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]2, or even nucleosomes [H2A/H2B-CenH3/H4]-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; Aillat 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 reverosomes for reverse nucleosomes) (Bancaud et al., 2007). We were able to model the transition steps and to propose an atomic structure for the reverosome (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 reverosome transition under positive torsional constraints (unpublished data). These results indicate that the right-handed centromeric particles could, in principle, be made of reverosomes. However, in yeast, where point and regional centromeres are both depleted of H2A-H2B histones (Mizuguchi et al., 2007), it is not reverosomes 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 reverosome (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; Dechassa 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 (Musgrave 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 (Fohtz 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, tetrameric (tetrasomes 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 a 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 hemisomes 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 “reversome” is an alternative possibility (Bancaud et al., 2007). Reversomes 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...
Supplemental Data
Right-Handed Nucleosome: Myth or Reality?
Christophe Lavelle, Pierre Recouvreux, Hua Wong, Aurélien Bancaud, Jean-Louis Viovy, Ariel Prunell, and Jean-Marc Victor
**Figure S1. Nucleosome chiral transition**

**Upper panel**
Selected snapshots (side and front views) show the changes in the molecular architectures of the canonical nucleosome in the course of its chiral transition towards the right-handed reversome (adapted from Bancaud et al., 2007; inverse cinematics was used to obtain this transition). In the first stage of the transition (a-b), dimers break their docking on the tetramer; then the tetramer undergoes the chiral transition (b-d); eventually, the particle folds into a right-handed helix (d-e). During this transition, the octamer is practically turned inside out. Considering the topological deformation of a right-handed tetrasome, we estimated that the linking number difference (writhe + twist) of the reversome is $\Delta L_k = W_r + \Delta Tw = 0.7 + 0.2 = 0.9$ (Sivolob et al., 2009). These values, compared with the topological deformation of a canonical nucleosome ($\Delta L_k = -1$), suggest the reversome is substantially less compact than the nucleosome, even if both particles fold a similar length of DNA. The reason is that dimers cannot dock as tightly on the reverse tetramer as a consequence of less favorable interfaces (the all-atom model shows the absence of strong interactions between the C-terminal portion of H2A and H3, the so-called “docking domain” in canonical nucleosomes). Moreover, H3 $\alpha_N$-extensions (and N-terminal tails) are no longer appropriately located to interact with, and stabilize, reversome entry-exit DNAs. *Histones H2A, H2B, H3 and H4 are shown in yellow, red, blue and green, respectively.*

**Lower panel**
Electrostatic potentials of left-handed (canonical) and right-handed (reverse) nucleosomes have been computed with GRID (http://www.moldiscovery.com/soft_grid.php). Residues are color coded by charge (red = negative; blue = positive; white = neutral). The negative charges that are present on the nucleosome front view (left) are clustered (red spot) and correspond to the so-called “acidic patch”. This patch ends up inside the reversome after the chiral transition, whereas the negative charges that were sparse inside the nucleosome end up on the outer face of the reversome (right). Accordingly, the docking domain of H2A that binds the dimer H2A/H2B to the tetramer (H3/H4)$_2$ in the nucleosome does not interact any longer with the tetramer in the reversome, thus leading to a more open structure. Moreover, the locations of H3 $\alpha_N$ helices on the outer faces of the reversome prevent them from clamping entry/exit DNAs, thus opening the particle further.

**Note on the nomenclature**
Due to increasing evidence in the literature for non-nucleosome histone-DNA functional particles (Lavelle and Prunell, 2007) a new nomenclature has recently been proposed. This nomenclature takes into account both the histone stoichiometry [octasome for the octameric particle containing the full complement of histones; tetrasome and hemisome, respectively, for (H3-H4)$_2$ and H2A-H2B-H3-H4 tetrameric particles] and the chirality of each particle [L for left-handed, R for right-handed] (Zlatanova et al, 2009). Nucleosomes and reversomes are called L-octasomes and R-octasomes, respectively; and tetrasomes/hemisomes can be either L- or R-tetrasomes/hemisomes. To avoid confusion, however, we have stuck to the traditional nomenclature used by Furuyama and Henikoff (2009) in their *Cell* paper.
Supplemental References


