THE EMBO JOURNAL

Have you seen?

The EMBO Journal (2013) 32, 2090–2092
www.embojournal.org

How two become one: HJURP dimerization drives CENP-A assembly

Dani L Bodor and Lars ET Jansen*

Instituto Gulbenkian de Ciência, Oeiras, Portugal
*Correspondence to: ljansen@igc.gulbenkian.pt

The EMBO Journal (2013) **32,** 2090–2092. doi:10.1038/emboj.2013.150; Published online 21 June 2013

CENP-A containing nucleosomes epigenetically specify centromere position on chromosomes. Deposition of CENP-A into chromatin is mediated by HJURP, a specific CENP-A chaperone. Paradoxically, HJURP binding sterically prevents dimerization of CENP-A, which is critical to form functional centromeric nucleosomes. A recent publication in *The EMBO Journal* (Zasadzińska *et al*, 2013) demonstrates that HJURP itself dimerizes through a C-terminal repeat region, which is essential for centromeric assembly of nascent CENP-A.

CENP-A containing nucleosomes have a well-established role in the epigenetic specification of centromere position. However, the composition of the CENP-A nucleosome has been the subject of intense investigation and debate (as has been extensively reviewed, e.g., in Black and Cleveland, 2011). X-ray crystallography data, biochemical interaction experiments and *in vivo* mutational analysis provide strong evidence that CENP-A nucleosomes are octameric (CENP-A/H4/H2A/H2B)$_2$, analogous to their histone H3-containing counterparts (Tachiwana *et al*, 2011; Bassett *et al*, 2012). Alternatively, based primarily on AFM data and nucleosome crosslinking assays, a tetrameric CENP-A/H4/H2A/H2B 'hemisome' has been proposed to be present at centromeres, at least during part of the cell cycle (Dalal *et al*, 2007; Bui *et al*, 2012). Whether both nucleosome types exist under specific conditions remains an unresolved question. However, recent studies by the Maddox and Black labs have reported single-molecule fluorescence measurements of CENP-A nucleosomes and high-resolution DNA protection assays of centromeric chromatin, respectively, both of which indicate that octamers are the predominant species of CENP-A *in vivo* (Hasson *et al*, 2013; Padeganeh *et al*, 2013).

HJURP is the centromeric histone chaperone that is responsible for timely assembly of CENP-A nucleosomes. HJURP binds to soluble CENP-A and is recruited to centromeric chromatin in early G1 phase, concurrently with nascent CENP-A (Stellfox *et al*, 2013). Importantly, HJURP facilitates

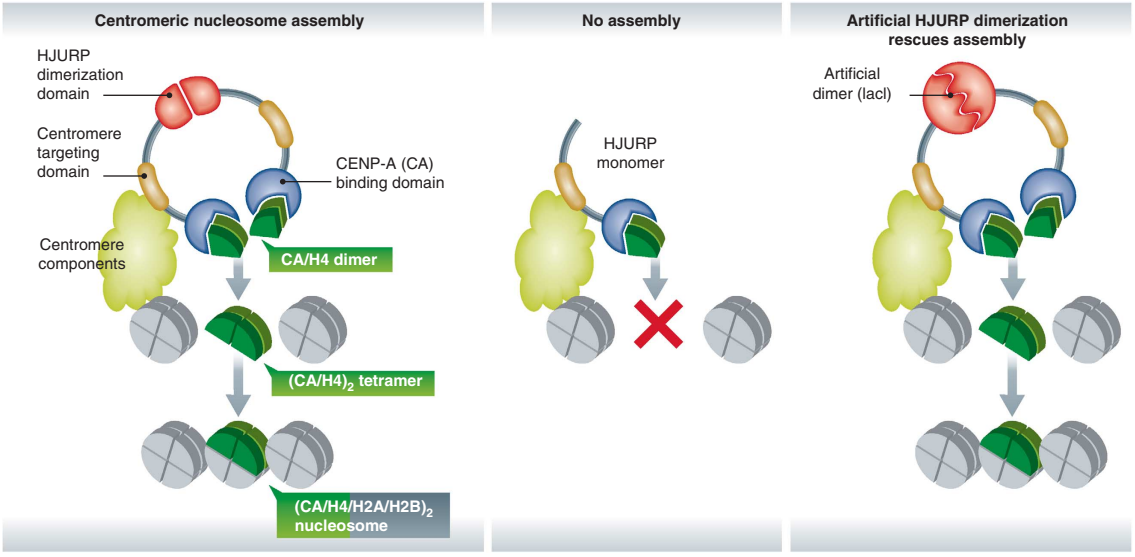

Figure 1 Human HJURP contains separate protein domains that are responsible for CENP-A/H4 binding (blue), centromere targeting (brown) and dimerization (red). Full-length HJURP containing all these domains is capable of assembling CENP-A nucleosomes at centromeres (left). Zasadzińska *et al* (2013) now show that HJURP lacking the dimerization domain is still able to localize to centromeres, but is unable to assemble CENP-A nucleosomes (middle). However, replacement of the HJURP dimerization domain by an exogenous dimerization domain fully rescues the capability to form CENP-A nucleosomes at centromeres (right). These findings show that HJURP dimerization is an essential feature in the process of nucleosome formation, and explain how (CENP-A/H4)$_2$ tetramers can be formed by a chaperone that exclusively binds to CENP-A/H4 dimers.

CENP-A nucleosome formation *in vitro* and its transient targeting to non-centromeric chromatin is sufficient to stably deposit CENP-A at these sites *in vivo* (Barnhart *et al*, 2011). Together, these observations identify HJURP as a *bona fide* centromeric CENP-A histone assembly factor.

However, there is an apparent discrepancy between the role of HJURP in CENP-A assembly and the octameric nature of CENP-A nucleosomes. The crystal structure of the human prenucleosomal complex clearly shows that HJURP binds to CENP-A/H4 dimers in a manner that precludes CENP-A/H4 hetero-tetramerization (Hu *et al*, 2011). Interestingly, however, mutational analysis of CENP-A has shown that tetramerization is crucial for centromere assembly (Bassett *et al*, 2012). Thus, a mechanism must exist to allow for two trimeric HJURP/CENP-A/H4 complexes to coordinately assemble a tetrameric $(CENP\text{-}A/H4)_2$ particle.

In this issue, a study by the Foltz lab sheds light on these paradoxical observations (Zasadzińska *et al*, 2013). Human HJURP contains two C-terminal repeat regions (HJURP C-terminal domains; HCTD). Expression of short fragments of HJURP containing either of these was sufficient to allow for centromere targeting. However, depletion of endogenous HJURP abolished centromere targeting of the C-terminally located HCTD2 fragment, without affecting the localization of the fragment containing HCTD1. These observations suggest that HCTD1 is required for centromere targeting, while HCTD2 allows for HJURP dimerization. Indeed, the authors go on to show that the latter fragment is both necessary and sufficient to form functional dimers of HJURP. RNAi replacement experiments show that HJURP lacking the HCTD2 dimerization domain is incapable of loading nascent CENP-A into centromeres. Importantly, Zasadzińska *et al* (2013) demonstrate that the defect in CENP-A loading can be directly attributed to a lack of HJURP dimerization. In an elegant experiment where the HCTD2 containing domain is replaced by an unrelated dimerization domain (that of bacterial LacI), CENP-A assembly is rescued to wild-type levels (Figure 1). This indicates that dimerization of HJURP is an essential step in centromeric chromatin assembly and provides a potential mechanism for the assembly of tetrameric $(CENP\text{-}A/H4)_2$ structures into chromatin as precursors to octameric nucleosomes.

While the composition of the HJURP complex suggests a likely mechanism for the formation of octameric nucleosomes, this poses a new challenge to the field. Future studies will be needed to dissect how the shielded HJURP-bound state of CENP-A/H4 can transition to a tetramer on DNA. Interestingly, HJURP is not the only histone chaperone that exclusively binds to histone dimers. Crystal structures of trimeric complexes of both Asf1a/H3.1/H4 (English *et al*, 2006) as well as DAXX/H3.3/H4 (Elsässer *et al*, 2012) clearly show sterical incompatibility between chaperone binding and histone tetramerization. It follows that efficient chromatin assembly requires a mode for two histone chaperones to deposit histone dimers in a coordinated fashion, e.g., through dimerization as has been shown for Nap1 (McBryant and Peersen, 2004) and now for HJURP. However, dimerization does not appear to be a universal feature for histone chaperones, as a single CAF1 chaperone is able to bind two H3/H4 dimers as well as $(H3/H4)_2$ tetramers (Winkler *et al*, 2012). Thus, while deposition of H3.1/H4 at the replication fork may be driven by the high density of pre-assembly complexes, assembly of nucleosomes containing the replacement variant H3.3, H3.1 nucleosomes at DNA damage sites, and CENP-A at the centromere would require a more active form of coordination. Histone chaperone dimerization may therefore be a common feature in the pipeline to chromatin formation.

In summary, Zasadzińska *et al* (2013) propose a solution to a paradox in the assembly pathway of CENP-A. They show that while each HJURP molecule can exclusively bind a single CENP-A/H4 dimer, HJURP itself dimerizes, ultimately allowing for the formation of tetrameric $(CENP\text{-}A/H4)_2$ structures in chromatin. Interestingly, exclusive dimer binding has been observed for a number of histone chaperones, suggesting that chaperone dimerization may be a more general process in the nucleosome assembly pathway.

Acknowledgements

We apologize to those authors whose work was not cited due to space restrictions. Work in our lab is supported by a fellowship (SFRH/BD/74284/2010) from the Fundação para a Ciência e a Tecnologia (FCT) to DLB, as well as FCT grants BIA-BCM/100557/2008 and BIAPRO/100537/2008 and an EMBO Installation Grant to LETJ.

Conflict of interest

The authors declare that they have no conflict of interest.

References

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

Bassett EA, DeNizio J, Barnhart-Dailey MC, Panchenko T, Sekulic N, Rogers DJ, Foltz DR, Black BE (2012) HJURP uses distinct CENP-A surfaces to recognize and to stabilize CENP-A/histone H4 for centromere assembly. *Dev Cell* **22:** 749–762

Black BE, Cleveland DW (2011) Epigenetic centromere propagation and the nature of CENP-A nucleosomes. *Cell* **144:** 471–479

Bui M, Dimitriadis EK, Hoischen C, An E, Quénet D, Giebe S, Nita-Lazar A, Diekmann S, Dalal Y (2012) Cell-cycle-dependent structural transitions in the human CENP-A nucleosome *in vivo*. *Cell* **150:** 317–326

Dalal Y, Wang H, Lindsay S, Henikoff S (2007) Tetrameric structure of centromeric nucleosomes in interphase *Drosophila* cells. *PLoS Biol* **5:** e218

Elsässer SJ, Huang H, Lewis PW, Chin JW, Allis CD, Patel DJ (2012) DAXX envelops a histone H3.3-H4 dimer for H3.3-specific recognition. *Nature* **491:** 560–565

English CM, Adkins MW, Carson JJ, Churchill MEA, Tyler JK (2006) Structural basis for the histone chaperone activity of Asf1. *Cell* **127:** 495–508

Hasson D, Panchenko T, Salimian KJ, Salman MU, Sekulic N, Alonso A, Warburton PE, Black BE (2013) The octamer is the major form of CENP-A nucleosomes at human centromeres. *Nat Struct Mol Biol* **20:** 687–695

Hu H, Liu Y, Wang M, Fang J, Huang H, Yang N, Li Y, Wang J, Yao X, Shi Y, Li G, Xu R-M (2011) Structure of a CENP-A-histone H4

heterodimer in complex with chaperone HJURP. *Genes Dev* **25:** 901–906

McBryant SJ, Peersen OB (2004) Self-association of the yeast nucleosome assembly protein 1. *Biochemistry (Mosc.)* **43:** 10592–10599

Padeganeh A, Ryan J, Boisvert J, Ladouceur A-M, Dorn JF, Maddox PS (2013) Octameric CENP-A nucleosomes are present at human centromeres throughout the cell cycle. *Curr Biol Cb* **23:** 764–769

Stellfox ME, Bailey AO, Foltz DR (2013) Putting CENP-A in its place. *Cell Mol Life Sci* **70:** 387–406

Tachiwana H, Kagawa W, Shiga T, Osakabe A, Miya Y, Saito K, Hayashi-Takanaka Y, Oda T, Sato M, Park S-Y, Kimura H, Kurumizaka H (2011) Crystal structure of the human centromeric nucleosome containing CENP-A. *Nature* **476:** 232–235

Winkler DD, Zhou H, Dar MA, Zhang Z, Luger K (2012) Yeast CAF-1 assembles histone (H3-H4)2 tetramers prior to DNA deposition. *Nucleic Acids Res* **40:** 10139–10149

Zasadzińska E, Barnhart-Dailey MC, Kuich PHJL, Foltz DR (2013) Dimerization of the CENP-A assembly factor HJURP is required for centromeric nucleosome deposition. *EMBO J* **32:** 2113–2124

