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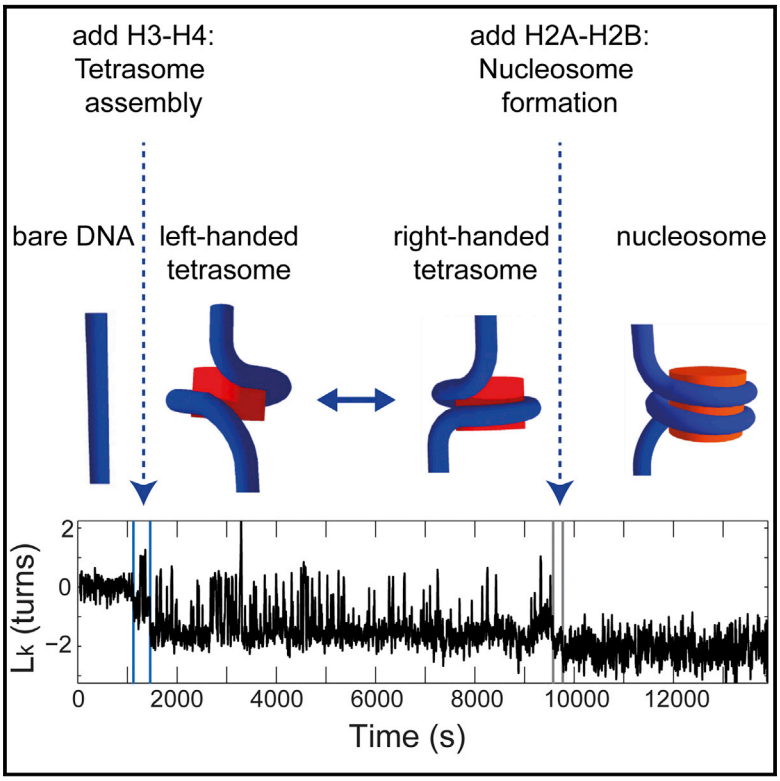
Nucleosome Assembly Dynamics Involve Spontaneous Fluctuations in the Handedness of Tetrasomes

Graphical Abstract



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In Brief

In eukaryotes, DNA is wrapped in a left-handed fashion around histone protein cores, forming nucleosomes. Vlijm et al. now use real-time monitoring of DNA length and linking number to show that tetrasomes, biologically relevant subnucleosomal structures, exhibit spontaneous flipping between a preferentially occupied left-handed and a right-handed state.

Highlights

- We monitor assembly of nucleosomes and tetrasomes by NAP1 on DNA in real time
- Tetrasomes spontaneously flip between a left- and right-handed conformation
- Addition of H2A/H2B to flipping tetrasomes generates stable left-handed nucleosomes
- Small positive torques drive tetrasomes from a left-handed into a right-handed state

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Nucleosome Assembly Dynamics Involve Spontaneous Fluctuations in the Handedness of Tetrasomes

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SUMMARY

DNA wrapping around histone octamers generates nucleosomes, the basic compaction unit of eukaryotic chromatin. Nucleosome stability is carefully tuned to maintain DNA accessibility in transcription, replication, and repair. Using freely orbiting magnetic tweezers, which measure the twist and length of single DNA molecules, we monitor the real-time loading of tetramers or complete histone octamers onto DNA by Nucleosome Assembly Protein-1 (NAP1). Remarkably, we find that tetrasomes exhibit spontaneous flipping between a preferentially occupied left-handed state ($\Delta Lk = -0.73$) and a right-handed state ($\Delta Lk = +1.0$), separated by a free energy difference of 2.3 k_BT (1.5 kcal/mol). This flipping occurs without concomitant changes in DNA end-to-end length. The application of weak positive torque converts left-handed tetrasomes into right-handed tetrasomes, whereas nucleosomes display more gradual conformational changes. Our findings reveal unexpected dynamical rearrangements of the nucleosomal structure, suggesting that chromatin can serve as a "twist reservoir," offering a mechanistic explanation for the regulation of DNA supercoiling in chromatin.

INTRODUCTION

Nucleosomes, the basic compaction unit of eukaryotic DNA (Kornberg, 1977; Olins and Olins, 1974), consist of 147 bp of DNA wrapped 1.7 times around a protein core called the histone octamer (Luger et al., 1997). Their assembly requires a precisely defined pathway: first two copies of the H3-H4 histones bind to the DNA, forming a tetrasome, followed by the two H2A-H2B dimers (Jorcano and Ruiz-Carrillo, 1979). In vivo, nucleosome assembly is facilitated by chaperones, such as NAP1 (Andrews et al., 2010; Ito et al., 1996; Zlatanova et al., 2007) and ATP-dependent chromatin-assembly factors (Lusser et al., 2005). In vitro, nucleosome assembly onto DNA fragments is often carried out using salt dialysis (Peterson, 2008). It has been established that nucleosome positioning is sensitive to the DNA sequence, with the binding affinity for a given 147 bp sequence varying over more than three orders of magnitude (Thåström et al., 1999). High-affinity binding to DNA sequences that contain 10 bp repeats of bendable AT/TA dinucleotides (Jiang and Pugh, 2009; Kaplan et al., 2009; Struhl and Segal, 2013; Zhang et al., 2009) has facilitated both high-throughput visualization of nucleosomes (Lee and Greene, 2011; Visnapuu and Greene, 2009) and the mapping out of the energy landscape for single nucleosomes or nucleosome arrays through mechanical disruption (Brower-Toland et al., 2002; Hall et al., 2009; Bancaud et al., 2007; Kruithof et al., 2009), providing quantitative insight into the underlying histone-DNA interactions.

It is becoming increasingly clear that nucleosomes exhibit structural dynamics that are key to understanding the mechanisms regulating genome accessibility in transcription, replication, and repair (Bell et al., 2011; Choy and Lee, 2012; Gansen et al., 2009; Simon et al., 2011; Zentner and Henikoff, 2013). For example, nucleosomes display dynamical "breathing" (Li et al., 2005), in which short stretches of DNA transiently unwrap from the octamer. In addition, active remodeling of nucleosomes alters their stability and positioning (Blosser et al., 2009; Clapier and Cairns, 2009). Several studies suggest that altered conformations of nucleosomes and tetrasomes may be associated with changes in the topology of the wrapped DNA (Bancaud et al., 2007; Hamiche et al., 1996; Peterson et al., 2007), which could have profound implications for cellular processes like transcription and replication (Liu and Wang, 1987). To date, however, no studies have addressed the dynamics of (sub)nucleosome chirality, which requires the ability to detect transient states in nucleosomal linking number.

Here, we directly monitor, in real time, the NAP1-mediated assembly of (sub-)nucleosomes onto bare DNA and its subsequent dynamics. In vivo, NAP1 has been found to predominantly interact with H2A/H2B and is considered responsible for the loading of H2A/H2B onto chromatin (Zlatanova et al., 2007). In vitro, NAP1 prevents histone aggregation and acts as a chaperone for both H2A/H2B and H3/H4, facilitating their stepwise loading onto DNA that is free of any strong positioning sequences that could impact subsequent dynamics (Andrews