Steroid Receptor Co-Activators and Chromatin Remodelling

In recent studies by Hager and co-workers, an array containing 200 copies of the MMTV-LTR was exploited to investigate the interaction of a green fluorescent protein (GFP)–GR construct in living cells [23]. The results highlight the dynamic nature of receptor–DNA/chromatin interactions and support a 'hit-and-run' mechanism of GR action (see Hager et al., this issue, pp. 405–410, for further discussion).

With regard to chromatin remodelling and nuclear receptor action a number of questions remain, for example:

1. What are the molecular details of the mechanism of action of chromatin remodelling complexes? Is it sufficient to ‘move’ key nucleosomes or is it necessary to displace or disrupt the entire histone–DNA complex?

2. Why are there so many remodelling complexes? Are they redundant or can we expect receptor/promoter specific interactions?

3. Do all proteins with HAT activity acetylate histones?

Answers to some of these questions are already beginning to emerge and we can expect further developments in this fast-moving field in the not too distant future. What is clear from the above studies and the recent two-day colloquium, held in Leeds as part of the Biochemical Society’s annual meeting, is that the dynamic nature of chromatin and its role in regulating nuclear processes are now on centre stage.

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References

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Helical repeat of DNA in the nucleosome core particle

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Abstract

Although the crystal structure of nucleosome core particle is essentially symmetrical in the vicinity of the dyad, the linker histone binds asymmetrically in this region to select a single high-affinity site from potentially two equivalent sites. To try to resolve this apparent paradox we mapped to base-pair resolution the dyads and rotational settings of nucleosome core particles reassembled on syn-
thetic tandemly repeating 20 bp DNA sequences. In agreement with previous observations, we observed (1) that the helical repeat on each side of the dyad cluster is 10 bp maintaining register with the sequence repeat and (2) that this register changes by 2 bp in the vicinity of the dyad. The additional 2 bp required to effect the change in the rotational settings is accommodated by an adjustment immediately adjacent to the dyad. At the dyad the hydroxyl radical cleavage is asymmetric and we suggest that the inferred structural asymmetry could direct the binding of the linker histone to a single preferred site.

**Introduction**

The fundamental subunit of chromatin is the nucleosome core particle in which the 146 bp of DNA are wrapped in 1.65 superhelical turns about the histone octamer [1]. This particle has a high degree of symmetry about the crystallographic dyad axis, yet the globular domain of the linker histone binds asymmetrically to the core particle, forming a bridge between one terminus of the bound DNA and the region close to the dyad [2]. In principle a symmetrical particle should contain two equivalent sites with which this globular domain could interact. On this argument, asymmetric binding could be achieved by random, yet mutually exclusive, binding at each of the two equivalent sites, or alternatively by an intrinsic asymmetry in the nucleosome core particle itself. Such an asymmetry need not be manifested in all particles.

One measure of structural symmetry is the organization of the helical repeat of the DNA in the nucleosome core particle. There is substantial evidence from DNA sequencing [3], photo-induced pyrimidine dimer formation [4,5] and cleavage by hydroxyl radicals [6] that the average relative helical repeat of the bound 145 bp is approx. 10.2–10.3 bp. Several studies have indicated that this average value reflects position-dependent differences. Early DNase I digestion profiles of core particles indicated that the number of base pairs between the cleavage maxima, i.e. the positions of maximum accessibility of the minor groove, in the central three turns is 10.7 bp and in the flanking bound DNA is approx. 10 bp [7–9]. Although these results were suggestive of a higher helical repeat in the dyad region, the possibility could not be excluded at that time that the cleavage periodicity might be influenced by exclusion of the enzyme from otherwise favoured cleavage sites by the local morphology of the particle. However, the equation of the cleavage periodicity with the helical repeat was firmly established by the demonstrations that the sequence periodicity of nucleosome core DNA matched the observed DNase I cleavage periodicity extremely well [10] and also that the hydroxyl radical cleavage periodicity of a core nucleosome assembled on *Xenopus borealis* somatic 5 S rDNA again matched the originally reported DNase I cleavage periodicity [6]. None of these results directly addressed the question of a possible asymmetry of the helical repeat because in both the DNase I cleavage studies and the sequence analysis the data were necessarily symmetrized about the midpoint of the bound DNA. The analysis of the hydroxyl-radical data was also complicated by the possibility of multiple dyad positions adopted by the histone octamer on the sequence used (discussed in [11]).

**Experimental system**

To investigate the organization of the helical repeat of nucleosome core DNA more directly, we used a model system based on a synthetic repetitive DNA sequence designed by Shrader and Crothers [12,13] to optimize rotational determinants. This construct, termed ‘TG’, contains direct repeats of a 20 bp sequence unit and is therefore polar in character. The use of such a sequence has two potential advantages: the repetitive nature means that any local structural sequence-dependent perturbations, such as those induced by long dA:dT tracts are minimized and the analysis is simplified (Figure 1). The repeated unit is composed of different alternating sequence motifs designed to adopt local conformations compatible with outwards-facing and inwards-facing minor grooves of the DNA wrapped around the histone octamer. These ‘out’ and ‘in’ blocks, defining the orientation of the minor groove relative to the histone octamer, are separated from each other by two nucleotides. Each repeat contains two different ‘out’ trinucleotides, CGG and GCC, and two different ‘in’ trinucleotides, TTA and AAT, with

**Figure 1**

The 20 bp sequence repeat in assembled nucleosome core particles

**TG**

| CGG TG TTA GA GCC TG TAA CT |

**Variant**

| CGG TG TTA CT CGG TG TAA CT |
three different dinucleotides, TA, GA and CT, between the positioning trinucleotides. In vitro this sequence has a high affinity for the histone octamer and adopts a preferred rotational setting on the nucleosome, as confirmed by DNase I and hydroxyl-radical footprinting [13]. However, in vitro the sequence seems not to direct precise positioning [14,15].

The sequence repeat in the TG sequences is an integral multiple of 10 bp and therefore differs from the preferred nucleosomal helical repeat of 10.2–10.3 bp. Nevertheless, Shrader and Crothers [13] observed that the histone octamer could bind DNA sequences with average sequence periodicities varying between 9.5 and 11 bp, although the affinity for sequences at both extremities of this range was substantially lower. This raises the additional question of how the core particle accommodates sequence repeats that differ significantly from the average observed helical repeat.

The helical repeat changes close to the dyad

To address these issues, we mapped both the dyad positions and the rotational settings of nucleosomes assembled on DNA fragments containing eight repeats of the original TG sequence and/or eight repeats of a variant sequence in which the GAGGC sequence of TG was replaced by CTTCGG. The dyad positions were determined by the method of Flaus et al. [16] and the rotational settings by cleavage with hydroxyl radicals. The site-directed cleavage shows that, within the resolution of the experiment, all the translational positions on TG8 are characterized by the same sequence at the dyad. In this context we define the dyad position as the maximum of the general cleavage by hydroxyl radicals between the site-directed cleavages. This position does not necessarily correspond precisely to the pseudodyad in the crystal structure of the core nucleosome but should represent the midpoint of the outwards-facing minor groove at the dyad and therefore defines the helical repeat. Similarly, all translational positions on the variant are characterized by the same dyad sequence. These positions are shifted by 2 bp relative to the positions adopted on the TG8 sequence. The dyad mapping also reveals that the positioning of the core particle on the TG8 sequence is not precise. Instead of the single major and two minor cleavages that are expected from the site-directed hydroxyl-radical reaction [16] we observe clusters of bands for both the major and minor cleavage sites. That for the major cleavage site can contain up to four successive bands with single nucleotide spacing. In contrast, the cleavage pattern on the variant sequence is much tighter. The results also show that the rotational settings with respect to the sequence repeat differ on each side of the dyad clusters. This result is particularly clear for the nucleosome assembled on the variant DNA sequence. We conclude (1) that the helical repeat on each side of the dyad cluster, with the exception of the outer two double-helical turns, which are not resolved in the gel, is 10 bp maintaining register with the sequence repeat and (2) that this register changes by 2 bp in the vicinity of the dyad. This change is confined to the central two turns. This result shows unambiguously that in the nucleosomes studied the helical repeat does change in the vicinity of the dyad and that the 2 bp difference in the rotational settings on the outer arms is, within experimental error, identical with the 2.1 bp determined from the analysis of core nucleosome sequences [10]. It further follows that the binding of sequences of different periodicities to the histone octamer [13] is accommodated by having different rotational settings on each side of the dyad, thereby incurring an energetic penalty. We conclude that the average helical repeat of DNA in the nucleosome can be varied only within very narrow limits, perhaps comparable with those observed by Luger et al. [1] in crystal structures of different nucleosomes.

An asymmetric nucleosome core particle?

The cleavage pattern of the assembled nucleosomes with hydroxyl radicals also revealed that for the variant sequence the cleavage maximum at the dyad, i.e. where the minor groove faces out, differs in character from the maxima in the remainder of the assembled particle. Notably this cleavage maximum is more extended and skewed relative to the immediate flanking maxima, indicating that the DNA at the dyad is more accessible to this reagent and more importantly that the distribution of cleavage sites is asymmetric with respect to the dyad position. If this skewing is indicative of a true asymmetry in the particle, this result implies that the two potential binding sites for globular domain on each side of the dyad would be structurally distinct, creating an asymmetry. Such an asymmetry could potentially resolve the problem of how histone H1 selects a single high-affinity, and necessarily asymmetric, site on the core particle [2,17,18] instead of
choosing between two equivalent sites on a completely symmetrical core particle. In this situation sequence-directed asymmetry in core particle structure could determine, at least in part, the affinity for histone H1 and the ability to form polar condensed nucleosome arrays [19,20].

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Mechanisms for ATP-dependent chromatin remodelling
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Abstract
Gene regulation involves the generation of a local chromatin topology that is conducive to transcription. Several classes of chromatin remodelling activity have been shown to play a role in this process. ATP-dependent chromatin-remodelling activities use energy derived from the hydrolysis of ATP to alter the structure of chromatin, making it more accessible for transcription factor binding. The yeast SWI–SWF complex is the founding member of this family of ATP-dependent chromatin-remodelling activities. We have developed a model system to study the ability of the SWI–SWF complex to alter chromatin structure. Using this system, we find that SWI–SWF is able to alter the position of nucleosomes along the DNA. This is consistent with recent reports that other ATP-dependent chromatin-remodelling activities can alter the positions of nucleosomes along DNA. This suggests that nucleosome mobilization may be a general feature of the activity of ATP-dependent chromatin-remodelling activities. Some of the mechanisms by which nucleosomes may be moved along DNA are discussed.

Introduction
The fundamental subunit of chromatin is the nucleosome [1]. The assembly of DNA into nucleosomes can impede the binding of factors and repress transcription [2]. This means that unfolding of chromatin is required to enable factors to gain access to the appropriate sites during gene regulation. The finding that chromatin structure becomes altered at many loci prior to, or concurrently with, transcription provides support for the idea that remodelling of chromatin structure plays an important role in gene regulation [3].

More recently, activities that function to modify or remodel chromatin structure during gene regulation have been identified. These include histone acetyltransferases and histone deacetylases, which catalyse the reversible modifi-