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The inheritance of centromere identity

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ABSTRACT

CENP-A (Centromere protein A) is a histone H3 variant that epigenetically determines the centromere position, but the mechanism of its centromere inheritance is obscure. We propose that CENP-A ubiquitylation, which is inherited through dimerization between rounds of cell division, is a candidate for the epigenetic mark of centromere identity.

Centromere protein A (CENP-A) is a centromere-specific histone H3 variant that plays an essential role in ensuring kinetochore assembly for proper chromosome segregation. CENP-A nucleosomes are required for active centromeres that recruit a constitutive centromere-associated network (CCAN) and the other kinetochore proteins in a DNA sequence–independent manner.[1] In most species, except budding yeast, centromere identity relies not on the DNA sequence but on the presence of a special nucleosome that contains CENP-A.[1] CENP-A nucleosomes in human chromosomes localize to the inner kinetochores and bind to the 171-bp α-satellite DNA.[1] Therefore, CENP-A is proposed to be the epigenetic mark of centromere identify. The key question is how human CENP-A functions as the epigenetic mark at the molecular level. Our previous studies showed that CUL4A (Cullin 4A)-RBX1 (Ring-box 1)-COPS8 (COP9 signalosome complex subunit 8) E3 ligase activity is required for ubiquitylation of lysine 124 (K124) in CENP-A and centromere localization of CENP-A during the M and G1 phases.[2]

In our recent article published in *Cell Reports*,[3] we demonstrated that CENP-A K124 ubiquitylation is epigenetically inherited through dimerization between rounds of cell division. Our results of in vivo and in vitro ubiquitylation assays using a constitutively ubiquitylated CENP-A mutant clearly showed that ubiquitylated CENP-A is required for ubiquitylation of nonubiquitylated CENP-A. Therefore, the heterodimer (i.e., a dimer of old CENP-A and new CENP-A) is presumably recognized by the CUL4A complex, and the new CENP-A is ubiquitylated and maintained at the centromeres.[2] Based on these results, we provide a model of epigenetic inheritance of CENP-A ubiquitylation for the control of CENP-A deposition and maintenance at centromeres (Fig. 1). CENP-A–containing nucleosomes are formed with canonical histones H2A, H2B, and H4 at the active centromeres, but the current model of interconversion between tetrameric and octameric CENP-A nucleosomes in the cell cycle remains controversial[1] Here, we have provided an octamer model of epigenetic inheritance of CENP-A ubiquitylation for simplicity (Fig. 1). H3.3 is deposited at centromeres during S phase as a placeholder for CENP-A that is newly assembled during G1 phase[4] (Fig. 1, S phase). In this octamer model, 2 CENP-A dimers in one nucleosome are split/diluted between the 2 daughter centromere-DNA sequences, and one CENP-A molecule is either replaced with one H3 molecule or leaves a molecule-free gap during replication/S phase (Fig. 1, S phase). Evidence from our studies and others[3] supports our proposed model in which Holliday junction recognition protein (HJURP) preferentially binds to ubiquitylated, preassembled "old" CENP-A, which resides predominantly in nucleosomes (Fig. 1, (1), anaphase/telophase). During this process, newly synthesized, free CENP-A targets ubiquitylated centromeric CENP-A through its attraction to HJURP (Fig. 1, (1), telophase/early G1). Subsequently, new CENP-A is ubiquitylated in the proximity of the nucleosome and/or inside the nucleosomes in a heterodimerization-dependent manner (old CENP-A–new CENP-A) (Fig. 1, (1), telophase/early G1), and HJURP partly contributes to ubiquitylation. Thus, in these models ubiquitylation and the location of the centromere are inherited epigenetically (Fig. 1, (1)).

If K124 ubiquitylation does not occur on newly synthesized CENP-A (Fig. 1, (2), first telophase/early G1), non-ubiquitylated CENP-A would occupy one of the duplicated/split nucleosomes when old CENP-A is distributed during the S phase (Fig. 1, (2), S
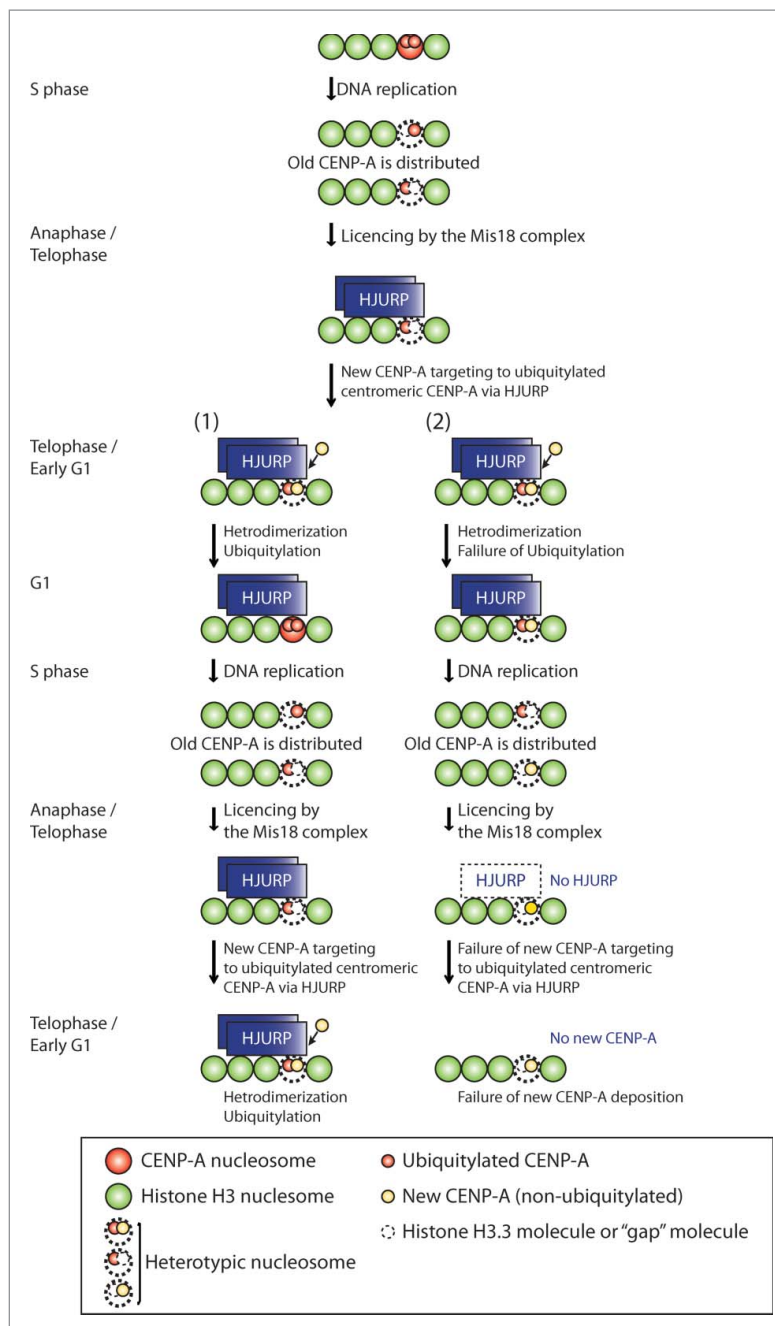

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Figure 1. Models of epigenetic inheritance of centromere protein A (CENP-A) ubiquitylation. In the octamer model, 2 CENP-A dimers in one nucleosome are split/diluted between the 2 daughter centromere-DNA sequences, and one CENP-A molecule is either replaced with one H3 molecule or leaves a molecule-free gap during replication/S phase. HJURP (Holliday junction recognition protein) preferentially binds to ubiquitylated, preassembled "old" CENP-A, which resides predominantly in nucleosomes. A new CENP-A monomer targets ubiquitylated centromeric CENP-A via preassembled HJURP. Note that histone H4 is omitted for simplicity. (1) New CENP-A is properly ubiquitylated in a heterodimerization-dependent manner (i.e., dimers of old CENP-A with new CENP-A). In this way, both ubiquitylation and the location of the centromere are inherited epigenetically. (2) If K124 ubiquitylation does not occur on newly synthesized CENP-A, the non-ubiquitylated CENP-A nucleosome distributed during the S phase does not recruit HJURP to the centromere because the affinity of non-ubiquitylated CENP-A to HJURP is low. Subsequently, this loss of localization of HJURP at the centromere leads to the failure of new CENP-A targeting to ubiquitylated centromeric CENP-A via HJURP, and eventually to the failure of new CENP-A deposition.

phase). This non-ubiquitylated CENP-A nucleosome does not recruit HJURP at the centromere because the affinity of non-ubiquitylated CENP-A to HJURP is low (Fig. 1, (2), second telophase). Subsequently, this loss of localization of HJURP at the centromere leads to a failure to target new CENP-A to ubiquitylated centromeric CENP-A via HJURP, and eventually to the failure of new CENP-A deposition (Fig. 1, (2), second telophase/early G1). Therefore, CENP-A ubiquitylation is necessary for epigenetic inheritance of the centromere location in humans.

More than 100 neocentromeres in human clinical samples have been described.[5] They form on diverse DNA sequences and are associated with CENP-A localization but not with α-satellite arrays. These findings provide strong evidence that human centromeres result from DNA sequence-independent epigenetic mechanisms. However, human neocentromeres have not yet been created experimentally; overexpression of CENP-A induces mislocalization of CENP-A but not the formation of functional neocentromeres.[6] Identification and analysis of

factors essential for the generation of human neocentromeres is important to clarify the mechanism of epigenetic inheritance of centromeres. In our study,[3] overexpression of the monoubiquitin fusion protein Flag-CENP-A K124R-Ub (K48R) led to sufficient recruitment of HJURP and central-outer kinetochore components to noncentromeric chromatin regions, and SKA1-positive putative neocentromeres were replicated and inherited epigenetically between rounds of cell division.

CENP-A has been proposed to be the epigenetic mark of the centromere identity in many studies.[1] However, we have shown that overexpression of CENP-A itself is not sufficient for the creation of a neocentromere at a noncentromeric region (Fig. 1) and that ubiquitylation of CENP-A is necessary for the formation of neocentromeres and for epigenetic inheritance of the centromere location in humans.[3] Considering that histone post-translational modifications are traditionally defined as "epigenetic marks", we propose that CENP-A ubiquitylation is a candidate for the epigenetic mark of centromere location, i.e., the centromere identity.

Overexpression of CENP-A and addition of neocentromere to a chromosome with an endogenous centromere results in aneuploidy, which can lead to cancer.[7-9] Thus, revealing the mechanism that controls quantitative amounts of CENP-A and neocentromere formation will contribute to our understanding of the mechanism of "cancer evolution" that results in resistance to cancer therapy.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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