Making a Point about Holocentromeres

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Faithful chromosome segregation is essential for proper cell division. Aberrant chromosome segregation leads to aneuploidy, and is linked to numerous pathologies (Siegel and Amon, 2012). The chromosomal segregation machinery assembles on specialized chromosomal regions called centromeres, which contain specialized chromatin including cenH3, a variant form of histone H3. In budding yeast, each chromosome has a single centromere defined by a well-characterized DNA sequence (point centromeres). However, the situation is more complex in most other eukaryotes. Metazoans such as fruit flies and humans have centromeric regions spanning kilobases to megabases (regional centromeres), and the highly repetitive nature of these sequences has made their analysis by sequencing-based methods difficult.

An intriguing form of centromeres is the holocentromere, in which centromeres span the entire length of the chromosome. Despite having been identified over 80 years ago, the molecular characteristics of holocentromeres have remained elusive due to the low resolution of the imaging methods used to characterize them. Two models are proposed: (1) a diffuse model, in which centromeres cover the entire chromosome, and (2) a polycentric model, in which discrete centromeric sites are spread along the entire length of the chromosome, giving the impression of full chromosomal coverage when analyzed by microscopy. To resolve the molecular nature of *C. elegans* holocentromeres, postdoctoral fellow Dr. Florian Steiner in the laboratory of Dr. Steven Henikoff (Basic Sciences Division) mapped the genome-wide distribution of the centromere-specific histone variant cenH3. As reported in a recent article in *eLife*, their results indicate that these holocentromeres are polycentric and that centromeric sites resemble budding yeast point centromeres.

The researchers first performed native chromatin immunoprecipitation and sequencing (N-ChIP-seq) for the centromeric histone variant cenH3. They found that cenH3 was localized at discrete, high-occupancy sites. In total, 707 discrete sites of cenH3 enrichment were found. To confirm that these cenH3 sites were bona fide centromeres, they performed ChIP-seq for CENP-C, a component of the kinetochore. They observed high correspondence between cenH3 and CENP-C ChIP signal, suggesting that these discrete sites of cenH3 enrichment are functional centromeres. CenH3 was also found to be weakly enriched within broad domains spread across large portions of chromosomes, which had been previously observed by crosslinking ChIP and microarray analysis

(X-ChIP-chip). These domains anti-correlate with active transcription and nucleosome turnover and are likely the result of low-level incorporation of cenH3 into canonical nucleosomes.

By analyzing chromatin structure around cenH3 sites, the authors found that, at each site, a single cenH3-containing nucleosome was flanked by nucleosomes containing canonical H3, similar to budding yeast point centromeres (Krassovsky et al., 2012). This observation led the authors to conclude that point centromeres are the basic unit of *C. elegans* polycentromeres.

As budding yeast point centromeres are genetically defined, the authors reasoned that *C. elegans* cenH3 sites might also contain specific sequences. Analysis of the sequences underlying these sites revealed a GA-rich motif. However, this motif also occurs ~60,000 times in the genome and is thus unlikely to specify functional centromeres. Additionally, this motif is strikingly similar to a sequence found at Highly Occupied Target (HOT) sites, which are sites bound by at least 15 transcription factors. The authors found that approximately half of previously identified HOT sites overlapped with high-occupancy cenH3 sites, and found that sites occupied by cenH3 in dividing cells were occupied by transcription factors in non-dividing muscle cells. These findings suggest that transcription factors occupy centromeric sites in post-mitotic cells.

These results resolve the long-standing question about the molecular nature of holocentromeres in the model nematode *C. elegans*. "We found that cenH3 nucleosomes are localized to dispersed sites along the length of each chromosome. Each of these sites contains just one cenH3-containing nucleosome and likely serves as an attachment site for a microtubule, leading us to the conclusion that *C. elegans* holocentromeres are organized as dispersed point centromeres," said Dr. Steiner. "Why and how these particular sites are selected remains an open question."

Citation:

Steiner FA, Henikoff S. 2014. Holocentromeres are dispersed point centromeres localized at transcription factor hotspots. *eLife* 3:e02025.

See also:

Siegel JJ, Amon A. 2012. New insights into the troubles of aneuploidy. *Annu Rev Cell Dev Biol* 28:189-214.

Krassovsky K, Henikoff JG, Henikoff S. 2012. Tripartite organization of centromeric chromatin in budding yeast. *Proc Natl Acad Sci USA* 109(1):243-248.

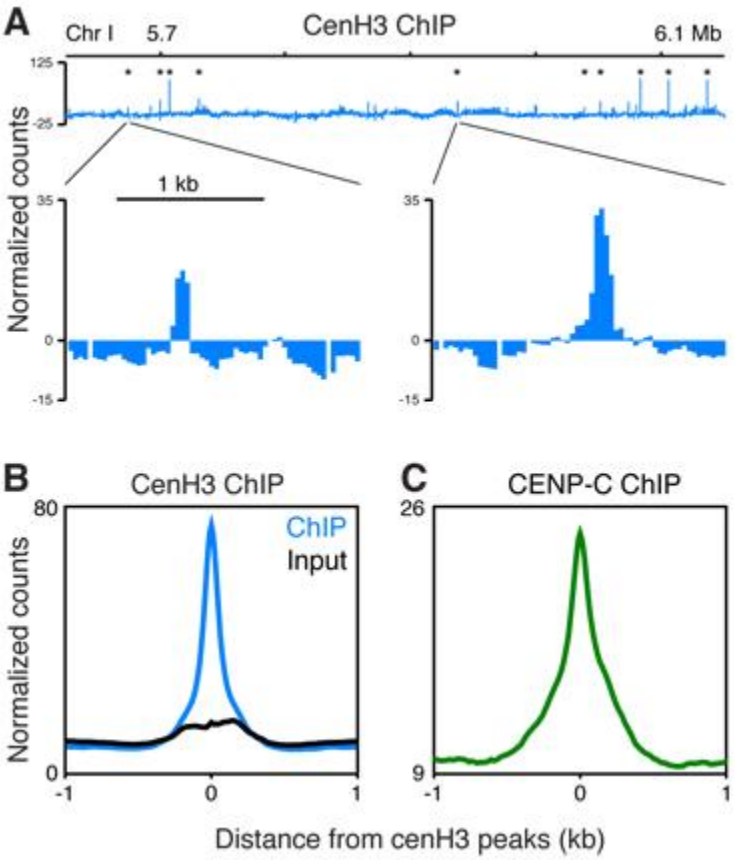

Image provided by Dr. Florian Steiner

Genome-wide profiling of the cenH3 histone variant reveals discrete, high-occupancy centromeric sites. (A) Genome browser view of 525 kb on Chr I for cenH3 ChIP-seq. CenH3 peaks are marked by asterisks (top). Two representative cenH3 peaks are enlarged (bottom). (B) Average plots of input and cenH3 ChIP signal at all 707 identified cenH3 peaks. (C) Average plots of CENP-C ChIP signal at all 707 identified cenH3 peaks.