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# The KAT's Out of the Bag: Histone Acetylation Promotes Centromere Assembly

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**Heterochromatin is incompatible with centromeric chromatin assembly and propagation. In this issue of *Developmental Cell*, Ohzeki et al. (2016) reveal that a critical role of the Mis18 complex is to transiently recruit the lysine acetyltransferase KAT7 to centromeres to facilitate the removal of H3K9me3 and the deposition of CENP-A.**

In eukaryotes, the accurate distribution of genetic material through cell division is dependent on the centromere, which is marked by the histone H3 variant CENP-A. Metazoan centromeric chromatin is composed of stretches of alternating CENP-A and H3 nucleosomes and is typically embedded within heterochromatin harboring H3K9 trimethylation (H3K9me3). Centromeric histones are hypoacetylated and marked by H3K4me2, H3K36me2/3, and H4K20me1, while lacking H3K9me3 (Sullivan and Karpen, 2004; McKinley and Cheeseman, 2016). Preventing heterochromatin from encroaching on the centromere is critical for centromere integrity and maintenance. An article in this issue of *Developmental Cell* by Ohzeki and colleagues (2016) identifies the mechanism by which histone acetylation antagonizes heterochromatin, allowing the deposition of CENP-A at the centromere.

In human HT1080 cells, centromeres can form de novo on human artificial chromosomes that harbor a synthetic centromere array composed of a dimeric α-satellite repeat with one natural monomer, containing the CENP-B DNA binding motif, and one artificial monomer, in which the CENP-B box is replaced by the tetracycline operator (alphoid$^{tetO}$). Recruitment of transcriptional silencers fused to the tetracycline repressor (tetR) to the array leads to the accumulation of heterochromatic marks and abolishes centromere formation (Nakano et al., 2008).

In HeLa cells, de novo CENP-A assembly on the alphoid$^{tetO}$ array is impaired due to high levels of H3K9me3. Tethering histone acetyl transferases (HATs) to the heterochromatic alphoid$^{tetO}$ array allows for stable assembly of CENP-A chromatin (Ohzeki et al., 2012). However, the significance of these findings in the context of endogenous centromere function and maintenance have remained elusive, particularly given the observation that human centromeric chromatin is generally hypoacetylated (Sullivan and Karpen, 2004).

In humans, deposition of nascent CENP-A to centromeres occurs during telophase/G1 and is mediated by the assembly factor HJURP, which is recruited by the Mis18 complex (composed of hMis18α, hMis18β, and Mis18BP1/M18BP1). Knockdown of hMis18α or Mis18BP1 leads to defective deposition of new CENP-A and to gradual loss of pre-existing CENP-A from centromeres. Surprisingly, global inhibition of histone deacetylation with Tricostatin A was reported to rescue the loss of CENP-A in cells depleted of hMis18α. These results led to the hypothesis that one function of the Mis18 complex is to mediate a centromere licensing event involving histone acetylation and preceding CENP-A deposition (Fujita et al., 2007).

In this new study, Ohzeki and colleagues (2016) set off to identify HATs that may interact with the Mis18 complex. Using an integrated alphoid$^{tetO}$/tetR tethering assay in HeLa cells, they screened nearly all known lysine acetyl transferases (KATs) for their ability to be recruited by tethered subunits of the Mis18 complex. The only KAT identified through these assays was KAT7, which is a component of the MYST family protein complexes. The authors found that KAT7 interacts specifically with Mis18BP1 and that tethering KAT7 to the alphoid$^{tetO}$ array recruits additional MYST complex subunits and the chromatin-remodeling factor RSF1, which is required for proper CENP-A chromatin incorporation (Perpelescu et al., 2009). Interestingly, tethering of another HAT, PCAF, to the alphoid$^{tetO}$ array also stimulates RSF1 recruitment, indicating that acetylated chromatin promotes RSF1 binding.

The Mis18 complex associates with centromeres only transiently, during telophase/G1. Ohzeki and colleagues (2016) found that KAT7 is enriched at native centromeres primarily during G1, consistent with Mis18BP1 mediating its recruitment. The authors also detected a KAT7-dependent increase in H3K14ac at the endogenous alphoid DNA of chromosomes X and 21 in G1, suggesting that KAT7 transiently acetylates centromeric chromatin around the time of new CENP-A deposition.

Importantly, KAT7 knockout (KAT7KO) or knockdown by siRNA resulted in defective CENP-A loading at native centromeres, and overexpression of KAT7 in these cells restored normal centromeric CENP-A levels. These data suggest that KAT7-mediated H3K14 acetylation, and possibly that of additional histone lysines, is required for proper incorporation of new CENP-A at centromeres.

Defects in centromere function lead to chromosome segregation errors that can result in the formation of micronuclei. Interestingly, loss of KAT7 in conjunction with overexpression of the H3K9me3 methyltransferase Suv39h1 resulted in even lower CENP-A levels at centromeres and in micronuclei formation. These findings suggest that KAT7 may antagonize the deleterious effects of

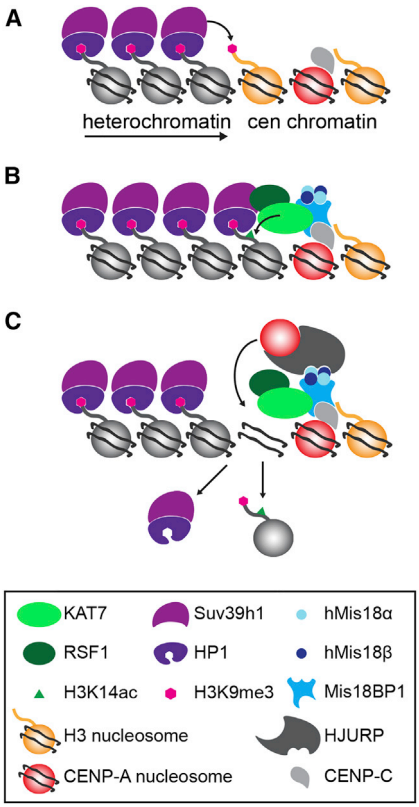


**Figure 1. Centromeric Chromatin Acetylation by KAT7 Antagonizes Heterochromatin Spreading**

(A) The presence of heterochromatin on alphoid DNA is incompatible with new CENP-A deposition. Heterochromatin (gray circles) surrounds centromeric chromatin and has the potential to spread when Suv39h1 is overexpressed.

(B) In telophase/G1, the Mis18 complex recruits KAT7, along with the chromatin remodeling factor RSF1, to the centromere. KAT7 acetylates lysine 14 of histone H3 (H3K14ac), and possibly additional lysines, and transcription is stimulated (not shown).

(C) RSF1, and possibly transcription, promotes the removal of H3 trimethylated at K9 (H3K9me3). The hMis18α/β heterotetramer recruits the CENP-A assembly factor HJURP, which deposits new CENP-A.

heterochromatin spreading onto centromeric chromatin.

To test whether KAT7 and RSF1 can indeed counteract heterochromatin, the authors assessed their ability to remove H3K9me3 from the alphoid$^{tetO}$ array in HeLa cells. Not only did the tethering of KAT7 or RSF1 result in the reduction of H3K9me3 at the alphoid$^{tetO}$ array, but it also promoted the deposition of nascent histone H3.3 and CENP-A, presumably via their respective assembly factors. Importantly, KAT7 or RSF1 tethering stimulated transcription of the alphoid$^{tetO}$ array. This finding is consistent with previous studies showing the importance of transcription for de novo CENP-A chromatin establishment in *S. pombe*, flies, and humans (Catania et al., 2015; Chen et al., 2015; Nakano et al., 2008).

In hMis18α siRNA cells, centromeric CENP-A levels can be restored by increasing global histone acetylation (Fujita et al., 2007), and Ohzeki and colleagues (2016) show that KAT7 overexpression partially ameliorates CENP-A centromeric localization under these conditions. Collectively, these findings suggest that a key role for the Mis18 complex is to recruit KAT7 to centromeres to promote transient chromatin acetylation. However, how HJURP is directed to centromeres in cells in which Mis 18 is knocked-down is unclear. The observation that new CENP-A recruitment via tethered KAT7 only occurs on synthetic alphoid$^{tetO}$ arrays, and not on synthetic non-alphoid$^{tetO}$ arrays, suggests that properties of alphoid DNA itself, such as the presence of the CENP-B DNA-binding motif, are required for de novo CENP-A deposition. Recently, CENP-B was found to stabilize CENP-A and fortify centromere function (Fachinetti et al., 2015). Perhaps, in the absence of the Mis18 complex, CENP-B can direct the CENP-A assembly machinery to centromeres, as long as histone acetylation can still occur to promote histone turnover.

Overall, the work of Ohzeki and colleagues (2016) elucidates the role of acetylation in CENP-A chromatin assembly at native centromeres, expanding upon previous studies that used synthetic centromeres assembled at ectopic synthetic arrays. These new findings support a model whereby acetylated centromeric chromatin recruits RSF1, which in turn reorganizes chromatin and promotes histone exchange, leading to recruitment of CENP-A (Figure 1). Since centromeric chromatin is generally hypoacetylated, we speculate that lysine deacetylases are recruited at the end of G1 to prevent excessive CENP-A loading. Further work will elucidate the respective contributions of lysine acetylation, RSF1-mediated chromatin remodeling, and transcription to CENP-A deposition.

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