Centromeric chromatin gets loaded

Christopher W. Carroll and Aaron F. Straight

Department of Biochemistry, Stanford University, Stanford, CA 94305

Centromeric nucleosomes contain a histone H3 variant called centromere protein A (CENP-A) that is required for kinetochore assembly and chromosome segregation. Two new studies, Jansen et al. (see p. 795 of this issue) and Maddox et al. (see p. 757 of this issue), address when CENP-A is deposited at centromeres during the cell division cycle and identify an evolutionally conserved protein required for CENP-A deposition. Together, these studies advance our understanding of centromeric chromatin assembly and provide a framework for investigating the molecular mechanisms that underlie the centromere-specific loading of CENP-A.

Centromeres are the specialized chromatin domains on each eukaryotic chromosome that direct kinetochore assembly during mitosis. Kinetochores mediate chromosome attachment to the mitotic spindle and are required for microtubule-dependent chromosome movement during mitosis. Kinetochores also act as signaling centers that activate the mitotic checkpoint, delaying the initiation of anaphase in response to improper chromosome attachments to the mitotic spindle (Maiato et al., 2004). CENP-A is a histone H3 variant that is present exclusively in centromeric nucleosomes and is required to direct kinetochore assembly (Cleveland et al., 2003; Carroll and Straight, 2006). Centromeric DNA is not well conserved even among closely related species, and, in humans, no individual DNA sequence is necessary or sufficient to encode centromere function. However, all functional centromeres contain CENP-A. As the key universally conserved element of centromeric nucleosomes throughout eukaryotic organisms, CENP-A is thought to provide the epigenetic mark that specifies centromere function. A central question, then, is how the specialized chromatin of the centromere is assembled and stably propagated.

Two recent studies published in this issue address this question. Applying a novel fluorescent technology in a series of elegant pulse-chase experiments, Jansen et al. (2007) demonstrate that the deposition of newly synthesized CENP-A is coupled to cell cycle progression. Somewhat surprisingly, however, these authors show that CENP-A deposition into chromatin occurs during telophase/early G1 phase (Fig. 1). This is in marked contrast to conventional histone H3, which is assembled concomitantly with DNA replication. Furthermore, by exploiting cell fusion experiments of the type pioneered in classic cell cycle studies by Rao and Johnson (1970), Jansen et al. (2007) show that exit from mitosis is required for the loading of nascent CENP-A into centromeric chromatin. Similar conclusions were recently reached for loading of the *Drosophila melanogaster* CENP-A (called *Cid*) during early embryonic cell cycles (Schuh et al., 2007), indicating that the timing of eukaryotic centromeric chromatin replication is likely conserved.

In a related study also published in this issue, Maddox et al. (2007) used a functional genomics strategy to identify genes required for CENP-A deposition in *Caenorhabditis elegans*. Disruption of a single gene called kinetochore null 2 (KNL-2) resulted in the dramatic loss of CENP-A from chromosomes, resulting in a phenotype that is indistinguishable from that of CENP-A depletion itself (Oegema et al., 2001). *C. elegans* chromosomes are holocentric, an interesting evolutionary variation in which centromeres and thus CENP-A span the entire length of the chromosome. This raised the possibility that the mechanism of CENP-A deposition might somehow be divergent from other organisms with monocentric chromosomes. However, Maddox et al. (2007) identified the human homologue of KNL-2 (also called Mis18BP1) and demonstrated that it was also required for CENP-A localization (Fig. 1). This suggests that mechanisms of centromere assembly are conserved in diverse species and that KNL-2 is a key factor in this process.

The studies highlighted here represent a fundamental advance in our understanding of how the integrity of centromeric chromatin is established and maintained. Nevertheless, several questions remain to be answered. For example, how is the existing centromere recognized as the site for incorporation of new CENP-A? One recent model has suggested that tension applied across the centromere as a result of bipolar spindle attachment during mitosis could condition centromeric chromatin for CENP-A loading (Mellone and Allshire, 2003), thereby ensuring that only active centromeres will be targeted for the deposition of new CENP-A. Jansen et al. (2007) addressed this question by forcing cells to exit mitosis in the absence of microtubule–chromosome attachments. Under these conditions, CENP-A loading occurs normally, indicating that tension across the centromere is not required for CENP-A loading. However, structural components of the kinetochore have been shown to be required for CENP-A loading, suggesting that interaction between kinetochore assembly and CENP-A deposition contributes to stable centromere maintenance (Kline et al., 2006; Okada et al., 2006).

Correspondence to Aaron F. Straight: astraight@stanford.edu

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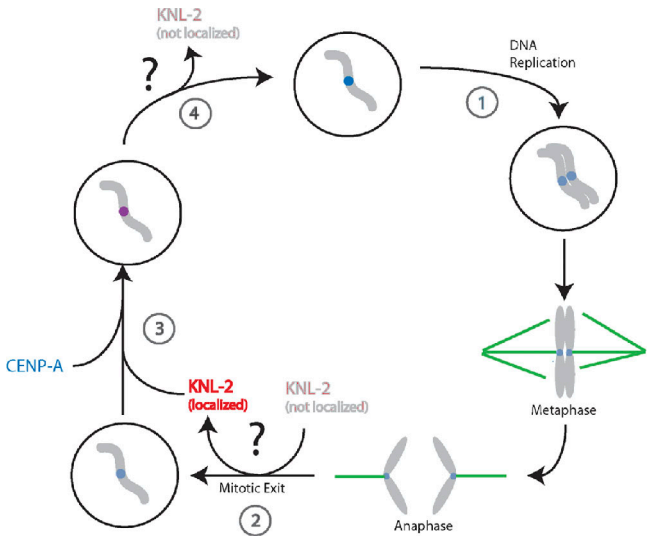

Figure 1. **A model for the centromere replication cycle for a single human chromosome.** During DNA replication (1), CENP-A–containing nucleosomes (blue dots) are randomly distributed to each sister chromatid, resulting in a twofold reduction in the amount of CENP-A at each centromere. Upon exit from mitosis (2), a change in the state of KNL-2 or within the chromatin itself targets KNL-2 to the centromere, which, in turn, recruits newly synthesized CENP-A for deposition (3). After centromere replication, KNL-2 dissociates from centromeres (4). The mechanisms that control KNL-2 centromere binding and dissociation are unknown.

Another important question regards the mechanism by which CENP-A is loaded into centromeric chromatin and the role of KNL-2 in this process. Conventional histone H3 and the histone variant H3.3 copurify with distinct histone chaperone complexes called chromatin assembly factor 1 (CAF1) and HIRA, respectively, which are required for the deposition of their associated histones (Tagami et al., 2004). Although no analogous histone chaperone complex specifically required for CENP-A loading has been characterized to date, several genes have been identified that are necessary for the centromere-specific localization of CENP-A. Of particular interest are the products of the *Schizosaccharomyces pombe* Mis16 and Mis18 genes (Hayashi et al., 2004). Mis16 is a homologue of the closely related human proteins RbAp46 and RbAp48, which are also required for CENP-A localization. RbAp48, the only common subunit of CAF1 and HIRA, was also found to associate with CENP-A purified from *Drosophila* cells (Furuyama et al., 2006). Two Mis18 homologues, α and β, were recently shown to be required for CENP-A loading in human cells and are found in a complex with human KNL-2 (Fujita et al., 2007). Interestingly, KNL-2 and Mis18α/β localize specifically to centromeric chromatin during telophase/early G1 phase. Thus, KNL-2 likely functions at chromatin during CENP-A loading, possibly as a targeting element for a CENP-A–containing histone chaperone complex.

CENP-A is the best candidate to provide the epigenetic mark that specifies centromere identity within eukaryotic chromosomes. Until recently, the molecular systems that control the centromere-specific loading of CENP-A were completely unknown. The two studies highlighted here along with several advances in diverse experimental systems have laid the foundation for further studies designed to elucidate a detailed molecular understanding of how centromeric chromatin is assembled and maintained. The continued identification of factors required for this process along with the development of in vitro systems to dissect the mechanisms of CENP-A deposition are now essential to understand the molecular underpinnings of centromeric chromatin assembly.

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