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In Vitro and *in Vivo* Nucleosome Positioning on the Ovine β-Lactoglobulin Gene Are Related

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Although positioned nucleosomes are known to play a direct, localised role in regulating access to DNA sequence, they also have the potential, through their long-range distribution, to affect the detailed structure of the higher-order chromatin fibre. To investigate this possibility, we firstly mapped, *in vitro*, the sequence-dependent positions that the core histone octamer adopts when reconstituted onto DNA containing the ovine β-lactoglobulin gene. These positioning sites are discussed in terms of their relative affinity for the histone octamer, their locations with respect to the gene sequence and their periodic distribution throughout the gene region. Secondly, we mapped, *in vivo*, the sites that nucleosomes occupy on the same sequence in liver nuclei, where the gene is transcriptionally inactive. Although the sequence is largely packaged into regularly spaced nucleosomes, reflecting a fibre of uniform higher-order structure, this organisation is disrupted by a number of unusual chromatin structures in a region stretching from the second to the third introns of the gene. A comparison of the *in vitro* and *in vivo* nucleosome positioning data shows that they are qualitatively and quantitatively related, suggesting that the structure of the higher-order chromatin fibre containing the β-lactoglobulin gene is determined, in part, by the long-range organisation of the non-coding sequences within which the gene is embedded.

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Introduction

DNA in eukaryotic nuclei is packaged by histones into chains of nucleosomes that are folded into a higher-order, 30 nm chromatin fibre.[1–5] This fibre is further organised into domains and subject to higher levels of packaging.[6] In most cells, much of the chromatin is packaged into transcriptionally inactive heterochromatin. Currently, we have little insight into how the 30 nm fibre is folded to this higher level, although it can involve modification of both the DNA and core histones, and the recruitment of various non-histone proteins and small RNAs.[7–9] The structure of the euchromatic chromatin fraction is also poorly understood. In this component, which includes sequences primed for, or engaged in, transcription, the higher-order chromatin fibre often displays evidence of localised disruption, epitomised by the occurrence of hypersensitivity sites that are frequently associated with the regulatory regions of genes.[10,11] Current models suggest that the control of gene expression may involve the interaction of these regulatory regions brought about through the folding or looping of the intervening chromatin fibre.[12,13] Nucleosomes in active regions of chromatin are also subject to distinctive patterns of core histone modification, often focussed within regulatory regions of genes.[14–16] However, transcribed sequences retain both core and linker histones,[17–20] and their conversion to a substantially unfolded conformation is only transient.[20] Thus, the repression and expression of DNA sequences takes place in the context of a higher-order chromatin fibre and, as such, these processes are likely to be influenced by, or demanding of, the detailed long-range organisation of this structure.

The disposition of nucleosomes over long stretches of DNA will influence the architecture and stability of

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Abbreviation used: OP-Cu, copper phenanthroline.

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the chromatin fibre because the packaging of the DNA into this higher-order state is sensitive to the spatial distribution of nucleosomes,[5,21] which in turn can be influenced by the underlying DNA sequence.[5,22–24] In a local context, such as on promoters, enhancers and other regulatory DNA sequences, the occurrence of positioned nucleosomes and their implications for function are well established.[22,23,25,26]

To investigate whether there are features contained within the genomic DNA sequence that might influence the higher-order packaging of chromatin, we developed an *in vitro* nucleosome positioning assay that can be applied to study long stretches of DNA.[27] Analysis of a 4.4 kb region encompassing the entire chicken β-globin gene revealed a large variation in the strength of histone octomer positioning, and demonstrated that the highest-affinity sites, which were phased throughout the region but tended to be contained within the non-coding regions, were arranged with a periodicity of about 200 bp.[24] Given that nucleosome positioning sites identified *in vitro* often reflect those available to be employed *in vivo*,[22,23,25,26] this periodic distribution of positioning sites suggested a role in packaging the chicken globin gene into a higher-order chromatin structure.[24] However, it remains to be seen whether our findings relate specifically to this gene, or reflect more general principles.

To expand our understanding of sequence-dependent nucleosomal positioning, we have now analysed the ovine β-lactoglobulin gene. Although not part of a developmentally-regulated gene locus, this milk protein gene is expressed in a specific temporal and spatial pattern during differentiation of the mammary gland.[28] When relatively small DNA fragments (~10 kb) containing the β-lactoglobulin sequence are used as transgenes, the gene is expressed in a position-independent, copy number-dependent manner in the mammary gland of mice,[28–30] suggesting that the sequence elements required to determine an active chromatin structure in the appropriate cell type are all present within a fragment of this size. However, although analysis of β-lactoglobulin chromatin structure has been performed, and the presence, location and temporal appearance of DNase I hypersensitive sites have been documented,[30–32] the nature of the sequences responsible for conferring its transgene-competent status have not been clearly identified. These properties made this ovine sequence an attractive prospect for further study. In the present work, we mapped the sites at which histone octamers bind to the β-lactoglobulin gene region *in vitro* and we have compared these to the sites occupied by nucleosomes in the nucleus, allowing us to assess directly the contribution of genomic DNA sequence to determining its long-range chromatin structure in the cell.

Results

Histone octamer positioning on the ovine β-lactoglobulin gene *in vitro*

To map the precise translational positions adopted by core histone octamers reconstituted onto a 10.7 kb DNA fragment encompassing the ovine β-lactoglobulin gene, we employed the technique of monomer extension.[24,27] This involves digesting reconstituted chromatin with micrococcal nuclease to produce a population of 147 bp core particle DNA fragments ("monomers") that reflect the preferred sites of octamer positioning on the DNA. These fragments are mapped by identifying the positions at which they anneal back to the β-lactoglobulin gene sequence. The 5′ end-labelled core particle DNA population is annealed to a single-stranded derivative (the mapping clone) of the β-lactoglobulin sequence and then extended with Klenow polymerase in the presence of a restriction enzyme that cuts the nascent double-stranded DNA at a unique location. When the products of this reaction are resolved on a sequencing gel (Figure 2), a set of

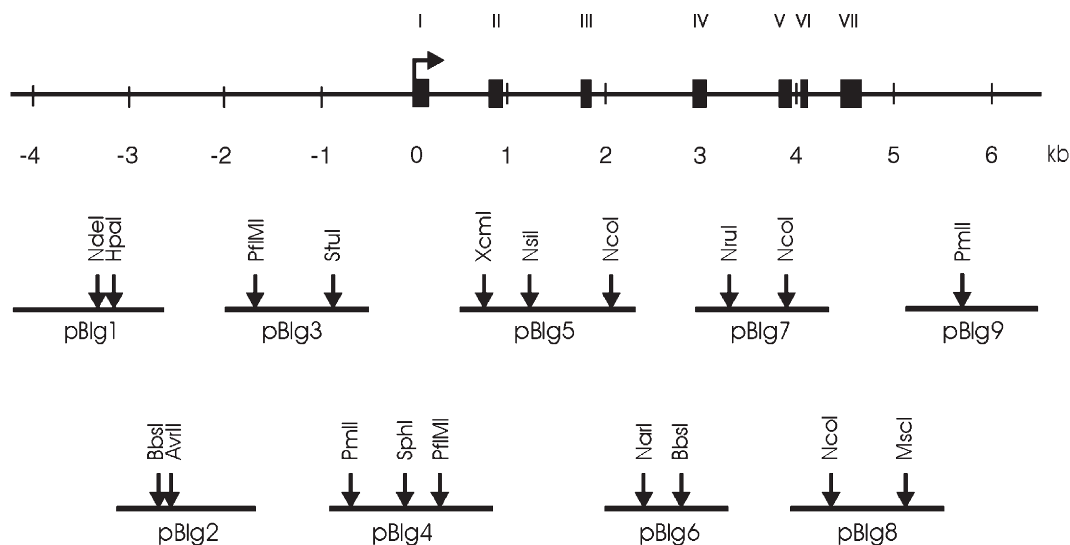

Figure 1. The 10.7 kb ovine β-lactoglobulin gene region. The seven exons of the gene (I–VII) are denoted by rectangles. Subclones employed in the monomer extension analysis are shown together with their relevant restriction sites.

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