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SPOTLIGHT

Going the distance: Neocentromeres make long-range contacts with heterochromatin

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Neocentromeres are ectopic centromeres that form at noncanonical, usually nonrepetitive, genomic locations. Nishimura et al. (2019. *J. Cell Biol.* https://doi.org/10.1083/jcb.201805003) explore the three-dimensional architecture of vertebrate neocentromeres, leading to a model for centromere function and maintenance via nuclear clustering with heterochromatin.

Centromeres are fundamental to genome inheritance and ensure that chromosomes are accurately and equally segregated each cell cycle. The centromere is the locus at which the multi-protein kinetochore is formed to facilitate spindle microtubule attachment and chromosome movement during cell division. The centromere-specific histone variant CENP-A is the epigenetic mark creating the platform needed for recruitment of over 100 proteins involved in kinetochore formation, microtubule binding, and chromosome movement. CENP-A nucleosomes are interspersed with H3 nucleosomes that contain euchromatic posttranslational modifications, creating the CENP-A domain or "centrochromatin" that is flanked by pericentric H3 nucleosomes containing heterochromatic modifications (1). CENP-A chromatin presumably creates centromere memory, in that newly synthesized CENP-A is incorporated near old CENP-A, thereby templating and maintaining the centromere long-term at the same genomic site (2). The presence of nearby heterochromatin is thought to limit erroneous incorporation or spreading of CENP-A beyond centromeres (3).

Ectopic centromeres, or neocentromeres, form spontaneously at noncanonical locations in the genome. In humans, they contribute to both acquired and congenital diseases (4). Neocentromeres were initially identified in humans (4), but they have since been experimentally produced in *Drosophila melanogaster*, chicken cells, and yeasts. When native centromeres are physically removed by genome engineering, neocentromeres arise elsewhere on the same chromosome (5, 6). Induced neocentromeres occur throughout the length of the targeted chromosome, although some neocentromeres can reproducibly form near the same genomic region. What features make genomic regions more amenable to ectopic centromere formation? Are there particular chromatin conformations that promote CENP-A incorporation and centromere formation? Neocentromeres in fungi and *Drosophila* often arise near heterochromatic regions at native centromeres or telomeres; however, neocentromeres in chicken cells are not located adjacent to heterochromatin (7). Clustering of pericentromeric regions into heterochromatic foci is common in many organisms, arguing for a functional requirement to sequester centromere regions into specific nuclear domains. It has not been clear if, or how, the nuclear organization of centromeres is a property or requirement of centromere function in vertebrates. In this issue, Nishimura et al. provide key insight into this question by analyzing the spatial organization of interphase chromatin and identifying regions that make contact with neocentromeres.

Nishumura et al. aimed to test if nuclear clustering with distant heterochromatic regions is a common feature of vertebrate centromeres (8). They used their established system of ectopic centromere assembly in DT40 chicken cells as a model to test if nuclear centromere dynamics differed between native centromeres and neocentromeres. Chicken chromosomes contain both repetitive (satellite) and nonrepetitive centromeres. Previously, this group had removed the nonrepetitive native Z centromere in DT40 cells and recovered a series of neocentromeres that formed throughout the Z chromosome (7). In Nishumura et al., the authors focused on three of the neocentromeres that arose at distinct genomic locations: near Z chromosome short arm telomere (genomic position 3.8 Mb), the middle of the Z short arm (genomic position 35 Mb), and the Z long arm (genomic position 55 Mb; 8). Each neocentromere location served as a distinct viewpoint, or region of interest, for capturing global DNA interactions using a targeted chromosome conformation capture approach (4C-seq). Each neocentromere region interacted with the same sites on the Z chromosome, specifically two genomic positions (8 and 26 Mb) that were enriched for H3K9 trimethylation (H3K9me3), a marker of heterochromatin. Notably, the neocentromere regions did not interact with the 8- and 26-Mb heterochromatic locations in the parental cells before centromere induction, indicating that the associations arose after ectopic centromere assembly occurred. The nonrepetitive native Z centromere, located at genomic position 42.6 Mb, also interacted with the 8- and 26-Mb genomic locations. Moreover, each neocentromere region was in contact with several other native, nonrepetitive centromeres.

Nishimura et al. (8) verified that their observations were not exclusive to the Z chromosome but also applied to a native repetitive centromere on another chromosome. Altogether, these results imply that interactions of repetitive, nonrepetitive, and induced centromeres with distantly located heterochromatin regions are inherent to centromere function. In addition

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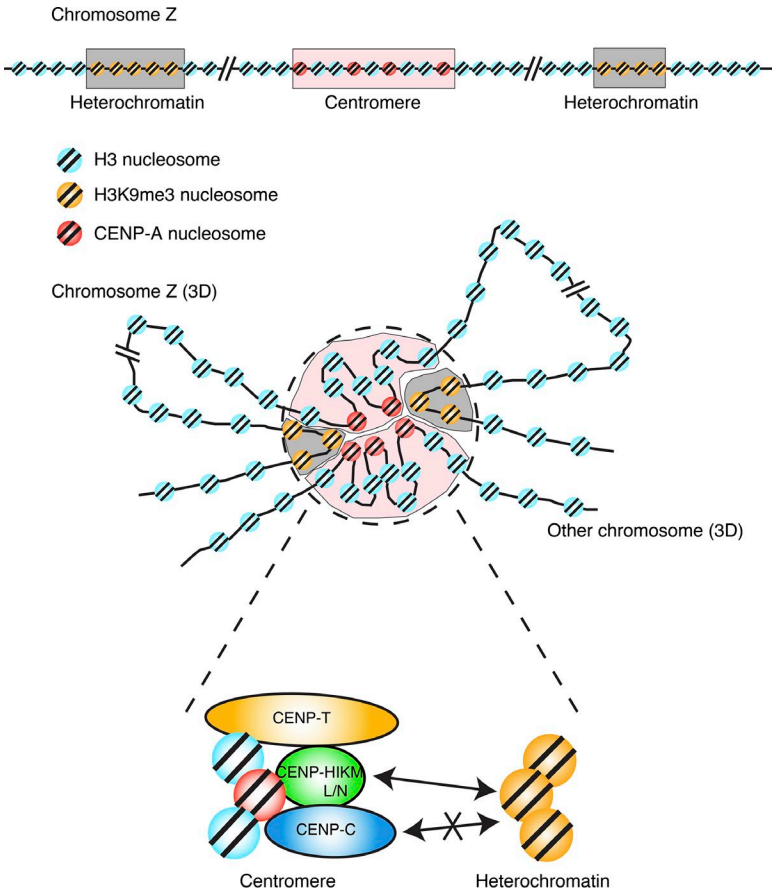

Figure 1. **Republished from Nishimura et al. (8).** In 3D interphase space, large-scale chromatin looping brings chicken centromeres (red circles) into proximity to heterochromatin (yellow circles) located distantly on a linear genomic scale. This nuclear architecture may serve to constrain centromeres to limited genomic positions. In the case of neocentromeres that lack flanking heterochromatin, positioning of centromeres near heterochromatin via long-range interactions may promote both centromere assembly and memory. Clustering of native centromeres and neocentromeres into heterochromatin-rich foci is facilitated by CENP-H, a component of the CCAN that links the inner and outer kinetochore domains.

to long-range chromosomal interactions, the authors also probed local interactions within the ∼40-kb neocentromeres. They found that each neocentromere exhibited multiple contact points within each ∼40-kb region, suggestive of folding of centromeric chromatin that is consistent with models of 3D chromatin organization in which CENP-A molecules distributed throughout centrochromatin are compacted or stacked in space (9).

Nishimura and colleagues' observations that native and neocentromeres are clustered with heterochromatin in interphase raised the possibility that the enzymes that add methyl groups to H3K9 might be involved in establishing the nuclear contacts. Therefore, they disrupted the H3K9 methyltransferases Suv39H1 and Suv39H2 in one neocentromere-containing line, but did not observe a significant reduction in the interactions between the neocentromere and the 8-Mb heterochromatic interaction location. Interpretation of these experiments was complicated by the retention of H3K9me3 within the knockout cells, possibly suggesting that other H3K9 methyltransferases might be partially redundant and/or that H3.3K9 methylation might compensate for reduced H3K9 methylation. Although it was not tested here, it would be interesting to know if pericentric heterochromatin or centromere function or structure were affected long-term after loss of Suv39H1/H2.

That the 3.8- and 35-Mb neocentromere regions did not interact with the 8- and 26-Mb heterochromatic locations until after neocentromere formation suggested that a stronger factor in creating and maintaining the genomic interactions was centromere assembly itself. The Constitutive Centromere-Associated Network (CCAN) is a multi-protein complex that links CENP-A chromatin at the centromere to the outer kinetochore through the assembly of distinct subdomains that recruit different subsets of outer kinetochore proteins (10). Nishimura et al. (8) used auxin-inducible degradation of CENP-C and CENP-H, centromere proteins that represent two separate pathways in CCAN assembly, to test the effect of kinetochore assembly on long-range centromere interactions. Removal of CENP-H, but not CENP-C, disrupted interactions between the neocentromere regions and the heterochromatic 8- and 26-Mb genomic locations. These findings support a model of centromere and kinetochore assembly through the CENP-H arm of the CCAN that subsequently drives long-range centromere–heterochromatin interactions (Fig. 1).

This interesting study has several implications for current models of centromere assembly. Since CENP-H is recruited before CENP-C, sequestration of immature centromeres/prekinetochores by heterochromatin may be required for proper centromere maturation and recruitment of additional kinetochore proteins. This may be particularly important for neocentromeres that arise on nonrepetitive DNA and are not inherently located near heterochromatin. The retention of centromeres into spatially constrained domains might also be important for new CENP-A loading, ensuring that CENP-A is not inappropriately introduced elsewhere in the genome and therefore preventing centromere drift. This model is supported by the fact that the 8- and 26-Mb heterochromatic regions did not interact with each other, but were brought into proximity to the compacted CENP-A chromatin/centrochromatin of each neocentromere region through 3D positioning.

Future experiments could test the spatial orientation of the 8- and 26-Mb heterochromatic regions and each neocentromere region using three-color FISH. Finally, an alternative, but not mutually exclusive, view is that clustering of centromeres reinforces heterochromatin function and stability, as has been described in *Drosophila* in which centromere clustering at the nucleolus contributes to heterochromatic silencing of repetitive elements (11). The DT40 system could be used in the future to test effects on heterochromatin function at the 8- and 26-Mb genomic locations when centromere clustering is disrupted by CENP-H removal.

Several questions about the role of 3D genome architecture in neocentromere formation and maintenance remain to be answered. Why do neocentromeres form at seemingly unrelated genomic locations? What is the initiating event for neocentromere formation—transcription, the activity of mobile elements, or proximity to native centromeres? While the 3.8-, 35-, and 55-Mb neocentromere regions did not contact the 8- and 26-Mb heterochromatic regions before ectopic centromere formation, these loci might have interacted with native centromere clusters, replication centers, or transcription factories that somehow allowed CENP-A to be mis-incorporated, thereby priming the region for neocentromere formation.

Overall, this study enhances our perspective on vertebrate centromeres and their relationship to each other, to the rest of the genome in the context of 3D nuclear architecture, and to the machinery required for their assembly. Uncovering the interplay between CENP-H and other CCAN components with heterochromatin and specific nuclear structures in DT40 cells, and testing these interactions in mammalian cells, are important next steps for advancing our understanding of centromere assembly and function.

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