Stressful situation if CENP-A not front and CENter

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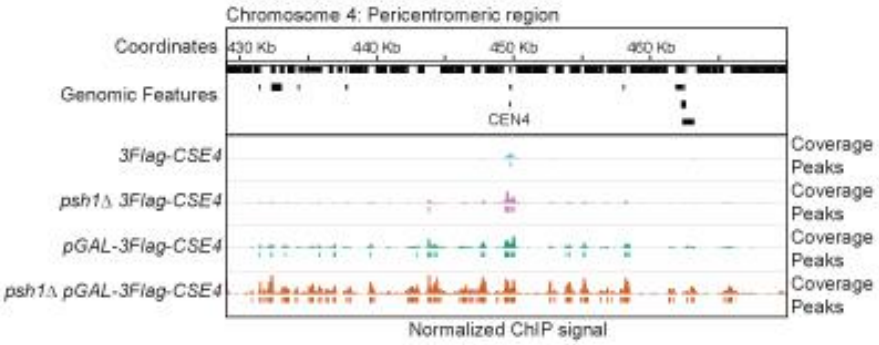

The location of CENP-A protein across the genome revealed by ChIP-seq. When CENP-A is overexpressed, and more so when it is stabilized by preventing degradation, it becomes enriched at non-centromeric, intergenic regions. This ultimately leads to errors in gene expression.

Image provided by Erica Hildebrand.

DNA is wound around protein complexes called nucleosomes in order to protect it from damage and allow precise regulation of gene expression. Nucleosomes contain the four core histone proteins H2A, H2B, H3, and H4. There are also histone proteins with specialized functions, such as an H3 variant called CENP-A that marks the centromere. Centromeres are the site on every chromosome where a protein complex called the kinetochore forms to carry out chromosome segregation during cell division. Maintaining CENP-A at the centromere, and only at the centromere, is important for chromosome segregation and may also protect cells from errors due to misregulation of chromatin (complex of DNA and protein) structure and function. In order to address this latter aspect of CENP-A regulation, graduate student Erica Hildebrand of the Biggins Lab (Basic Sciences Division) set out to characterize the genomic location of mislocalized CENP-A in budding yeast, a model organism for studying the highly conserved process of cell division. The results of her investigation were recently published in *PLOS Genetics*.

Previous research in the Biggins Lab uncovered that CENP-A protein is targeted for proteosomal degradation by an ubiquitin ligase called Psh1. When Psh1 is inactivated, excess CENP-A mislocalizes throughout chromosomes rather than just at centromeres. Hildebrand and her colleagues analyzed whether CENP-A mislocalizes to specific places in the genome by performing chromatin immunoprecipitation followed by sequencing (ChIP-seq). The location of CENP-A was

tracked in four different genetic backgrounds: wild-type, Psh1 mutant, overexpressing CENP-A, and overexpressing CENP-A and Psh1 mutant (see figure). Consistent with previous observations, they noted that the most dramatic mislocalization appeared in the strain with mutant Psh1 and overexpressing CENP-A. They performed a genome-wide analysis of the sequences that associated with CENP-A and found that the majority of mislocalized CENP-A mapped to intergenic regions. Further spatial characterization of intergenic regions showed that mislocalized CENP-A was enriched in gene promoters relative to terminators.

The scientists noted that overexpressed, mislocalized CENP-A was highly enriched in the -1 and +1 nucleosomes that surround the transcription start site (TSS) in promoters. Given this association with the TSS, the scientists wondered whether mislocalized CENP-A was found in front of highly transcribed genes, which have a more dynamic ("open") chromatin structure in their promoters to allow for transcription factors and RNA polymerase to bind. Interestingly however, they found no significant correlation between level of gene expression and whether a gene had mislocalized CENP-A in its promoter. This suggests that the mislocalization does not solely depend on the process of transcription or "open" chromatin per se.

The localization of CENP-A to the nucleosomes surrounding the TSS is similar to the localization pattern of another histone variant, called H2A.Z. To explore if CENP-A location was dependent on H2A.Z, the researchers mutated H2A.Z and performed ChIP-seq on CENP-A. Interestingly, the CENP-A pattern was largely unchanged. Another hypothesis the scientists had was that chromatin remodelers involved in H2A.Z localization might also deposit excess CENP-A. They explored two of these chromatin remodelers: SWR-C and the Ino80 complex. While SWR-C was not required for the mislocalization of CENP-A, loss of Ino80 activity partially protected cells from the growth defect caused by overexpression of CENP-A in the Psh1-mutant background. By performing co-immunoprecipitation analysis, they determined that CENP-A associates with Ino80 and that the association is increased when Psh1 is mutated. Thus the Ino80 complex may be involved in regulating CENP-A nucleosome localization.

Altering nucleosomes ultimately changes the process of gene expression. To trace this, total RNA sequencing was performed comparing wild-type and cells with highly mislocalized CENP-A. This revealed a large number of genes that were up- and down-regulated in the mutant background. A significant portion of the misregulated genes had been identified by other groups as misregulated when H2A.Z is mutated. "For the first time in any organism we link mislocalization of the centromeric nucleosome to transcriptional defects—suggesting that controlling the placement of the centromere is important not only for cell division but also for maintaining euchromatic function," said Hildebrand.

Hildebrand EM, Biggins S. 2016. "Regulation of Budding Yeast CENP-A Levels Prevents Misincorporation at Promoter Nucleosomes and Transcriptional Defects." *PLOS Genetics*. 12(3): e1005930. doi:10.1371/journal.pgen.1005930.

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