Reductionism at the vertebrate kinetochore

Ana Stankovic and Lars E.T. Jansen

Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal

The kinetochore forms the site of attachment for mitotic spindle microtubules driving chromosome segregation. The interdependent protein interactions in this large structure have made it difficult to dissect the function of its components. In this issue, Hori et al. (2013. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201210106) present a novel and powerful methodology to address the sufficiency of individual proteins for the creation of a functional de novo centromere.

The centromere is the chromosomal locus responsible for kinetochore nucleation and chromosome segregation in mitosis (Cheeseman and Desai, 2008). In most organisms, centromere position is specified epigenetically through a unique chromatin structure marked by the presence of the histone H3 variant CENP-A and a constitutive complex of centromere proteins (Foltz et al., 2006; Okada et al., 2006). CENP-A is a particularly promising candidate for the epigenetic marking of the centromere, as targeted deposition of CENP-A has been shown to lead to the formation of a functional kinetochore, which can be heritably maintained (Barnhart et al., 2011; Guse et al., 2011; Mendiburo et al., 2011). How CENP-A is recruited to the centromere site and how the associated centromere complex defines the kinetochore are key current questions.

Understanding the kinetochore puzzle

The constitutive centromere-associated network (CCAN) of 16 proteins is thought to form a functional bridge, linking centromeric CENP-A chromatin to the kinetochore. In a study reported in this issue, T. Fukagawa and his team dissect the direct contribution of different CCAN components to the nucleation of centromeric chromatin as well as to recruitment of the microtubule-binding complex of the outer kinetochore. They expand on a methodology recently used by the same group in collaboration with I. Cheeseman's laboratory. In that study, truncated versions of CENP-C and CENP-T, two proteins that are part of the CCAN, were fused to the Lac repressor protein (LacI) and tethered to chromosomally integrated arrays of bacterial Lac operator (LacO) sequences (Gascoigne et al., 2011). These engineered chromosomal foci consisting of both CENP-C and CENP-T led to recruitment of the outer kinetochore and generated transient functional microtubule attachments.

In the study in this issue, Hori et al. take this approach a major step further. Now, the authors combined the LacI–LacO tethering system with conditional deletion of an endogenous, loxP-flanked centromere, which they previously developed (Fig. 1 A; Shang et al., 2010). By deleting the centromere of the only Z chromosome in chicken DT40 cells, the authors were able to test directly which LacI fusion proteins can rescue chromosome segregation and viability. In human cells, de novo centromere formation has been promoted on ectopically introduced alphoid DNA arrays to which CENP-A deposition factors were tethered (Ohzeki et al., 2012). A strategy to build artificial kinetochores on whole chromosomes has previously been achieved in yeast (Kiermaier et al., 2009; Lacefield et al., 2009) but had thus far not seen its counterpart in a vertebrate cell system.

Strikingly, targeting of the CCAN components CENP-C, CENP-I, or the CENP-A–specific chaperone HJURP is sufficient to initiate a heritable centromere at the LacO array. This ectopic centromere functionally replaced the endogenous one and faithfully maintained chromosome Z ploidy in the cell population. Consistently, the NDC80 complex, the principal microtubule-binding module, and other components of the kinetochore known as the KMN (KNL-1/Mis12/Ndc80) network (Cheeseman et al., 2006) were corecruited to the ectopic site, as well as the chromosomal passenger complex (CPC), components at the inner centromere. Remarkably, CENP-A centromeric chromatin was assembled not only after deposition of its chaperone HJURP but also after tethering of CENP-C or CENP-I (Fig. 1 B). Although these two CCAN components have been shown to be required for CENP-A assembly at endogenous centromeres (Okada et al., 2006; Erhardt et al., 2008; Carroll et al., 2010), these results now show they can also be sufficient for de novo recruitment of CENP-A on naive chromatin. Importantly, this implies that although CENP-A chromatin provides a stable heritable core, its propagation involves a positive epigenetic feedback mechanism in which other CCAN components, themselves dependent on CENP-A, play an active role in CENP-A recruitment.

Building a minimal kinetochore

LacI-mediated targeting of CENP-T or the N terminus of CENP-C, both of which make contacts to the outer kinetochore, proved also sufficient to generate functional ectopic kinetochores. Importantly, analysis of their architecture revealed that these lacked CCAN components, including CENP-C and CENP-A, but recruited the CPC components, providing a functional link between the centromere complex and the inner centromere (Fig. 1 B).

Correspondence to Lars E.T. Jansen: ljansen@igc.gulbenkian.pt

The Rockefeller University Press
J. Cell Biol. Vol. 200 No. 1 7–8
www.jcb.org/cgi/doi/10.1083/jcb.201212005

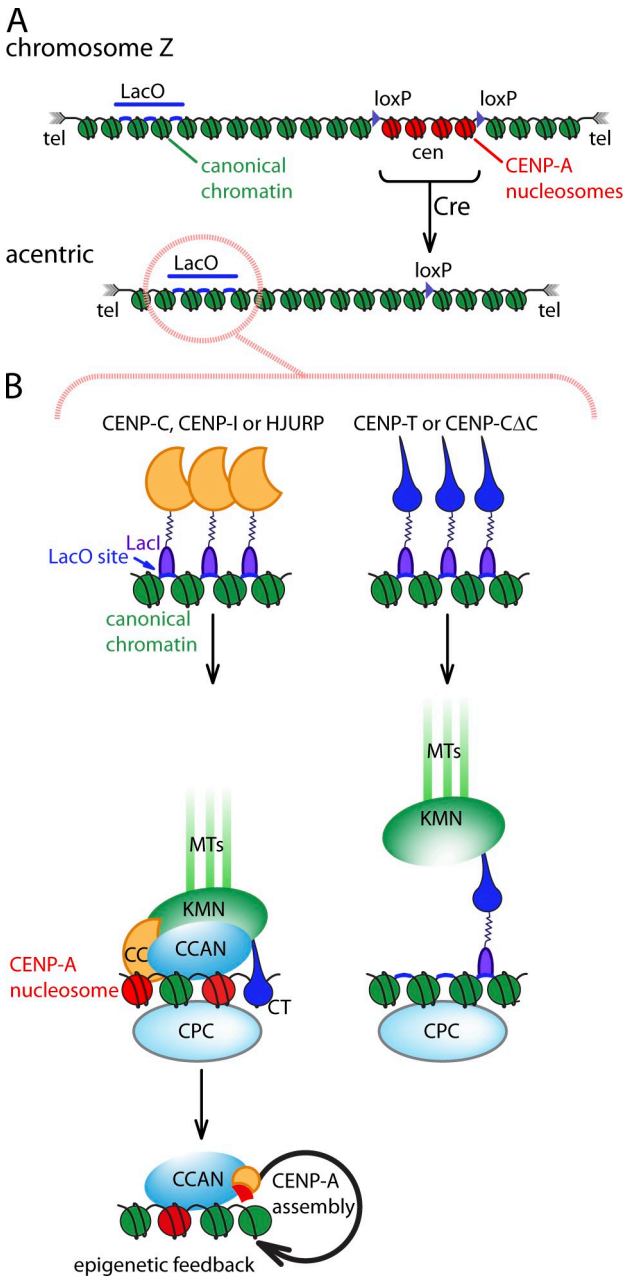

Figure 1. **Engineering vertebrate centromeres.** (A) The endogenous centromere from DT40 chromosome Z is deleted by Cre-loxP–mediated excision. The chromosome is engineered to carry an array of LacO sites. tel, telomere. (B) Fusion of CENP-C, CENP-I, or the CENP-A chaperone HJURP to the Lac repressor (LacI) tethers these proteins to the LacO array and leads to functional replacement of the endogenous centromere through recruitment of centromeric chromatin (CENP-A) and centromere complex (CCAN), the inner centromere (CPC), and the kinetochore (KMN). The CCAN factors CENP-C (CC) and CENP-I are sufficient for CENP-A chromatin establishment, indicating they play a direct role in the maintenance of a heritable centromere core. Tethering of CENP-T (CT) or the CENP-C N terminus (CENP-CΔC) leads to functional LacI tether-dependent kinetochore formation through recruitment of KMN components and the CPC but lacking the remainder of the CCAN. MTs, microtubules.

Despite the lack of centromeric chromatin, these ectopic kinetochores maintained chromosome Z segregation, although not quite as efficiently as a full-fledged neocentromere carrying the remainder of the CCAN and CENP-A chromatin. Consequently, these kinetochores are continuously dependent on the LacI–LacO interaction, as allosteric disruption of LacI binding with IPTG led to rapid loss of chromosome Z. In contrast, CENP-A chromatin/CCAN-containing neocentromeres are independent of the initial seeding event and, once formed, can be weaned from LacI.

These results provide an important functional insight in the role of the centromere complex. On the one hand, it specifies the site of recruitment of kinetochore proteins, whereas on the other, it forms an integral component of a heritable self-replicating protein complex that provides a stable chromosomal anchor. Finally, the system developed by Hori et al. (2013) offers exciting prospects. The construction of highly simplified vertebrate artificial chromosomes in comparison to those currently available, which typically rely on the use of large arrays of centromere-associated DNA repeats, will likely help the field answer critical questions ahead.

Submitted: 3 December 2012
Accepted: 10 December 2012

References

Barnhart, M.C., P.H.J.L. Kuich, M.E. Stellfox, J.A. Ward, E.A. Bassett, B.E. Black, and D.R. Foltz. 2011. HJURP is a CENP-A chromatin assembly factor sufficient to form a functional de novo kinetochore. *J. Cell Biol.* 194:229–243. http://dx.doi.org/10.1083/jcb.201012017

Carroll, C.W., K.J. Milks, and A.F. Straight. 2010. Dual recognition of CENP-A nucleosomes is required for centromere assembly. *J. Cell Biol.* 189:1143–1155. http://dx.doi.org/10.1083/jcb.201001013

Cheeseman, I.M., and A. Desai. 2008. Molecular architecture of the kinetochore-microtubule interface. *Nat. Rev. Mol. Cell Biol.* 9:33–46. http://dx.doi.org/10.1038/nrm2310

Cheeseman, I.M., J.S. Chappie, E.M. Wilson-Kubalek, and A. Desai. 2006. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell*. 127:983–997. http://dx.doi.org/10.1016/j.cell.2006.09.039

Erhardt, S., B.G. Mellone, C.M. Betts, W. Zhang, G.H. Karpen, and A.F. Straight. 2008. Genome-wide analysis reveals a cell cycle–dependent mechanism controlling centromere propagation. *J. Cell Biol.* 183:805–818. http://dx.doi.org/10.1083/jcb.200806038

Foltz, D.R., L.E.T. Jansen, B.E. Black, A.O. Bailey, J.R. Yates III, and D.W. Cleveland. 2006. The human CENP-A centromeric nucleosome-associated complex. *Nat. Cell Biol.* 8:458–469. http://dx.doi.org/10.1038/ncb1397

Gascoigne, K.E., K. Takeuchi, A. Suzuki, T. Hori, T. Fukagawa, and I.M. Cheeseman. 2011. Induced ectopic kinetochore assembly bypasses the requirement for CENP-A nucleosomes. *Cell*. 145:410–422. http://dx.doi.org/10.1016/j.cell.2011.03.031

Guse, A., C.W. Carroll, B. Moree, C.J. Fuller, and A.F. Straight. 2011. In vitro centromere and kinetochore assembly on defined chromatin templates. *Nature*. 477:354–358. http://dx.doi.org/10.1038/nature10379

Hori, T., W.-H. Shang, K. Takeuchi, and T. Fukagawa. 2013. The CCAN recruits CENP-A to the centromere and forms the structural core for kinetochore assembly. *J. Cell Biol.* 200:45–60.

Kiermaier, E., S. Woehrer, Y. Peng, K. Mechtler, and S. Westermann. 2009. A Dam1-based artificial kinetochore is sufficient to promote chromosome segregation in budding yeast. *Nat. Cell Biol.* 11:1109–1115. http://dx.doi.org/10.1038/ncb1924

Lacefield, S., D.T.C. Lau, and A.W. Murray. 2009. Recruiting a microtubule-binding complex to DNA directs chromosome segregation in budding yeast. *Nat. Cell Biol.* 11:1116–1120. http://dx.doi.org/10.1038/ncb1925

Mendiburo, M.J., J. Padeken, S. Fülöp, A. Schepers, and P. Heun. 2011. *Drosophila* CENH3 is sufficient for centromere formation. *Science*. 334:686–690. http://dx.doi.org/10.1126/science.1206880

Ohzeki, J., J.H. Bergmann, N. Kouprina, V.N. Noskov, M. Nakano, H. Kimura, W.C. Earnshaw, V. Larionov, and H. Masumoto. 2012. Breaking the HAC Barrier: histone H3K9 acetyl/methyl balance regulates CENP-A assembly. *EMBO J.* 31:2391–2402. http://dx.doi.org/10.1038/emboj.2012.82

Okada, M., I.M. Cheeseman, T. Hori, K. Okawa, I.X. McLeod, J.R. Yates III, A. Desai, and T. Fukagawa. 2006. The CENP-H-I complex is required for the efficient incorporation of newly synthesized CENP-A into centromeres. *Nat. Cell Biol.* 8:446–457. http://dx.doi.org/10.1038/ncb1396

Shang, W.-H., T. Hori, A. Toyoda, J. Kato, K. Popendorf, Y. Sakakibara, A. Fujiyama, and T. Fukagawa. 2010. Chickens possess centromeres with both extended tandem repeats and short non-tandem-repetitive sequences. *Genome Res.* 20:1219–1228. http://dx.doi.org/10.1101/gr.106245.110