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Spontaneous Access to DNA Target Sites in Folded Chromatin Fibers

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DNA wrapped in nucleosomes is sterically occluded from many protein complexes that must act on it; how such complexes gain access to nucleosomal DNA is not known. *In vitro* studies on isolated nucleosomes show that they undergo spontaneous partial unwrapping conformational transitions, which make the wrapped nucleosomal DNA transiently accessible. Thus, site exposure might provide a general mechanism allowing access of protein complexes to nucleosomal DNA. However, existing quantitative analyses of site exposure focused on single nucleosomes, while the presence of neighbor nucleosomes and concomitant chromatin folding might significantly influence site exposure. In this work, we carried out quantitative studies on the accessibility of nucleosomal DNA in homogeneous nucleosome arrays. Two striking findings emerged. Organization into chromatin fibers changes the accessibility of nucleosomal DNA only modestly, from ~3-fold decreases to ~8-fold increases in accessibility. This means that nucleosome arrays are intrinsically dynamic and accessible even when they are visibly condensed. In contrast, chromatin folding decreases the accessibility of linker DNA by as much as ~50-fold. Thus, nucleosome positioning dramatically influences the accessibility of target sites located inside nucleosomes, while chromatin folding dramatically regulates access to target sites in linker DNA.

Keywords: chromatin; nucleosome; higher-order structure; site exposure; gene regulation

Introduction

Eukaryotic genomes are organized into repeating arrays of nucleosomes that occlude most of the genomic DNA from the many protein complexes required for genome function.[1] How such complexes gain access to their DNA target sites *in vivo* is not known. Genomes encode an intrinsic nucleosome organization in which transcription factor binding sites that need to be accessible have a relatively lower probability of being occluded inside a nucleosome,[2] but these are probabilistic biases only; they do not keep critical target sites nucleosome free.[3–9] Nucleosomal DNA needs to be unwrapped at times to function.[10–13]

Two broad mechanisms that provide access to buried nucleosomal target sites have been characterized. One mechanism involves ATP-dependent nucleosome remodeling factors,[14,15] which disassemble nucleosomes or allow nucleosomes to redistribute their locations in response to changing constellations of DNA binding proteins. These factors are recruited to particular chromatin regions by site-specific DNA binding proteins,[14,16,17] raising the question of how those DNA binding proteins themselves gain access to their own target sites. One idea is that some ATP-dependent remodeling factors may act on nucleosomes ubiquitously, without a requirement for specific binding, thereby rendering

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Abbreviations used: RE, restriction enzyme; cpDNA, core particle DNA; AFM, atomic force microscopy; PL, poly-L-lysine.

chromatin inherently "fluid" for protein binding.[18] Such a ubiquitous activity has not yet been demonstrated *in vivo*.

We focus here on a second mechanism for site accessibility in nucleosomes that is known to occur, is intrinsic to the nucleosomes themselves, and has been implicated in chromatin function *in vivo*. Nucleosomes spontaneously undergo large-scale conformational fluctuations ("site exposure") in which stretches of their wrapped DNA partially unwrap off the histone protein surface, starting from one end. Site exposure provides spontaneous access to the entire nucleosomal DNA length. Access to the more outer stretches of the DNA is particularly rapid (spontaneous opening as often as every ~250 ms)[19] and efficient (the DNA ends are unwrapped as much as ~1%–5% of the time),[20,21] depending on the DNA sequence.[22]

Two lines of evidence suggest that intrinsic nucleosomal site exposure is important for chromosome function *in vivo*. First, one consequence of site exposure is to confer a novel nucleosome-dependent positive cooperativity on the binding of pairs of arbitrary site-specific DNA binding proteins when their DNA target sites are contained within the same nucleosome.[23] This phenomenon occurs *in vivo*[24–26] and is now thought to contribute to cooperativity in transcription factor action, genome-wide.[27] Second, an analysis of rates of DNA repair by photolyase *in vivo* concluded that repair occurs too quickly to be explained by known ATP-dependent remodeling activities and suggested instead that rapid repair is facilitated by intrinsic nucleosome site exposure.[28]

Existing studies characterized the site exposure process only in isolated nucleosomes, but nucleosomes *in vivo* occur in long arrays, which, in physiological conditions, compact into higher-order structures that could hinder site exposure. Indeed, such chromatin compaction occurring *in vivo* has long been proposed to contribute to transcriptional gene silencing by sterically occluding DNA from access to activators or polymerases,[29,30] although a recent study has challenged this interpretation.[31]

Here, we ask how the presence of nucleosome neighbors and concomitant chromatin folding influence site exposure. Two striking findings emerged. First, despite the visible compaction of a model 17-nucleosome chromatin fiber, access to target sites within the central nucleosome is only modestly affected relative to access to the same sites in an isolated nucleosome, and these modest changes range from ~3-fold decreases to ~8-fold increases in accessibility. This means that nucleosome arrays are intrinsically dynamic and accessible even when they are visibly condensed. This finding helps explain how upstream activators may have access to their DNA target sites even in transcriptionally silenced chromatin *in vivo*.[31] Second, in contrast to the modest changes in accessibility it causes within nucleosomes, chromatin folding greatly decreases the accessibility at sites in the linker DNA between nucleosomes, by as much as ~50-fold. Thus, the genome's intrinsic nucleosome positioning ("chromatin primary structure") strongly influences the accessibility of target sites that, on average, are located inside nucleosomes, while chromatin fiber folding ("chromatin secondary structure") regulates access to target sites in regions that, on average, are located in linker DNA.

Results

Reconstitution and characterization of nucleosome arrays

Restriction enzymes (REs) face the same steric problems for access to target sites inside nucleosomes as do many eukaryotic regulators and enzymes and are convenient probes for quantitative analyses of nucleosomal DNA accessibility. Site exposure occurs as a rapid preequilibrium[21]; thus, REs digest nucleosomal DNA at a rate equal to their digestion rate on naked DNA multiplied by the (small) fraction of time that the nucleosomal target sites look like naked DNA, which is the equilibrium constant for exposure of the restriction site in the nucleosomes (Fig. 1). To assess how organization of nucleosomes into compact chromatin fibers influences DNA accessibility, we created two new model systems, having one "test" nucleosome ("mp2") that is flanked by one distinct "mp1" nucleosome on one side only (a dinucleosome) or one mp2 nucleosome flanked by eight other mp1 nucleosomes on each side (a nucleosome 17-mer) (Fig. 2). For the resulting chromatin fibers to have homogeneous nucleosome locations, each mp1 or mp2 nucleosome derives from the 147-bp nucleosome positioning region of sequence 601,[32] with an exactly repeating length of 30 bp of linker DNA separating consecutive nucleosome core particles. Thus, our constructs reproduce essential features of the 177-bp nucleosome repeat systems used in recent biophysical and structural studies in the laboratories of Richmond and Rhodes[33–36] and are novel only in their inclusion of a single distinguishable variant of 601, which allows us to probe accessibility within a particular central nucleosome embedded within a highly positioned nucleosome array.

The nucleosome array reconstitutions were carried out similarly to the work reported in Refs. 33–36, using excess nucleosome core particle DNA (cpDNA) as a histone buffer. When used together with high-affinity nucleosome positioning arrays, the cpDNA allows the preparation of arrays in which every high-affinity sequence is occupied by a nucleosome, without significant aggregation occurring. Optimal dimer reconstitution conditions were determined by titration with increasing concentrations of histone octamer (Fig. 3a). Native gel electrophoresis of the products reveals two distinct shifted single histone octamer-containing bands that converge to a single dinucleosome band by the highest histone octamer concentration used (0.8:1 mass of histone to mass of total DNA, 2.4 mol histone octamer per mole of nucleosome positioning sequence). Products obtained