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SPOTLIGHT

Truly epigenetic: A centromere finds a "neo" home

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Murillo-Pineda and colleagues (2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202007210) use CRISPR-Cas9–based genetic engineering in human cells to induce a new functional centromere at a naive chromosomal site. Long-read DNA sequencing at the neocentromere provides firm evidence that centromere establishment is a truly epigenetic event.

The centromere is the unique site on each chromosome that orchestrates accurate chromosome segregation at cell division. Human centromeres comprise large arrays of repetitive α satellite DNA sequences. Yet, this α satellite DNA is neither necessary nor sufficient for centromere function. Rather, it is the incorporation the histone H3 variant CENP-A that determines centromere identity and function in an epigenetic manner (1).

Neocentromeres are centromeres that are spontaneously formed at chromosomal positions void of α satellite DNA (2). Naturally occurring neocentromeres, mostly derived from chromosomal rearrangements, have been observed in human patients (3). Yet, their biogenesis remains unclear as they are typically studied long after formation. Previous experiments in model systems have successfully induced neocentromeres through the deletion of endogenous centromeres (4, 5, 6), providing insight into species-specific features. However, this had not yet been achieved for more complex mammalian centromeres. Making use of innovative CRISPR-Cas9–based chromosome engineering, Murillo-Pineda and colleagues have now overcome this barrier. They describe, for the first time, the induction of a spontaneous neocentromere in human cultured cells in vitro (7). This technical breakthrough involves using CRISPR-Cas9 to target and excise an 8-megabase (Mb) region of human chromosome 4 containing the endogenous centromere and flanking pericentric regions. Following deletion, based on the cytological examination of CENP-A recruitment, cells harboring a centromere at a novel site on chromosome 4 were isolated at a frequency of about one in eight million (Fig. 1 A). A major advantage of the approach is that it enables analysis of the chromosomal site "before" and "after" neocentromere induction. This allows study not only of the birth of the neocentromere relatively soon after it is established, but also its maturation over time. Such experiments are not possible in human patients carrying neocentromeres and indeed permit investigation into the role of new centromeres in the development of diseases, such as cancer (8).

By performing long-read DNA sequencing of the locus before and after neocentromere induction, Murillo-Pineda and colleagues report no changes at the sequence level and a complete lack of α satellite DNA. Moreover, no sequence changes were detected upon neocentromere maturation after ∼200 cell divisions (7). Sequencing of patient-derived neocentromeres previously indicated the absence of α satellite repeats, so this is perhaps an expected result. However, what was not possible at that time was to examine the ancestral DNA state within a short time frame before neocentromere formation. This powerful system now allows such a comparison. In doing so, it provides an elegant and robust confirmation that human centromere establishment is a truly epigenetic event.

Beyond DNA sequence, the authors examine the epigenetic and chromatin landscape of the neocentromere and make a number of unexpected observations (Fig. 1 B). They find that it forms in a region enriched for the heterochromatin marker histone H3 lysine 9 trimethylated (H3K9me3; 7). Hence, heterochromatin itself appears to be permissive for neocentromere formation, at least initially. Moreover, proximal heterochromatin does not appear to be a required feature, different from observations of yeast neocentromeres (4). This result does beg the question as to why centromeric chromatin is commonly flanked by pericentric heterochromatin in many organisms. One possibility is that when challenged, the neocentromere might prove unstable, perhaps revealing a requirement for proximity to heterochromatin in this instance. In any case, centromeres do appear to prefer certain loci, and it is not simply an "anywhere will do" scenario for building a centromere from scratch.

The authors also find that the neocentromere region is gene poor. However, it is transcriptionally active (7). Therefore, neocentromere establishment and maintenance is compatible with transcription. Transcripts derived from centromere sequences have been detected in many systems (9), including human. Indeed, this system will be useful to robustly test transcription's contribution to human centromere function and long-term stability. Lastly, despite structural defects in the inner kinetochore, the neocentromere chromosome segregates accurately and is

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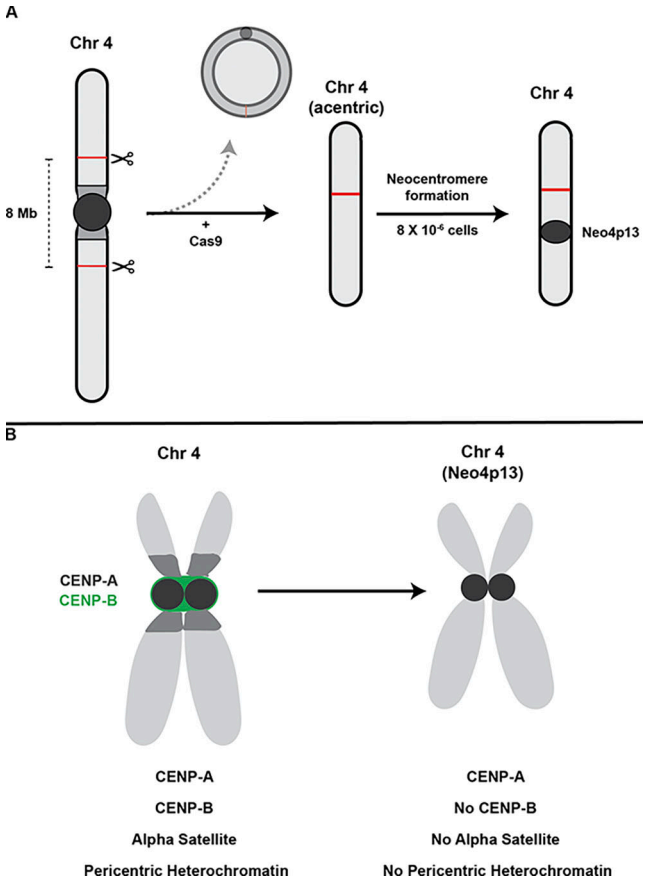

Figure 1. **Establishment of a human neocentromere on chromosome 4 (Chr4). (A)** CRISPR-Cas9 is used to remove an 8-Mb region of Chr4 containing the endogenous centromere and surrounding pericentric heterochromatin. This approach generates an acentric Chr4. These cells are cultured and screened by immunofluorescent microscopy for neocentromere formation. A stable neocentromere, Neo4p13, is established on the *p* (short) arm at a frequency of one in eight million cells. **(B)** Comparison of the features of the canonical centromere versus neocentromere Neo4p13. Chr4 (left) normally contains a centromere that is epigenetically specified by the histone H3 variant CENP-A. The underlying centromeric DNA contains a higher order array of α satellite repeats, flanked by pericentric heterochromatin. CENP-B binds to α satellite DNA via the CENP-B box, and it is sufficient to reform a centromere (via CENP-C) in the absence of CENP-A (11). In contrast, de novo centromere formation at Neo4p13 (right) occurs at sites void of α satellite DNA, CENP-B binding, and proximal heterochromatin. This indicates that centromere establishment is truly an epigenetic event, independent of centromeric DNA contribution.

mitotically stable. Intriguingly, it acquires an increasing assembly of the chromosomal passenger complex as it matures, suggesting an adaption and stabilization over time. Perhaps surprising is a gradual depletion of the core centromere component CENP-C over time (7). Indeed, how stable this neocentromere is with a depleting maintenance machinery and lacking the α satellite binding partner CENP-B remains an open question.

Overall, this study reports an exciting development in the centromere field, as it is the first viable method for purposefully engineering human neocentromeres. The ability to time-stamp its biogenesis will undoubtably add to our understanding of centromere formation, maturation, and stability. It also raises a number of poignant questions to be pursued. In particular, why are canonical human centromeres associated with repetitive α satellite DNA, and what is its function in addition to CENP-B binding? Perhaps it is required to ensure optimal function, particularly under stress conditions, which now would be feasible to test. In theory, one could engineer α satellites into these chromosomes before deletion of the endogenous centromere. This would test the preference of CENP-A for said DNA sequences if available at another chromosomal locus. Over 90 different neocentromeres have been isolated in human patients (3). It would be interesting to delete the endogenous centromere of additional chromosomes to examine commonalities and exceptions, or indeed to determine if neocentromeres on chromosome 4 always form at the same site. Adaptation of this technique to other chromosomal domains is another important outcome of this study and should not be underestimated. The heroic efforts toward achieving the telomere-to-telomere assembly of human chromosomes, which includes repeated arrays at centromeres and pericentromeres, now stands as a critical resource (10). It might soon be possible to delete pericentric heterochromatin or other repetitive DNA sequences from human chromosomes, allowing the function of erroneously labeled "junk" DNA to be determined. Ultimately, Murillo-Pineda and colleagues have opened the field to avenues previously inexplorable for many aspects of centromere biology. To this end, a "neo" age for centromere research is upon us.

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References

1. McKinley, K.L., and I.M. Cheeseman. 2016. *Nat. Rev. Mol. Cell Biol.* https://doi.org/10.1038/nrm.2015.5
2. Fukagawa, T., and W.C. Earnshaw. 2014. *Curr. Biol.* https://doi.org/10.1016/j.cub.2014.08.032
3. Marshall, O.J., et al. 2008. *Am. J. Hum. Genet.* https://doi.org/10.1016/j.ajhg.2007.11.009
4. Ishii, K., et al. 2008. *Science.* https://doi.org/10.1126/science.1158699
5. Ketel, C., et al. 2009. *PLoS Genet.* https://doi.org/10.1371/journal.pgen.1000400
6. Shang, W.H., et al. 2013. *Dev. Cell.* https://doi.org/10.1016/j.devcel.2013.02.009
7. Murillo-Pineda, M., et al. 2021. *J. Cell Biol.* https://doi.org/10.1083/jcb.202007210
8. Burrack, L.S., and J. Berman. 2012. *Chromosome Res.* https://doi.org/10.1007/s10577-012-9296-x
9. Perea-Resa, C., and M.D. Blower. 2018. *Mol. Cell. Biol.* https://doi.org/10.1128/MCB.00263-18
10. Miga, K.H., et al. 2020. *Nature.* https://doi.org/10.1038/s41586-020-2547-7
11. Hoffmann, S., et al. 2020. *EMBO J.* https://doi.org/10.15252/embj.2020105505