New insights into how chromatin remodellers direct CENP-A to centromeres

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Centromeric nucleosomes contain the unique histone H3 variant centromere protein A (CENP-A). How CENP-A is specifically loaded onto centromeres and maintained is an active area of investigation and several SNF2 family chromatin remodellers have been implicated in this process. In this issue of *The EMBO Journal*, Gkikopolus *et al* (2011) demonstrate a novel function for the SWI/SNF complex in removal of Cse4 (CENP-A in *Saccharomyces cerevisiae*) from ectopic chromosomal locations. Here we highlight this finding and the role of different remodeling complexes in CENP-A targeting to centromeres.

Centromeres are regions present in each eukaryotic chromosome that are required for faithful segregation of chromosomes during cell division. The centromere is epigenetically specified by nucleosomes bearing a unique histone H3 variant. Homologues of this variant are present in all eukaryotes and are called Cse4 in budding yeast, Cnp1 in fission yeast, CID in flies and CENP-A in humans. CENP-A-containing nucleosomes impart identity to the centromere and provide a platform for the assembly of kinetochore proteins that drive chromosome segregation. Despite extensive research in this area, it is still unclear exactly how CENP-A is specifically targeted to the centromere. Current models for the propagation of CENP-A chromatin involve a series of maturation steps to form a functional centromere at mitosis, including a reduction of the CENP-A amount by half during DNA replication (Black and Cleveland, 2011). ATP-dependent chromatin remodellers belonging to the SNF2 family of helicases can contribute to disassembly (eviction) and assembly of nucleosomes. This can lead to changed composition and repositioning of nucleosomes. Thus, SNF2 enzymes are good candidates for being involved in CENP-A targeting.

How do chromatin remodellers contribute to CENP-A propagation?

The remodelling and spacing factor (RSF) interacts with CENP-A chromatin and knock-down of RSF in HeLa cells

Figure 1 The role of chromatin remodelling complexes at the centromeres. (A) Concerted action of histone chaperones and the RSF remodeller in CENP-A targeting. (B) Transcription-coupled disassembly of canonical nucleosomes by the Chd1 remodeller, allowing for assembly of CENP-A nucleosomes. (C) Maintenance of centromere structure by the action of the Fft3 remodeller at boundary elements. (D) Removal of CENP-A by SWI/SNF from ectopic locations.

leads to a reduction of the CENP-A levels at centromeres. RSF can reconstitute regularly spaced CENP-A nucleosomes *in vitro*. It was suggested that CENP-A assembly occurs in two steps during the G1 stage of the cell cycle. First a weak association of CENP-A to the centromeres is mediated by a histone chaperone, followed by RSF remodelling to enhance CENP-A stability at the centromeres (Figure 1A) (Perpelescu *et al*, 2009). Remodelling factors have also been suggested to contribute to CENP-A targeting by evicting H3. In fission yeast, Hrp1 (a Chd1 homologue) was shown to be required for maintaining appropriate levels of Cnp1 (CENP-A in fission yeast) (Walfridsson *et al*, 2005). It is clear from genome-wide studies that Hrp1 is responsible for lowering the histone H3 density at gene promoters (Walfridsson *et al*, 2007). More recently we have found that Hrp1 remodelling occurs at cryptic RNAPII-dependent promoters in Cnp1 chromatin at centromeres and that a low level of Cnp1 is present at gene promoters where H3 is depleted by Hrp1, suggesting that Hrp1 has a similar role at centromeres and gene promoters (Choi ES, Stralfors A, Castillo GA, Durand-Dubief M, Ekwall K and Allshire RC, unpublished data). In chicken DT40 cells, targeting of Chd1 to the centromeres and CENP-A loading have been shown to be physically associated with SSRP1, a subunit of the FACT (Facilitates chromatin transcription) complex, and knockdown of Chd1 in HeLa cells results in CENP-A loading defects (Okada *et al*, 2009). Thus, it is conceivable that Chd1 plays a similar role in transcription-coupled nucleosome disassembly steps to promote CENP-A targeting in these cases (Figure 1B).

Chromatin remodellers may also contribute to CENP-A targeting more indirectly by stabilizing higher-order chromatin structures at centromeres. The chromatin remodeller Fft3 is localized to the boundaries of centromeric and telomeric chromatin domains in fission yeast. Interestingly, there is a reduction of the Cnp1 levels in *fft3Δ* cells and an increase of H3, H2A.Z and acetylated H4, leading to defects in centromere function (Stralfors *et al*, 2011). It is likely that Fft3

remodelling of boundary element chromatin leads to protection of the centromeric domain (Figure 1C).

Since CENP-A provides a functional identity to centromeres, mis-localization of CENP-A to non-centromeric sites also needs to be prevented. Previously, one such mechanism was described as involving ubiquitin-mediated degradation of non-centromeric CENP-A (Ranjitkar *et al*, 2010). In this issue of *The EMBO Journal*, Gkikopolus *et al* (2011) describe a novel role for a chromatin remodelling factor, SWI/SNF, in preventing ectopic CENP-A localization. These authors show that SNF2 removes Cse4 from non-centromeric locations in *S. cerevisiae*. Deletion of *snf2* results in a significant increase in non-centromeric Cse4, correlated with reduced Cse4 at centromeres and defects in chromosome segregation (Figure 1D). Furthermore, *in vitro* experiments demonstrated that SNF2 specifically disassembles Cse4-containing nucleosomes. Curiously, SNF2 is localized at centromeres and may also be directly involved in regulating centromere structure. An interesting question, therefore, is how the centromeric Cse4 is protected from eviction by SNF2. Intriguingly, another chromatin remodeller, RSC, is also physically associated with the budding yeast centromere but is dispensable for Cse4 deposition (Hsu *et al*, 2003). A challenge now is to understand how the joint activities of SNF2 and RSC are connected to centromere function in budding yeast.

To conclude, CENP-A targeting seems rather complex with multiple chromatin remodelling mechanisms involved, including the removal of CENP-A from non-centromeric locations. It remains to be determined to what extent the roles of chromatin remodellers in this process are conserved between species. The multitude of chromatin remodelling mechanisms could reflect an evolutionary plasticity in CENP-A targeting mechanisms.

Conflict of interest

The authors declare that they have no conflict of interest.

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