Starting from scratch: *de novo* kinetochore assembly in vertebrates

Sarion R Bowers and Barbara G Mellone*

Department of Molecular and Cell Biology, University of Connecticut, Storrs, CT, USA

*Correspondence to: barbara.mellone@uconn.edu

The EMBO Journal (2011) 30, 3882–3884. doi:10.1038/emboj.2011.329; Published online 6 September 2011

Centromeres are epigenetically defined by the presence of the histone H3 variant, CENP-A. Up until now, the question of how CENP-A nucleosomes mediate kinetochore nucleation was poorly understood. In a study just published in *Nature*, a unique cell-free system was used to demonstrate that *in vitro* assembled CENP-A chromatin arrays recapitulate the assembly of *bona fide* kinetochores through the essential C-terminal tail of CENP-A.

Accurate chromosome segregation requires stable attachments between spindle microtubules and kinetochores, which assemble on centromere templates to mediate chromosome movement during mitosis and meiosis. Centromeres are present throughout the cell cycle and direct kinetochore assembly in a cell-cycle-regulated manner. In most eukaryotes, the centromere locus is inherited independently from the underlying DNA sequence and relies upon the presence of specialized nucleosomes that contain the histone H3 variant CENP-A (Allshire and Karpen, 2008). Because of its essential role in promoting centromere and kinetochore assembly, CENP-A has been proposed to provide the physical foundation onto which additional centromeric and kinetochore proteins are recruited (Allshire and Karpen, 2008; Black and Cleveland, 2011). However, how CENP-A chromatin mediates kinetochore assembly remains elusive. Much attention has been paid to the sequence and structural differences between canonical histone H3 and CENP-A. The region spanning loop 1 and α 2-helix of the histone-fold domain in CENP-A forms the so-called centromere targeting domain (CATD), which is sufficient for centromere targeting of a histone H3-CATD chimera (Black *et al.*, 2007). The CATD is required for CENP-A recognition by HJURP, a chaperone that assembles CENP-A at centromeres (Dunleavy *et al.*, 2009; Foltz *et al.*, 2009; Barnhart *et al.*, 2011). The C-terminal tail of CENP-A, which consists of a unique six amino-acid long chain, was recently shown to be required for the interaction between CENP-C and CENP-A nucleosomes (Carroll *et al.*,

Figure 1 Model of stepwise kinetochore specification. The CATD of CENP-A directs centromere propagation through the specific association with the CENP-A assembly factor HJURP, which is recruited to the centromere by Mis18 (not depicted; Barnhart *et al.*, 2011). Once CENP-A is assembled, CENP-C is recruited via the CENP-A C-terminus and subsequently recruits centromere proteins CENP-K and CENP-N, KMN components, checkpoint proteins CENP-E, ZW10, Mad2, and Rod and microtubules.

2010). CENP-C then provides a physical link between centromeric chromatin and the kinetochore through high affinity binding to the Mis12 complex in humans (Screpanti *et al.*, 2011).

Dissection of the stepwise events that culminate in the assembly of fully functional kinetochores can be hampered by the existence of repetitive DNA sequences underlying centromeric chromatin and by the difficulty in interpreting the effects of depletion of essential proteins on endogenous centromeres. A recent study used ectopic mislocalization to a discrete ectopic chromosomal site to analyse the ability of two key centromere components, CENP-C and CENP-T, to form ectopic kinetochores. Gascoigne *et al.* (2011) showed that ectopically localized CENP-C or CENP-T were sufficient to mediate kinetochore assembly, bypassing the requirement for CENP-A. Ectopic CENP-C and CENP-T recruited subunits of the Knl1–Mis12–Ndc80 complexes (KMN), associated with microtubules and supported segregation of the Z chromosome in chicken DT-40 cells, demonstrating that CENP-C and CENP-T function to direct kinetochore formation in vertebrates. Interestingly, overexpression of CENP-A did not cause ectopic kinetochore formation and only mislocalized a subset of centromeric proteins (Gascoigne *et al.*, 2011). Although CENP-A is recognized as the key upstream factor required for kinetochore specification, these observations suggest that its presence alone is not sufficient to direct new kinetochore formation on non-centromeric templates in humans. A new article by Guse *et al.* (2011) now re-evaluates CENP-A's ability to initiate kinetochore assembly *de novo* using a newly developed cell-free experimental approach.

To determine how CENP-A chromatin promotes to kinetochore assembly, Guse *et al.* generated nucleosome arrays *in vitro* that were designed to mimic the CENP-A arrays present in complex vertebrate centromeres. These arrays utilized 19 repeats of a 147-bp high affinity nucleosome positioning sequence onto which nucleosomes containing either CENP-A, H3 or CENP-A/H3 chimeras were assembled. Magnetic beads coated with these nucleosomal arrays were tested for their ability to recruit centromere and kinetochore components in *Xenopus* egg extracts. Using quantitative immunofluorescence, the authors demonstrated that CENP-A arrays specifically recruit the centromere proteins CENP-C, CENP-N and CENP-K, and the kinetochore protein Ndc80 in a cell-cycle-independent manner, whereas CENP-T displays equal binding to CENP-A and H3 arrays. The CENP-A arrays not only recapitulated essential steps of centromere formation, but were also able to initiate a spindle attachment checkpoint response, as judged by the presence of the checkpoint proteins CENP-E, Mad2, Rod and ZW10 upon addition of nocodazole, and made stable connections with microtubules. Excitingly, with this pliable system, the authors have created the first *in vitro* kinetochore and used it to dissect the requirements for kinetochore specification.

To investigate the contributions of different domains of CENP-A in kinetochore assembly, nucleosome arrays containing CENP-A/H3 chimeras were tested for their ability to recruit CENP-C and CENP-K. Strikingly, a chimera of histone H3 with the CENP-A C-terminal tail (H3^{CAC}) were equally able to recruit CENP-C and CENP-K in this assay. Interestingly, the H3^{CAC} chimera was also able to recruit CENP-N, even though CENP-N was previously shown to require the CATD to bind to CENP-A nucleosomes *in vitro* (Carroll *et al.*, 2009). In con-

trast, the binding of CENP-N to chimeric arrays comprising histone H3 with the CENP-A CATD (H3^{CATD}) or CENP-A with the H3 C-terminal tail (CENP-A^{H3C}) was highly inefficient, indicating that the CENP-A C-terminus is also required for CENP-N association with CENP-A chromatin. Depletion experiments confirmed that in fact CENP-N's ability to bind CENP-A and H3^{CAC} arrays depended upon the presence of CENP-C, leading to the proposal that CENP-C somehow alters the centromeric chromatin to allow CENP-N binding to CENP-A nucleosomes. Alternatively, the requirements for CENP-N recognition of CENP-A nucleosomes may be different in *in vitro* binding assays and in the context of these reconstituted centromeres.

Next, Guse *et al.* showed that, as was observed for the CENP-A arrays, H3^{CAC} arrays efficiently recruited checkpoint proteins, were able to delay exit from mitosis after microtubule depolymerization, and established stable connections with microtubules, whereas H3^{CATD} and CENP-A^{H3C} could not mediate these functions. These results clearly demonstrate that the CENP-A C-terminus is necessary and sufficient for the assembly of *bona fide* kinetochores through recruitment of CENP-C and CENP-N.

An H3^{CATD} chimera was previously shown to support kinetochore function upon CENP-A depletion in HeLa cells (Black *et al.*, 2007). However, in the light of the study by Guse *et al.*, it is unclear how this occurs given that H3^{CATD} nucleosomes presumably cannot recruit CENP-C and the KMN components to endogenous centromeres. One possibility is that there is some type of redundancy *in vivo* whereby recruitment of CENP-C can be maintained even in the absence of the CENP-A C-terminus, albeit transiently. Perhaps such functional compensation is facilitated in the context of pre-existing centromere templates, such as in cells where CENP-A is gradually depleted, through CENP-C dimerization or oligomerization, whereas *de novo* kinetochore nucleation absolutely requires the CENP-A C-terminus. Guse *et al.* propose that the two domains within CENP-A mediate distinct processes: the CATD specifies the interaction with centromere-specific chaperone HJURP, and therefore the maintenance of centromere identity, whereas the C-terminus mediates kinetochore assembly (Figure 1). It will be interesting to determine whether H3^{CATD+CAC} nucleosomes will be sufficient to replace endogenous CENP-A in mediating long-term centromere heritability and faithful kinetochore assembly *in vivo*.

It is worth noting that while CENP-A arrays are sufficient to form functional kinetochores in *Xenopus* extracts (Guse *et al.*, 2011), CENP-A overexpression in human cells is not (Van Hooser *et al.*, 2001; Gascoigne *et al.*, 2011). One possibility is that CENP-A overexpression does not effectively mimic a chromatin template sufficient to support kinetochore assembly whereas continuous CENP-A nucleosomes on an array do. Consistent with this, CENP-A foci generated by the ectopic targeting of HJURP are indeed sufficient to form functional kinetochores (Barnhart *et al.*, 2011).

Future work will define the role of interspersed H3-containing chromatin and chromatin modifications, which are absent in cell-free assays, in contributing to correct kinetochore geometry, bi-orientation and chromosome segregation. For now, these novel cell-free (Guse *et al.*, 2011) and ectopic targeting approaches (Barnhart *et al.*, 2011; Gascoigne *et al.*, 2011) have provided, and will continue to

provide, invaluable mechanistic insights into the incredibly complex nature of centromere and kinetochore specification in vertebrates.

References

- Allshire RC, Karpen GH (2008) Epigenetic regulation of centromeric chromatin: old dogs, new tricks? *Nat Rev Genet* **9**: 923–937
- Barnhart MC, Kuich PHJL, Stellfox ME, Ward JA, Bassett EA, Black BE, Foltz DR (2011) HJURP is a CENP-A chromatin assembly factor sufficient to form a functional *de novo* kinetochore. *J Cell Biol* **194**: 229–243
- Black BE, Cleveland DW (2011) Epigenetic centromere propagation and the nature of CENP-a nucleosomes. *Cell* **144**: 471–479
- Black BE, Jansen LET, Maddox PS, Foltz DR, Desai AB, Shah JV, Cleveland DW (2007) Centromere identity maintained by nucleosomes assembled with histone H3 containing the CENP-A targeting domain. *Mol Cell* **25**: 309–322
- Carroll CW, Milks KJ, Straight AF (2010) Dual recognition of CENP-A nucleosomes is required for centromere assembly. *J Cell Biol* **189**: 1143–1155
- Carroll CW, Silva MCC, Godek KM, Jansen LET, Straight AF (2009) Centromere assembly requires the direct recognition of CENP-A nucleosomes by CENP-N. *Nat Cell Biol* **11**: 896–902
- Dunleavy EM, Roche D, Tagami H, Lacoste N, Ray-Gallet D, Nakamura Y, Daigo Y, Nakatani Y, Almouzni-Pettinotti G (2009) HJURP is a cell-cycle-dependent maintenance and deposition factor of CENP-A at centromeres. *Cell* **137**: 485–497
- Foltz DR, Jansen LET, Bailey AO, Yates III JR, Bassett EA, Wood S, Black BE, Cleveland DW (2009) Centromere-specific assembly of CENP-a nucleosomes is mediated by HJURP. *Cell* **137**: 472–484
- Gascoigne KE, Takeuchi K, Suzuki A, Hori T, Fukagawa T, Cheeseman IM (2011) Induced ectopic kinetochore assembly bypasses the requirement for CENP-A nucleosomes. *Cell* **145**: 410–422
- Guse A, Carroll CW, Moree B, Fuller CJ, Straight AF (2011) *In vitro* centromere and kinetochore assembly on defined chromatin templates. *Nature* **477**: 354–358
- Scrapanti E, De Antoni A, Alushin GM, Petrovic A, Melis T, Nogales E, Musacchio A (2011) Direct binding of Cenp-C to the Mis12 complex joins the inner and outer kinetochore. *Curr Biol* **21**: 391–398
- Van Hooser AA, Ouspenski II, Gregson HC, Starr DA, Yen TJ, Goldberg ML, Yokomori K, Earnshaw WC, Sullivan KF, Brinkley BR (2001) Specification of kinetochore-forming chromatin by the histone H3 variant CENP-A. *J Cell Sci* **114**(Part 19): 3529–3542

Conflict of interest

The authors declare that they have no conflict of interest.