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The histone variant CENP-A and centromere specification

Ben E Black and Emily A Bassett

The centromere is the chromosomal locus that guides faithful inheritance. Centromeres are specified epigenetically, and the histone H3 variant CENP-A has emerged as the best candidate to carry the epigenetic centromere mark. Recent advances demonstrate the physical basis for this epigenetic mark whereby CENP-A confers conformational rigidity to the nucleosome it forms with other core histones. This nucleosome is recognized by a multisubunit complex of constitutive centromere proteins, termed the CENP-A^{NAC}. Evidence from two CENP-A relatives in diverse eukaryotes suggests that the histone complexes they form adopt highly unconventional arrangements on DNA. Centromere identity, itself, is propagated during mitotic exit and early G1, and it relies upon a *cis*-acting targeting domain within CENP-A and a proposed centromere 'priming' reaction.

Addresses
Department of Biochemistry and Biophysics and Program in Biochemistry and Molecular Biophysics, University of Pennsylvania, Philadelphia, PA 19104, United States

Corresponding author: Black, Ben E (blackbe@mail.med.upenn.edu)

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Introduction

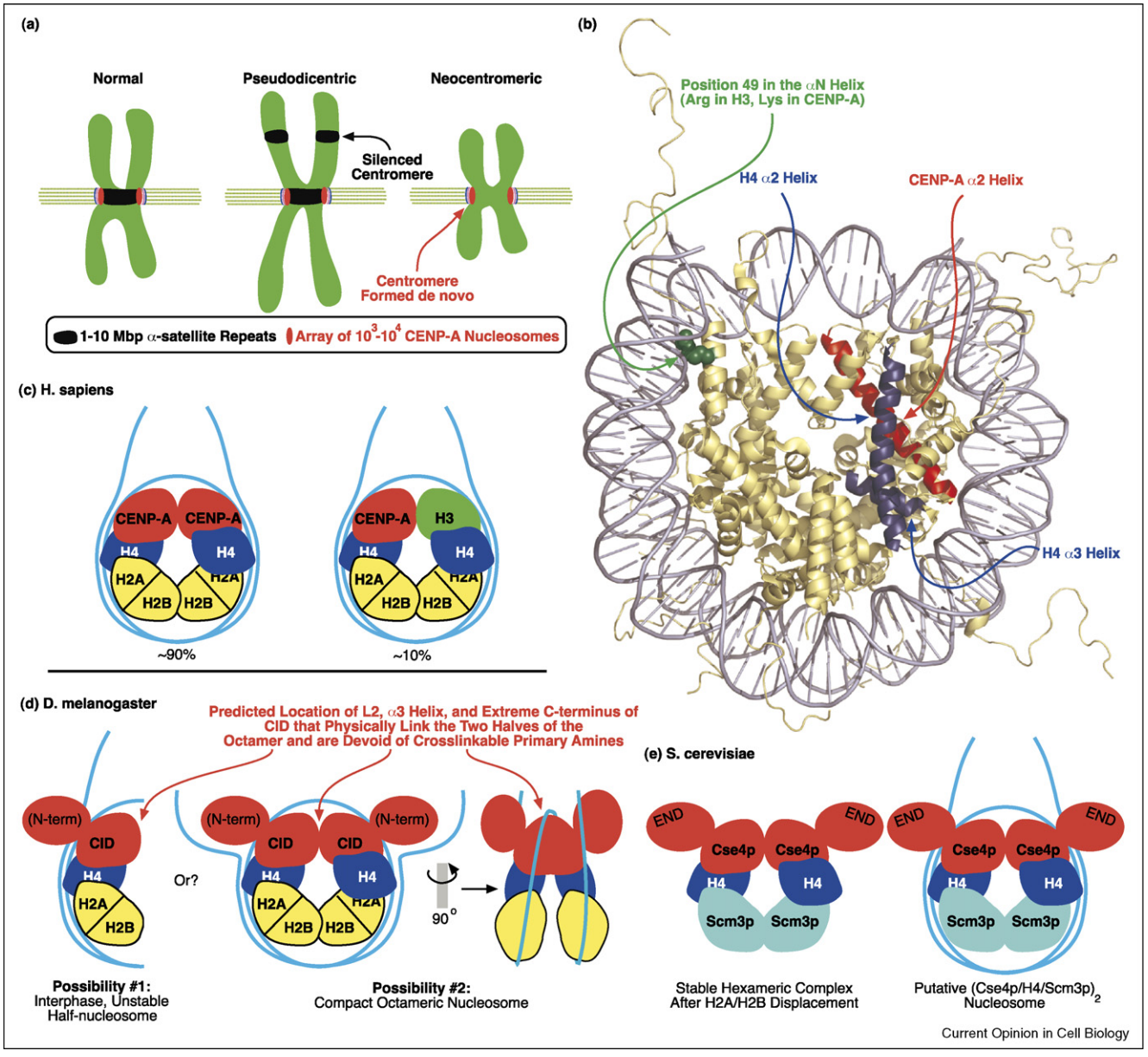
The faithful transmission of the genome relies upon a cell division mechanism whereby each replicated chromosome pair, termed sister chromatids, are separated and equally partitioned into daughter cells. This process involves bi-orientation of mitotic chromosomes on the microtubule-based spindle. The centromere is the locus on the chromosome that serves as the spindle connection point, and lessons from chromosomal rearrangements that result in either the duplication or elimination of the centromere strongly suggest the rule that one-and-only-one centromere is tolerated per chromosome (Figure 1a). More than one centromere will lead to chromosome breakage by the spindle. Conversely, the lack of a centromere will lead an entire chromosome to missegregate. Either of these events is a genetic disaster, and as such, epigenetic mechanisms are in place to maintain centromere identity [1,2].

Nucleosomes in which CENP-A replaces canonical H3 are the fundamental units of the chromatin at the foundation of the kinetochore, the mitotic protein assembly that mediates attachment to the mitotic spindle and generates a mitotic checkpoint signal that arrests cells from entering anaphase until all chromosomes are properly aligned on the metaphase plate. CENP-A nucleosomes are found at active but not at inactive centromeres and are strong candidates to carry the epigenetic centromere mark. Many other chromatin-based post-translational modifications and pericentromeric heterochromatin domains are required for full centromere function, which includes controlling sister chromatin cohesion [3]. In this review, however, we will focus on recent advances in understanding the physical basis of how CENP-A-containing nucleosomes distinguish themselves from bulk chromatin as the site for centromere and kinetochore formation, and we will highlight emerging work from diverse eukaryotic systems that sheds light on the cellular pathway leading to cell-cycle-coupled replenishment of the CENP-A mark.

How does CENP-A mark centromeres?

CENP-A physically marks centromeres by assembling into a nucleosomal structure that is distinguishable from its canonical counterparts that contain histone H3. In the human version of the CENP-A nucleosome, the α2-helix (Figure 1b, red) and the preceding loop (L1) form the CENP-A targeting domain (CATD) that confers conformational rigidity — a 10-fold slowing of hydrogen exchange along the peptide backbone — to the interface it forms with its binding partner histone H4 (Figure 1b, the α2 and α3 helices of H4 are shown in blue) [4••]. The CATD also confers centromere targeting and an essential mitotic function to CENP-A, and together this supports a model where a structurally divergent nucleosome containing CENP-A is the fundamental unit of the chromatin that specifies centromere location [5••]. The centromere must accommodate extraordinary physical constraints: firstly, specialized higher-order chromatin folding; secondly, assembly of the mitotic kinetochore complex; and thirdly, mitotic spindle forces [6,7••,8]. One particular site in the CENP-A nucleosome that may be important for accommodating these constraints is the entry/exit DNA of the nucleosome. Indeed, Lys49 of CENP-A causes a weakening of its interaction with the entry/exit DNA of the nucleosome compared to within the canonical nucleosome where H3 has an arginine at the corresponding position in its αN helix (Figure 1b; Arg49 in green) [9]. The steady-state unwrapping of 7 bp of DNA at the entry/exit may provide centromeric chromatin arrays with the ability to adopt atypical higher-

Figure 1



CENP-A nucleosomes are the fundamental unit of centromeric chromatin. **(a)** CENP-A is found at all active centromeres including those on neocentromeric marker chromosomes (right) that lack the α-satellite repeats typically found in megabase stretches at normal human centromeres (left) [62]. In addition, CENP-A vacates inactive centromeres, such as those found epigenetically silenced in the case of pseudodicentric chromosomes (center). **(b)** Model of the canonical H3-containing nucleosome (Protein Data Bank number 1kx5; [63]) with the corresponding region highlighted that displays conformational rigidity when assembled with CENP-A in place of histone H3 (the α2 helix of CENP-A in red; the α2 and α3 helices of H4 in blue). Only one pair of CENP-A and H4 is highlighted for reasons of clarity. The side-chain of Arg49 from histone H3 (green) that intercalates into the entry/exit DNA is changed to a lysine in the corresponding position in CENP-A and leads to steady state unwrapping of 7 bp on nucleosomes assembled onto DNA minicircles [9]. Recent studies have indicated diverse arrangements of nucleosomes and histone complexes containing CENP-A (or one of its relatives) in humans **(c)**, flies **(d)**, and budding yeast **(e)**. Although human CENP-A has a tail of identical length to canonical H3, in its counterparts CID (d) and Cse4p (e), large domains that do not display evolutionary conservation extend >100 amino acids outside the predicted nucleosome core. The N-terminal extension includes the END domain in Cse4p that provides an essential function to budding yeast centromeres [64]. Scm3p is an essential component of budding yeast centromeres [13••,15•,16•] that has the ability to displace H2A/H2B dimers from $(Cse4p/H4)_2$ heterotetramers [13••], raising the possibility that $(Cse4p/H4/Scm3p)_2$ heterohexamers (panel e, left) wrap centromeric DNA into a nucleosome-like structure (panel e, right).