Nuclease digestion patterns as a criterion for nucleosome orientation in the higher order structure of chromatin

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Nuclease digestion patterns have been used to discriminate between possible orientations of nucleosomes in the higher order structure of chromatin. Computer simulations were done assuming 3 basically different orientations of nucleosomes which include all proposed models for the '30 nm fibre'. It is found that only alternating exposure of consecutive nucleosomes can explain the DNase I and DNase II digestion patterns.

Nucleosome *Chromatin structure* *Nuclease digestion*

The nuclease digestion pattern of whole nuclei or high-M_r chromatin fragments must reflect accessibility of the DNA. Small molecules like micrococcal nuclease (M_r 16800) can probably penetrate the flexible fibre and digest the protein-free DNA independently of whether it is on the outside or not. On the contrary, large molecules like DNase I (M_r 31000), DNase II (M_r 38000) or nucleases crosslinked to big carrier molecules will digest only that part of DNA which is exposed on the surface of the fibre. Thus the DNA digestion pattern will depend strongly on the orientation of the nucleosomes and the position of the linker DNA.

Micrococcal nuclease predominantly digests the linker DNA, although a careful examination of the products shows that mononucleosomes are over-represented and di- to tetranucleosomes are under-represented in the digest [1]. This suggests that there is some preference for digestion from the ends of the fibre or that the released oligomers are rapidly converted into mononucleosomes.

DNase II digests the thick fibre in the so-called '100 bp mode' [2] which in fact results from cleavage at positions 2 and 12 (20 bp from the ends) inside the core particle [3,4]. This can be seen only if cleavage is more probable at these points than anywhere in the linker region. These results suggest that the linker DNA is probably buried inside the fibre and that the positions 2 and 12 are exposed on the surface (see fig.1).

Digestion of whole nuclei with free DNase I [5–7] or DNase I immobilised on ferritin carrier [8,9] produces a 'double nucleosome' repeat length pattern. On low resolution single- and double-stranded gels, the even multiples size nucleosome peaks ($2aN$) are seen together with $2aN$ 70 nucleotides (n) peaks symmetrical to them, where N is the number of nucleotides per repeating unit and a is an integer. The straightforward explanation put forward for this pattern was that the basic periodic unit is a dinucleosome (nucleodisome) [6] or that every second nucleosome is inaccessible to DNase I [8]. However, the low resolution of the gels does not allow a definite conclusion to be drawn on whether the odd multiples are weak or they do not exist at all. Ten nucleotide-resolution gels of chicken erythrocyte DNase I digests up to 300 n show in fact that instead of a 210 n peak there are two peaks of approximate sizes 180 and 250 n [10]. Such splitting of the odd multiples pro-

Abbreviations: n, nucleotides; bp, basepairs; N, nucleosomal repeat length in bp

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bably makes them undetectable in low resolution gels.

As with the 'phase problem' in diffraction studies, from the digestion pattern one cannot directly obtain the exposed regions of DNA on the surface of the fibre, but one can calculate the digestion pattern of any assumed configuration of exposed regions. In fig.1 are shown two consecutive nucleosomes along the helix of the thick fibre, representing three basically different orientations: (a) with radial and 'parallel' axes of symmetry; (b) with radial and 'antiparallel' axes; (c) with alternating oblique axes. The numbers represent the distances in nucleotides from the 5'-end of the core particle which are exposed on the surface of the fibre. For simplicity only one strand of the DNA is marked.

The simplest case of radial and 'parallel' axes of symmetry (fig.1 A1) represents the model of Thoma et al. [11] of uniformly exposed nucleosomes in which all the linker DNA is inside the fibre. Assuming that the nuclease molecule reaches each nucleosome through an 'accessibility window' of 90° (see B1), only positions 20, 30, 40, 100, 110 and 120 are accessible. Taking the relative probabilities for DNase I cutting at these positions from the data on core particles in [12] one can calculate the mass-average distribution of the single-stranded DNA fragments for a chosen time of digestion. In fig.1, A2, A3 and A4 are shown such distributions for 3 different repeat lengths (190, 200 and 210 bp). Calculations were done for a 10 n approximation. The dotted profile above the distribution A4 shows a 40 n half-width resolution. It is seen that the distributions resemble the 'half nucleosome' or '100 bp' repeat pattern which was observed by digestion of whole nuclei with DNase II [2–4]. In fact, this pattern changes very little when the probabilities for cutting by DNase II are used instead of those for DNase I (not shown). Apparently the 'half nucleosome' repeat is caused not by the specificity of a particular DNase but by the quasi-periodical appearance of the DNA on the surface of the fibre after every 80 n and one repeat length minus 80 n. This double periodicity causes a partial splitting of every second peak. When the repeat length was chosen to be 160 bp all peaks appeared with the same widths (not shown). However, the distributions for model A do not show any enhancement of even multiple length peaks over the odd multiples, independent of the repeat length or the size of the 'windows' chosen.

'Antiparallel' nucleosomes with folded linker between them are shown in fig.1 B1 and represent the models of Finch and Klug [13], Worcel and Benyajati [14] and McGhee et al. [15]. The calculations for this case are accompanied with some uncertainties because:

(1) The data available for the probabilities of DNA cutting inside the core particle [12] were obtained with core particles lacking H1-histone. The presence of H1 probably modifies these probabilities for positions 60, 70 and 80 and the 5'- and 3'-ends.

(2) There are no available data for the probabilities for cutting the linker relative to the DNA inside the core particle.

(3) We do not know the actual path of the linker DNA and what length of it to consider as accessible to nuclease attack. Initially the simplifying assumption was made that linker DNA is also cut at 10 n intervals and that all these positions on the linker DNA are accessible. As one might expect, this assumption leads to a micrococcal nuclease-like digestion pattern with the small difference that all multiple size peaks are partially split into two, with sizes 30 n (see fig.1 B2). The splitting of the odd multiples depends on the repeat length, but the character of the pattern does not depend on the repeat length. Variation of the probabilities for cutting at the positions which are probably affected by the presence of H1-histone do not change the character of the pattern. However, this picture changes completely if one assumes that the linker DNA is folded in a regular manner and is reached by the nuclease molecule through the same '90° window' as shown in fig.1 B1. (Positions marked A are cut and positions marked O are not.) Fig.1 B3 shows a calculated pattern with an assumed cutting probability at positions A equal to 0.05. One can see that double nucleosome repeat size fragments ($2aN$) form well-defined peaks with minor peaks of $2aN$ 85 n symmetrical to them, whilst the odd multiples are split into $(2a+1)N$ 45 n. Assuming different probabilities for cutting at positions A changes the relative abundances of the peaks but has minimal effect on their positions. Fig.1 B4 shows a pattern calculated on the assumption that positions A are cut with a very low pro-

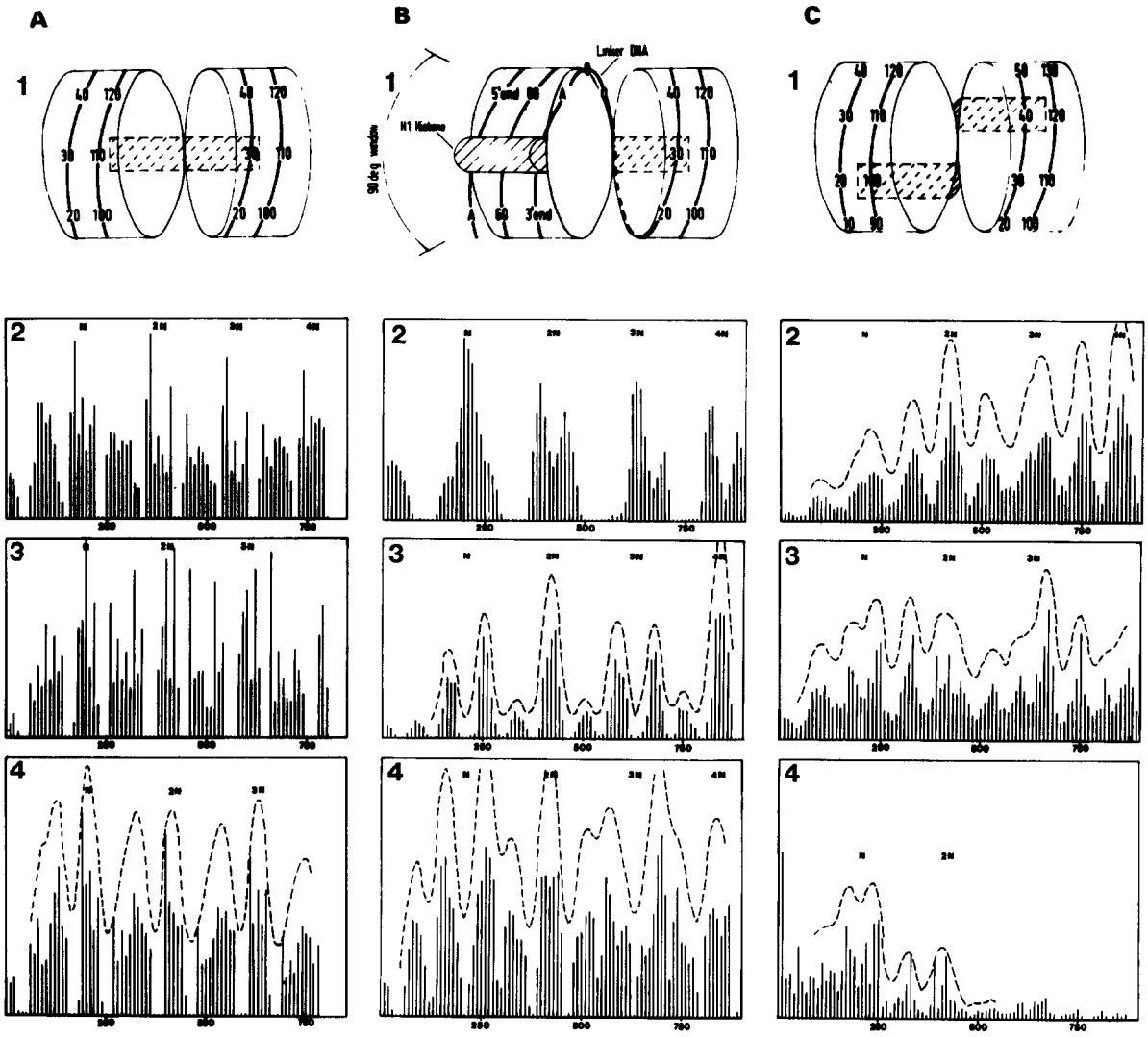

Fig.1. A schematic representation of two nucleosomes with three mutual orientations showing the exposed DNA on the 'surface' of the thick fibre and the predicted DNase I digestion patterns for each orientation. (A) Nucleosomes with radial and 'parallel' axes of symmetry. Predicted digestion patterns for repeat lengths of 190 bp (2); 200 bp (3); and 210 bp (4). Digestion time 10 s under the conditions in [12]. (B) 'Antiparallel' nucleosomes with folded linker between them which is also digested at '10 nucleotide' intervals. Repeat length 210 bp. (2) All positions on the linker DNA are fully accessible to the nuclease; (3) positions marked A are accessible with a cutting probability of 0.05 and positions marked O are inaccessible; (4) as (3) but positions marked A are cut with probability 0.01. (C) Alternating oblique nucleosomes with inaccessible linkers and repeat length 210 bp. Digestion times: (2) 0.3 s; (3) 3 s; (4) 30 s. Calculations were made by computer with a 10 n resolution for all possible fragments from a 4000 bp length of DNA.

bability (0.01). In this case also the character of the pattern does not depend on variation of the cutting probabilities for the positions affected by the presence of H1-histone.

The nucleosomes with alternating oblique axes shown in Fig.1 C1 represent the nonsequential model of Staynov [16]. Since the linker DNA and the parts of the core particles which interact with

H1-histone are inside the fibre, according to this model, all accessible positions have known probabilities for cutting and one does not need to vary any of their values. Fig.1 C2, C3 and C4 shows the calculated patterns for 3 different times of digestion for 210 bp repeat lengths. It is seen that the calculated pattern is of the 'double nucleosome' character, having the characteristic $2aN$ 90 n peaks and partially split odd multiple peaks. The splitting increases with the time of digestion.

Two more possibilities were considered. An alternative was suggested by Worcel and Benyajati [14] and McGhee et al. [15] that DNA may fold into a regular helix of 80 bp/turn (in fact 90 bp was assumed in [14]) and that nucleosomes do not have a preferable orientation but follow according to the repeat length. For a 200 bp repeat length this model is the same as that shown in fig.1 B1. All other repeat length calculations gave a regular pattern of 80 n periodicity (not shown), which is very different from the experimental results. The double-helical or 'ribbon' model of Worcel et al. [17] has a groove, the size of which depends on the linker length and for a 210 bp repeat is 15 nm wide. Linker DNA is fully accessible to nuclease attack and the digestion pattern for such a structure would be similar to that shown in fig.1 B2.

For the case of alternating oblique nucleosomes it has been shown [16] that when the calculations were done for DNase II instead of DNase I the general character of the pattern did not change but the peaks of $2aN$ 90 n shifted away from even multiples to $2aN$ 100 n and thus the pattern looked more like 'half nucleosome' repeat pattern of DNase II [1–3]. These data suggest that the 'double nucleosome' and the 'half nucleosome' repeat patterns have a common origin determined by the higher order structure of chromatin and not by the mode of digestion of the nucleases. Indeed when nuclei were digested with micrococcal nuclease at 0°C, two distinct shoulders were seen between mono- and dinucleosome peaks with lengths of 260 and 320 bp [18] corresponding to $N + 50$ and to $2N - 100$ n.

It is to be expected that only some kind of regular structure (or regular exposure) can produce a 'double nucleosome repeat' digestion pattern and it cannot be of the kind shown in fig.1 A1 with all nucleosomes uniformly exposed. However, as is seen in fig.1B,C this pattern can be obtained from two principally different structures and the only requirement is that the nucleosomes alternate. The small differences in the sizes of the fragments are beyond the experimental accuracy. Therefore, although the low resolution digestion pattern rules out some of the proposed models, it cannot discriminate among models with alternating nucleosomes, i.e., Worcel and Benyajati [14], McGhee et al. [15] and Staynov [16].

The 10 nucleotide-resolution gels of DNase I digestion pattern rarely reach beyond 250 n. However, the small size fragments show a characteristic pattern. For example chicken erythrocyte chromatin digests have strong 80 and 110 n fragments. If one assumes a regular folding of DNA with 80 bp per turn, the strong 80 n fragment can be expected but 110 n fragment will come from $1\frac{3}{8}$ turns and should be very weak, as seen in fig.1 B3 and B4. This pattern, however, can be explained by the non-sequential model of Staynov [16] (fig.1C) or some other structure in which the linker is not folded. A proper trial of such models will be possible only after accurate determination (with end labelling) of the positions of nuclease attack on core particles obtained after DNase I digestion of whole nuclei.

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REFERENCES

[1] LaFond, R.E., Goguen, J., Einck, L. and Woodcock, C.L.F. (1981) Biochemistry 20, 2127–2132.
[2] Altenburger, W., Horz, W. and Zachau, H.G. (1976) Nature 264, 517–522.
[3] Horz, W. and Zachau, H.G. (1980) J. Mol. Biol. 144, 305–327.
[4] Horz, W., Miller, F., Klobeck, G. and Zachau, H.G. (1980) J. Mol. Biol. 144, 329–351.
[5] Arceci, R.J. and Gross, P.R. (1980) Dev. Biol. 80, 210–224.
[6] Khachatrian, A.T., Pospelov, V.A., Svetlikova, S.B. and Vorob'ev, V.I. (1981) FEBS Lett. 128, 90–92.
[7] Pospelov, V.A. and Svetlikova, S.B. (1982) FEBS Lett. 146, 157–160.

[8] Burgoyne, L.A. and Skinner, J.D. (1981) Biochem. Biophys. Res. Commun. 99, 893–899.
[9] Burgoyne, L.A. and Skinner, J.D. (1982) Nucleic Acids Res. 10, 665–673.
[10] Lohr, D., Tatchell, K. and Van Holde, K.E. (1977) Cell 12, 829–836.
[11] Thoma, F., Koller, Th. and Klug, A. (1979) J. Cell Biol. 83, 403–427.
[12] Lutter, L.C. (1978) J. Mol. Biol. 124, 391–420.
[13] Finch, J.T. and Klug, A. (1976) Proc. Natl. Acad. Sci. USA 73, 1897–1901.
[14] Worcel, A. and Benyajati, C. (1977) Cell 12, 83–100.
[15] McGhee, J.D., Rau, D.C., Charney, E. and Felsenfeld, G. (1980) Cell 22, 87–96.
[16] Staynov, D.Z. (1983) Int. J. Biol. Macromol. 5, 3–9.
[17] Worcel, A., Strogatz, S. and Riley, D. (1981) Proc. Natl. Acad. Sci. USA 78, 1461–1465.
[18] Greil, W., Igo-Kamenes, T. and Zachau, H.G. (1976) Nucleic Acids Res. 3, 2633–2644.