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# The Split Personality of CENP-A Nucleosomes

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## Abstract

The composition and structure of centromeric nucleosomes, which contain the histone H3 variant CENP-A, is intensely debated. Two independent studies in this issue, in yeast and human cells, now suggest that CENP-A nucleosomes adopt different structures depending on the stage of the cell cycle.

A specialized chromatin domain, called the centromere, ensures accurate chromosome segregation during mitosis. Centromeres are the foundation for the assembly of the kinetochore, the site on each chromosome that acts as the primary interface between the chromosomes and the microtubules of the mitotic spindle. Maintaining the centromere is therefore essential for chromosome stability. The chromatin mark that determines centromere identity is a specialized histone H3 variant called CENP-A (called Cse4 in the budding yeast *Saccharomyces cerevisiae*). Budding yeast assemble Cse4 into chromatin during each round of DNA replication, whereas vertebrate CENP-A nucleosome assembly is replication independent, occurring during telophase and G1.

A recent, controversial question is whether CENP-A nucleosomes differ in structure and composition compared to H3 nucleosomes. Structural and biochemical studies of both reconstituted and purified CENP-A nucleosomes have demonstrated that CENP-A and H4 can form a soluble histone tetramer $(CENP\text{-}A/H4)_2$ and, in the presence of DNA and H2A/H2B, an octameric nucleosome $(CENP\text{-}A/H4/H2A/H2B)_2$ akin to H3 nucleosomes (reviewed in Maddox et al., 2012). However, other studies have suggested a more unique organization of the CENP-A nucleosome. In particular, characterization of yeast, fly, and human CENP-A chromatin by cross-linking, atomic force microscopy, and topological analysis indicated that CENP-A nucleosomes can adopt a "hemisomal" structure, consisting of a single copy each of H2A/H2B/CENP-A/H4, and wrapping less DNA in a right, rather than left-handed super-helix (reviewed in Henikoff and Furuyama, 2012). Using a combination of quantitative imaging and biochemical analysis of CENP-A chromatin recovered from cells, two papers in this issue argue that the structure of the CENP-A nucleosome is not constant but changes with the cell cycle.

An obvious distinction between octameric nucleosomes and hemisomes is that one should contain two molecules of CENP-A, whereas the other only one. However, determining the absolute number of CENP-A molecules at a centromere is difficult. Studies based on chromatin immunoprecipitation (ChIP) have found that budding yeast centromeres contain a single Cse4 nucleosome (Furuyama and Biggins, 2007), whereas quantitative imaging studies have indicated that the number of Cse4 molecules might be as high as five (Coffman et al., 2011; Lawrimore et al., 2011). To attempt to resolve these differences, Shivaraju et al. (2012) developed a method to count the number of enhanced green fluorescent protein Cse4-



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EGFP molecules at a centromere. The authors calibrate cytosolic GFP fluorescence by using fluorescence correlation spectroscopy (FCS) and apply that standard to estimate the number of Cse4-EGFP molecules at the cluster of 16 centromeres in a yeast cell. By comparing these measurements to GFP-labeled nuclear pore complex proteins, which have a defined protein stoichiometry, Shivaraju et al. argue that 16 molecules of Cse4 are present in a 16 centromere cluster and that this number doubles to 32 after replication, when sister centromeres are juxtaposed. Surprisingly, the number of Cse4 molecules also increases to 32 when cells go through anaphase before returning to 16 in G1. These data suggest that yeast centromeres contain a single copy of Cse4 throughout the cell cycle, with the exception being anaphase, when the copy number rises to two (Figure 1A). This study also indicates that budding yeast recruit Cse4 to centromeres during two distinct phases of the cell cycle.

Does Cse4 recruitment to anaphase centromeres cause a change in the composition of chromatin? Using a diploid yeast cell harboring one allele of Cse4-EGFP and one of Cse4-mCherry, Shivaraju et al. demonstrate that energy transfer between Cse4-EGFP and Cse4-mCherry increases when cells are in anaphase. Moreover, in a diploid yeast strain expressing both Mycand FLAG-tagged Cse4, sequential immunoprecipitation of Myc-Cse4 and FLAG-Cse4 from nuclease-digested chromatin reveals an anaphase-specific enrichment of the 125 base pair sequence that defines centromeres in *S. cerevisiae*. These experiments suggest an anaphase transition from a nucleosome containing one copy of Cse4 to a nucleosome with two copies and thus potentially a transition from a hemisome to an octameric nucleosome.

Could changes in CENP-A nucleosome composition also occur in other organisms? Using methods previously developed for isolating endogenous CENP-A chromatin from cells, Bui et al. (2012) collected centromeric nucleosomes from human cells in different cell-cycle stages and analyzed the morphology of those nucleosomes by atomic force microscopy. They report that isolated CENP-A nucleosomes increase in height and volume specifically during S phase, and thus may switch between hemisomes and octameric nucleosomes in a process coordinated with DNA replication (Figure 1B). Bui et al. then use a similar assay as Shivaraju et al. to determine the composition of CENP-A nucleosomes. Immunoprecipitates of GFP-CENP-A from nuclease-digested chromatin preferentially co-precipitates endogenous CENP-A during S phase, suggestive of nucleosomes containing two copies of CENP-A. Taken together, Bui et al. argue that human CENP-A nucleosomes also undergo a structural transition from hemisomes to octameric nucleosomes, but in a distinctly different stage of the cell cycle than that observed for yeast cells.

What is the mechanism for the cell-cycle-dependent change in centromeric nucleosome organization? A conserved chaperone protein required for CENP-A assembly is Scm3/HJURP, which forms a stable complex with a dimer of CENP-A and histone H4, and transiently localizes to centromeres in cells during the time of new CENP-Aassembly (reviewed in (Black and Cleveland, 2011; Maddox et al., 2012)). Previous localization studies in yeast have shown that Scm3 localizes to the centromere in anaphase (Mizuguchi et al., 2007). Surprisingly, Shivaraju et al. quantify Scm3 ChIP to show that the association of Scm3 with chromatin decreases during anaphase, concurrent with the doubling of Cse4-EGFP centromere levels. This contradiction perhaps reflects an increase in Scm3 turnover at centromeres in anaphase, which might reduce the amount of Scm3 associated chromatin observed by ChIP, but would be consistent with activation of Scm3-mediated Cse4 loading.

Consistent with the observations made in yeast, Bui and colleagues report that the HJURP:CENP-A interaction, and the presence of HJURP on chromatin fibers, decreases during S phase, concurrent with the change in the CENP-A nucleosome. Although these observations suggest Scm3/HJURP association may stabilize hemisomes, structural studies indicate that Scm3/HJURP binding to CENP-A/H4 dimers would preclude nucleosomal

incorporation of CENP-A/H4 (Maddox et al. 2012). Thus, more investigation is required to establish how Scm3/HJURP association affects centromeric chromatin, or vice versa.

In humans, CENP-A assembly during telophase/G1 is required to restore CENP-A levels after DNA replication. However, Cse4 is assembled in a DNA replication-dependent manner in *S. cerevisiae*. The purpose of anaphase Cse4 assembly is not obvious, especially as Cse4 returns to preanaphase levels during telophase/G1. It is unclear whether Cse4 is actually incorporated into chromatin during this anaphase window, but if it is, one interesting possibility is that this could provide a mechanism to remove "old" Cse4 from chromatin during telophase. Importantly, these data also suggest that DNA-replication-independent assembly of CENP-A during mitotic exit may be conserved between budding yeast and metazoan organisms.

In summary, both Shivaraju et al. and Bui et al. present a model of hemisomes acting as the "default" CENP-A nucleosome structure, with temporary transitions to octameric nucleosomes during anaphase (in yeast) and early S phase (in humans). However, the recent observation that both histone H3 and Cse4 are present at centromeres in budding yeast suggests that octameric nucleosomes containing CENP-A/H3/$H4_2$ "heterotetramers" could also represent a nucleosomal state containing one Cse4 molecule at the centromere (Lochmann and Ivanov, 2012). Furthermore, a significant population of CENP-A/H3 heterotetrameric nucleosomes can be isolated from human chromatin (Foltz et al., 2006), suggesting that heterotetrameric nucleosomes may also play a role in humans. Further studies are required to distinguish between models for alternative nucleosome structures at eukaryotic centromeres. Moreover, characterizing the cell-cycle timing of CENP-A nucleosome reorganization in different organisms, and how these changes are mediated, remain key immediate goals for the centromere field.

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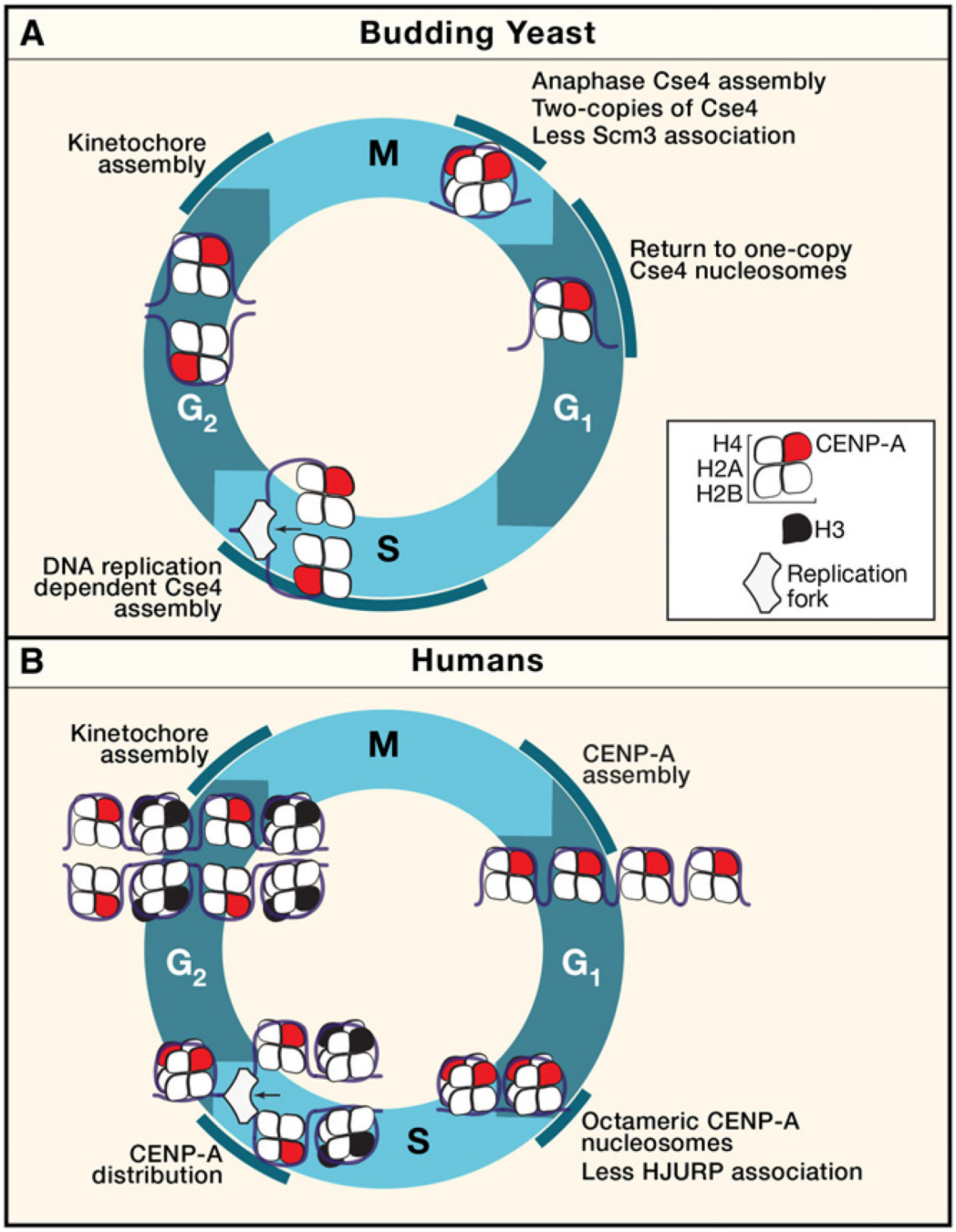


**Figure 1. Cell-Cycle-Dependent Transitions in Centromeric Nucleosomes**

(A) Representation of the changes in an individual Cse4 nucleosome during the cell cycle in *S. cerevisiae*. Shivaraju et al. propose that, at budding yeast centromeres, there is one copy of Cse4 during G1, S phase, and G2, and that Cse4 exists within a hemisome during these phases. The number of Cse4 molecules doubles at centromeres during S phase, concomitant with DNA-replication. Shivaraju et al. report that the number of Cse4 molecules also doubles during anaphase, and suggest that this reflects the formation of octameric Cse4 nucleosomes. During anaphase, Shivaraju et al. observe reduced association of Scm3 with centromeres. How octameric nucleosomes revert back to hemisomes remains unclear.

(B) Representation of the changes in an array of CENP-A nucleosomes during the cell cycle in human cells. Bui et al. propose that, in humans, CENP-A exists within a hemisome throughout the cell cycle except at the G1/S transition, when CENP-A nucleosomes become octameric. In this model, new CENP-A assembly in telophase/G1 is uncoupled from the reorganization of the CENP-A nucleosome at G1/S and is correlated with a reduction in HJURP association with chromatin. How CENP-A returns to hemisomes is unclear but is suggested to involve equal distribution of CENP-A during DNA replication. The splitting and equal distribution of octameric CENP-A nucleosomes to daughter DNA strands is depicted here, but the segregation of intact octameric nucleosomes to one DNA strand followed by subsequent reorganization is also consistent with the Bui et al.'s observations. Importantly, no new CENP-A is assembled during DNA replication, but histone H3 is incorporated into chromatin as depicted.