Year of publication 2019


Summary

CENP-A is the histone H3 variant necessary to specify the location of all eukaryotic centromeres via its CENP-A targeting domain and either one of its terminal regions. In humans, several post-translational modifications occur on CENP-A, but their role in centromere function remains controversial. One of these modifications of CENP-A, phosphorylation on serine 7, has been proposed to control centromere assembly and function. Here, using gene targeting at both endogenous CENP-A alleles and gene replacement in human cells, we demonstrate that a CENP-A variant that cannot be phosphorylated at serine 7 maintains correct CENP-C recruitment, faithful chromosome segregation and long-term cell viability. Thus, we conclude that phosphorylation of CENP-A on serine 7 is dispensable to maintain correct centromere dynamics and function.

Year of publication 2018


Summary


Summary

Centromeres are the chromosomal domains required to ensure faithful transmission of the genome during cell division. They have a central role in preventing aneuploidy, by orchestrating the assembly of several components required for chromosome separation. However, centromeres also adopt a complex structure that makes them susceptible to being sites of chromosome rearrangements. Therefore, preservation of centromere integrity is a difficult, but important task for the cell. In this review, we discuss how centromeres could
potentially be a source of genome instability and how centromere aberrations and rearrangements are linked with human diseases such as cancer.

Sebastian Hoffmann, Daniele Fachinetti (2018 Aug 4)
Real-Time De Novo Deposition of Centromeric Histone-Associated Proteins Using the Auxin-Inducible Degradation System.

Summary

Measuring protein dynamics is essential to uncover protein function and to understand the formation of large protein complexes such as centromeres. Recently, genome engineering in human cells has improved our ability to study the function of endogenous proteins. By combining genome editing techniques with the auxin-inducible degradation (AID) system, we created a versatile tool to study protein dynamics. This system allows us to analyze both protein function and dynamics by enabling rapid protein depletion and reexpression in the same experimental setup. Here, we focus on the dynamics of the centromeric histone-associated protein CENP-C, responsible for the formation of the kinetochore complex. Following rapid removal and reactivation of a fluorescent version of CENP-C by auxin treatment and removal, we could follow CENP-C de novo deposition at centromeric regions during different stages of the cell cycle. In conclusion, the auxin degradation system is a powerful tool to assess and quantify protein dynamics in real time.

M Dumont, D Fachinetti (2017 Aug 26)
DNA Sequences in Centromere Formation and Function.
Progress in molecular and subcellular biology : 305-336 : DOI: 10.1007/978-3-319-58592-5_13

Summary

Faithful chromosome segregation during cell division depends on the centromere, a complex DNA/protein structure that links chromosomes to spindle microtubules. This chromosomal domain has to be marked throughout cell division and its chromosomal localization preserved across cell generations. From fission yeast to human, centromeres are established on a series of repetitive DNA sequences and on specialized centromeric chromatin. This chromatin is enriched with the histone H3 variant, named CENP-A, that was demonstrated to be the epigenetic mark that maintains centromere identity and function indefinitely. Although centromere identity is thought to be exclusively epigenetic, the presence of specific DNA sequences in the majority of eukaryotes and of the centromeric protein CENP-B that binds to these sequences, suggests the existence of a genetic component as well. In this review, we will highlight the importance of centromeric sequences for centromere formation and function, and discuss the centromere DNA sequence/CENP-B paradox.
A time out for CENP-A.

Summary

Proper chromosome segregation relies on a functional centromere-kinetochore interface. We showed that chromatin containing CENTromere Protein A (CENP-A) is essential for centromere assembly, but dispensable for chromosome segregation in the presence of CENP-B-bound DNA sequences. This demonstrates the existence of two contact points between the DNA and the kinetochore to mediate successful chromosome segregation.

Centromeres are maintained by fastening CENP-A to DNA and directing an arginine anchor-dependent nucleosome transition.

Summary

Maintaining centromere identity relies upon the persistence of the epigenetic mark provided by the histone H3 variant, centromere protein A (CENP-A), but the molecular mechanisms that underlie its remarkable stability remain unclear. Here, we define the contributions of each of the three candidate CENP-A nucleosome-binding domains (two on CENP-C and one on CENP-N) to CENP-A stability using gene replacement and rapid protein degradation. Surprisingly, the most conserved domain, the CENP-C motif, is dispensable. Instead, the stability is conferred by the unfolded central domain of CENP-C and the folded N-terminal domain of CENP-N that becomes rigidified 1,000-fold upon crossbridging CENP-A and its adjacent nucleosomal DNA. Disrupting the ‘arginine anchor’ on CENP-C for the nucleosomal acidic patch disrupts the CENP-A nucleosome structural transition and removes CENP-A nucleosomes from centromeres. CENP-A nucleosome retention at centromeres requires a core centromeric nucleosome complex where CENP-C clamps down a stable nucleosome conformation and CENP-N fastens CENP-A to the DNA.

α-amino trimethylation of CENP-A by NRMT is required for full recruitment of the centromere.

Summary

Centromeres are unique chromosomal domains that control chromosome segregation,
are epigenetically specified by the presence of the CENP-A containing nucleosomes. CENP-A governs centromere function by recruiting the constitutive centromere associated network (CCAN) complex. The features of the CENP-A nucleosome necessary to distinguish centromeric chromatin from general chromatin are not completely understood. Here we show that CENP-A undergoes α-amino trimethylation by the enzyme NRMT in vivo. We show that α-amino trimethylation of the CENP-A tail contributes to cell survival. Loss of α-amino trimethylation causes a reduction in the CENP-T and CENP-I CCAN components at the centromere and leads to lagging chromosomes and spindle pole defects. The function of p53 alters the response of cells to defects associated with decreased CENP-A methylation. Altogether we show an important functional role for α-amino trimethylation of the CENP-A nucleosome in maintaining centromere function and faithful chromosomes segregation.

Yael Nechemia-Arbely, Daniele Fachinetti, Karen H Miga, Nikolina Sekulic, Gautam V Soni, Dong Hyun Kim, Adeline K Wong, Ah Young Lee, Kristen Nguyen, Cees Dekker, Bing Ren, Ben E Black, Don W Cleveland (2017 Feb 26)

Human centromeric CENP-A chromatin is a homotypic, octameric nucleosome at all cell cycle points.

The Journal of cell biology: 607-621: DOI: 10.1083/jcb.201608083

Summary

Chromatin assembled with centromere protein A (CENP-A) is the epigenetic mark of centromere identity. Using new reference models, we now identify sites of CENP-A and histone H3.1 binding within the megabase, α-satellite repeat-containing centromeres of 23 human chromosomes. The overwhelming majority (97%) of α-satellite DNA is found to be assembled with histone H3.1-containing nucleosomes with wrapped DNA termini. In both G1 and G2 cell cycle phases, the 2-4% of α-satellite assembled with CENP-A protects DNA lengths centered on 133 bp, consistent with octameric nucleosomes with DNA unwrapping at entry and exit. CENP-A chromatin is shown to contain equimolar amounts of CENP-A and histones H2A, H2B, and H4, with no H3. Solid-state nanopore analyses show it to be nucleosomal in size. Thus, in contrast to models for hemisomes that briefly transition to octameric nucleosomes at specific cell cycle points or heterotypic nucleosomes containing both CENP-A and histone H3, human CENP-A chromatin complexes are octameric nucleosomes with two molecules of CENP-A at all cell cycle phases.

Fachinetti D, Logsdon GA, Abdullah A, Selzer EB, Cleveland DW, Black BE (2017 Jan 9)

CENP-A Modifications on Ser68 and Lys124 Are Dispensable for Establishment, Maintenance, and Long-Term Function of Human Centromeres.


Summary
Selective Y centromere inactivation triggers chromosome shattering in micronuclei and repair by non-homologous end joining.

**Summary**

Chromosome missegregation into a micronucleus can cause complex and localized genomic rearrangements known as chromothripsis, but the underlying mechanisms remain unresolved. Here we developed an inducible Y centromere-selective inactivation strategy by exploiting a CENP-A/histone H3 chimaera to directly examine the fate of missegregated chromosomes in otherwise diploid human cells. Using this approach, we identified a temporal cascade of events that are initiated following centromere inactivation involving chromosome missegregation, fragmentation, and re-ligation that span three consecutive cell cycles. Following centromere inactivation, a micronucleus harbouring the Y chromosome is formed in the first cell cycle. Chromosome shattering, producing up to 53 dispersed fragments from a single chromosome, is triggered by premature micronuclear condensation prior to or during mitotic entry of the second cycle. Lastly, canonical non-homologous end joining (NHEJ), but not homology-dependent repair, is shown to facilitate re-ligation of chromosomal fragments in the third cycle. Thus, initial errors in cell division can provoke further genomic instability through fragmentation of micronuclear DNAs coupled to NHEJ-mediated reassembly in the subsequent interphase.

CENP-A Is Dispensable for Mitotic Centromere Function after Initial Centromere/Kinetochore Assembly.

**Summary**

Human centromeres are defined by chromatin containing the histone H3 variant CENP-A assembled onto repetitive alphoid DNA sequences. By inducing rapid, complete degradation of endogenous CENP-A, we now demonstrate that once the first steps of centromere assembly have been completed in G1/S, continued CENP-A binding is not required for maintaining kinetochore attachment to centromeres or for centromere function in the next mitosis. Degradation of CENP-A prior to kinetochore assembly is found to block deposition of CENP-C and CENP-N, but not CENP-T, thereby producing defective kinetochores and failure of chromosome segregation. Without the continuing presence of CENP-A, CENP-B binding to alphoid DNA sequences becomes essential to preserve anchoring of CENP-C and the kinetochore to each centromere. Thus, there is a reciprocal interdependency of CENP-A chromatin and the underlying repetitive centromere DNA sequences bound by CENP-B in the
maintenance of human chromosome segregation.

**Year of publication 2015**

Daniele Fachinetti, Joo Seok Han, Moira A McMahon, Peter Ly, Amira Abdullah, Alex J Wong, Don W Cleveland (2015 May 4)

**DNA Sequence-Specific Binding of CENP-B Enhances the Fidelity of Human Centromere Function.**

*Developmental cell*: 314-27 : [DOI: 10.1016/j.devcel.2015.03.020]

**Summary**

Human centromeres are specified by a stably inherited epigenetic mark that maintains centromere position and function through a two-step mechanism relying on self-templating centromeric chromatin assembled with the histone H3 variant CENP-A, followed by CENP-A-dependent nucleation of kinetochore assembly. Nevertheless, natural human centromeres are positioned within specific megabase chromosomal regions containing α-satellite DNA repeats, which contain binding sites for the DNA sequence-specific binding protein CENP-B. We now demonstrate that CENP-B directly binds both CENP-A’s amino-terminal tail and CENP-C, a key nucleator of kinetochore assembly. DNA sequence-dependent binding of CENP-B within α-satellite repeats is required to stabilize optimal centromeric levels of CENP-C. Chromosomes bearing centromeres without bound CENP-B, including the human Y chromosome, are shown to mis-segregate in cells at rates several-fold higher than chromosomes with CENP-B-containing centromeres. These data demonstrate a DNA sequence-specific enhancement by CENP-B of the fidelity of epigenetically defined human centromere function.

**Year of publication 2014**

Joo Seok Han, Benjamin Vitre, Daniele Fachinetti, Don W Cleveland (2014 Sep 24)

**Bimodal activation of BubR1 by Bub3 sustains mitotic checkpoint signaling.**

*Proceedings of the National Academy of Sciences of the United States of America*: E4185-93 : [DOI: 10.1073/pnas.1416277111]

**Summary**

The mitotic checkpoint (also known as the spindle assembly checkpoint) prevents premature anaphase onset through generation of an inhibitor of the E3 ubiquitin ligase APC/C, whose ubiquitination of cyclin B and securin targets them for degradation. Combining in vitro reconstitution and cell-based assays, we now identify dual mechanisms through which Bub3 promotes mitotic checkpoint signaling. Bub3 enhances signaling at unattached kinetochores not only by facilitating binding of BubR1 but also by enhancing Cdc20 recruitment to kinetochores mediated by BubR1’s internal Cdc20 binding site. Downstream of kinetochore-produced complexes, Bub3 promotes binding of BubR1’s conserved, amino terminal Cdc20
binding domain to a site in Cdc20 that becomes exposed by initial Mad2 binding. This latter Bub3-stimulated event generates the final mitotic checkpoint complex of Bub3-BubR1-Cdc20 that selectively inhibits ubiquitination of securin and cyclin B by APC/C(Cdc20). Thus, Bub3 promotes two distinct BubR1-Cdc20 interactions, involving each of the two Cdc20 binding sites of BubR1 and acting at unattached kinetochores or cytoplasmically, respectively, to facilitate production of the mitotic checkpoint inhibitor.

Year of publication 2013

Joo Seok Han, Andrew J Holland, Daniele Fachinetti, Anita Kulukian, Bulent Cetin, Don W Cleveland (2013 Jun 25)

**Catalytic assembly of the mitotic checkpoint inhibitor BubR1-Cdc20 by a Mad2-induced functional switch in Cdc20.**

*Molecular cell*: 92-104 : [DOI : 10.1016/j.molcel.2013.05.019](http://dx.doi.org/10.1016/j.molcel.2013.05.019)

**Summary**

The mitotic checkpoint acts to maintain chromosome content by generation of a diffusible anaphase inhibitor. Unattached kinetochores catalyze a conformational shift in Mad2, converting an inactive open form into a closed form that can capture Cdc20, the mitotic activator of the APC/C ubiquitin ligase. Mad2 binding is now shown to promote a functional switch in Cdc20, exposing a previously inaccessible site for binding to BubR1’s conserved Mad3 homology domain. BubR1, but not Mad2, binding to APC/C(Cdc20) is demonstrated to inhibit ubiquitination of cyclin B. Closed Mad2 is further shown to catalytically amplify production of BubR1-Cdc20 without necessarily being part of the complex. Thus, the mitotic checkpoint is produced by a cascade of two catalytic steps: an initial step acting at unattached kinetochores to produce a diffusible Mad2-Cdc20 intermediate and a diffusible step in which that intermediate amplifies production of BubR1-Cdc20, the inhibitor of cyclin B ubiquitination, by APC/C(Cdc20).