Centromeric Nucleosomes Induce Positive DNA Supercoils

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INTRODUCTION

Genomic DNA of eukaryotes is wrapped around octameric nucleosomes containing the CenH3 histone variant in place of canonical H3. Although H3 nucleosomes wrap DNA in a left-handed manner and induce negative supercoils, we show here that CenH3 nucleosomes reconstituted from Drosophila histones induce positive supercoils. Furthermore, we show that CenH3 likewise induces positive supercoils in functional centromeres in vivo, using a budding yeast minichromosome system and temperature-sensitive mutations in kinetochore proteins. The right-handed wrapping of DNA around the histone core implied by positive supercoiling indicates that centromere nucleosomes are unlikely to be octameric and that the exposed surfaces holding the nucleosome together would be available for kinetochore protein recruitment. The mutual incompatibility of nucleosomes with opposite topologies could explain how centromeres are efficiently maintained as unique loci on chromosomes.

SUMMARY

Centromeres of higher eukaryotes are epigenetically maintained; however, the mechanism that underlies centromere inheritance is unknown. Centromere identity and inheritance require the assembly of nucleosomes containing the CenH3 histone variant in place of canonical H3. Although H3 nucleosomes wrap DNA in a left-handed manner and induce negative supercoils, we show here that CenH3 nucleosomes reconstituted from Drosophila histones induce positive supercoils. Furthermore, we show that CenH3 likewise induces positive supercoils in functional centromeres in vivo, using a budding yeast minichromosome system and temperature-sensitive mutations in kinetochore proteins. The right-handed wrapping of DNA around the histone core implied by positive supercoiling indicates that centromere nucleosomes are unlikely to be octameric and that the exposed surfaces holding the nucleosome together would be available for kinetochore protein recruitment. The mutual incompatibility of nucleosomes with opposite topologies could explain how centromeres are efficiently maintained as unique loci on chromosomes.

The presence of a variant histone within a nucleosome has the potential to profoundly alter chromatin structural properties and impact chromosomal processes. At centromeres, the CenH3 variant replaces canonical H3 in centromeric nucleosomes (Buchwitz et al., 1999; Henikoff et al., 2000; Meluh et al., 1998; Palmer et al., 1991; Takahashi et al., 2000) and is essential for the recruitment of other kinetochore components (Heun et al., 2006). Relative to canonical H3, which is one of the most highly conserved proteins known, CenH3s from different organisms are surprisingly diverged, even within the histone core, with N-terminal tails that can be of very different lengths (Malik and Henikoff, 2003). Despite these differences, the function of CenH3 nucleosomes in organizing the kinetochore appears to be invariant. For example, CenH3 from budding yeast (Cse4p) can substitute for human CenH3 (CENP-A) (Wieland et al., 2004), even though only a single Cse4p nucleosome occupies each budding yeast centromere (Furuyama and Biggins, 2007), whereas human centromeres comprise long arrays consisting of thousands of CENP-A nucleosomes (Lam et al., 2006; Schueler et al., 2001). These observations suggest that there are general structural features of CenH3 nucleosomes responsible for their conserved role in forming the foundation of the kinetochore and for their faithful assembly at centromeres every cell cycle (Bloom and Carbon, 1982; Dalal et al., 2007b; Polizzi and Clarke, 1991; Takahashi et al., 1992). Indeed, CenH3 nucleosomes have been found to differ profoundly from their canonical counterparts. Micrococcal nuclease (Mnase) cleaves between canonical nucleosomes to yield familiar nucleosomal ladders with periodicities reflecting internucleosomal distances, but no such ladders were observed for fission yeast CenH3 (Cnp1) (Bloom and Carbon, 1982; Polizzi and Clarke, 1991). Mnase sensitivity was also seen for native Drosophila melanogaster CenH3 (Cid) nucleosomes (Dalal et al., 2007b), in which centromeric DNA was deduced to be wrapped around tetramers of CenH3, H4, H2A, and H2B. The tetrameric organization of Drosophila CenH3 nucleosomes observed in chromatin extracts was confirmed by direct measurement of purified native particle heights using atomic force microscopy and suggested that interphase CenH3 nucleosomes are stable heterotypic tetramers, for which a “hemisome” model has been proposed (Dalal et al., 2007b).

Reconstituted (H3/H4)2 tetramers can be wrapped in either direction (Hamiche et al., 1996), and only the addition of H2A/H2B dimers locks them in the left-handed configuration (Allat et al., 1999). Many archaea package DNA into nucleosomes, which are tetrameric and appear to wrap DNA in a left- or right-handed manner in vitro depending on the salt conditions.
used (Marc et al., 2002; Musgrave et al., 2000; Musgrave et al., 1991). Intriguingly, topological analysis of a yeast minichromosome suggested that deletion of the centromere resulted in more negatively supercoiled DNA, an observation made prior to the discovery of CenH3s and not interpreted as unusual by the authors of the study (Bloom et al., 1984).

Here, we examine the topological state of centromeric nucleosomes in vitro and in vivo to determine the direction of supercoiling induced by substitution of CenH3 for H3 within nucleosomes. We show that Drosophila CID induces positive supercoils when reconstituted into nucleosomes with partner histones in vitro. We confirm this observation in vivo, using wild-type and mutant budding yeast minichromosomes maintained in the presence of temperature-sensitive mutations in kinetochore components. Our findings suggest that positive supercoiling is a general feature of centromeric nucleosomes that has important implications for maintaining centromeres as uniquely defined loci that organize kinetochores.

**RESULTS**

**Reconstituted Drosophila CenH3 Nucleosomes Induce Positive Supercoils**

In previous work, we identified the abundant histone chaperone, RbAp48, as the single nonhistone stoichiometric component of native CID complexes isolated from soluble Drosophila extracts (Furuyama et al., 2006). Although the RbAp48-CID/H4 complex by itself was unable to assemble nucleosomes on DNA, the addition of H2A/H2B dimers led to the assembly of chromatin particles in vitro, as evidenced by electron microscopy, DNaseI digestion, and plasmid supercoiling (Furuyama et al., 2006). In the standard plasmid supercoiling assay, reconstitution of assembled particles onto a closed circular plasmid DNA is performed in the presence of topoisomerase I, which relaxes the compensatory torsional stress on DNA during nucleosome assembly. Subsequent removal of proteins yields a closed circular DNA, in which additional “turns,” each originally induced by the wrapping of DNA around one histone core particle, are now irreversibly trapped (Prunell, 1998). When these plasmids are electrophoretically separated, each additional full turn of nucleosome-wrapped DNA contributes to compaction relative to relaxed “open” circles, yielding a ladder of topoisomers. This assay is indicative of the number of nucleosomes assembled on the plasmid, but not the direction of induced writhe, because both positive and negative supercoils cause compaction relative to relaxed circles.

To ascertain the direction of supercoiling induced by CenH3 core particles assembled by RbAp48, we electrophoresed the deproteinized plasmids in the presence of the intercalating drug chloroquine, which reduces the twist of DNA (see Figure S1 available online). Because the linking number (Lk) is fixed in a covalently closed plasmid, the reduction in twist (Tw) must be compensated for by an increase in writhe (Wr), (\(\Delta Lk = \Delta Tw + \Delta Wr\)) (Prunell, 1998). At a chloroquine concentration of 1 \(\mu g/ml\), plasmids isolated from Escherichia coli migrate more slowly (Figure 1, bottom panel and Figure S2), because they...
are indicated. The relatively low nucleosome density of 2000 bp/8 nucleosomes = 250 bp/nucleosome (yeast average: 165 bp/nucleosome (Nelson and Fangman, 1979)) results from the presence of several well-positioned nucleosomes and several nucleosome-free regions on the minichromosome.

(C) The CEN3+cen6 double centromere minichromosome construct used in Figure 4 (see Table S1). (D) Same as (C) except that CEN6 carries the 2 bp substitution shown in (A). (E) Same as (C) except that both CEN3 and CEN6 carry the 2 bp substitution shown in (A).

are negatively supercoiled and so are relaxed by the additional positive writhe. Similarly, the additional positive writhe induced by chloroquine causes plasmids that are relaxed by pretreatment with topoisomerase to migrate faster than nicked circular DNA (Figures 1A and 1B, bottom panels). The relative position of topoisomers induced with assembled chromatin relative to that of relaxed DNA indicates their topological states (Figure S1). Topoisomers induced by H3-containing nucleosomes are known to be negatively supercoiled; therefore, topoisomers obtained upon chromatin assembly using RbAp48, H3 and its histone partners migrate more slowly in chloroquine-containing gels than initially relaxed plasmids (Figure 1A, bottom panel). In striking contrast, chloroquine intercalation causes topoisomers induced by RbAp48-assembled CID chromatin to migrate faster than initially relaxed plasmids (Figure 1B, bottom panel); therefore, these topoisomers must have had net positive supercoils compared to relaxed plasmids, just the opposite of supercoiling induced by H3 chromatin.

The DNA that we used in supercoiling assays contains a 3 kb segment of a 359 bp D. melanogaster satellite repeat array inserted into a plasmid vector (Furuyama et al., 2006). To ascertain whether DNA sequence might influence supercoiling behavior when CID nucleosomes are assembled, we cloned random 3 kb Drosophila DNA into the same plasmid vector and chose four of the resulting plasmids for supercoiling analysis. In all four cases, we observed positive supercoils induced by RbAp48-assembled CID nucleosomes (Figure 1C), and additional random plasmids yielded the same result (Figure S2). In contrast, reconstituted H3 nucleosomes induced only negative supercoils on a wide variety of DNAs (Bates and Maxwell, 2005). Therefore, we conclude that the direction of supercoiling depends on the presence of CID-containing nucleosomes rather than structural properties of specific DNA sequences.

Loss of CenH3 Reduces Positive Supercoiling in Budding Yeast In Vivo

Topology assays in vivo require small covalently closed DNA circles of a defined sequence that can be distinguished from endogenous sequences in the genome. Higher eukaryotes lack plasmid systems that can be manipulated to yield small closed DNA circles with active centromeres. Furthermore, centromeres of most higher eukaryotes are embedded in long tandem arrays of satellite sequences that contain interspersed blocks of both CenH3 and H3 nucleosomes (Blower and Karpen, 2001; Lam et al., 2006), and sequences known to support centromere function are often hundreds of kilobases in length. This situation is even more challenging in the case of Drosophila, where no single centromeric DNA satellite is common to all chromosomes. Because of these considerations, topological assays cannot be practically performed in Drosophila, and probably other complex eukaryotes, using available technologies. In contrast, each Saccharomyces cerevisiae centromere is specified by a ~125 bp centromere-determining element (CDE, Figure 2A) that contains a single Cse4p nucleosome (Furuyama and Biggins, 2007) and supports regular segregation of a plasmid that carries it (Clarke and Carbon, 1980).

We used a ~2 kb minichromosome derived from the well-characterized TRP-ARS1 construct (Thoma et al., 1984), into which both the Chromosome 3 centromere (CEN3) and a short stretch of bacterial sequence (for Southern blot probing) had been inserted (Figure 2B). The resulting minichromosome contains approximately eight total nucleosomes: one CenH3

Figure 2. Schematic Diagrams of Minichromosomes Used in the Study

(A) A diagram of a 190 bp CEN region. It consists of CDEI (small box), CDEII (large box), and CDEIII between the boxes. The conserved CDEIII sequence with two single base pair substitutions that abolish centromere functions are shown. (B) The CEN3 minichromosome construct used in Figure 3 (a gift from S. Biggins and T. Tsukiyama). This ~2 kb minichromosome contains a TRP selectable marker (dotted arrow indicates the location of ORF), CEN3 (solid arrow), and a short stretch of bacterial DNA (green bar), which was used as the Southern blot probe target, because it does not cross-hybridize with yeast genomic DNA (data not shown). The diagram is approximately to scale. The known locations of H3 nucleosomes that were previously mapped (Thoma et al., 1984) are shown as blue circles. Red circles indicate the presumed locations of Cse4p nucleosomes. We do not know whether a nucleosome is present over the bacterial sequence used for Southern blotting (dashed circle). Expected numbers of H3 and Cse4p nucleosomes (assuming that there are no nucleosomes corresponding to the dashed circle)
nucleosome at CEN3 plus seven previously mapped well-positioned H3 nucleosomes (Thoma et al., 1984) (although in what follows, the absolute number of nucleosomes on the minichromosome is not important). To observe this minichromosome in a configuration that lacks a centromere, we maintained it in an ndc10-1 background. ndc10-1 is a temperature-sensitive mutation in a component of the CBF3 complex, which binds to the CDEIII cis-acting element within the 125 bp CDE, and is required for the localization of Cse4p to CEN (Ortiz et al., 1999; Pearson et al., 2003). Both ndc10-1 and wild-type strains carrying the minichromosome were arrested at the G1/S phase boundary with α factor and then released into S phase at the restrictive temperature (37°C). As expected, loss of Cse4p in ndc10-1 results in eight left-handed H3 nucleosomes, each of which induces a negative supercoil. However, if Cse4p induces positive supercoiling as we observed in vitro, seven left-handed H3 nucleosomes and one right-handed CenH3 nucleosome in wild-type would contribute six net negative supercoils, for a difference of −2 supercoils between ndc10-1 and wild-type (For an explanation of the in vivo DNA topology assay, see Figure S3). We find that the topoisomer distribution of the minichromosome isolated from wild-type is shifted up (becomes less negative) by an average of 1.33 supercoils (−2.86), compared with the identical minichromosome isolated from the ndc10-1 mutant strain (−4.19) (Figure 3). The average number of negative supercoils in wild-type might have been overestimated, because the band corresponding to a topoisomer with no net writhe in the presence of chloroquine comigrates with a nicked circle (Figure S4) and so was not included in the calculation. Therefore, the difference between wild-type and the ndc10-1 mutant might be closer to 2 than 1.33, which would imply that loss of the centromere results in loss of a positive supercoil induced by the CenH3 nucleosome and gain of a negative supercoil induced by the H3 nucleosome that replaces it. The observed shift in topoisomer distribution between wild-type and ndc10-1 is similar to the shift seen when the centromere was deleted from a minichromosome (Bloom et al., 1984).

**The Number of Positive Supercoils Corresponds to the Number of Functional Centromeres**

A complication of comparing yeast plasmids with and without a functional centromere is that the proteinaceous yeast kinetochore is present throughout the cell cycle (McAinsh et al., 2003), and this evidently protects a region of ≈200 bp from MNase digestion, compared to≈160 bp protection afforded by the presence of a typical H3 nucleosome (Bloom and Carbon, 1982). Consequently, if Cse4p induces a negative supercoil, then the 1.33 negative supercoil gain with centromere loss might be attributable to the gain of two H3 nucleosomes that would replace the lost Cse4p nucleosome. Therefore, the observed topoisomer shifts do not rule out the possibility that Cse4p induces a negative supercoil.

To address this alternative interpretation, we constructed minichromosomes of identical size with zero, one, or two centromeres (Figure 2; Table S1), which effectively increases the resolution of the plasmid supercoiling assay (Figure S3). Yeast sequences taken from CEN3 and CEN6 were inserted as close as possible next to one another (CEN3+CEN6) (Figure 2) to reduce the dicentric chromosome instability, which is known to increase with distance between the two centromeres (Koshland et al., 1987). To eliminate centromere function without changing the size of the minichromosome, two base-pair substitutions were made in the critical CDEIII region, which causes loss of CBF3 complex binding and abolition of centromere function (Jehn et al., 1991). Minichromosomes that contain these base pair substitutions in one or both centromeres were also constructed (CEN3+CEN6mut and CEN3mut+CEN6mut, respectively). The resulting minichromosomes of identical size with zero, one, or two functional centromeres were used to transform wild-type and ndc10-1 strains.

To confirm the dicentricity of our CEN3+CEN6 construct, we measured characteristics previously documented for dicentric minichromosomes (Koshland et al., 1987). Strains carrying a dicentric minichromosome grow more slowly than strains carrying a monocentric minichromosome in selective media. Similar growth delays were consistently observed for our CEN3+CEN6 construct (Table 1). In addition, CEN3+CEN6 displayed a lower mitotic stability phenotype with a value very similar to

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**Figure 3. A Yeast Minichromosome Loses Negative Supercoils with a Functioning Centromere**

Total DNA was isolated from either a wild-type (WT) or a mutant ndc10-1 strain carrying the same CEN3 minichromosome (Figure 2B), released from α factor arrest at the restrictive temperature (37°C), and purified DNA was resolved on an agarose gel containing 0.3 μg/ml chloroquine. A Southern blot to detect the minichromosome is shown on the left. A densitometry trace of each lane is shown on the right. The most slowly migrating band contains both a nicked circle (N) and a topoisomer with no net writhe in the presence of chloroquine (0). The numbers correspond to the value of net writhe in this chloroquine gel. The minichromosome has fewer negative supercoils in the wild-type strain with Cse4p present at the centromere, compared with the ndc10-1 mutant strain, which loses Cse4p at the restrictive temperature. The arrow in the trace indicates the location of the mean topoisomer distribution. The asterisks mark the bands containing nicked and “0” topoisomers, which was omitted from calculating the mean distribution. Nicked and “0” topoisomers were resolved by two-dimensional electrophoresis, where similar results were obtained (Figure S4).
that reported previously for a dicentric minichromosome (Koshland et al., 1987) (Table 1).

We also tested our CEN3mut+CEN6mut constructs for loss of centromere function. When placed under selection for tryptophan, minichromosomes without a functional centromere are known to be maintained at elevated copy numbers relative to CEN plasmids (Hill and Bloom, 1987). As expected for a minichromosome without centromere function, the CEN3mut+CEN6mut minichromosome is maintained at a higher copy number (~10 copies per cell), as determined by probing Southern blots for both the endogenous TRP locus and the TRP locus on the minichromosome (Table 1; Figures S5 and S6A). A minichromosome with a single functional centromere is known to be maintained at ~2–3 copies per cell (Resnick et al., 1990), and our results are consistent with that observation. The CEN3+CEN6 minichromosome is maintained at lower copy number than a minichromosome with one functional centromere, which probably reflects dicentric chromosome instability. Taken together, the genetic characterization of strains carrying zero, one, or two centromeres show that each wild-type centromere is functional and each mutated centromere is not.

These minichromosome constructs display the topological differences that are expected if each CenH3 nucleosome induces one positive supercoil. The CEN3mut+CEN6mut minichromosome, which contains no functional centromeres, is the most negatively supercoiled construct in wild-type cells, whereas the addition of one or two functional centromeres results in progressively fewer supercoils (Figure 4A, lanes 1–6; Figure S5; Table 2). Small topological differences were also observed for minichromosomes obtained from wild-type cells grown at 25°C and 37°C, as expected from the known partial unwinding of DNA at higher temperature; this produces a compensatory increase in positive writhe that is removed by cellular topoisomerases in vivo, and results in net negative supercoils (Saavedra and Huberman, 1986) (Figure 4, lanes 1 versus 2 and 3 versus 4; Table 2).

Table 1. Doubling Time, Copy Number, and Mitotic Stability of Di-, Mono-, and A-Centric Minichromosomes

<table>
<thead>
<tr>
<th>Genetic Background</th>
<th>No. of CEN</th>
<th>Doubling Time(min)a</th>
<th>Mitotic Stability per Generationb</th>
<th>Copy Number per Cellc</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>2</td>
<td>325.1 ± 8.7</td>
<td>0.740 ± 0.029</td>
<td>1.53 ± 0.06</td>
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<tr>
<td></td>
<td>1</td>
<td>219.7 ± 16.7</td>
<td>0.958 ± 0.057</td>
<td>4.01 ± 0.71</td>
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<tr>
<td></td>
<td>0</td>
<td>219.1 ± 7.8</td>
<td>0.952 ± 0.026</td>
<td>9.96 ± 2.62</td>
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<tr>
<td>ndc10</td>
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<td>321.0 ± 23.2</td>
<td>0.793 ± 0.028</td>
<td>1.50 ± 0.17</td>
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<td>266.6 ± 0</td>
<td>0.942 ± 0.034</td>
<td>3.36 ± 0.31</td>
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<tr>
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<td>0</td>
<td>217.0 ± 11.4</td>
<td>0.938 ± 0.031</td>
<td>11.52 ± 1.20</td>
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<tr>
<td>ndc80</td>
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<td>0.456 ± 0.396</td>
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<tr>
<td></td>
<td>1</td>
<td>281.1 ± 6.7</td>
<td>0.850 ± 0.001</td>
<td>1.63 ± 0.12</td>
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<td></td>
<td>0</td>
<td>230.1 ± 6.7</td>
<td>0.948 ± 0.044</td>
<td>8.39 ± 2.79</td>
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a The average doubling time (±SD) at 25°C of early- to mid-log cultures for three independent transformants of the indicated genotype.
b The mitotic stability (±SD) from three independent transformants (Experimental Procedures).
c The average copy number (±SD) of minichromosomes per cell in three independent transformants (Experimental Procedures; Figure S4).

We can quantify the net supercoil change attributable to centromere mutations. The most slowly migrating band in each lane corresponds to nicked circles and topoisomers with no net writhe (0). The second most slowly migrating band corresponds to one with a net writhe value of ~1, and so on. In the CEN3mut+CEN6mut construct, which is expected to have ~9 total nucleosomes (Figure 2E), we observed at least eight topological isomers. However, in the CEN3+CEN6 construct, which has two functional centromeres, we can reliably count only five topoisomers, approximately four fewer than observed for the same construct with mutated centromeres (Figures 4 and S3). It is not plausible that two functional centromeres remove four H3 nucleosomes from other regions of the minichromosome. It is also not plausible that mutating both centromeres results in a gain of four additional H3 nucleosomes, because a gain from 9 to 13 nucleosomes would exceed the maximum capacity of this 2 kb minichromosome for octamers. If two out of nine total nucleosomes are positively supercoiled, we expect net ~5 supercoils rather than ~9 supercoils predicted from 9 nucleosomes that are negatively supercoiled (Figure S3). Analysis of the densitometry trace of the gel reveals that the mean distribution of topoisomers in lane 1 and lane 5 is shifted by approximately three to four supercoils (Figure 4B WT panel; Table 2). The CEN3+CEN6mut minichromosome with one functional centromere is intermediate between the two as we expected, showing approximately seven topoisomers, and the mean distribution is shifted by two to three supercoils from one with no functional centromeres. Therefore, we conclude that each functional centromeric nucleosome containing Cse4p induces one positive supercoil into the minichromosome, canceling one negative supercoil induced by a canonical H3-containing nucleosome.

A Defective Centromeric Nucleosome, but Not a Defective Kinetochore, Causes Progressive Loss of Positive Supercoils

To show that the differences in topological states of these minichromosomes depend on the presence of Cse4p nucleosomes, minichromosomes were isolated from ndc10-1 mutant cells at either the permissive (25°C) or restrictive (37°C) temperature. As discussed above, Cse4p fails to localize to centromeres when the ndc10-1 mutant is allowed to pass through S phase at the restrictive temperature (Ortiz et al., 1999; Pearson et al., 2003). As expected, all three constructs behave similarly to their respective constructs in the wild-type background at the permissive temperature; however, all three constructs become virtually indistinguishable at the restrictive temperature, reaching the most negatively supercoiled states of the CEN3mut+CEN6mut minichromosome with no functional centromere (Figure 4, lanes 7–12; Table 2). This indicates that in the ndc10-1 mutant at the restrictive temperature, Cse4p nucleosomes that induce positive supercoils are replaced by H3 nucleosomes, which in turn induces negative supercoils into minichromosomes.

Because ndc10-1 mutants at the restrictive temperature disrupt both Cse4p nucleosomes and kinetochore functions in general, it is formally possible that kinetochore function, which is to attach the centromere to the mitotic spindle, can directly or indirectly alter the level of supercoiling by physically pulling
on DNA (Gore et al., 2006). In this case, changes in linking number would occur with kinetochore loss, complicating the interpretation of our experiments. To rule out this possibility, we determined the topological states of minichromosomes isolated from cells that carry a temperature-sensitive mutation in the ndc80 gene, which encodes a component of the central kinetochore (Welburn and Cheeseman, 2008). This ndc80-1 mutation does not affect the localization of Cse4p; however, it will cause failure of the mitotic spindle to attach to centromeres. Topological analysis shows that the ndc80-1 mutation has a much smaller effect on the topological states of minichromosomes relative to ndc10-1 (Figure 4), indicating that microtubule attachment does not substantially change topological states of minichromosomes (see also Table 2). It has been reported that the ndc80-1 mutation genetically interacts with ndc10-1 (Wigge and Kilmartin, 2001); therefore, the small changes in the supercoiling states in a more negative direction in the ndc80-1 mutant strain relative to wild-type at the restrictive temperature might be attributed to a marginally increased loss of Cse4p from centromeres. Importantly, ndc80-1 mutant cells released from a restrictive temperature arrest at mitosis, so that the observed shift in the topoisomer distribution occurs during mitosis, when kinetochores function. Therefore, positive supercoiling induced by Cse4p nucleosomes is a feature of functional kinetochores.

Only kinetochore proteins that come into contact with centromeric DNA are candidates for affecting DNA topology. Condensin from Xenopus has been shown to induce positive supercoils in vitro (Kimura and Hirano, 1997), although budding yeast condensin lacks this in vitro activity (Stray et al., 2005). We observed no changes in the distribution of topoisomers on CenH3 minichromosomes in a condensin ycg1 mutant (Figure S7), which indicates that condensin plays no role in inducing positive supercoils on these minichromosomes. Rather, our data indicate that either the Cse4p nucleosome or the CBF3 complex, which contains Ndc10, is responsible for the observed positive supercoils. Because of the small footprint of CBF3 on DNA, it is highly unlikely that this complex alone can change topology by one linking number. Therefore, we conclude that changes in the topological states of minichromosomes result from the presence or absence of Cse4p nucleosomes.

**DISCUSSION**

We have shown that CenH3 nucleosomes induce positive supercoils, both when *D. melanogaster* CID is reconstituted into nucleosomes in vitro and when *S. cerevisiae* Cse4p is assembled at functional minichromosome centromeres in vivo. This behavior is in stark contrast to canonical nucleosomes, in which the left-handed wrapping leads to induction of negative supercoils in topological assays. Our observations of positive supercoiling induced by CenH3 from eukaryotic taxa as different as animals and fungi can be explained by either of two general models: over-twisting with left-handed wrapping or right-handed wrapping.

In a covalently closed circle, overtwisting of DNA (positive ΔTw) causes compensatory negative writhe that is removed by
The mean supercoiling density was calculated from the densitometry trace in Figure 4B. The area under each peak except for the peak corresponding to a nicked circle and a topoisomer with no net writhe was determined using ImageJ. The supercoiling value corresponding to a nicked circle and a topoisomer with no net writhe was presumed to be 0.

Table 2. Mean Supercoiling Densities of Minichromosomes

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*The mean supercoiling density was calculated from the densitometry trace in Figure 4B. The area under each peak except for the peak corresponding to a nicked circle and a topoisomer with no net writhe was determined using ImageJ. The supercoiling value is calculated as the mean supercoiling density of minichromosomes.

Table 2. Mean Supercoiling Densities of Minichromosomes

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*The mean supercoiling density was calculated from the densitometry trace in Figure 4B. The area under each peak except for the peak corresponding to a nicked circle and a topoisomer with no net writhe was determined using ImageJ. The supercoiling value is calculated as the mean supercoiling density of minichromosomes.

denominating, resulting in a net positive \( \Delta Lk \) after deproteination (Malcolm and Snounou, 1983). If CenH3 nucleosomes are left-handed octamers (\( \Delta Lk = 1 \)), they would need to be +2 in order to result in a \( \Delta Lk \) of +1 (\( \Delta Lk = \Delta Tw + \DeltaWr \), where \( \Delta Wr \) is the reported value of \( \Delta Wr \) for left-handed octamers varies (Bancaud et al., 2006; Prunell, 1998). With the most conservative cited value of \( \Delta Wr \) to calculate the degree of overtwisting consistent with left-handed wrapping, the change required in the helical periodicity of DNA (\( \Delta h \)) to gain \( \Delta Tw = +2 \) and cancel one negative writh induced by a left-handed nucleosome can be calculated as \( \Delta h = -h^2 \times \Delta Tw/N \), where \( h = N/Tw \), and where \( N \) is the number of base pairs wraped around the nucleosome. If we assume an octameric CenH3 nucleosome (\( N = 150 \) bp), \( \Delta h \) equals \( -1.47 \) for \( \Delta Tw = +2 \). This corresponds to a helical periodicity of 9.03 bp/turn (whereas \( h = 10.5 \) bp/turn for B-DNA free in solution). The situation is even more extreme for CenH3 hemisomes, which wrap 80–120 bp of DNA, because the same amount of twist must be taken up by the shorter span of DNA (helical periodicity of 7.74–8.66 bp/turn). These estimated values for helical twist are conservative in that they assume that the extra twist is distributed over the whole nucleosome, including the DNA that wraps H2A/H2B dimers, whereas in the crystal structure of the H3 nucleosome core particle, the twist of DNA wrapping H2A/H2B is similar to that in free solution (Luger et al., 1997). In addition, DNeasel digestion of Cid chromatin assembled in vitro resulted in a normal helical periodicity estimate of ~10 bp/turn (Furuyama et al., 2006), and electron microscopy of Cid chromatin revealed a beads-on-a-string appearance (Dalal et al., 2007b; Furuyama et al., 2006), suggesting entry/exit crossing. Thus, existing data are inconsistent with positive \( \Delta Tw \) being the reason for the observed positive supercoiling.

The implausibility of such strongly overtwisted DNA wrapping around a left-handed nucleosome leads us to conclude that positive supercoiling instead indicates a right-handed wrap. A right-handed nucleosome would satisfy the observed positive supercoiling of approximately one supercoil per CenH3 nucleosome without a significant change in B-DNA periodicity. Tetrameric archaeal nucleosomes also wrap DNA in a right-handed configuration, with a helical periodicity of 10–11 bp/turn (Musgrave et al., 1991). Also, in the absence of H2A/H2B dimers, (H3/H4)2 tetramers are capable of spontaneously shifting between both left- and right-handed configurations (Hamiche et al., 1996), presumably without significant changes in helical twist.

Histone octamers capable of wrapping DNA into a right-handed configuration have never been observed. Because H3/ H4 tetramers can wrap DNA in either direction, it is the creation of a left-handed ramp by addition of two H2A/H2B dimers that is incompatible with the right-handed structure (see Figures 5A and 5B). The crystal structure of the H3 nucleosome (H2A'- H2B'-H4'-H3-H4-H2B-H2A plus DNA) reveals that the N-terminal helix of H3, as well as the C terminus of H4, contact the C-terminal docking domain of H2A', which are essential interactions that hold the octamer together (Luger et al., 1997). In addition, the interaction between H2A and H2A' within the octamer through their Loop 1 regions holds together the two gyres of the DNA superhelix (Luger et al., 1997). These interactions that hold the octamer together are expected to be disrupted in a right-handed nucleosome because they would face away from each other in the right-handed structure (Figures 5C and 5D); therefore, there is a strong structural basis for the absence of right-handed octameric nucleosomes in eukaryotes. Without altering the twist of DNA significantly, the only structures that yield \( \Delta Lk = +1 \) other than a right-handed octamer are right-handed hemisomes with right entry/exit crossing, and left-handed hemisomes with right entry/exit crossing. A single superhelical turn of DNA around a hemisome results in a closer physical distance between the entry/exit DNA, compared with that in an octameric structure, which has an additional turn between the two entry/exit sites (compare Figure 5B with 5F). Therefore, it is structurally very difficult to make a left-handed hemisome with a right-handed crossing. In budding yeast, various models of Cse4p nucleosomes have been suggested, including octamers (H2A/H2B/H4/Cse4p/Cse4p/H4/H2B/H2A) (Meluh et al., 1998), hemisomes (Cse4p/Cse4p/H4/H2B/H2A) (Dalal et al., 2007a), and nucleosomes containing the nonhistone Scm3 protein substituting for H2A/H2B dimers (H4/Cse4p/Cse4p/H4)(Scm3)1–2 (Mizuguchi et al., 2007). Given our finding that Cse4p nucleosomes induce positive supercoils, it is unlikely that they can exist as octamers. Furthermore, the observation that Scm3 binds to the region of Cse4p required for the four-helix bundle homodimerization interface of the octameric particle (Stoler et al., 2007) would be a priori against a stable octameric particle. That leaves either Cse4p/H4/H2A/ H2B hemisomes or Cse4p/H4/Scm3 particles as candidate yeast CenH3 nucleosomes. Both of these models are consistent with the localization of Cse4p to a small ~80 bp CDEII region of
It is attractive to suggest that right-handed hemisomes are conserved in all eukaryotes, because Cse4p can functionally replace human CENP-A (Wieland et al., 2004). There are several structural implications of right-handed hemisomes. The strong H3/H3 four-helix bundle at the dyad axis and the weak H4/H2B four-helix bundles linking the central tetramer to flanking dimers precludes formation of H3/H4/H2B/H2A hemisomes, and indeed no stable H3 hemisomes have been observed. Therefore, the existence of CenH3 hemisomes suggests that CenH3 induces structural alterations that stabilize the tetrameric particle. The crossing of entry/exit DNA in the CenH3 hemisome may be an important feature, because it can potentially stabilize the hemisome. In contrast, the entry/exit DNA of H3 octameric nucleosomes does not cross most of the time, but rather is occupied by a linker histone (Bancaud et al., 2006; Prunell, 1998). Consistent with this difference, the H1 linker histone is depleted from centromeric chromatin (Maresca et al., 2005), and the H5 linker histone is incapable of associating with human CENP-A nucleosomes in vitro (Conde e Silva et al., 2007). In addition, surfaces involved in contacts within left-handed octameric nucleosomes will be exposed in right-handed nucleosomes, such as the C-terminal docking domain of H2A and the N-terminal helix of H3 (Figures 5A and 5B). A right-handed configuration also changes the relative position of these domains (Figures 5C and 5D). The combination of additional exposed surfaces and altered presentation of the same surfaces might provide essential interaction domains for kinetochore proteins to assemble functional centromeres. Our finding that CenH3 nucleosomes are right-handed also might help explain why key residues involved in H3/H3 four-helix bundle formation are invariant in CenH3s, despite considerable divergence elsewhere in the core. This observation suggests that the CenH3 dimerization interface is occupied under at least some circumstances. We suggest that this interface has been retained to permit CenH3/H3 hybrid formation (Foltz et al., 2006), which would result in left/right core particles that should be unable to stably wrap DNA. Misincorporation of CenH3 outside of centromeres occurs under many circumstances (Blower and Karpen, 2001; Henikoff et al., 2000; Tomonaga et al., 2003; Van Hooser et al., 2001), yet is potentially catastrophic, causing dicentric formation, chromosome loss, and dominant lethality (Heun et al., 2006; Tomonaga et al., 2003). By retaining the ability to dimerize with H3, misincorporated CenH3s would predominantly form structurally defective nucleosomes, thus helping to maintain the extraordinary fidelity of centromere maintenance.

At the boundary between CenH3 and H3 nucleosomal arrays, the change in the direction of DNA around histones from left-handed to right-handed might also have profound implications for maintaining functional centromeres. The uniform packaging of H3 nucleosomes in pericentric heterochromatin, induced in part by the uniform size of centromeric satellite repeats, is expected to be disturbed by the sudden change in the direction of DNA wrapping around CenH3. This would result in a higher-order structural transition from near-crystalline rigid...
heterochromatin to less densely packaged centromeric chromatin as implied by the unusually long linker DNA found in Drosophila centromeric chromatin (Dalal et al., 2007b). The octameric form of canonical H3 nucleosomes is believed to represent a critical evolutionary leap in being able to more densely package the genome, whereas tetrameric archaeal nucleosomes fail to condense into a comparable higher order packaging (Pereira et al., 1997; Sandman et al., 1998). Therefore, the presence of a CenH3 hemisome array that packages DNA in a right-handed orientation and resists octameric packaging would provide a singular location that remains decondensed during mitosis and accessible to binding by kinetochore proteins. The mutual incompatibility of nucleosome cores that wrap DNA in opposite directions suggests a novel mechanism for perpetual maintenance of the centromere within a chromosomal landscape that is dominated by conventional chromatin.

**EXPERIMENTAL PROCEDURES**

**In Vitro Chromatin Assembly**

In vitro chromatin assembly reactions were performed as previously described (Furuyama et al., 2006). Briefly, 0.5 µg of plasmid pCR4-360x8 was relaxed with Topoisomerase I. Purified C/H4, RbAp48, and H2A/H2B were added and incubated for 2 hr at room temperature in the presence of Topoisomerase I. Plasmid DNA was deproteinized and purified by standard methods, and then topoisomers were resolved in agarose gels ± 1 µg/ml chloroquine. Some reactions were done using plasmids containing randomly cloned Taara/C14 using a radiolabeled oligonucleotide (ACTAGCAATTGTGAGCGGA).

**Yeast Genetic Analysis**

All yeast strains (Table S1) were cultured in either YPD media for nonselective growth or yeast nitrogen base without amino acids supplemented with TRP dropout powder (Sigma) and 2% glucose for selection of strains carrying minichromosomes. Doubling times were determined as follows. Three independent transformants were selected at random and cultured to late log overnight. All yeast strains (Table S1) were cultured in either YPD media for nonselective growth or yeast nitrogen base without amino acids supplemented with TRP dropout powder (Sigma) and 2% glucose for selection of strains carrying minichromosomes. Doubling times were determined as follows. Three independent transformants were selected at random and cultured to late log overnight. Cultures were diluted to OD595 = 0.2, and OD600/OD595 measurements were taken every hour. The data from early- to mid-log phase were fitted to an exponential curve, and then the doubling time was calculated from the exponent of the best fit curve using Microsoft Excel. Copy numbers were determined by quantifying the intensity of the TRP selectable marker on the minichromosomes to that of the endogenous TRP locus by Southern blot analysis. Mitotic stability measurements were done essentially as previously described (Kosherland et al., 1987) from three independent transformants. Briefly, each strain was cultured in YPD for ~10 generations, then plated on YPD or TRP-dropout plates. The tenth root of the ratio of the number of resulting colonies on selective versus nonselective plates approximates the probability of minichromosome loss per generation.

**Topological Assays of Minichromosomes**

All strains were grown at 25°C to mid-log phase, then α factor was added to 1 µg/ml for strains in the wild-type SBY3 background and 10 µg/ml for the others, depending on their genotypes at the barT locus. Cultures were incubated for 1.5–2 hr to arrest at the G1/S phase boundary and were spun, and cell pellets were washed to remove α factor and then resuspended in fresh media. Half of each culture was incubated at 25°C while the other half was incubated at 37°C for 2 hr. Total DNA was purified by standard methods and then electrophoresed on 15% Tris-Borate-EDTA gels containing 0.3 µg/ml chloroquine. The value of 0.3 µg/ml was empirically determined for this minichromosome to obtain maximal resolution of all topoisomers. Two-dimensional gel electrophoresis was also performed (Figure S4). Approximately 5- to 10-fold less DNA was used for strains carrying the CEN3mut-CEN6mut construct because of its high copy number. Southern blotting was performed at 57°C using a radiolabeled oligonucleotide (ACTAGCAATTGTGAGCGGAGA TAAACATT, which is present in multiple tandem copies on the minichromosomes). Blots were autoradiographed using X-ray film or scanned using a Typhoon PhosphorImager, then analyzed using ImageJ software to obtain densitometrict traces of each lane and areas under each peak. The uppermost band in each lane, which includes topoisomers with zero net writhe in the chloroquine gel and nicked circles, was omitted from the calculation because of varying amounts of nicked species in different DNA preparations.

**SUPPLEMENTAL DATA**

Supplemental Data include seven figures and one table and can be found with this article online at http://www.cell.com/supplemental/0009-2874(09)00510-6.

**ACKNOWLEDGMENTS**

We thank Kerry Bloom for describing previous work that encouraged our yeast minichromosome study, Sue Biggins for helping us with the yeast experiments, Yamini Dalal for discussions regarding positive supercoiling, and Sue Biggins, Bungo Akiyoshi, and Toshi Tsukiyama for yeast plasmids and strains. We thank Sue Biggins, Yamini Dalal, Roger Deal, Paul Talbert, Toshi Tsukiyama, and Danielle Vermaak for critical readings of the manuscript.

Received: December 13, 2008

Revised: March 4, 2009

Accepted: April 14, 2009

Published: July 9, 2009

**REFERENCES**


Right-Handed Nucleosome: Myth or Reality?

In their recent paper in Cell, Furuyama and Henikoff (2009) report that nucleosomes in centromeres may be right-handed, that is, they wrap DNA in a right-handed manner and induce positive supercoils. This raises intriguing new questions, such as how centromeric histone variants may be assembled into right-handed particles, and why chromatin would retain negative supercoiling in chromosome arms but positive supercoiling in centromeres. We wish to comment on these new findings in the context of topological insights that we have gained from recent in vitro experiments with centromeric nucleosomes and single chromatin fibers submitted to torsional constraints and from 3D modeling of chromatin dynamics. We will also discuss alternative compositions of centromeric nucleosome particles and suggest potential mechanisms by which local positive supercoiling may be established.

Henikoff and coworkers previously identified half-nucleosomes—containing only one copy each of H2A, H2B, and H4 histones and the CenH3 histone variant—in the centromeres of Drosophila interphase nuclei (Dalal et al., 2007). In their new work, Furuyama and Henikoff (2009) propose that these tetrameric particles, now called hemisomes (Lavelle and Prunell, 2007), wrap DNA in a right-handed manner compared to “canonical” nucleosomes that wrap DNA in a left-handed manner. To reach that conclusion, these authors analyzed centromeric particles obtained from the in vitro reconstitution of purified components of Drosophila cells and from direct assembly in budding yeast in vivo. Because the histone composition of these particles was not determined, the question remains as to whether they are truly composed of right-handed hemisomes (H2A/H2B-CenH3/H4), or whether they are composed of right-handed tetrasomes [(CenH3/H4)2], hexasomes [H2A/H2B-(CenH3/H4)3], or even nucleosomes [H2A/H2B-(CenH3/H4)2-h2A/H2B].

Based on topological assays of nucleosomes reconstituted on DNA minicircles, Prunell and colleagues provided the first in vitro evidence for the existence of right-handed (H3-H4)2 tetrasomes (Hamiche et al., 1996; Aillett et al., 1999). We later showed that nucleosome arrays subjected to a large positive torsional stress transiently trap approximately one positive turn of DNA supercoiling per particle. We suggested that this may reflect the chiral transition of nucleosomes, resulting in the formation of metastable octameric particles built on the right-handed tetrasome (so-called reversomes for reverse nucleosomes) (Bancaud et al., 2007). We were able to model the transition steps and to propose an atomic structure for the reversome (Figure S1 and Movie S1 available online). The absence of docking domains between the H2A/H2B dimers and the (H3-H4)2 flipped tetramer, as made clear by the all-atom structure, results in a more open architecture with relatively destabilized dimers (Figure S1). Moreover, chromatin fibers reconstituted from CENP-A-containing nucleosomes were also shown to undergo the reversome transition under positive torsional constraints (unpublished data). These results indicate that the right-handed centromeric particles could, in principle, be made of reversomes. However, in yeast, where point and regional centromeres are both depleted of H2A-H2B histones (Mizuguchi et al., 2007), it is not reversomes or hemisomes, but rather tetrasomes or hexasomes, that are the likely candidates.

Whatever the composition of the centromeric particles, the question is: how is right-handedness generated and stabilized? Right-handedness is not expected to be an intrinsic property of CenH3-containing particles alone. Topological assays of human CENP-A nucleosomes in vitro have shown that they are left-handed, although their conformational dynamics were somewhat different from those of canonical H3-containing nucleosomes (Conde e Silva et al., 2007). In fact, the chiral transition is energetically unfavorable, by ~1 kcal.mol−1 for the tetrasome and by ~5 kcal.mol−1 for the reversome (Sivolob et al., 2000; Bancaud et al., 2007). It follows that during nucleosome assembly, right-handedness is likely to be mediated by histone chaperones such as RbAp48 or Scm3 (Furuyama and Henikoff, 2009; Dechaussé et al., 2009). Further stabilization through particle-particle interactions may eventually be required, as exemplified by tetrasomes formed by archaeal HM histones, which trap positive supercoils when assembled in arrays (Mustaço et al., 1991) but fail to do so as individual particles on DNA minicircles (A.P., unpublished data). Tetrasomes are indeed capable of stacking on top of each other (Lavelle and Prunell, 2007), and it remains to be seen if such a mechanism also applies to hemisomes. The precise path of assembly of centromeric particles may also be important, as suggested by the intriguing observation that Nap1-mediated deposition of H3/H4 as either a tetramer or a dimer results in negative or positive supercoils, respectively, in the ensuing tetrasome array (Peterson et al., 2007).

One might also ask whether centromeric nucleosomes are right-handed in all organisms or throughout the cell cycle of a single organism. The normal histone composition of the human CENP-A nucleosome-associated complex (Foltz et al., 2006) suggests that this is not the case. Centromeric nucleosomes might for example be left-handed after DNA replication. The positive supercoiling waves generated at a distance by tracking enzymes, such as helicases or polymerases, might then help to trigger partial or complete release of H2A-H2B, followed by flipping to the right-handed conformation and stabilization by further protein binding and/or particle interactions. Such H2A-H2B release under positive stress is supported by the observed higher lability of CENP-A nucleosomes (Conde e Silva et al., 2007). Notably, when DNA is submitted to extensive mechanical torques, supercoiled structures will react to those torques according to their supercoiling polarity. Hence, whereas a positive torque would destabilize left-handed particles such as canonical nucleosomes, it would lock right-handed particles provided that they are stabilized by some (intra-particle) docking domains or (inter-particle) stacking interactions. Stable particles with positive supercoiling would thus provide a natural block to transcription, suggesting an evolutionary reason for the presence of constitutive positive supercoiling constraints in centromeres.
In conclusion, the composition of centromeric particles—octameric, hexameric, tetramer (tetrosomes or hemisomes), or a combination of these—as well as the mechanisms by which centromeric chromatin acquires stable positive supercoiling certainly need further clarification. However, the results of Furuyama and Henikoff confirm that nucleosomes are not mere repetitive “canonical” chromatin entities: not only do they have a well-acknowledged polymorphism stemming from the existence of histone variants, histone posttranslational modifications, and sequence-dependent properties of the wrapped DNA, but they also come in different histone/DNA stoichiometries and opposite chiralities. Overall, the coexistence of negative and positive chromatin supercoiling at specific loci of chromosomes enhances the physiological importance of DNA topology.

**Supplemental Data**

Supplemental Data include one figure and one movie and can be found with this article online at http://www.cell.com/supplemental/S0092-8674(09)01561-X.

**REFERENCES**


Response

**Right-Handed Half-Nucleosomes at Centromeres**

Our study in *Cell* demonstrated that centromeric nucleosomes induce positive supercoils. We showed this using both the *Drosophila* CenH3 histone variant assembled in vitro by its native chaperone (RbAp48) and yeast minichromosomes in vivo (Furuyama and Henikoff, 2009). To verify the in vivo results, we used yeast centromere mutants and conditional mutant kinetochore proteins. We showed that positive supercoiling is an inherent property of functional centromeres at mitosis and depends on deposition of CenH3 (Cse4). In their Correspondence, Lavelle et al. accept our conclusions with respect to the topology of centromeric DNA and agree that this is a provocative result. However, they raise a key question that was only indirectly addressed by our study, namely, which protein core structure can impose such an extraordinary reversal of DNA wrapping? Our views and theirs on this subject are largely in agreement, in that conventional octameric nucleosomes are inconsistent with the right-handed wrapping that can lead to positive supercoiling. However, we favor nucleosomes as the candidate core structure, based on previous direct in vivo biochemical evidence. The *Drosophila* CenH3 histone variant is a stoichiometric component of stable tetrameric nucleosomes purified in their native form, which are proposed to be hemisomes based on protein content, DNA wrapping, and direct measurements of dimensions and conformation at the single-molecule level (Dalal et al., 2007; Wang et al., 2008). In contrast, Lavelle et al. argue that a particle derived from a “reverseosome” is an alternative possibility (Bancaud et al., 2007). Reverseosomes are transient structures that require sustained high torsional stress in order to flip a left-handed octamer into a right-handed configuration. Lavelle et al. propose that such an unstable intermediate might become stabilized upon loss of H2A/H2B dimers via unknown protein-protein interactions. However, our ability to induce positive supercoils using purified histones, RbAp48, and relaxed plasmid circles, without the addition of any machinery for generating torsional stress, demonstrates that no such elaborate mechanism is required. In addition, key features of
CenH3 nucleosomes that have been documented in vitro and in vivo strongly argue against structures for CenH3 particles other than hemisomes.

Lavelle et al. refer to an important in vitro finding by the Prunell group in which the human CenH3 histone variant (CENP-A) and histone H4 failed to assemble into distinct tetrasomes (H4/CenH3/CenH3/H4-containing particles), despite the fact that these particles are readily formed using H3 instead of CenH3 (Conde e Silva et al., 2007). They concluded that the addition of H2A and H2B provides the hydrophobic environment necessary for CenH3 octamers to form under conditions of 2M salt. This implies that the assembly intermediate for CenH3 octameric nucleosomes, namely the tetrasome, is inherently unstable. Therefore, there is no plausible assembly pathway for CENP-A tetrasomes in vivo, which according to their flipping model would be an obligatory intermediate. So, although we accept the possibility that flipped particles may be transient intermediate structures produced by torsional stress, we find their existence at centromeres to be implausible.

In addition, Lavelle et al. refer to evidence in yeast that argues for a non-canonical Cse4 structure based in part on depletion of H2A/H2B (Mizuguchi et al., 2007). The original interpretation of these experiments was that the non-histone protein Scm3 took the place of H2A/H2B dimers. However, recent work shows that viable yeast with CenH3 at their centromeres can be obtained in the absence of Scm3 (Camahort et al., 2009). Although it is conceivable that a tetrasome instead occupies the yeast centromere, we are struck by the fact that yeast Cse4 can support centromere function in human cells depleted of CENP-A (Wieland et al., 2004). H2A/H2B has been observed in human centromeric chromatin (Foltz et al., 2006), such that Lavelle et al. would need to suppose that Cse4 is part of a right-handed tetrasome in yeast but can nevertheless replace its orthologous copy in humans to form a completely different yet functional particle. Rather than consider such an unlikely possibility, we note that centromeres with CenH3 histones likely shared a common evolutionary origin (Malik and Henikoff, 2009), so that the most parsimonious interpretation is that all eukaryotic CenH3 nucleosomes have similar structures and so wrap DNA in a right-handed manner.

In summary, Lavelle et al. accept our surprising discovery of a right-handed wrap around centromeric nucleosomes but disagree as to the nature of the histone core that is responsible. However, we find that their evidence for flipping of a left-handed octamer does not apply to CenH3 nucleosomes, and we await further biochemical evidence to definitively resolve this issue. Although we recognize that there is a lively debate in the centromere field concerning this issue (Dechassa et al., 2009; Hill and Williams, 2009), we note that there is general agreement that the extraordinary epigenetic inheritance of centromeres depends ultimately on the properties of CenH3 nucleosomes (Bernad et al., 2009). We are excited about the prospects for resolution of perhaps the oldest unsolved problem in genetics (Flemming, 1882).

REFERENCES


