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The DNase I generated disomal series is coherent to 16*N*

Implications for coiling models of chromatin structure

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The disomal series generated by the digestion of chromatin with DNase I has been followed to its highest orders using Klenow end-labelling and field-inversion gel electrophoresis to maximise the resolution of large DNA fragments. The series is coherent to the 16*N* level and as such is incompatible with the most structurally acceptable coiling models. We propose that this is evidence for the general unsuitability of coiling models and is support for the existence of simple 'back-to-back' double-stranded structures within chromatin.

Chromatin; DNase I; Disomal series; Structural model; Field-inversion gel electrophoresis

1. INTRODUCTION

The length of the disomal series generated by digesting chromatin with DNase I-based probes places important limitations on the various models of chromatin structure. The very existence of a 2*N* series is incompatible with many models and other models imply definite lengths for the series.

Models have tended to displace one another as a greater understanding of the nucleosome has been obtained and, as a result, there are now a number of generations of coiling models designed to explain the nucleosomal organization of the 30 nm fibre. Of the most recent generation of coiling models some propose that the internucleosomal bridges cross the axis of the coil (e.g. [1,2]) and thereby expose the alternate nucleosomes of the nucleosomal 'zig-zag' to different sides of the coil. In this way, it has been proposed [1], a disomal series would be generated by the asymmetric exposure of the coil to the nuclease. However, according to this class of models both sides of the zig-zag are exposed at alternate cycles of the coil [1]. Thus, the first cyclic change in exposure would result in a break in the coherence of the 2*N* series. Moreover, such exposure would result in the preferential generation of a class of fragments, the so-called 'coil signature', with a length approximately equal to the cycle length of the coil.

Here, we have addressed the possibility that the disomal series is generated from coils containing nucleosomal bridges that cross the axis. Thus, a search was made for the predicted break in coherence, and for the coil signature using procedures that maximise the electrophoretic resolution of large DNase I-generated DNA fragments.

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2. EXPERIMENTAL

Previously, the necessity to load relatively large amounts of DNA onto gels for staining with ethidium bromide has led to overloading problems which have tended to obscure the higher orders of the disomal series. This was avoided by loading small amounts of DNA [^{32}P]dATP end-labelled with the Klenow fragment of polymerase I. Resolution was further enhanced by employing field-inversion gel electrophoresis, a technique

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recently developed to separate large DNA fragments [3].

Chicken erythrocytes were permeabilised and probed with ferritin-DNase I as in [4]. 75-ng samples of DNA end-labelled with [^{32}P]dATP using the Klenow fragment of polymerase I were electrophoresed in 1% agarose, 10 mM Tris, 5 mM sodium acetate, 0.5 mM EDTA, 0.1% SDS, pH 7.8, under the standard conditions of a 5 V/cm constant field or in the presence of an inverting field ramped from 0.6 to 3 s forwards at 4.2 V/cm and 0.3 to 1 s backwards at 2.1 V/cm. Autoradiographs of the dried gels were analysed by computer-assisted densitometry.

3. RESULTS

The results of such an analysis are shown in fig.1. The disomal series clearly continues up to 16*N* without any signs of a break: there is no degeneration into a 1*N* series. Models with axis-crossing characteristics would predict that this series would break at or about 6*N* depending on the details of the assumptions used in constructing the model. Moreover, there is no preferential generation of a class of fragments which might correspond to the cycle length of the putative coil and represent the coil signature.

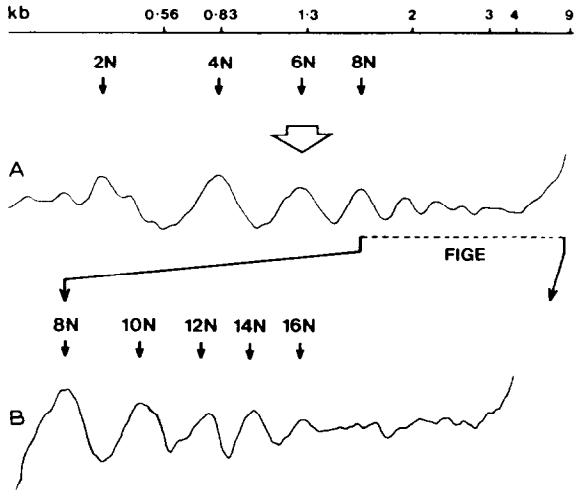

Fig.1. Standard and field-inversion gel electrophoresis of chromatin fragments from chicken erythrocytes permeabilised and probed with ferritin-DNase I. The DNA was end-labelled with [^{32}P]dATP using the Klenow fragment of polymerase I. Autoradiographs of the gels were analysed by computer-assisted densitometry.

4. DISCUSSION

There is a large variety of coiling models for chromatin structure. However, these models are either difficult to reconcile with current understanding of nucleosome structure, or, if they are consistent with the facts, they tend not to be able to explain the nuclease digestion data, in particular, the long disomal repeats generated by DNase I. There are two separate features of the nuclease digests that must be considered: the length and coherence of the disomal repeats and the absence of a coil signature.

The length and coherence of the disomal repeats is incompatible with the explanation of disomal susceptibility implied by the 'bridge-crossing' models [1,2]. Coiling models that propose a maintenance of 'disomal properties' by crossing the bridges over the coil centre would need to have at least the series maximum of nucleosomes per turn (i.e. 14–16 nucleosomes) in order to be compatible with the lengths of the 2*N* series observed here. Such coils would have diameters considerably larger than the commonly accepted 30 nm and would require inordinately long linker DNA.

The absence of a coil signature, i.e. of a preferentially generated class of fragments with a length of the order of the size of the coil turn, is not conclusive evidence against some sort of coil. It is, however, strong circumstantial evidence against a regular coil, since it requires the model to generate some special explanation for how the nuclease approach to these regular coils is always locally symmetric. It should be noted that irregular coils do not have this problem.

In generating the most recent models of chromatin structure workers have tended to concentrate on the structure of a single strand of nucleosomal chromatin and to base conclusions upon this assumption of single strandedness. However, there are well established claims, from electron-microscopic studies, that chromatin is commonly double stranded [5–7] and there is a report [8] that indicates how 'back-to-back' double strandedness might explain in a simple manner the long coherent disomal repeats that have been

observed here. In accord with common observation, such an uncoiled or an irregularly coiled double strand would not generate a coil signature. We propose that models of chromatin structure that are based on linear folds of DNA are much more compatible with the length and coherence of the disomal series and the absence of a coil signature than are the various coiling models.

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