Histone H3K4 methylation keeps centromeres open for business

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Eukaryotic centromeres are composed of a combination of nucleosomes containing the histone H3 variant CENP-A and canonical H3 di-methylated at lysine 4 (H3K4me2). Many questions exist over the functional importance of H3K4me2 nucleosomes within the centromere region. In this issue of *The EMBO Journal*, Bergmann *et al* (2011) reveal a role for H3K4me2 and transcription in CENP-A maintenance. They also extend the profile of centromeric histone modifications to include H3K36 methylation, typically found at transcribed regions of the genome.

The centromere is an essential chromosomal locus required for chromosome segregation during cell division. CENP-A is an important marker of the centromere, distinguishing it from the rest of the genome independent of DNA sequence. After replication and mitosis, the complement of CENP-A at each centromere is reduced (Jansen *et al*, 2007). However, centromere identity is maintained through addition of newly synthesized CENP-A by the chaperone HJURP (Holliday Junction Recognition Protein) (Foltz *et al*, 2009). Chromatin containing CENP-A is periodically interrupted by nucleosomes containing H3K4me2, a modification characteristic of euchromatin (Blower *et al*, 2002; Santos-Rosa *et al*, 2002; Sullivan and Karpen, 2004). CENP-A/H3K4me2 chromatin is distinct from flanking heterochromatin characterized by di- and tri-methylated H3K9, H3K27 and H4K20. The functional significance of a chromatin landscape at centromeres that includes open chromatin has been puzzling. The new work of Bergmann *et al* (2011) shows that open chromatin and satellite DNA transcription are linked to centromere maintenance.

Figure 1 Endogenous human centromeres are assembled on α satellite (alphoid) DNA composed of tandem repeat arrays of 171 bp monomers, some of which include a CENP-B-binding motif. CENP-A-containing nucleosomes are interspersed with H3K4me2 and H3K36me2 euchromatin and assembled across a portion of the alphoid array. The new human artificial chromosome, comprised of alternating monomers of synthetic alphoid DNA containing CENP-B boxes or tetO sequences, exhibits chromatin features similar to endogenous centromeres. Initial tetO sequence-mediated recruitment of LSD1-EYFP-TetR fusion protein decreased H3K4me2 and H3K36me2 levels on the alphoid^{tetO} HAC, and diminished alphoid transcription and HJURP recruitment. New CENP-A assembly did not occur, although the HAC retained kinetochore function. Long-term LSD1-EYFP-TetR tethering to alphoid^{tetO} HAC resulted in CENP-A depletion, undetectable transcription of α satellite DNA, as well as reduction in euchromatin and mitotic instability.

Centromeres and chromatin organization are difficult to study globally, as depletion of centromere or chromatin factors adversely affects the entire genome. To circumvent

Endogenous centromere

Alphoid^{tetO} human artificial chromosome (Alphoid^{tetO} HAC)

this problem, Bergmann *et al* used a non-essential human artificial chromosome (HAC) previously established by these groups (Nakano *et al*, 2008). This HAC has a dimeric structure, composed of a 171 bp repeat unit (monomer) of centromeric α satellite (alphoid) DNA and a monomer in which the CENP-B box, a 17-bp protein-binding motif within α satellite DNA, was replaced by tet operator (tetO) repeats (Nakano *et al*, 2008). This synthetic HAC (called alphoid^{tetO}) has a fully functional centromere and exhibits mitotic stability comparable to endogenous chromosomes. An advantage of this approach is that tet repressor (TetR)-gene fusions can be targeted to alphoid^{tetO} HAC without affecting centromeres of other chromosomes. Bergmann *et al* showed that alphoid^{tetO} HAC contained histone modifications previously identified at human centromeres, such as H3K4me2, H3K9me3 and H3K27 methylation (Sullivan and Karpen, 2004; Ribeiro *et al*, 2010) (Figure 1). However, they also found enrichment for H3K36 methylation, a modification normally associated with transcriptional elongation. H3K36me2 chromatin spanned a large portion of the centromere, partially overlapping with CENP-A and H3K4me2 chromatin (Figure 1). Furthermore, transcripts were detected from both α satellite DNA on alphoid^{tetO} HAC and the endogenous chromosome 21 centromeres. These results confirm that centromeres are transcriptionally active and have properties of euchromatin.

Is chromatin environment and transcription a product or promoter of centromere structure and function? To address this question, Bergmann *et al* created the expression construct TetR-EYFP-LSD1 that could be tethered to the tetO sites on alphoid^{tetO} HAC. LSD1 is a histone demethylase that targets H3K4me2. When introduced into cells containing the HAC, TetR-EYFP-LSD1 caused depletion of H3K4me2 as well as loss of centromere proteins CENP-A and CENP-C on alphoid^{tetO} HAC. Extended exposure to TetR-EYFP-LSD1 for 7 days resulted in complete loss of H3K4me2. CENP-A and CENP-C were greatly reduced and alphoid^{tetO} HAC became mitotically unstable. Centromeric H3K36me2 and the number of alphoid^{tetO} transcripts also decreased upon H3K4me2 demethylation. Thus, active H3K4me2 demethylation adversely impacts α satellite transcription, centromeric chromatin organization and chromosome stability.

Does demethylation of H3K4 destabilize the existing chromatin landscape on alphoid^{tetO} HAC, or prevent incorporation of

newly synthesized CENP-A (CENP-A^{new})? To address this issue, Bergmann *et al* assessed incorporation of CENP-A^{new} in the absence of H3K4me2. CENP-A was expressed as a SNAP-HA-tagged fusion protein in cells containing TetR-EYFP-LSD1. Existing tagged CENP-A was masked using non-fluorescent BG substrate, and tagged CENP-A^{new} was detected using fluorescent substrate (Jansen *et al*, 2007). Demethylation of H3K4me2 correlated with reduced levels of CENP-A^{new} on alphoid^{tetO} HAC. Co-transfection experiments of RFP-tagged HJURP and TetR-EYFP-LSD1 demonstrated that HJURP was undetectable on alphoid^{tetO} HAC in the absence of H3K4me2. These findings suggest that H3K4me2 and/or open chromatin are important for recruitment of HJURP and proper incorporation of CENP-A^{new} at centromeres.

The work of Bergmann *et al* adds new details to the existing chromatin profile of human centromeres and underscores similarities with regions of euchromatin. It also provides a transcription-based mechanism for the location of CENP-A nucleosomes within open chromatin. This study, along with others (Topp *et al*, 2004; Carone *et al*, 2009; Wong *et al*, 2006), highlights the complex chromatin composition and transcriptional properties of eukaryotic centromeres. This study provokes further questions about centromeres as well. Too much euchromatin or heterochromatin disrupts centromere function (Nakano *et al*, 2008). How, then, is an equilibrium of open chromatin to CENP-A chromatin achieved? Do transcripts arise from CENP-A or H3K4me2/H3K36me2 portions of the array, or both? What does HJURP recognize—centromeric transcripts, transcription factors, a chromatin signature and/or nucleosome conformation? Finally, alphoid^{tetO} HAC is a synthetic model of a human chromosome, albeit one that apparently recapitulates human centromere organization and behaviour. Future studies should address if H3K4me2 and transcription are similarly important for CENP-A maintenance and centromere structure on endogenous chromosomes. Nevertheless, the work by Bergmann *et al* is an exciting advance that emphasizes the importance of core histone modifications at the centromere and the epigenetic nature of this fascinating chromosomal locus.

Conflict of interest

The authors declare that they have no conflict of interest.

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