Year of publication 2021

Daniel Jeffery, Alberto Gatto, Katrina Podsypanina, Charlène Renaud-Pageot, Rebeca Ponce Landete, Lorraine Bonneville, Marie Dumont, Daniele Fachinetti, Geneviève Almouzni (2021 Mar 26)
CENP-A overexpression promotes distinct fates in human cells, depending on p53 status

Summary

Simona Giunta, Solène Hervé, Ryan R White, Therese Wilhelm, Marie Dumont, Andrea Scelfo, Riccardo Gamba, Cheng Kit Wong, Giulia Rancati, Agata Smogorzewska, Hironori Funabiki, Daniele Fachinetti (2021 Mar 3)
CENP-A chromatin prevents replication stress at centromeres to avoid structural aneuploidy.
*Proceedings of the National Academy of Sciences of the United States of America* : [DOI: e2015634118]

Summary

Chromosome segregation relies on centromeres, yet their repetitive DNA is often prone to aberrant rearrangements under pathological conditions. Factors that maintain centromere integrity to prevent centromere-associated chromosome translocations are unknown. Here, we demonstrate the importance of the centromere-specific histone H3 variant CENP-A in safeguarding DNA replication of alpha-satellite repeats to prevent structural aneuploidy. Rapid removal of CENP-A in S phase, but not other cell-cycle stages, caused accumulation of R loops with increased centromeric transcripts, and interfered with replication fork progression. Replication without CENP-A causes recombination at alpha-satellites in an R loop-dependent manner, unfinished replication, and anaphase bridges. In turn, chromosome breakage and translocations arise specifically at centromeric regions. Our findings provide insights into how specialized centromeric chromatin maintains the integrity of transcribed noncoding repetitive DNA during S phase.

CENP-A overexpression promotes aneuploidy with karyotypic heterogeneity.
Summary

Chromosomal instability (CIN) is a hallmark of many cancers. Restricting the localization of centromeric histone H3 variant CENP-A to centromeres prevents CIN. CENP-A overexpression (OE) and mislocalization have been observed in cancers and correlate with poor prognosis; however, the molecular consequences of CENP-A OE on CIN and aneuploidy have not been defined. Here, we show that CENP-A OE leads to its mislocalization and CIN with lagging chromosomes and micronuclei in pseudodiploid DLD1 cells and xenograft mouse model. CIN is due to reduced localization of proteins to the kinetochore, resulting in defects in kinetochore integrity and unstable kinetochore-microtubule attachments. CENP-A OE contributes to reduced expression of cell adhesion genes and higher invasion of DLD1 cells. We show that CENP-A OE contributes to aneuploidy with karyotypic heterogeneity in human cells and xenograft mouse model. In summary, our results provide a molecular link between CENP-A OE and aneuploidy, and suggest that karyotypic heterogeneity may contribute to the aggressive phenotype of CENP-A-overexpressing cancers.

Marina Murillo-Pineda, Luis P Valente, Marie Dumont, João F Mata, Daniele Fachinetti, Lars E T Jansen (2021 Jan 14)
Induction of spontaneous human neocentromere formation and long-term maturation.
The Journal of cell biology: DOI: e202007210

Summary

Human centromeres form primarily on α-satellite DNA but sporadically arise de novo at naive ectopic loci, creating neocentromeres. Centromere inheritance is driven primarily by chromatin containing the histone H3 variant CENP-A. Here, we report a chromosome engineering system for neocentromere formation in human cells and characterize the first experimentally induced human neocentromere at a naive locus. The spontaneously formed neocentromere spans a gene-poor 100-kb domain enriched in histone H3 lysine 9 trimethylated (H3K9me3). Long-read sequencing revealed this neocentromere was formed by purely epigenetic means and assembly of a functional kinetochore correlated with CENP-A seeding, eviction of H3K9me3 and local accumulation of mitotic cohesin and RNA polymerase II. At formation, the young neocentromere showed markedly reduced chromosomal passenger complex (CPC) occupancy and poor sister chromatin cohesion. However, long-term tracking revealed increased CPC assembly and low-level transcription providing evidence for centromere maturation over time.

Year of publication 2020

Daniele Fachinetti, Hiroshi Masumoto, Natalay Kouprina (2020 Sep 27)
Artificial chromosomes.
Experimental cell research : 112302 : DOI : S0014-4827(20)30551-6
Summary

Sebastiaan Jw van den Berg, Lars Et Jansen (2020 Sep 22)
Centromeres: genetic input to calibrate an epigenetic feedback loop.
The EMBO journal : e106638 : DOI: 10.15252/embj.2020106638

Summary

Centromeres are chromatin domains maintained by a self-templating feedback loop based on nucleosomes bearing the histone H3 variant CENP-A. The underlying centromeric DNA sequence is largely dispensable, yet paradoxically, it has highly conserved features. Hoffmann et al (2020) now uncover that when the epigenetic chromatin cycle falters, a genetically hardwired mechanism offers robustness to a dynamic epigenetic feedback loop ensuring long-term centromere inheritance.

Sebastian Hoffmann, Helena M Izquierdo, Riccardo Gamba, Florian Chardon, Marie Dumont, Veer Keizer, Solène Hervé, Shannon M McNulty, Beth A Sullivan, Nicolas Manel, Daniele Fachinetti (2020 Sep 18)
A genetic memory initiates the epigenetic loop necessary to preserve centromere position.
The EMBO journal : e105505 : DOI: 10.15252/embj.2020105505

Summary

Centromeres are built on repetitive DNA sequences (CenDNA) and a specific chromatin enriched with the histone H3 variant CENP-A, the epigenetic mark that identifies centromere position. Here, we interrogate the importance of CenDNA in centromere specification by developing a system to rapidly remove and reactivate CENP-A (CENP-A). Using this system, we define the temporal cascade of events necessary to maintain centromere position. We unveil that CENP-B bound to CenDNA provides memory for maintenance on human centromeres by promoting de novo CENP-A deposition. Indeed, lack of CENP-B favors neocentromere formation under selective pressure. Occasionally, CENP-B triggers centromere re-activation initiated by CENP-C, but not CENP-A, recruitment at both ectopic and native centromeres. This is then sufficient to initiate the CENP-A-based epigenetic loop. Finally, we identify a population of CENP-A-negative, CENP-B/C-positive resting CD4 T cells capable to re-express and reassembles CENP-A upon cell cycle entry, demonstrating the physiological importance of the genetic memory.

Marie Dumont, Daniele Fachinetti (2020 Sep 18)
Centromere strength: just a sense of proportion.
**Summary**

The overall structure and composition of human centromeres have been well reported, but how these elements vary between individual chromosomes and influence the chromosome-specific behavior during mitosis remains untested. In our study, we discover the existence of heterogeneity of centromeric DNA features that dictates the chromosome segregation fidelity during mitosis.

Riccardo Gamba, Daniele Fachinetti (2020 Mar 17)

*From evolution to function: Two sides of the same CENP-B coin?*

*Experimental cell research*: 111959 : [DOI : S0014-4827(20)30167-1](https://doi.org/S0014-4827(20)30167-1)

**Summary**

The centromere is the nucleoproteic chromosomal structure necessary for accurate chromosome segregation during cell division. One of the earliest centromeric proteins to be discovered was CENP-B, the only one capable of recognizing a specific centromeric DNA binding motif. The phylogenetic history of this protein and of its DNA binding site shows independent events of function acquisition across different species and raises questions on the evolutionary dynamics of CENP-B, including what may be the selective advantage provided by its role at the centromere. Recent results have provided insight into potential functions of CENP-B in chromosome dynamics, however, its function is still object of debate. The recurrent appearance of CENP-B centromeric activity along phylogensis, together with its dispensability, represent strictly intertwined facets of this controversy. This chapter focuses on the evolution, function and homeostasis of CENP-B and its importance in centromere biology.

**Year of publication 2019**


*Human chromosome-specific aneuploidy is influenced by DNA-dependent centromeric features.*

*The EMBO journal*: e102924 : [DOI : 10.15252/embj.2019102924](https://doi.org/10.15252/embj.2019102924)

**Summary**

Intrinsic genomic features of individual chromosomes can contribute to chromosome-specific aneuploidy. Centromeres are key elements for the maintenance of chromosome segregation fidelity via a specialized chromatin marked by CENP-A wrapped by repetitive DNA. These long stretches of repetitive DNA vary in length among human chromosomes. Using CENP-A genetic inactivation in human cells, we directly interrogate if differences in the centromere length reflect the heterogeneity of centromeric DNA-dependent features and whether this, in
turn, affects the genesis of chromosome-specific aneuploidy. Using three distinct approaches, we show that mis-segregation rates vary among different chromosomes under conditions that compromise centromere function. Whole-genome sequencing and centromere mapping combined with cytogenetic analysis, small molecule inhibitors, and genetic manipulation revealed that inter-chromosomal heterogeneity of centromeric features, but not centromere length, influences chromosome segregation fidelity. We conclude that faithful chromosome segregation for most of human chromosomes is biased in favor of centromeres with high abundance of DNA-dependent centromeric components. These inter-chromosomal differences in centromere features can translate into non-random aneuploidy, a hallmark of cancer and genetic diseases.

Reiho Watanabe, Masatoshi Hara, Ei-Ichi Okumura, Solène Hervé, Daniele Fachinetti, Mariko Ariyoshi, Tatsuo Fukagawa (2019 Nov 3)  
**CDK1-mediated CENP-C phosphorylation modulates CENP-A binding and mitotic kinetochore localization.**  
*The Journal of cell biology* : DOI : jcb.201907006

**Summary**

The kinetochore is essential for faithful chromosome segregation during mitosis. To form a functional kinetochore, constitutive centromere-associated network (CCAN) proteins are assembled on the centromere chromatin that contains the centromere-specific histone CENP-A. CENP-C, a CCAN protein, directly interacts with the CENP-A nucleosome to nucleate the kinetochore structure. As CENP-C is a hub protein for kinetochore assembly, it is critical to address how the CENP-A-CENP-C interaction is regulated during cell cycle progression. To address this question, we investigated the CENP-C C-terminal region, including a conserved CENP-A-binding motif, in both chicken and human cells and found that CDK1-mediated phosphorylation of CENP-C facilitates its binding to CENP-A in vitro and in vivo. We observed that CENP-A binding is involved in CENP-C kinetochore localization during mitosis. We also demonstrate that the CENP-A-CENP-C interaction is critical for long-term viability in human RPE-1 cells. These results provide deeper insights into protein-interaction network plasticity in centromere proteins during cell cycle progression.

**Centromere Dysfunction Compromises Mitotic Spindle Pole Integrity.**  
*Current biology : CB* : 3072-3080.e5 : DOI : S0960-9822(19)30932-7

**Summary**

Centromeres and centrosomes are crucial mitotic players. Centromeres are unique chromosomal sites characterized by the presence of the histone H3-variant centromere protein A (CENP-A) [1]. CENP-A recruits the majority of centromere components, collectively
named the constitutive centromere associated network (CCAN) [2]. The CCAN is necessary for kinetochore assembly, a multiprotein complex that attaches spindle microtubules (MTs) and is required for chromosome segregation [3]. In most animal cells, the dominant site for MT nucleation in mitosis are the centrosomes, which are composed of two centrioles, surrounded by a protein-rich matrix of electron-dense pericentriolar material (PCM) [4]. The PCM is the site of MT nucleation during mitosis [5]. Even if centromeres and centrosomes are connected via MTs in mitosis, it is not known whether defects in either one of the two structures have an impact on the function of the other. Here, using high-resolution microscopy combined with rapid removal of CENP-A in human cells, we found that perturbation of centromere function impacts mitotic spindle pole integrity. This includes release of MT minus-ends from the centrosome, leading to PCM dispersion and centriole mis-positioning at the spindle poles. Mechanistically, we show that these defects result from abnormal spindle MT dynamics due to defective kinetochore-MT attachments. Importantly, restoring mitotic spindle pole integrity following centromere inactivation lead to a decrease in the frequency of chromosome mis-segregation. Overall, our work identifies an unexpected relationship between centromeres and maintenance of the mitotic pole integrity necessary to ensure mitotic accuracy and thus to maintain genetic stability.

Robert F Lera, Roshan X Norman, Marie Dumont, Alexandra Dennee, Joanne Martin-Koob, Daniele Fachinetti, Mark E Burkard (2019 Aug 31)

**Plk1 protects kinetochore-centromere architecture against microtubule pulling forces.**

*EMBO reports* : e48711 : [DOI : 10.15252/embr.201948711](https://doi.org/10.15252/embr.201948711)

**Summary**

During mitosis, sister chromatids attach to microtubules which generate ~700 pN pulling force focused on the centromere. We report that chromatin-localized signals generated by Polo-like kinase 1 (Plk1) maintain the integrity of the kinetochore and centromere against this force. Without sufficient Plk1 activity, chromosomes become misaligned after normal condensation and congression. These chromosomes are silent to the mitotic checkpoint, and many lag and mis-segregate in anaphase. Their centromeres and kinetochores lack CENP-A, CENP-C, CENP-T, Hec1, Nuf2, and Knl1; however, CENP-B is retained. CENP-A loss occurs coincident with secondary misalignment and anaphase onset. This disruption occurs asymmetrically prior to anaphase and requires tension generated by microtubules. Mechanistically, centromeres highly recruit PICH DNA helicase and PICH depletion restores kinetochore disruption in pre-anaphase cells. Furthermore, anaphase defects are significantly reduced by tethering Plk1 to chromatin, including H2B, and INCENP, but not to CENP-A. Taken as a whole, this demonstrates that Plk1 signals are crucial for stabilizing centromeric architecture against tension.

Andrea Scelfo, Daniele Fachinetti (2019 Aug 21)

**Keeping the Centromere under Control: A Promising Role for DNA Methylation.**

*Cells* : [DOI : E912](https://doi.org/10.1002/201909250)
Summary

In order to maintain cell and organism homeostasis, the genetic material has to be faithfully and equally inherited through cell divisions while preserving its integrity. Centromeres play an essential task in this process; they are special sites on chromosomes where kinetochores form on repetitive DNA sequences to enable accurate chromosome segregation. Recent evidence suggests that centromeric DNA sequences, and epigenetic regulation of centromeres, have important roles in centromere physiology. In particular, DNA methylation is abundant at the centromere, and aberrant DNA methylation, observed in certain tumors, has been correlated to aneuploidy and genomic instability. In this review, we evaluate past and current insights on the relationship between centromere function and the DNA methylation pattern of its underlying sequences.

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

Summary

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.