Publications de l’équipe

Mécanismes moléculaires de la dynamique des chromosomes

Année de publication : 2019

Yael Nechemia-Arbely, Karen H Miga, Ofer Shoshani, Aaron Aslanian, Moira A McMahon, Ah Young Lee, Daniele Fachinetti, John R Yates, Bing Ren, Don W Cleveland (2019 Jun 5)
DNA replication acts as an error correction mechanism to maintain centromere identity by restricting CENP-A to centromeres.
*Nature cell biology*: 743-754 : DOI : 10.1038/s41556-019-0331-4

Résumé

Chromatin assembled with the histone H3 variant CENP-A is the heritable epigenetic determinant of human centromere identity. Using genome-wide mapping and reference models for 23 human centromeres, CENP-A binding sites are identified within the megabase-long, repetitive α-satellite DNAs at each centromere. CENP-A is shown in early G1 to be assembled into nucleosomes within each centromere and onto 11,390 transcriptionally active sites on the chromosome arms. DNA replication is demonstrated to remove ectopically loaded, non-centromeric CENP-A. In contrast, tethering of centromeric CENP-A to the sites of DNA replication through the constitutive centromere associated network (CCAN) is shown to enable precise reloading of centromere-bound CENP-A onto the same DNA sequences as in its initial prereplication loading. Thus, DNA replication acts as an error correction mechanism for maintaining centromere identity through its removal of non-centromeric CENP-A coupled with CCAN-mediated retention and precise reloading of centromeric CENP-A.

The N-Terminal Domain of cGAS Determines Preferential Association with Centromeric DNA and Innate Immune Activation in the Nucleus.
*Cell reports*: 3798 : DOI : S2211-1247(19)30365-1

Résumé

Viviana Barra, Glennis A Logsdon, Andrea Scelfo, Sebastian Hoffmann, Solène Hervé, Aaron Aslanian, Yael Nechemia-Arbely, Don W Cleveland, Ben E Black, Daniele Fachinetti (2019 Jan 13)
Phosphorylation of CENP-A on serine 7 does not control centromere function.
*Nature communications*: 175 : DOI : 10.1038/s41467-018-08073-1

Résumé

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.

**Résumé**

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.
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.

Année de publication : 2017

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

Résumé

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.

S Hoffmann, D Fachinetti (2017 Jun 16)
A time out for CENP-A.

Résumé

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.

*Nature communications*: 15775 : [DOI : 10.1038/ncomms15775](https://doi.org/10.1038/ncomms15775)

Résumé

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.

*Nature communications*: 14678 : [DOI : 10.1038/ncomms14678](https://doi.org/10.1038/ncomms14678)

Résumé

Centromeres are unique chromosomal domains that control chromosome segregation, and 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.
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.**

**Résumé**

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.**

**Résumé**

**Année de publication : 2016**

Peter Ly, Levi S Teitz, Dong H Kim, Ofer Shoshani, Helen Skaletsky, Daniele Fachinetti, David C Page, Don W Cleveland (2016 Dec 6)
**Selective Y centromere inactivation triggers chromosome shattering in micronuclei and repair by non-homologous end joining.**
*Nature cell biology* : [DOI : 10.1038/ncb3450]

**Résumé**

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.

Sebastian Hoffmann, Marie Dumont, Viviana Barra, Peter Ly, Yael Nechemia-Arbely, Moira A McMahon, Solène Hervé, Don W Cleveland, Daniele Fachinetti (2016 Nov 24)  
CENP-A Is Dispensable for Mitotic Centromere Function after Initial Centromere/Kinetochore Assembly.  
Cell reports : 2394-2404 : DOI : 10.1016/j.celrep.2016.10.084

Résumé

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.

Année de 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

Résumé

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.