Cell Cycle 12:19, 3133–3134; October 1, 2013; © 2013 Landes Bioscience

# An evolving tail of centromere histone variant CENP-A

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The reproduction and development of all organisms depends upon the accurate segregation of chromosomes during mitosis. Chromosome segregation is facilitated by the assembly of the kinetochore complex and its attachment to mitotic spindle microtubules at the chromosomal centromere. The centromere is specified by the nucleosome in which canonical histone H3 is replaced by its variant CENP-A. A key question in centromere biology is how the kinetochore complex recognizes the centromere nucleosome. Earlier studies have shown that human centromere proteins C (CENP-C) and N (CENP-N), components of the kinetochore complex, specifically recognize the CENP-A nucleosome,[1,2] and CENP-C is found in all model systems for centromere studies.[3] It is also known that the CENP-C central region (residues 426–537) and the CENP-C motif (residues 736–758) are required for CENP-C targeting to the centromere.[2,4] The CENP-C central region directly binds to the CENP-A nucleosome in vitro,[2] whereas the targeting mechanism for the CENP-C motif is unknown.

In the recent issue of *Science*,[5] we reported a conserved structural mechanism for centromeric nucleosome recognition by CENP-C. We discovered that both the CENP-C central region and the CENP-C motif dock on the acidic patch region of histone H2A and H2B in the nucleosome through electrostatic interactions, which facilitates their recognition of the CENP-A C-terminal tail through hydrophobic interactions (**Fig. 1A and B**). Our study provides the structural basis for the specific link between centromeric chromatin and the kinetochore complex and explains a number of diverse experimental results, including the lack of CENP-C localization to the centromere of *Xenopus* sperm chromatin in which H2A and H2B are replaced by protamines. In addition, our results favor an octameric nucleosome for attachment of the kinetochore core during M phase of the cell cycle over various other structural forms. This is because CENP-C exists as a dimer that can bind both sides of the octameric nucleosome, and there seems to be only one centromeric nucleosome in the budding yeast *S. cerevisiae*.

The most surprising feature of the CENP-A nucleosome recognition by CENP-C is the widely conserved nature of the CENP-C motif in terms of its amino acid sequence, which the C-terminal tail of CENP-A lacks (**Fig. 1C and D**). The only conserved feature of the C-terminal tail of CENP-A is its higher hydrophobicity in comparison with that of H3. The C-terminal tail of human CENP-A also includes Gly and Glu residues, whereas they are absent in the CENP-A (Cse4) of *S. cerevisiae*. Based on the structure of the CENP-C/CENP-A nucleosome complex, the lack of a side chain in Gly seems important for the formation of a turn structure that allows the hydrophobic residues in the CENP-A C-terminal tail to establish interactions upon binding of CENP-C. However, the role of the Glu residues is not obvious from the structure of the complex. In particular, when Glu is mutated to Ala, the binding affinity between CENP-C and the CENP-A nucleosome increases, indicating that the CENP-A C-terminal tail has not evolved for high binding affinity.

We hypothesize that the Glu residues in the C-terminal tail of CENP-A have evolved to bind CENP-A chaperones. Our hypothesis is based on the observation that human CENP-A and budding yeast Cse4 bind to their corresponding chaperones HJURP and Scm3 differently (**Fig. 1E and F**). In the structure of the human HJURP/CENP-A/H4 complex, the HJURP helix is highly positively charged and interacts with the acidic residues of an extended α-helix in the C-terminal tail of CENP-A.[6] In other words, the C-terminal tail of CENP-A, which includes only 6 residues, is required to interact with 2 different partners, CENP-C and HJURP, using hydrophobic and electrostatic interactions, respectively. Sequence alignment of the C-terminal tail of CENP-A shows that in addition to the Gly and hydrophobic residues, most vertebrates have acidic residues (**Fig. 1C**), suggesting that the interactions between CENP-A tail and HJURP are likely conserved in vertebrates. In contrast, the structure of the Scm3/Cse4/H4 complex[7,8] shows that the disordered C-terminal region of Cse4 is not required for interaction with Scm3. In this regard, it is interesting to note that in most yeasts, only hydrophobic residues are a conserved feature. Although exceptions exist, Gly and acidic residues are lacking. Thus, it is likely that the Gly and acidic residues were acquired later during evolution to interact with CENP-A chaperones. The interaction of HJURP with the C-terminal tail of CENP-A also suggests that the tail also plays a role in CENP-A deposition at the centromere. Finally, the observation that the short C-terminal tail of human CENP-A can bind to more than one protein is consistent with the fact that in higher eukaryotes intrinsically disordered proteins often interact with multiple proteins.

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Submitted: 07/04/2013; Accepted: 07/12/2013
http://dx.doi.org/10.4161/cc.26353
Comment on: Kato H, et al. Science 2013; 340:1110-3; PMID:23723239; http://dx.doi.org/10.1126/science.1235532

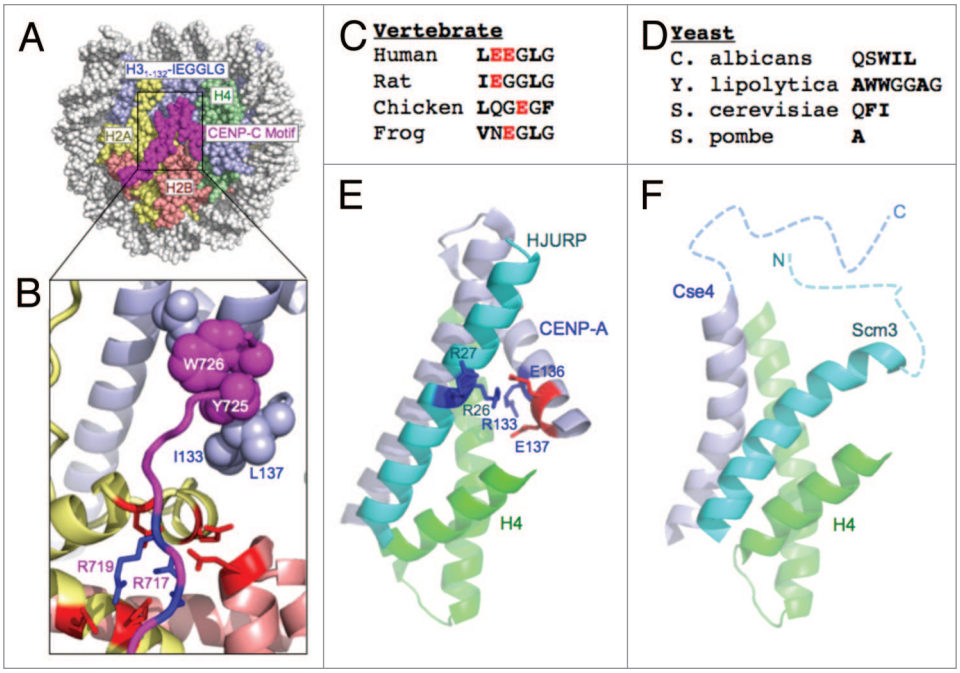


**Figure 1.** The C-terminal tail of CENP-A binds to centromere protein CENP-C and chaperone HJURP. (**A**) Space filling presentation of the overall structure of the rat CENP-C motif in complex with a nucleosome containing the $H3_{1–132}$-IEGGLG chimera. (**B**) Enlarged illustration of the interactions between the CENP-C motif and the $H3_{1–132}$-IEGGLG nucleosome. Blue and red sticks represent the positively charged Args in the CENP-C motif and the acidic residues in histones H2A and H2B. (**C**) Amino acid sequences of the CENP-A C-terminal tails in several vertebrate species. (**D**) Amino acid sequences of the CENP-A C-terminal tails of several yeast species. (**E**) A structural region of the HJURP/CENP-A/H4 complex, illustrating the electrostatic interactions between HJURP and the C-terminal tail of CENP-A. (**F**) A region of the Scm3/Cse4/H4 complex corresponding to that in (**E**), showing that the N-terminal region of Scm3 and the C-terminal region of Cse4 are disordered.

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