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Centromeres Poised En Pointe: CDKs Put a Hold on CENP-A Assembly

Kaitlin M. Stimpson[1,2] and Beth A. Sullivan[1,2,*]
[1]Institute for Genome Sciences & Policy
[2]Department of Molecular Genetics and Microbiology
Duke University School of Medicine, Durham, NC 27708 USA
*Correspondence: beth.sullivan@duke.edu
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Eukaryotic centromeres are propagated by incorporation of the centromere-specific histone CENP-A into centromeric chromatin. Silva et al. (2012) now show that cyclin-dependent kinases (CDKs) hold the CENP-A assembly machinery in an inactive state until mitotic exit and entry into G1, at which time new CENP-A is loaded.

Chromosome inheritance and genome stability depend on proper assembly and function of the centromere, an essential chromosomal locus that is the site of kinetochore formation and spindle microtubule attachment during cell division. Centromeres contain a unique type of chromatin containing interspersed nucleosomes composed of either the centromeric histone CENP-A or canonical H3 that is thought to mark the centromere for maintenance and replenishment after replication and cell division. During replication, CENP-A is semiconservatively distributed to both DNA strands and placeholder H3 or H3.3 fills the gaps left by this distribution (Dunleavy et al., 2011). Replication of centromeric DNA is uncoupled from the synthesis and assembly of CENP-A into centromeric chromatin (Shelby et al., 2000). Only after passage through mitosis and entry into the next G1 phase is newly synthesized CENP-A presented to chromatin by the chaperone HJURP (Dunleavy et al., 2009; Foltz et al., 2009; Jansen et al., 2007). G1 incorporation of CENP-A at centromeres depends on the presence of both HJURP and the Mis18 complex that collectively contains Mis18α, Mis18β, and Mis18BP1^{HsKNL2} (Barnhart et al., 2011; Foltz et al., 2009; Moree et al., 2011). However, the molecular signal that controls CENP-A assembly during the cell cycle has been unclear. In this issue of *Developmental Cell*, a new study from Lars Jansen's lab (Silva et al., 2012) addresses how cell cycle factors molecularly control CENP-A assembly. They show that the cyclin-dependent kinases CDK1 and CDK2 (CDK1/2) hold the CENP-A assembly machinery in an inactive state until completion of mitosis.

Cell cycle progression is tightly regulated by phosphorylation of various substrates by multiple cyclin-CDK complexes. During G1, there is an increase in the amount of cyclin E bound to CKD2. When cyclin E is degraded, levels of cyclin A-CDK1 activity rise so that cells enter S phase for replication and genome duplication. The cell moves into G2/M when cyclin A is degraded and mitotic cyclin B-CDK1 activity increases. Finally, the anaphase-promoting complex (APC) initiates mitotic exit by degrading cyclin B and decreasing CDK2 activity (Figure 1A).

Silva et al. first ask if CDKs controlled loading of new CENP-A after mitosis. They monitor nascent CENP-A using the SNAP-tagging technology previously employed to study CENP-A loading in G1 (Jansen et al., 2007). To test if CDKs control CENP-A assembly independently of cyclin activation, destruction of APC/C targets, or other aspects of mitosis, the authors block CDK activity in several human cell lines using CDK inhibitors Roscovitine and Purvalanol A. In each case, cyclin B levels remain high, indicating that the cells have not completed mitosis, and SNAP-CENP-A is loaded at centromeres in G2 (Figure 1B), suggesting that the assembly machinery is capable of loading CENP-A prior to mitosis, but is normally prevented from doing so. Since the small molecule inhibitors lack specificity, Silva et al. genetically dissect the roles of Cdks in CENP-A assembly in chicken DT40 cells using specific mutations in Cdk1 and Cdk2. In *Cdk2*$^{-/-}$ cells, CENP-A assembly is unaffected, but in *Cdk1*$^{-/-}$ and Cdk1/Cdk2 double mutant cells, unscheduled CENP-A assembly occurs in S and G2. Based on these studies, Cdk1 appears to suppress CENP-A assembly prior to mitotic exit.

CENP-A assembly depends on the canonical CENP-A assembly pathway, specifically, the Mis18 complex (Hayashi et al., 2004; Moree et al., 2011). Silva et al. find that CDK inhibition causes premature recruitment of Mis18α and Mis18BP1^{HsKNL2} to centromeres. Importantly, G1 and G2 assembly of CENP-A does not occur when CDK inhibition is combined with Mis18α, Mis18BP1^{HsKNL2}, or HJURP depletion. In addition, partial depletion of the Mis18 complex prevents nascent CENP-A recruitment, suggesting that these CENP-A assembly factors are rate limiting.

These chemical and genetic studies implicate Cdk activity in suspending CENP-A loading machinery by holding the Mis18 complex in an inactive state until mitotic exit. Given that CDKs control protein function or degradation via phosphorylation, Silva et al. explore the phosphorylation status of the Mis18 complex. Each protein in the complex has one or more amino acid positions that are potential targets for CDKs. Mutation of serine/threonine (S/T) sites in Mis18α or Mis18β does not affect CENP-A loading, but conversion of all 24 S/T sites to alanine in Mis18BP1^{HsKNL2} results in premature recruitment of Mis18BP1^{HsKNL2} to centromeres during G2 or M. Mis18BP1^{HsKNL2} is maximally phosphorylated in mitosis,

suggesting that its modification, presumably by CDKs, keeps it dormant until mitotic exit. However, preventing phosphorylation of Mis18BP1^{HsKNL2} does not lead to premature recruitment of nascent SNAP-CENP-A to centromeres. Thus, while Mis18BP1^{HsKNL2} phosphorylation is important for sequestering the assembly machinery from centromeres prior to G1, other events that impact Mis18α, Mis18β, HJURP, CENP-A, or possibly even features of the centromeric chromatin itself are also important for centromere assembly. Future experiments are necessary to test if CDKs directly phosphorylate Mis18BP1^{HsKNL2} and to determine if other centromere proteins, such as HJURP with three target residues for phosphorylation, are similarly regulated.

This exciting work from Silva and colleagues presents a model that places regulation of centromere assembly in the context of normal cell cycle control, with CDKs dictating specific timing of replication versus centromere propagation. Still, key questions remain. During replication, when the CENP-A loading machinery is inactive, H3 and H3.3 fill gaps created when old CENP-A is distributed between the newly replicated DNA strands. The unique mixture of centromeric nucleosomes (CENP-A:H4 versus H3:H4 versus H3.3:H4) is thought to recruit other CENPs to non-CENP-A regions in late S/G2 in order to build a proper kinetochore. Delaying CENP-A assembly may allow centromeric chromatin containing H3 nucleosomes to switch to a mitotic state for chromosome segregation (Prendergast et al., 2011). If H3-associated versus CENP-A-associated kinetochore assembly must occur distinctly, it will be interesting to determine if unscheduled CENP-A assembly affects centromere size, prevents H3.1/H3.3 from being loaded, or interferes with recruitment of other centromere/kinetochore proteins.

Mis18BP1 alone does not promote CENP-A recruitment. Recent work in *Xenopus laevis* revealed that CENP-C, another component of the centromere nucleosome-associated complex, recruits Mis18BP1 to *Xenopus* centromeres prior to CENP-A assembly (Moree et al., 2011). Mis18BP1 binds to the C terminus of CENP-C, and it is unclear if or how CDK activity impacts this interaction. CENP-C is constitutively present at centromeres, yet in humans it exhibits distinct interphase and metaphase dynamics. Future studies should address whether CENP-C recruits Mis18BP1^{HsKNL2} in human cells, and if the interaction is regulated by phosphorylation and/or the cell cycle. How HJURP recruitment occurs, particularly during unscheduled localization of mutant Mis18BP1^{HsKNL2} at centromeres, also merits investigation. Phosphorylation of Mis18BP1^{HsKNL2} may prevent HJURP recruitment or inhibit remodeling factors that affect accessibility of chromatin to centromere assembly machinery. Defining how the cell cycle regulates interactions between centromere proteins, CENP-A chaperones, and nucleosome assembly factors will provide deeper insight into CENP-A loading and centromere propagation.

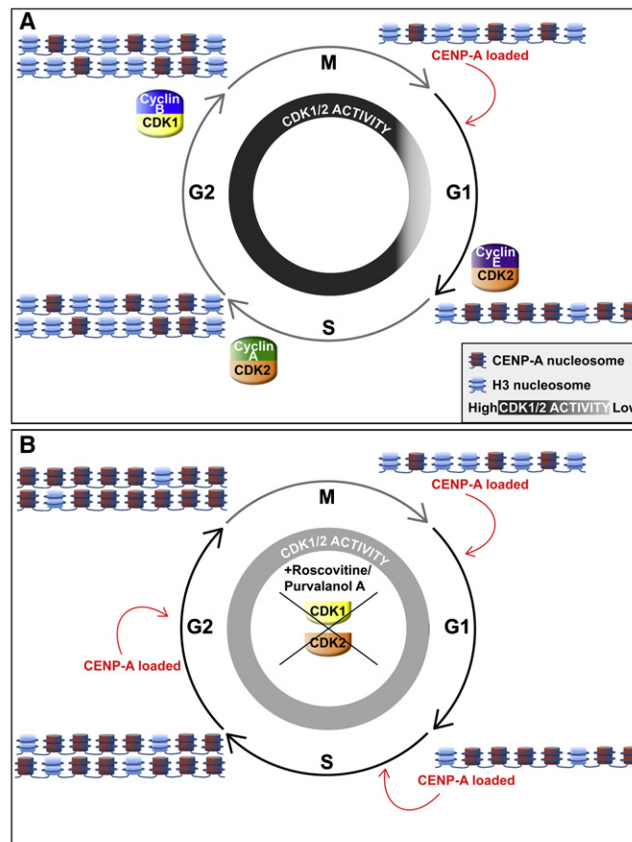

Figure 1. CENP-A Loading Is Regulated by CDKs
Centromeric chromatin is composed of interspersed CENP-A-containing (red) and H3-containing (blue) nucleosomes. (A) Normal CENP-A loading occurs at late mitosis/early G1 when CDK1/2 activity decreases. (B) When CDKs are inhibited, unscheduled CENP-A loading occurs throughout the cell cycle.

REFERENCES

Barnhart, M.C., Kuich, P.H., Stellfox, M.E., Ward, J.A., Bassett, E.A., Black, B.E., and Foltz, D.R. (2011). J. Cell Biol. *194*, 229–243.

Dunleavy, E.M., Roche, D., Tagami, H., Lacoste, N., Ray-Gallet, D., Nakamura, Y., Daigo, Y., Nakatani, Y., and Almouzni-Pettinotti, G. (2009). Cell *137*, 485–497.

Dunleavy, E.M., Almouzni, G., and Karpen, G.H. (2011). Nucleus *2*, 146–157.

Foltz, D.R., Jansen, L.E., Bailey, A.O., Yates, J.R., 3rd, Bassett, E.A., Wood, S., Black, B.E., and Cleveland, D.W. (2009). Cell *137*, 472–484.

Hayashi, T., Fujita, Y., Iwasaki, O., Adachi, Y., Takahashi, K., and Yanagida, M. (2004). Cell *118*, 715–729.

Jansen, L.E., Black, B.E., Foltz, D.R., and Cleveland, D.W. (2007). J. Cell Biol. *176*, 795–805.

Moree, B., Meyer, C.B., Fuller, C.J., and Straight, A.F. (2011). J. Cell Biol. *194*, 855–871.

Prendergast, L., van Vuuren, C., Kaczmarczyk, A., Doering, V., Hellwig, D., Quinn, N., Hoischen, C., Diekmann, S., and Sullivan, K.F. (2011). PLoS Biol. *9*, e1001082.

Shelby, R.D., Monier, K., and Sullivan, K.F. (2000). J. Cell Biol. *151*, 1113–1118.

Silva, M.C.C., Bodor, D.L., Stellfox, M.E., Martins, N.M.C., Hochegger, H., Foltz, D.R., and Jansen, L.E.T. (2012). Dev. Cell *22*, this issue, 52–63.