The Sequence of Nucleosomal DNA Modulates Sliding by the Chd1 Chromatin Remodeler

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http://dx.doi.org/10.1016/j.jmb.2017.02.002
Edited by Sepideh Khorasanizadeh

Abstract

Chromatin remodelers are ATP-dependent enzymes that are critical for reorganizing and repositioning nucleosomes in concert with many basic cellular processes. For the chromodomain helicase DNA-binding protein 1 (Chd1) remodeler, nucleosome sliding has been shown to depend on the DNA flanking the nucleosome, transcription factor binding at the nucleosome edge, and the presence of the histone H2A/H2B dimer on the entry side. Here, we report that Chd1 is also sensitive to the sequence of DNA within the nucleosome and slides nucleosomes made with the 601 Widom positioning sequence asymmetrically. Kinetic and equilibrium experiments show that poly(dA:dT) tracts perturb remodeling reactions if within one and a half helical turns of superhelix location 2 (SHL2), where the Chd1 ATPase engages nucleosomal DNA. These sequence-dependent effects do not rely on the Chd1 DNA-binding domain and are not due to differences in nucleosome affinity. Using site-specific cross-linking, we show that internal poly(dA:dT) tracts do not block the engagement of the ATPase motor with SHL2, yet they promote multiple translational positions of DNA with respect to both Chd1 and the histone core. We speculate that Chd1 senses the sequence-dependent response of DNA as the remodeler ATPase perturbs the duplex at SHL2. These results suggest that the sequence sensitivity of histones and remodelers occur at unique segments of DNA on the nucleosome, allowing them to work together or in opposition to determine nucleosome positions throughout the genome.

Introduction

A core feature of eukaryotic genomes is the extensive packaging of DNA into nucleosomes. Many nucleosomes are specifically positioned throughout the genome by chromatin remodelers, which dictate the remarkably predictable placement of nucleosomes in gene promoters and can pack intragenic nucleosomes into evenly spaced arrays [1]. Chromatin remodelers can be categorized into distinct families, which exhibit unique biochemical properties and appear specialized for particular biological settings [2]. These remodelers are uniquely influenced by a variety of inputs including transcription factor binding, DNA linker length, and DNA sequence, often competing and collaborating with each other [3–7]. Key questions are therefore how different remodelers select preferred nucleosome substrates and what nucleosome features either activate or inhibit remodeler actions.

In recent years, there has been a growing appreciation that DNA sequence directly impacts chromatin remodeling. In one study, seven different remodelers were found to reposition a starting pool of mononucleosomes into different products, with each remodeler showing characteristic patterns of nucleosome repositioning [8]. For ATP-dependent chromatin-assembly factor (ACF), an ISWI-type remodeler, the authors found that the remodeling pattern was strongly influenced by a particular sequence of DNA within the nucleosome. A separate study on ACF, however, failed to observe evidence of sequence-directed sliding [9]. Although the reason for this discrepancy is unclear, one possibility is that remodeling is biased by the sequence context of a particular DNA segment on the nucleosome. One example where sequence context

appears paramount is for the INO80 remodeler. In a genome-wide study of nucleosome positioning in *Saccharomyces cerevisiae*, INO80 was discovered to be responsible for the precise positioning of +1 nucleosomes and could recapitulate *in vivo* nucleosome positions through DNA sequence alone [6]. Although further work will be needed to tease out the relationship between DNA sequence and INO80 action, nucleosome positioning by INO80 correlated with a predicted greater twist of DNA over the dyad of the +1 nucleosome, suggesting a mechanism of responding to DNA shape on the nucleosome. DNA sequence also guides the action of a SWI/SNF-type complex named for its ability to remodel the structure of chromatin (RSC). In *S. cerevisiae*, RSC plays a primary role in removing and/or shifting nucleosomes away from nucleosome-free regions in gene promoters [10–12]. Nucleosome-free regions in yeast promoters were shown to be commonly associated with poly(dA:dT) tracts [13,14]. The unique structural characteristics of poly(dA:dT) tracts, such as a narrow minor groove, bifurcated hydrogen bond base pairing and increased twist [15–18], gave rise to the idea that nucleosomes would intrinsically disfavor assembly on DNA containing poly(dA:dT) tracts [19,20]. However, poly(dA:dT) tracts were found to wrap into nucleosomes with surprisingly little or no energetic penalty [21–23], and nucleosomes possessing these tracts exhibited rather canonical conformations of DNA [24,25]. Poly(dA:dT) tracts were instead found to exert their effects by directing RSC, resulting in the preferential shifting of nucleosomes off of poly(dA:dT) tracts [6,26].

In this study, we investigate the effects of DNA sequence on the chromodomain helicase DNA-binding protein 1 (Chd1) chromatin remodeler. Chd1 is known for preferentially shifting mononucleosomes toward the center of short DNA fragments, which is believed to underlie its ability to evenly distribute nucleosomes in arrays [27–29]. To date, directional sliding by Chd1 has been shown to arise from responding to three distinct nucleosome characteristics: the length and availability of DNA flanking the nucleosome [28,30], the presence of a transcription factor bound at the nucleosome edge [4], and the absence of the histone H2A/H2B dimer on the entry side of hexasomes [31]. Our work here reveals that Chd1 activity is also impacted by the DNA sequence adjacent to SHL2. As we demonstrate using internal poly(dA:dT) tracts, responding to sequence elements within nucleosomal DNA was not solely due to binding defects and did not require the Chd1 DNA-binding domain. While the Chd1 ATPase was not blocked from engaging DNA at SHL2, cross-linking experiments suggested that internal poly(dA:dT) tracts promoted multiple translational positions of nucleosomal DNA. We speculate that the influence of DNA sequence on remodeling reflects a sensitivity of Chd1 to DNA energetics at intermediate steps in the catalytic cycle. The location of sequence sensitivity appears to be distinct from regions where histones most tightly grip DNA within the nucleosome. On a genome-wide scale, this independence would allow thermodynamically preferred positions to be either reinforced or overridden by chromatin remodelers such as Chd1.

Results

The Chd1 remodeler shows a directional preference in sliding nucleosomes made with the Widom 601 sequence

Chd1 has been shown to preferentially slide histone octamers of end-positioned mononucleosomes toward the center of short DNA fragments [28,30]. Since Chd1 acts throughout the genome and its DNA-binding domain appears to lack sequence specificity [32], we expected that nucleosome sliding with equal lengths of DNA flanking each side of the nucleosome would produce a symmetric distribution of the histone octamer about the center of the DNA fragment. Using the Widom 601 sequence to initially position nucleosomes, we unexpectedly found that Chd1 sliding was biased in one direction (Fig. 1). Nucleosome positions were determined using a cross-linking technique called histone mapping [33]. With this technique, an introduced cysteine (H2B-S53C) that is adjacent to nucleosomal DNA is labeled with the photoactivatable cross-linker 4-azidophenacyl bromide (APB). The formation of an APB adduct on DNA bases allows for the generation of abasic sites, resulting in cleavage of the DNA backbone and thus revealing the locations of cross-linked sites at base pair resolution [34]. The Widom 601 positioning sequence is asymmetric, with a G:C base pair on the dyad. Here, we refer to the orientation of the Widom 601 with the dyad C base on the top strand and with G on the bottom strand (Fig. 1a). DNA sequence influences the efficiency of histone–DNA cross-linking and may therefore bias the interpretation of histone mapping experiments. For histone mapping with H2B(S53C), however, each copy of H2B cross-links to only one DNA strand ~ 19 bp from the nucleosome edge [33], thereby allowing the two sides of the nucleosome to be independently monitored. For each side, nucleosome sliding reactions with Chd1 produced cross-linking patterns with ~ 10- to 11-bp periodicity, as expected for the strong rotational dominance of the Widom 601. As shown with double-labeled DNA, both sides of the nucleosome reported a similar and marked bias for the histone octamer shifting toward the right side of the 601 sequence (Fig. 1b and c).

Poly(dA:dT) tracts in the vicinity of SHL2 alter equilibrium nucleosome positions resulting from Chd1 remodeling

To investigate the role of DNA sequence in sliding directionality, we introduced poly(dA:dT) tracts into