used was 10 µg. The remaining protocol was the same as for CENP-A ChIP.

### **Cross-linked ChIP**

For CENP-C ChIP, embryos were homogenized with a metal Dounce homogenizer using 10 strokes each of the loose- and tight-fitting pestle in PBS containing 0.5 mM PMSF and 1x Complete Protease Inhibitor Cocktail (Roche). Nuclei were cross-linked with 2% formaldehyde for 10 min, the reaction was quenched with 125 mM glycine for 10 min on ice, and chromatin pelleted by centrifugation at 2,000×g for 10 min. The pellet was washed again twice in PBS, re-suspended in ChIP lysis buffer (20 mM Tris pH 8.0, 0.1% SDS, 0.1% Deoxycholate, 1% Triton X-100, 150 mM NaCl, 2 mM EDTA, 0.5 mM PMSF, and 1x Complete Protease Inhibitor Cocktail [Roche]), incubated 10 min at room temperature, and sonicated with a Bioruptor (Diagenode) for 30 min with 30s on/off at high amplitude. Debris removal, pre-clearing and CENP-C ChIP were done using a protocol modified from Patel *et al* (Patel et al., 2014) with the 20 µg of affinity purified CENP-C antibody. Beads were washed twice with 20 mM Tris pH 8.0, 0.1% SDS, 1% Triton X-100, 150 mM NaCl, 2 mM EDTA, and once with 10 mM Tris pH8.0, 1% NP40, 1% sodium deoxycholate, 0.25M LiCl, 2 mM EDTA and then with TE buffer twice. Crosslinks were reversed overnight at 65°C, chromatin was treated with RNase and Proteinase K, and DNA was isolated by phenol:chloroform extraction and precipitated with ethanol in the presence of 10 µg of glycogen.

### **Illumina sequencing and data processing**

Sequencing libraries were prepared using standard methods and Illumina protocols and sequenced on the Illumina Hi-Seq platforms. UCSC genome browser track data hubs (Raney et al., 2014) and utilities (<http://hgdownload.soe.ucsc.edu/admin/exe/>), SAMtools (Li et al., 2009), and BEDtools (Quinlan and Hall, 2010) were used throughout the data processing and analysis. Data were first mapped to an *Ascaris* germline draft genome (AgBionano\_v1.9) using bowtie2 (Langmead and Salzberg, 2012). For

CENP-A paired-end reads, we only used reads that aligned concordantly to the genome (on average 77% with insertion size < 1,500 bp) for further analysis. For these concordantly aligned paired-end reads, the entire sequences bordered by the paired-end reads were used for genome mapping and coverage analysis. For CENP-A single-end libraries, the reads were extended to the average nucleosome DNA sizes for data analysis. The total reads coverage for each developmental stage (including input and CENP-A ChIP-seq) is normalized to 3 Gb (~ 10x of the genome) for data comparison in the genome browser.

### **Definition of *Ascaris* CENP-A peaks and domains**

For each stage, the Input vs. CENP-A ChIP-seq data were compared using the MACS2 (Zhang et al., 2008) callpeak module (-p 1e-3 --broad --broad-cutoff 0.1) to identify CENP-A enriched regions. The summits for each CENP-A enriched regions were also identified using the callpeak module (-p 1e-3 --call-summits). Overlapping peak regions from two biological replicates were defined as CENP-A peaks (peak size cutoff  $\geq 1$  kb). These CENP-A peak regions and their coverage are shown in Table S1. CENP-A peaks that are within 2 kb of each other and present in at least one of the stages examined were merged into CENP-A domains (size cutoff  $\geq 5$  kb) to facilitate comparisons of CENP-A localization across all these developmental stages. The changes of CENP-A localization in these CENP-A domains during development are shown in Table S2.

### **Genome-wide ChIP-seq data comparison and correlation analysis**

To compare CENP-A, CENP-C, and histone mark (H3K27me3, H3K36me3, H3K4me3, H3K9me2, H3K9me3, and H4K20me1) ChIP-seq data within and among different stages, we first normalized the read numbers for each input and ChIP-seq library to 10 million. For each ChIP-seq vs. input pair, we scanned the entire genome using a 2-kb window to obtain the reads coverage and the ChIP-seq vs. input ratios. The ratios were transformed to log2 and were standardized by the mean and the standard deviation to generate genome-wide (2-kb window) z-score values. Genome-wide scatter plots and Pearson correlations between all ChIP-seq datasets and replicates were plotted and computed using R software. To compare CENP-A ChIP-seq and RNA-seq data, instead of using the 2-kb window, we used



genomic regions for 14,355 expressed *Ascaris* genes defined by RNA-seq data using StringTie (Pertea et al., 2015). The RNA levels (RPKMs) were log2 transformed for comparison.

### **CENP-A and H3 nucleosome analysis**

To characterize the DNA that wraps around CENP-A nucleosomes, we used paired-end sequencing for both input and CENP-A ChIP-seq data for 12 developmental stages. The normalized size distributions of the insertion size for all concordantly aligned paired-end reads were plotted using R software (Fig. S2D). For each stage, the DNA insertion size with the highest read frequency was considered as the major DNA size that wraps around the CENP-A or input (mainly H3) nucleosomes. We used the average of 3 stages with the most consistent CENP-A localization (5day, 7day, and L1) to evaluate the sizes for mono-, di-, tri-, and tetra-nucleosome differences between CENP-A and input (Fig. 2C).

### **CENP-A localization and DNA elimination analysis**

Genomic regions that are eliminated in somatic cells from the germline genome were identified using our previously established method (Wang et al., 2012). To compare the amount of CENP-A in the retained vs. eliminated chromosome regions, we first defined CENP-A peak regions in the retained and eliminated regions at each developmental stages (see Definition of *Ascaris* CENP-A peaks). We then compared the number of CENP-A peaks, the genomic regions covered by the CENP-A peaks, and the amount of sequencing reads in the CENP-A peaks between retained and eliminated regions. For CENP-A level comparison between different stages, we calculated the values of CENP-A levels (peak numbers, coverage, and read numbers) relative to Testis-1 (Fig. 3C). To illustrate the CENP-A changes in the eliminated regions, we also used Circos (Krzywinski et al., 2009) to plot the CENP-A z-score values for germinal mitotic regions (testis-1 and ovary-1) and the 4-cell embryo (60hr) in a chromosome assembly (> 4 Mb) that contains an eliminated region of at least 0.8 Mb (Fig. 3A).

### **Analysis of RNA changes and their association with CENP-A deposition during development**

We used our previous staged developmental RNA-seq datasets ((Wang et al., 2011; Wang et al., 2014; Wang et al., 2012) to define transcripts in the genome and to establish gene expression profiles during development using StringTie (Pertea et al., 2015). To compare CENP-A changes throughout development, we took the average values of three datasets for the following four stages: testis (testis1,

testis2, and testis3), ovary (ovary1, ovary2, and ovary3), early embryo (0hr, 46hr, and 64hr), and late embryo (5day, 7day, and L1). The RNA changes for the corresponding stages were used for comparisons and correlation studies (Fig. 4B, Fig. S4 and Table S3). Comparisons were done on both CENP-A domains and on genomic regions with differential RNA expressions (Fig. S4 and Table S3).

## Supplemental Figures

**Figure S1. CENP-A, CENP-C, and NDC80 identification, antibodies generation and staining during mitosis. Related to Figure 1.** **A.** Multiple sequence alignment of *Ascaris* CENP-A and their CENP-A orthologs. CENP-A is distinguished from other histone H3 variants by the non-conserved and extended N-terminal region and the marked regions. Note that the CENP-A Targeting Domain (CATD, black arrow) is also characteristic of CENP-A proteins. **B.** Multiple sequence alignment of *Ascaris* CENP-C and their orthologs. Region demarcated by black arrow is characteristic of CENP-C proteins. **C.** Multiple sequence alignment of *Ascaris* NDC80 and their orthologs. Region marked in blue is the key region identified by NDC80 pfam. **D.** Western blots for CENP-A (19 KD), CENP-C1 (135 KD) and CENP-C2 (113 KD), and NDC80 (70 KD). Note that the expected CENP-C bands run at higher molecular weights than predicted as observed for the *C. elegans* protein. **E.** CENP-A, CENP-C, and NDC80 staining during mitosis. Staining of centromere/kinetochore components during *Ascaris* early embryo mitosis. Note that the centromere/kinetochore components are highly reduced on the DNA that will be eliminated. Oval embryos are ~40 x 75  $\mu$ m in size. **F.** Normalized RNA expression level (RPKM) of CENP-A, CENP-C, and NDC80 orthologs in *Ascaris*. Zygotes 1-4 represent maturation of the fertilized oocyte in the female uterus where pronuclear fusion does not occur. 24 hr = pronuclear fusion; 46 hr = 2-cell embryo; 64 hr = 4-cell embryo and beginning of DNA elimination; 96 hr = end of DNA elimination; 116 hr or 5 day = 32-64 cell embryo; carcass represents the entire adult worm with the reproductive system removed.

**Figure S2. Reproducibility of *Ascaris* CENP-A ChIP-seq and nucleosome characterization. Related to Figure 2.** **A.** Genome-wide (2kb window) correlation plots of CENP-A localization from two biological replicates for the 12 developmental stages analyzed (see Fig. 3A). For each ChIP-seq experiment, the CENP-A and input ChIP-seq data were compared to generate the genome-wide z-score values for CENP-A localization (see Supplemental Experimental Procedures). The mean and medium Pearson correlation coefficient ( $r$ ) values for the 12 ChIP-seq datasets are 0.74 and 0.82, respectively. **B.** Genome-wide correlation plot of CENP-C ChIP-seq in two biological replicates from 5-day embryos. **C.**

CENP-A and CENP-C ChIP-seq correlation plots for *Ascaris* embryos (0 hr, 60 hr and 5 day). **D.** Comparison of the DNA length that wraps around CENP-A and H3 nucleosomes from 12 *Ascaris* developmental stages. Dashed lines indicate the DNA length for the major CENP-A (red) and input (blue) mononucleosomes.

**Figure S3. CENP-A localization is dynamic during *Ascaris* gametogenesis and early development. Related to Figure 3.** **A.** *Ascaris* reproductive systems and early embryo development are illustrated with color-coded regions or stages examined. **B.** Heatmap illustrating the genome-wide CENP-A localization correlation values (lower left half) and the % of overlapping CENP-A peaks (upper right half) between different stages.

**Figure S4. Relationship between CENP-A deposition, RNA transcription, and histone marks in *Ascaris*. Related to Figure 4.** **A.** Correlation plot illustrating a strong inverse relationship between *Ascaris* RNA transcripts and CENP-A deposition. **B.** Correlation plots showing the relationships between *Ascaris* CENP-A and several histone marks. **C.** Heatmap showing the genome-wide pairwise comparison of CENP-A deposition and histone marks. All data were derived from 32-64 cell (5 day) embryos. **D.** Transcripts derived from regions of the genome with CENP-A domains are expressed at very low levels. Shown are the average expression levels (derived from all *Ascaris* tissues/stages examined) for three groups of RNAs that are fully, partially, or not overlap with *Ascaris* CENP-A domains in the genome. **E.** Highly dynamic RNA transcription changes show a negative correlation with changes in *Ascaris* CENP-A deposition during development. Highly expressed RNAs (RNA RPKM  $\geq 50$  in at least one of the four stages) were divided into 3 groups based on the RNA changes between the four stages: 1). Non-dynamic expression = 1-10-fold change; 2). Dynamic expression = 10-100-fold change; 3). Highly dynamic expression =  $> 100$ -fold change. The RNA levels for these 3 groups of RNAs and their corresponding CENP-A levels are illustrated in two heatmaps for comparison. For each group of RNAs, the data were sorted based on the germline (testis and ovary) / embryo (early and late) RNA level, with germline enriched RNAs on the top (see Table S3).

#### **Supplemental Tables**



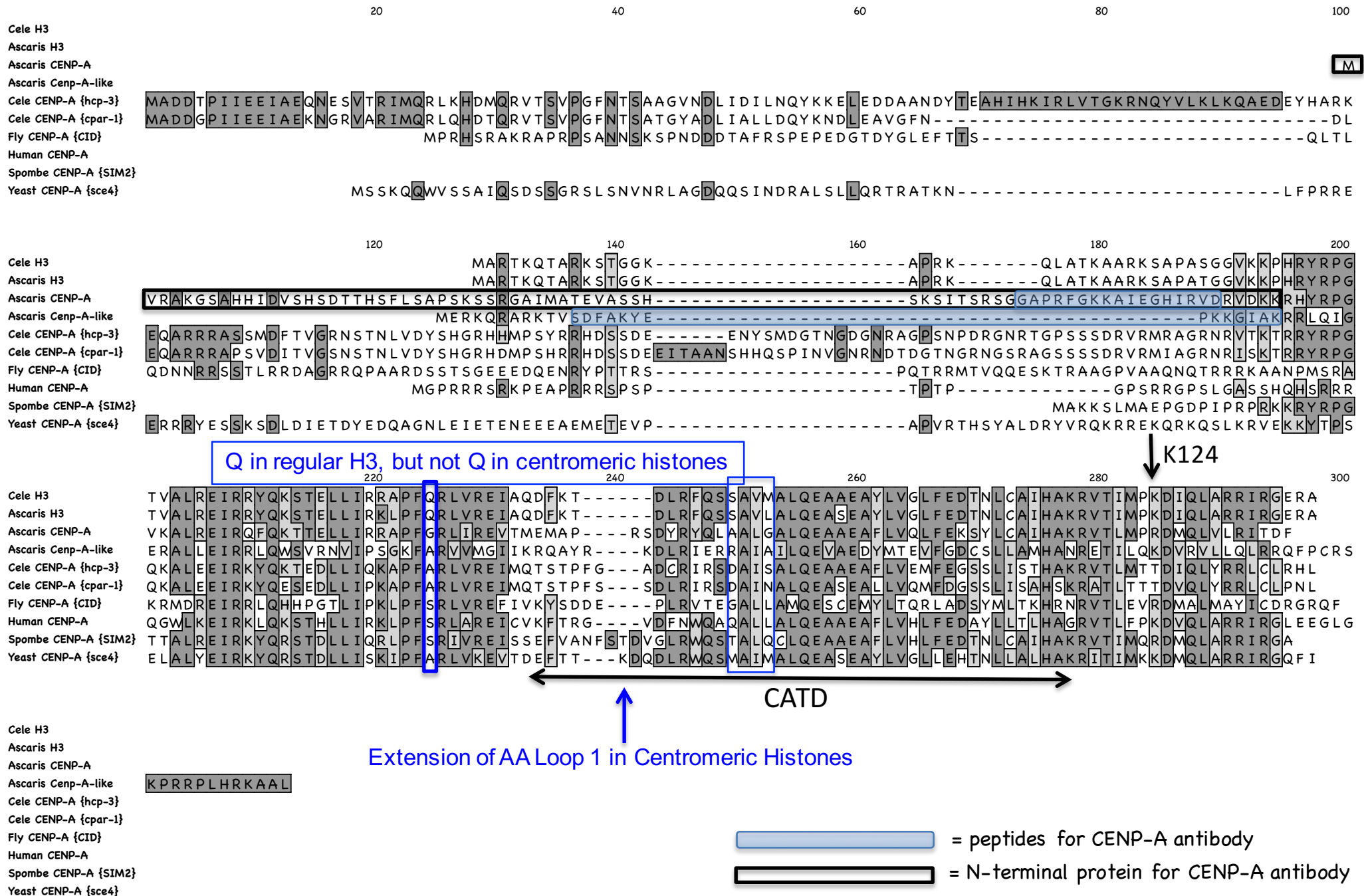
Table S1. CENP-A peaks in 12 developmental stages and DNA elimination. Related to Figure 3.

Table S2. CENP-A domains and CENP-A localization dynamics during development. Related to Figure 3.

Table S3. Relationship between CENP-A dynamics and RNA transcription. Related to Figure 4.

## Supplemental References

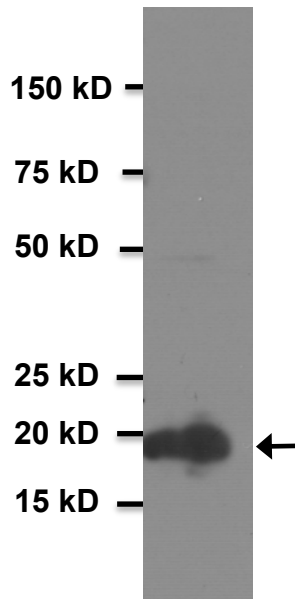
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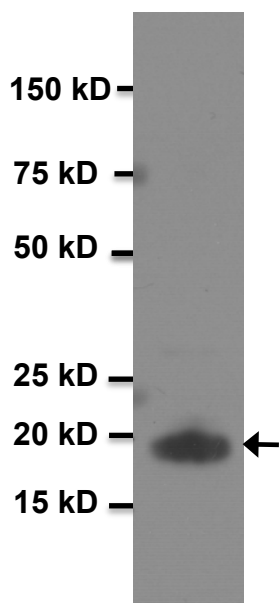




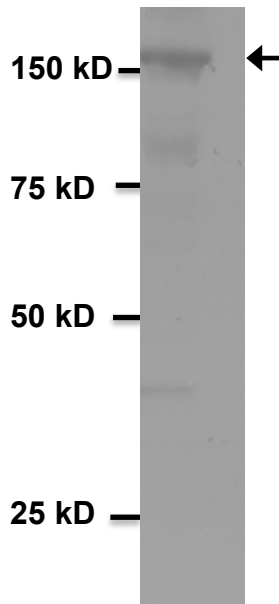
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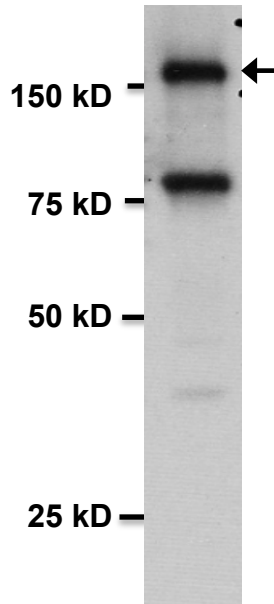
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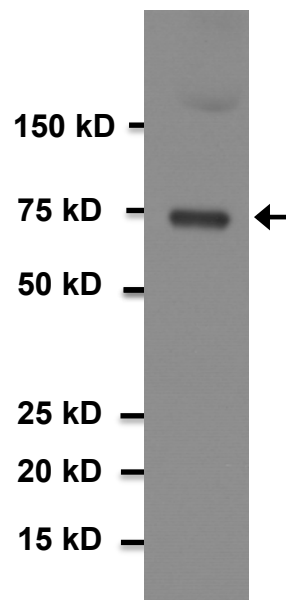
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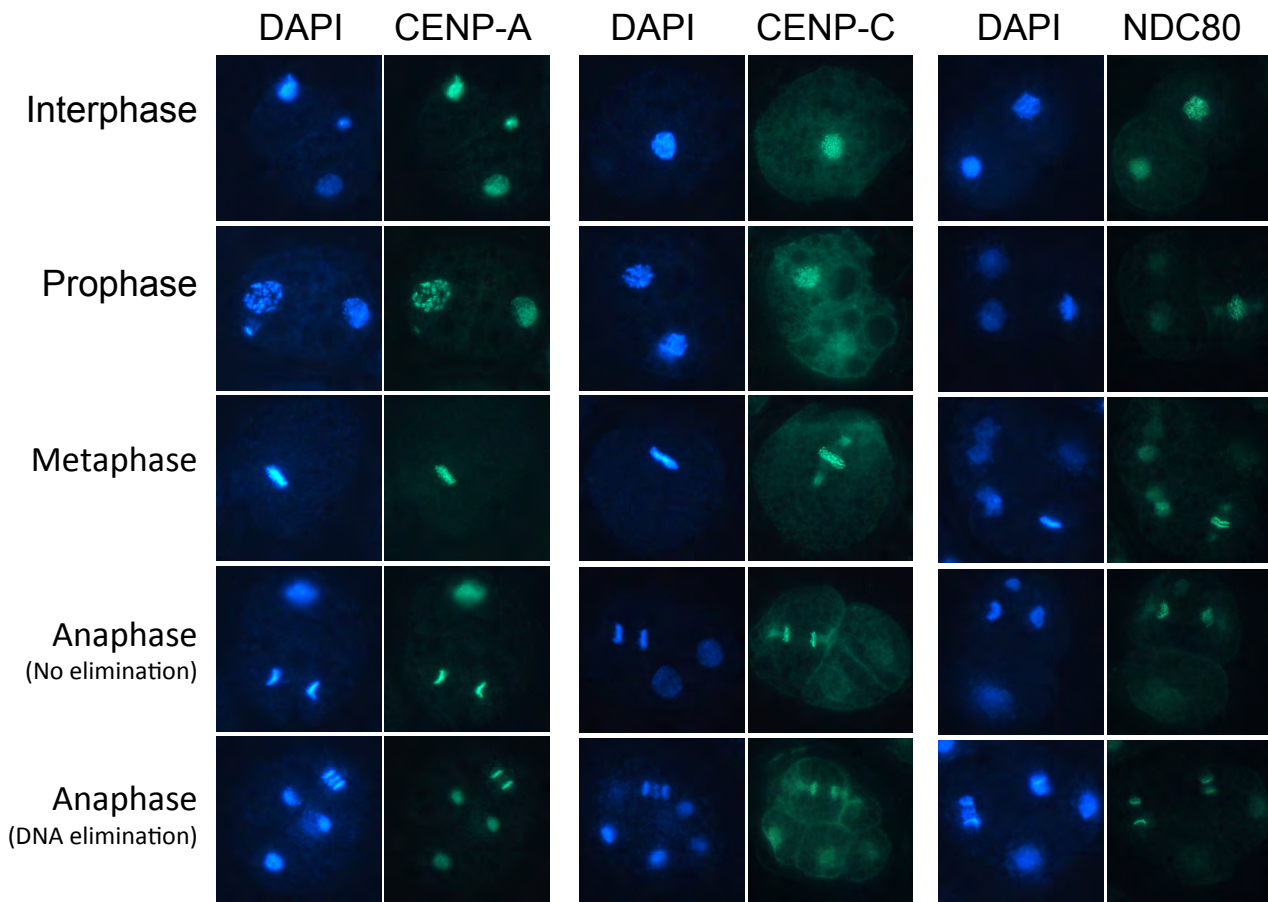


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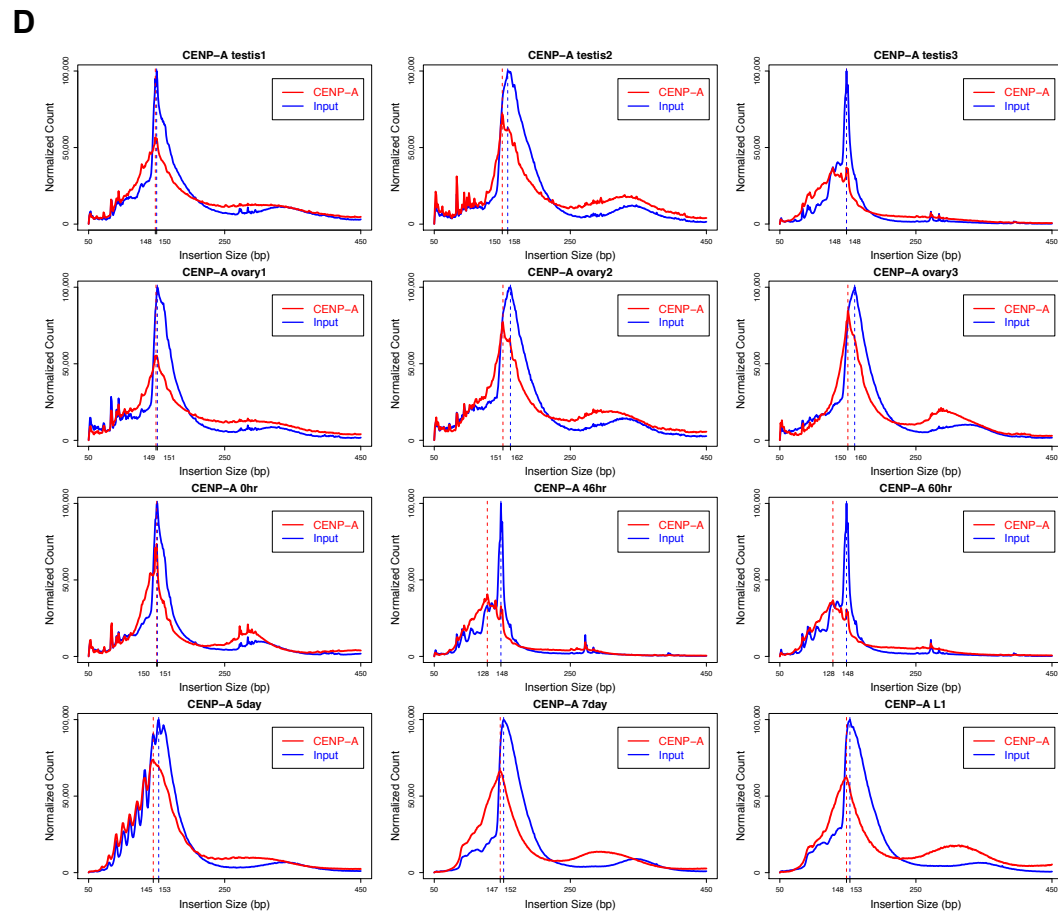
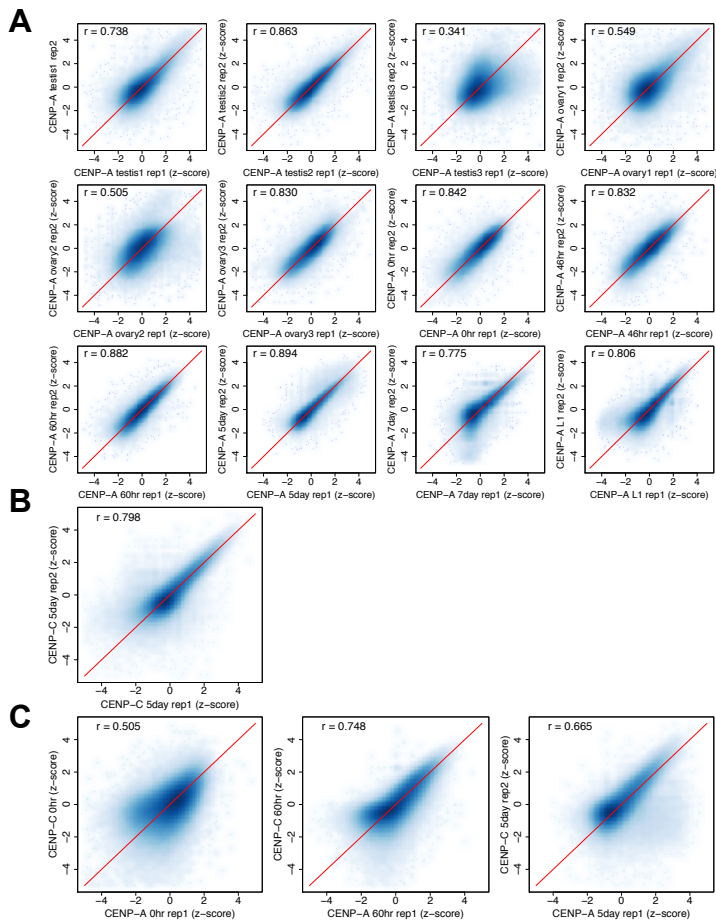
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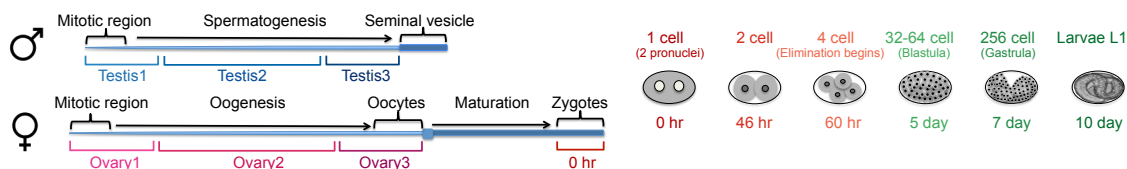


Accession	Protein	Testis	Ovary	Oocyte	Zygote1	Zygote2	Zygote3	Zygote4	24hr	46hr	64hr	96hr	116hr	7D	L1	L2	Intestine	Muscle	Male Carcass	Female Carcass
ASU_14124	Cenp-A	3.0	12.9	3.1	14.7	16	20.7	34.5	48.2	38.8	56.1	68.1	92.4	65.1	22.3	1.7	0.1	0.3	1.7	6.4
ASU_11276	Cenp-A-like	65.7	9.9	4.6	8.7	7	6.3	8.1	3.1	1.5	0.2	0.1	0	0.1	0.1	0	0.0	0.0	0.4	0.5
ASU_05506	Cenp-C1	6.8	24.2	18	14.2	17	32.6	23.1	52.5	74.4	111.9	73.4	87	65.5	20.8	1.6	0.2	0.3	1.0	4.2
ASU_05420	Cenp-C2	81.0	19.7	6.3	17.2	27.6	41.6	53	99.5	173.7	294.1	115	20	5.5	4.3	0.5	0.5	0.4	1.4	11.8
ASU_08074	Cenp-C3	148.1	4.6	0	0.1	0.1	0.1	0.4	0.5	0.4	0.3	0.4	0.6	0.1	0.4	0	0.3	0.1	0.9	0.2
ASU_03131	NDC80	92.0	21.2	19.5	12	13	17.2	21.4	36.4	35.8	30.9	41.1	57.2	31.1	15.4	0	0.2	0.7	0.8	4.6







**A****B**