CHROMATIN

Nucleosomal dynamics at centromeres

The proper functioning of centromeres is essential for the accurate segregation of chromatids and thus for protection against aneuploidy. Centromere-specific nucleosomes contain histone H3 variants — Cse4 in yeast or centromeric protein A (CENPA) in humans — but their histone stoichiometry is debated. Two new papers help to resolve this debate by showing that centromeric histone composition is dynamically regulated through the cell cycle.

Shivaraju *et al.* tracked the centromeric loading of GFP-tagged Cse4 through the *Saccharomyces cerevisiae* cell cycle by using fluorescence microscopy. In addition to the expected loading at S phase (which is required to maintain Cse4 levels in the newly replicated sister chromatids), they found a doubling of centromeric Cse4 content at mid-anaphase that persisted through to telophase.

Centromeric nucleosomes have been proposed to be either tetramers, hexamers or octamers, and to contain one or two Cse4 molecules. The authors quantified the number of Cse4 molecules through the cell cycle by normalizing the Cse4–GFP intensities to either monomeric cytosolic GFP or to GFP-tagged nuclear pore components of known stoichiometries. They concluded that for most of the cell cycle, each centromere had one Cse4 subunit, which they propose was present in a hemisomal tetramer of H2A–H2B–Cse4–H4, whereas during anaphase each centromere contained two Cse4 subunits, which they propose were in a full octameric nucleosome. In line with this, fluorescence resonance energy transfer (FRET) experiments showed an intranucleosomal physical interaction between Cse4 monomers only during anaphase.

In a related study, Bui *et al.* immunoprecipitated CENPA-containing nucleosomes from human cells in different cell cycle phases. Various analyses were consistent with a cell cycle-regulated oscillation between tetramers and octamers, including the nucleosomal dimensions (as assessed by atomic force microscopy) and the lengths of the associated DNA. Also concordant with the yeast study, microscopy and biochemical analyses showed an increase in intranucleosomal CENPA–CENPA interactions at cell cycle timepoints for which octameric nucleosomes were present. However, reflecting known differences in the timing of CENPA loading in human cells compared with Cse4 loading in yeast, the centromeric octameric nucleosomes were present throughout S phase rather than at anaphase.

What is the regulatory mechanism of these transitions? Scm3 is a chaperone that is involved in assembling Cse4-containing nucleosomes. Shivaraju *et al.* showed that Scm3 can assemble Cse4-containing nucleosomes *in vitro* that have a size, biochemical composition and DNA supercoiling that is consistent with an H2A–H2B–Cse4–H4 tetramer. Conversely, the same subunits assembled by the generic chaperone Nap1 typically resulted in octamers. Intriguingly, in cycling cells a decrease in the association of Scm3 with centromeric chromatin was coincident with the anaphase transition to octameric nucleosomes, perhaps as a result of the removal of this tetramer-favouring activity. Similarly, Bui *et al.* showed that HJURP — the human chaperone for CENPA — loses association with centromeric chromatin during the G1–S transition to octameric nucleosomes, and they also identified histone acetylations in CENPA-containing nucleosomes at this transition that might have a functional role.

These studies shed light on the cell cycle-regulated stoichiometry of centromeric nucleosomes. The precise cellular roles of centromeric nucleosomes in different species and at different cell cycle phases remain to be characterized.

Darren J. Burgess, Assistant Editor,
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ORIGINAL RESEARCH PAPERS Shivaraju, M. *et al.* Cell-cycle-coupled structural oscillation of centromeric nucleosomes in yeast. *Cell* **150**, 304–316 | Bui, M. *et al.* Cell-cycle-dependent structural transitions in the human CENP-A nucleosome *in vivo*. *Cell* **150**, 317–326