Review of "CENP-A associated lncRNAs influence chromosome segregation in human cells"

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Study conducted by Delphine Quénet[1,2], David Sturgill[1], Marin Olson[1], Yamini Dalal[1]

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General Assessment

This study makes a convincing case that centromeric noncoding RNAs play an important role in chromosomal integrity, and also provides an RNA transcript set that will facilitate future studies of centromere transcription. Given the difficulty of targeting highly repetitive centromeric sequence, the presented data and interpretations make impressive demonstrations on cenRNA localization and function. In particular, the *in vivo* immunofluorescence experiments clearly demonstrate that an individual cenRNA (cenRNA#4) localizes near centromeres. If the authors choose to make an addition to the study, they may consider adding an IF image of cells experiencing chromosomal segregation defects, as it would help illustrate the direct observations to readers who are unfamiliar with the field. In addition, future readers with backgrounds in genomics will likely be interested in knowing the defining feature of each of the four identified cenRNA classes, and whether cenRNA loci are generally near canonical promoters and/or polyadenylation signals. Overall, this work contributes exciting findings in support of a fascinating model that explains how centromeric RNAs target and load CENP-A protein to the centromere, ensuring proper chromosomal segregation during mitosis.

Research Summary

The paper presents a characterization of processed and functionally important lncRNAs deriving from human centromeres that bind the centromeric histone variant CENP-A. A RIP-seq library targeting CENP-A provided sequence reads that were used to construct 432 transcripts of heterogeneous length (300-2500 bases). These transcripts mapped confidently to almost every human chromosome, are enriched in ?-satellite repeats, and are unique enough to permit separation into four classes through two different clustering methods. Visualizing a subset of these transcripts via RNA FISH in synchronized HeLa cells revealed that multiple and unique ?-satellite-containing RNAs are present in the cell nucleus, and that individual cenRNAs (eg. cenRNA#4) can be localized near one centromere via co-IF with centromere protein B (CENP-B) in a fraction of cells. Both ?-satellite-containing RNAs and cenRNA#4 are likely polyadenylated as they can be amplified from cDNA using poly-dT primers. Finally, siRNA-knockdown of ?-satellite RNAs or cenRNA#4 individually resulted in a higher rate of chromosomal segregation defects compared to a scrambled siRNA target, lending support to a functional role of these transcripts in maintaining chromosome stability.

Significance

Past work of the field has demonstrated that transcription at centromeres of various eukaryotes is an important and conserved event for centromere function and chromosomal integrity. As this topic of research progresses, it will be important to characterize the molecular mechanism of cenRNAs, which are not understood completely. This research lays groundwork for investigating further questions on the molecular mechanisms of cenRNA-mediated CENP-A loading to centromeres. In particular, the finding that centromere-localizing cenRNAs are of heterogeneous length and sequence identity invites one to think whether they could be involved in important RNA-RNA interactions (eg. RNAi-based methods) that are important for CENP-A loading.

Observations

RNA localization images with immunofluorescence are accurately and clearly described. Alternative explanations for probe localization such as RNA-DNA hybrids are properly acknowledged with RNase control experiments. However, in the case of the cenRNA-CENP-B co-IF images, there is no mention or explanation of apparent nonspecific staining that is occurring in the ?-satellite (red) channel, which could make these figures harder to interpret on a first read-through. For readers who are non-experts in IF techniques, descriptions of IF images in Figures 2A and 3B will be easy to follow, however the authors may consider elaborating on what they consider to be a 'juxtaposed' localization of cenRNAs and CENP-B in reference to their model, and whether this was a general observation.

Interpretations

One major claim of this paper is that the knockdown of cenRNA#4 leads to chromosomal segregation defects. The explanatory model is that the cenRNA is responsible for binding and loading CENP-A to the centromere, and that segregation defects occur upon depletion of the cenRNA due to a lack of CENP-A. In Figure 4, the authors demonstrate that a higher proportion of cells experience segregation defects in HeLa cells by IF with ?-tubulin and CENP-B co-staining. If it were observed in an IF experiment that CENP-A was depleted or found at lower levels in the knockdown conditions, it would provide more support for this mechanistic hypothesis. A more speculative claim of the paper is that because the authors observe fewer foci of ?-satellite RNAs in HeLa nuclei than centromeres, cenRNAs may bind to centromeres in trans. This hypothesis is sound, yet could incorporate more support from further IF experiments that definitively show that one cenRNA deriving from one chromosome is localized to a different chromosome.

Clarity

The content of this paper has logical flow, and each topic is well encapsulated and discussed. Moving from RIP-seq to transcript mapping, and onwards to immunofluorescence experiments, there was no particular point at which the train of logic or experimentation took an irrelevant turn. Figure-wise, Figure 2A appear to be missing column labels that specify which channel the HeLa cells are being viewed through.