# Super-coil me:
Sizing up centromeric nucleosomes

by Emma Hill and Ruth Williams

Every chromosome needs a centromere for proper segregation during cell division. Centromeric chromatin wraps around histones, providing an anchor for kinetochore proteins and spindle attachment. It is clear why cells need centromeres, but how they form and what they look like is less so. Recent reports extend our understanding of chaperones involved in centromere formation. And other accounts of half-sized, right-handed nucleosomes have created an unexpected twist.
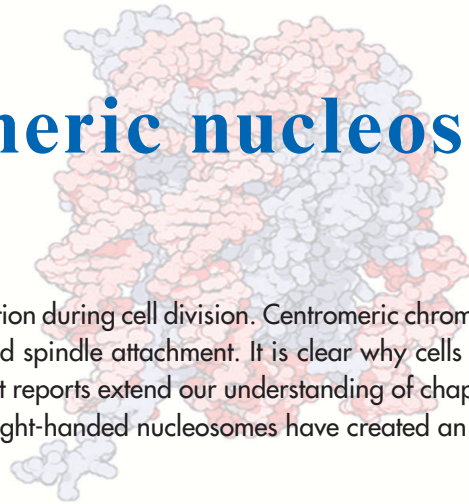
Replicated chromosomes line up neatly at the center of dividing cells, then in perfect synchrony, sister chromatids are pulled to opposite poles. Centromeres make segregation possible by recruiting a complex of kinetochore proteins, which acts as an attachment site for the cell's segregation machinery—the mitotic spindle microtubules (Fig. 1).

Despite their importance, centromeres remain something of a mystery: How they are distinguished from the reams of DNA present in a chromosome, and why kinetochore proteins only attach at these sites is not known. As centromeric DNA is highly repetitive and not conserved across species, researchers investigate the centromeres' chromatin environment and scaffold in an attempt to uncover their secrets.

Centromeric DNA wraps around nucleosomes, making them a good place to start. Nucleosomes contain two copies of each of the canonical histones H2A, H2B, H3, and H4 and the octameric structure they form is encased by genomic DNA (1) (Fig. 2). But in centromeric nucleosomes, histone H3 is replaced by a related protein, centromere protein A (CENP-A; also known as CenH3, or CID in *Drosophila*, and Cse4p in yeast). As the distinguishing feature of centromeric nucleosomes, CENP-A has been placed under the spotlight: "The histone variant is the key and so that's what we focus on," says Steven Henikoff, a long-time centromere researcher from the Fred Hutchinson Cancer Research Center in Seattle.

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BUILDING A CENTROMERE

Before a centromere can form, epigenetic modifications create a heterochromatin platform that allows nucleosome incorporation. This involves numerous proteins and probably the RNAi machinery to prevent transcription of satellite repeats. Nucleosomes are usually incorporated into DNA during replication, but CENP-A–containing centromeric nucleosomes are deposited at centromeres after cell division. It is not clear why this deposition is separated from replication, but recent studies have identified proteins that deliver the specialized histone.

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> *-Steven Henikoff*

Two studies, one lead by Geneviève Almouzni from the Institut Curie in Paris, and a second by Don Cleveland of the University of California, San Diego, identified a chaperone protein called HJURP (Holliday junction recognition protein) that associates with human CENP-A before it is incorporated into chromatin (2, 3). HJURP transiently localizes to centromeres immediately after cell division, according to Almouzni, and without HJURP, CENP-A doesn't arrive at the centromere. However, unlike previously identified CENP-A chaperone candidates, HJURP does not bind to canonical H3 (2). Similarly, in yeast, a distant HJURP relative called Scm3 selectively binds the CENP-A/H4 dimer and helps to deliver it to the centromere (4, 5). Scm3 might even displace H2A/H2B dimers to form a [CENP-A/H4/Scm3]$_2$ hexameric nucleosome complex at the inner kinetochore in yeast (4).

Chaperones may escort CENP-A to the centromere, but other factors convince it to stay. According to Perpelescu et al., a protein called RSF (remodeling and spacing factor) associates with centromeric chromatin and is required for normal mitotic progression by stabilizing CENP-A's association with chromatin (6).

HJURP interacts with CENP-A via the CATD (CENP-A targeting domain), which consists of the α helix 2 and the loop between α helices 1 and 2. Despite having a core sequence region in common with CENP-A, canonical histone H3 lacks the CATD domain, perhaps explaining H3's absence from centromeres. Indeed, CATD is indispensable for delivering CENP-A to the centromere, and chimeric proteins with the CATD domain inserted into H3 can substitute for CENP-A in centromeric nucleosomes (7).

The CATD domain of CENP-A may provide extra rigidity to centromeric nucleosomes according to studies by Cleveland's group. Histone H4 and the CATD domain of CENP-A intertwine their helices, decreasing the flexibility

of the complex (8). This rigidity might serve as an epigenetic mark to specify centromere location and for recognition by the kinetochore. The essential kinetochore assembly protein CENP-N selectively binds to CENP-A–containing nucleosomes, or to H3/CATD chimeras, but not to canonical H3 nucleosomes (9).

HENIKOFF'S HEMISOMES

Although extra rigidity may confer strength to the centromeric chromatin, in 2007 Henikoff and then-postdoc Yamini Dalal discovered another striking structural difference in centromeres (10). Centromeric nucleosomes had always been assumed to consist of a stable octameric complex like their canonical counterparts. But Dalal and Henikoff presented evidence for hemisomes—centromeric nucleosomes formed from a tetramer of H2A, H2B, CENP-A, and H4. The initial evidence for half-sized nucleosomes came when CENP-A nucleosomes extracted from fly cells were found to weigh only half of their expected weight. The evidence amassed when analyses using atomic force microscopy (AFM) showed that CENP-A nucleosomes were also half the expected height. Their findings created a stir in the field.

Other researchers, although intrigued, are more circumspect about the hemisome model. Tetrameric nucleosomes have been documented in Archaea but have not been found in eukaryotic chromatin. However, reconstitution studies from humans and flies suggest that their centromeric nucleosomes adopt the standard octameric structure (8, 11–13).

The discovery of hemisomes was thus surprising. "It's very provocative," says Almouzni. Cleveland expressed skepticism, pointing out technical concerns with Henikoff's results. Echoing this viewpoint, Ben Black, now at the University of Pennsylvania, recently suggested that the cross-linking method Henikoff used to isolate nucleosomes might have favored a tetrameric form rather than the full octamer (14). Successful cross-linking requires two lysines that are present in the standard H3 protein but are absent in *Drosophila* CENP-A. Thus the octamers may be there, but the cross-linking would fail to detect them. "Henikoff could be right," says Black. "I just don't think there's enough evidence to say definitively that the hemisome is the important form."

Henikoff rebuts the suggestion of methodological error, noting that the method used can cross-link H2A, H2B, and H4 residues that are very far apart and should therefore be able to cross-link CENP-A–containing nucleosomes. And cross-linking aside, Dalal and Henikoff's AFM measurements were performed on native nucleosomal particles, and the presence of hemisomes is a straightforward interpretation from those data.

Two other centromere investigators, Owen Marshall and Andy Choo of the Murdoch Childrens Research Institute in Melbourne, are positive but cautious about hemisomes. Henikoff reports a 10-nm chromatin conformation that could be the result of centromeric chromatin with a different topology resisting condensation. But Marshall and Choo recently found that centromeres in mitotic human chromosomes appear to exist as 30-nm fibers (15), which Marshall says, "would be inconsistent with a 10-nm hemisome structure." Marshall points out, however, that his study was performed on mitotic chromosomes only. Henikoff's hemisomes might occur at a different stage of the cell cycle. It's also possible that a 10-nm hemisome-containing fiber somehow folds into a 30-nm fiber at mitosis, concedes Marshall.

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Choo noted another possibility that would explain Henikoff's hemisomes. "Henikoff's work was done in flies," he says, "but it has yet to be shown whether it's going to be true for other species."

COILING CHROMATIN

Another recent study from Takehito Furuyama, a senior postdoctoral fellow with Henikoff, provides a new twist in favor of hemisomes, and extends the group's observations from flies to yeast (16). This study shows that CENP-A nucleosomes wrap DNA in a right-handed direction, forming positive supercoils. This is unexpected as DNA assumes a left-handed negative supercoil around canonical H3-containing nucleosomes (Fig. 2).

Furuyama and Henikoff used an in vitro supercoiling assay to determine the direction of DNA wrapping around CENP-A–containing nucleosomes. The assay is simple. The DNA-intercalating agent chloroquine is added to DNA and run on an electrophoresis gel: Negatively supercoiled DNA is relaxed by chloroquine and thus moves slowly on the gel, whereas positively supercoiled DNA is tightened by chloroquine and moves more quickly.
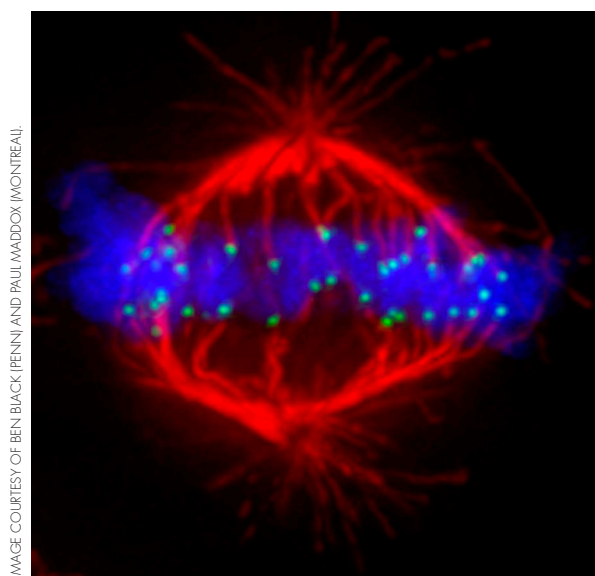

Figure 1. **Human metaphase cell showing spindle microtubules (red) that connect to the chromosomes (blue) at the array of CENP-A nucleosomes at the centromere (CENP-A stained green).**

The assay revealed that chromatin containing *Drosophila* CENP-A adopted positive supercoils, which infers right-handed DNA. These data directly contradict studies using human CENP-A, which demonstrated that in vitro assembled CENP-A–containing nucleosomes induced negative supercoils, albeit somewhat less tightly wrapped than those of canonical H3-containing nucleosomes (11).

Obtaining in vivo evidence of centromere conformation is tricky: Human centromeric DNA has long, tandem repeat sequences, and *Drosophila* do not have any common centromeric DNA sequences. Additionally, both humans and flies form their centromeres around multiple CENP-A–containing nucleosomes interspersed with regular nucleosomes. In yeast, however, the setup is simpler as their centromeres form around a single CENP-A–containing nucleosome at a conserved genomic location—the centromeric element. Furuyama and Henikoff thus made minichromosomes carrying zero, one, or two yeast centromeric elements, and inserted them into yeast. The resulting DNA was extracted and assayed for positive supercoils using chloroquine—inclusion of one centromeric element corresponded with the detection of a positive supercoil. "It's very mathematical," says Dalal, now at the National Cancer Institute in Bethesda. "For every centromeric DNA element included you get the same number of positive supercoils. It's really elegant—these DNA topology experiments are a beautiful complimentary approach [to AFM] to examine nucleosome assembly."

Right-handed wrapping, Henikoff explains, is inconsistent with octameric nucleosomes. Octameric nucleosomes impart a strict left-handed superhelicity to the DNA (Fig. 2), whereas tetramers can be bound by either right- or left-handed DNA,
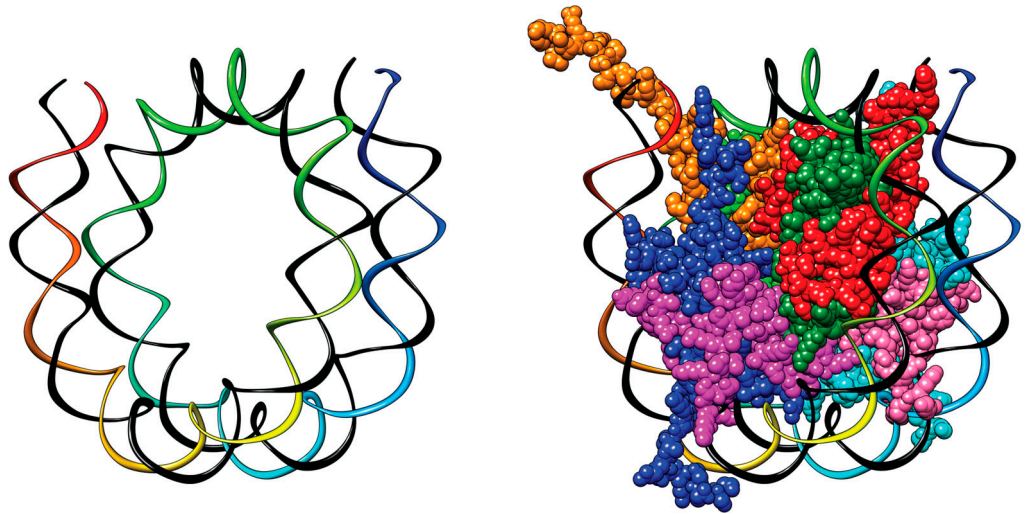

Figure 2. **Canonical H3-containing octameric nucleosome is depicted wrapped with left-handed, negative supercoiling DNA. Henikoff's new study infers a hemisomal CENP-A nucleosome with right-handed, positive supercoiling DNA.**

(Structural representations were created using Chimera and PDBID: 1aoi (1). Left side shows the DNA as ribbons, with one strand colored by rainbow; the right side shows the nucleosome wrapped with DNA. The nucleosome is colored by histone: the two H3 components are colored in red and orange, H4 is represented in dark green and light green, H2A is dark blue and cyan, H2B is purple and pink.)

with a steric preference for right-handed DNA (17). The unexpected positive supercoils could potentially arise from overtwisting of left-handed DNA, but Henikoff rules out this alternative explanation, arguing that the helical twist needed to achieve this over-twisting, combined with the dimensions of a nucleosome, would generate a structurally impossible crossover. Thus by process of elimination, the positive supercoiling they observe indicates right-handed nucleosomes and implies the presence of hemisomes (or at least not octamers).

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TOPOLOGY OF THE TWIST

Henikoff suggests that right-handed DNA at the centromere is a defining topological feature that might expose kinetochore-binding nucleosomal residues that would

otherwise be masked by the DNA. However, "the generality [of Henikoff's model] is an open issue," counters Black. "The physicality issue is completely open and the two sides of the debate could emanate from differences in the proteins of the different species." He points out that a comparison of the amino acid sequences of H3 and CENP-A "provides no indication of changes that would alter the CENP-A/CENP-A interface that links the two halves of the octamer together." Henikoff's model, he argues, offers no explanation for what might happen to this interface in the hemisomal form, and all existing evidence from humans points toward octameric nucleosomes.

It is difficult to reconcile the centromere hemisome model with octameric nucleosomes in which an [H3/H4]$_2$ tetramer assembles first as an intermediate before forming the nucleosome's central core (17). However, in vitro work found that the same process used to identify these [H3/H4]$_2$ tetramers fails to find [CENP-A/H4]$_2$ tetramers (11). Adding to the debate is the mystery of where the [CENP-A/H4/Scm3]$_2$ hexamers fit into the mix.

Despite sequence variation between CENP-As from different species, the proteins can functionally compensate for one another; yeast CENP-A can function in place of human CENP-A. But, things are often more complicated in humans. "When we pull out human CENP-A proteins, they are loaded with other proteins that decorate the nucleosome," says Black. "Some mystery factor X could disrupt the CENP-A interface and lead to hemisome formation, but there is no clue as to what that might be." Black echoes Choo's point about species specificity, stating that "what is now being proposed [by Henikoff] in invertebrates and yeast has not been shown in any vertebrate system at all." In other words, a conserved nucleosome structure may not be necessary for kinetochore assembly.

Despite these issues, says Henikoff, "it's hard for me to imagine the topology being any different in humans… topology is the most basic property of DNA. The next thing we have to do is get a structure, because that would settle it." In spite of these different views in the field, Dalal is positive: "It's a great time to be [working] in the centromere field," she says. "I think everyone will agree on that."

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The transparent image shown was adapted from the RCSB's PDB Molecule of the Month by David Goodsell using PDBID: 1aoi (1) doi:10.2210/rcsb_pdb/mom_2000_7.