Influence of DNA superstructural features and histone amino-terminal domains on nucleosome positioning

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Received 1 March 1995

Abstract Nucleosome positioning has been studied on a strongly curved 268 bp DNA fragment from a *Crithidia fasciculata* kinetoplast, complexed with a histone octamer either normal or lacking amino-terminal domains. A very similar nucleosome multiple positioning, with the same rotational phasing, has been found, by Exo III mapping, in both cases. The experimental positioning is in fairly good agreement with that predicted using a theoretical method based on DNA distortion energy, derived from the nucleotide sequence. Taking into account that nucleosomes, without histone amino-terminal domains, lack thirty percent of electrostatic interactions, these results suggest a dominant role on nucleosome positioning of DNA distortion energy with respect to modifications in histone domains.

Key words: DNA superstructure; Nucleosome positioning; Histone amino-terminal domain

1. Introduction

Recently, non-random nucleosome positioning on specific DNA sequences has been shown in many different biological systems as well as in synthetic DNAs [1,2]. In most cases, the main determinant of specific nucleosome positioning is associated with DNA curvature or flexibility [3,4]. The role of DNA superstructural features appears well established, making it possible, in many cases, to predict virtual nucleosome positioning from the nucleotide sequence in fairly good agreement with experimental mapping [5,6].

In contrast, the role of histone octamer different domains has been, so far, an elusive research topic, although their relevance on nucleosome organisation has been suggested from the beginning of chromatin structural and functional studies [7]. Namely, an important role of the N-terminal domains of histone core has been generally assumed, since these domains present a high concentration of positively charged residues and are the major sites of histone modifications such as acetylation and phosphorylation [8,9]. However, recent results by Dong et al. [10] and by Hayes et al. [11] suggest that, at least in the examined systems, 'tailless' octamers and the $(H3–H4)_2$ tetramer could recognize the same nucleosome positioning signals as the intact octamer. On the other hand, in supercoiled circular DNA the influence of histone amino-terminal domains has been shown to be relevant to nucleosome organization [12].

In this paper, taking advantage of a strongly curved 268 bp DNA fragment from *Crithidia fasciculata* kinetoplast which acts as a nucleosome 'attractor' [13], we have compared the influence of DNA superstructural features and histone amino-terminal domains on nucleosome positioning. The *Crithidia* fragment, in fact, is practically a circle, as shown by electron microscopy visualisation [14] and by theoretical analysis of DNA curvature [6] and a large number of energetically quasi equivalent nucleosome positions are predictable (see Fig. 1B). Thus the experimental mapping of nucleosomes on this fragment, using either normal histone octamer as histone octamer lacking amino-terminal domains, allows a stringent analysis of the respective influence on nucleosome positioning of DNA distortion energy (comparison between theoretical prediction and experimental nucleosome mapping) and histone amino-terminal domains (comparison between normal and trypsinized nucleosomes positioning).

2. Materials and methods

2.1. Materials

Restriction endonucleases and Bacteriophage T4 polynucleotide kinase were from USB and Sigma.

Exonuclease III was from PROMEGA.

Radiochemicals were from Amersham.

2.2. DNAs

The DNA fragment used was obtained by *Eco*RI–*Hin*dIII digestion from pPK201/CAT (3230 bp) plasmid kindly provided by E. Di Mauro.

Plasmid pPK201/CAT contains the *Stu*I–*Acc*I 211 bp bent segment from the kinetoplast DNA of the Trypanosomatidae Protozoon *C. fasciculata* cloned in the *Bam*HI linkers [15], the sequence of the fragment obtained with the restriction enzyme *Eco*RI and *Hin*dIII is reported in Fig. 1A.

5′-Terminal labelling was performed according to a standard procedure. Fragments labelled at one extremity were obtained as follows: plasmid DNA was restricted with 5′ protruding enzyme labelled and restricted with the appropriate secondary enzyme.

The fragments were separated by 1.5% (w/v) agarose gel electrophoresis and the band of interest was recovered by electroelution.

Nucleosomal DNA was obtained from nucleosomes at 2.5 M NaCl, treated with Proteinase K and phenol/chloroform extracted.

2.3. Nucleosomes

Nucleosome core particles were obtained from chicken erithocyte chromatin digested with micrococcal nuclease and purified through 5–20% sucrose gradient ultracentrifugation according to Forte et al. [16].

Modified nucleosomes, namely nucleosomes lacking histone NH_2 domains, were obtained from nucleosomes by digestion with immobilized Trypsin according to the method developed by Ausio et al. [17].

The polyacrylamide gel electrophoresis of trypsinized histones is shown in Fig. 2A in comparison with normal histones.

2.4. Reconstitution procedure

The 268 bp DNA fragment was reconstituted with normal nucleo-

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SSDI 0014-5793(95)00343-6

somes or trypsinized nucleosomes according to the salt dilution protocol described by Drew and Travers [3].

The reconstitutes were analyzed for resistance to micrococcal nuclease digestion. It was observed the normal nucleosome pattern (data not shown).

Reconstitution was monitored by gel electrophoresis in 0.8 % (w/v) agarose in 0.5 × TBE buffer (0.045 M Tris-borate, 0.001 M EDTA, pH 8).

2.5. Exonuclease III

Reconstituted samples were made 66 mM Tris-HCl (pH 8), 1.66 mM $MgCl_2$, 1 mM 2-mercaptoethanol and equilibrated at 30°C for the Exonuclease III reaction (Exo III). Exo III was added at 30°C, samples were withdrawn at appropriate intervals into an equal volume of 15 mM EDTA, 1% (w/v) sodium dodecylsulfate and held at 100 °C for 2 min. Following extraction with phenol and precipitation with ethanol the samples were resuspended in formamide and analyzed with autoradiography. Autoradiograph were analyzed with an LKB laser densitometer.

2.6. Theoretical prediction of nucleosome positioning

Theoretical prediction of nucleosome positioning was derived from the distortion energy profiles of 268 bp *Crithidia* fragment, by calculating for successive 145 bp tracts along the sequence the vectorial difference between the local curvature of the DNA tract and the corresponding curvature of the nucleosomal form, with the method developed by De Santis et al. [6,18].

3. Results

3.1. Monitoring the exchange between normal and trypsinized nucleosomes and the curved DNA fragment

The monitoring of the exchange between normal (nc) or

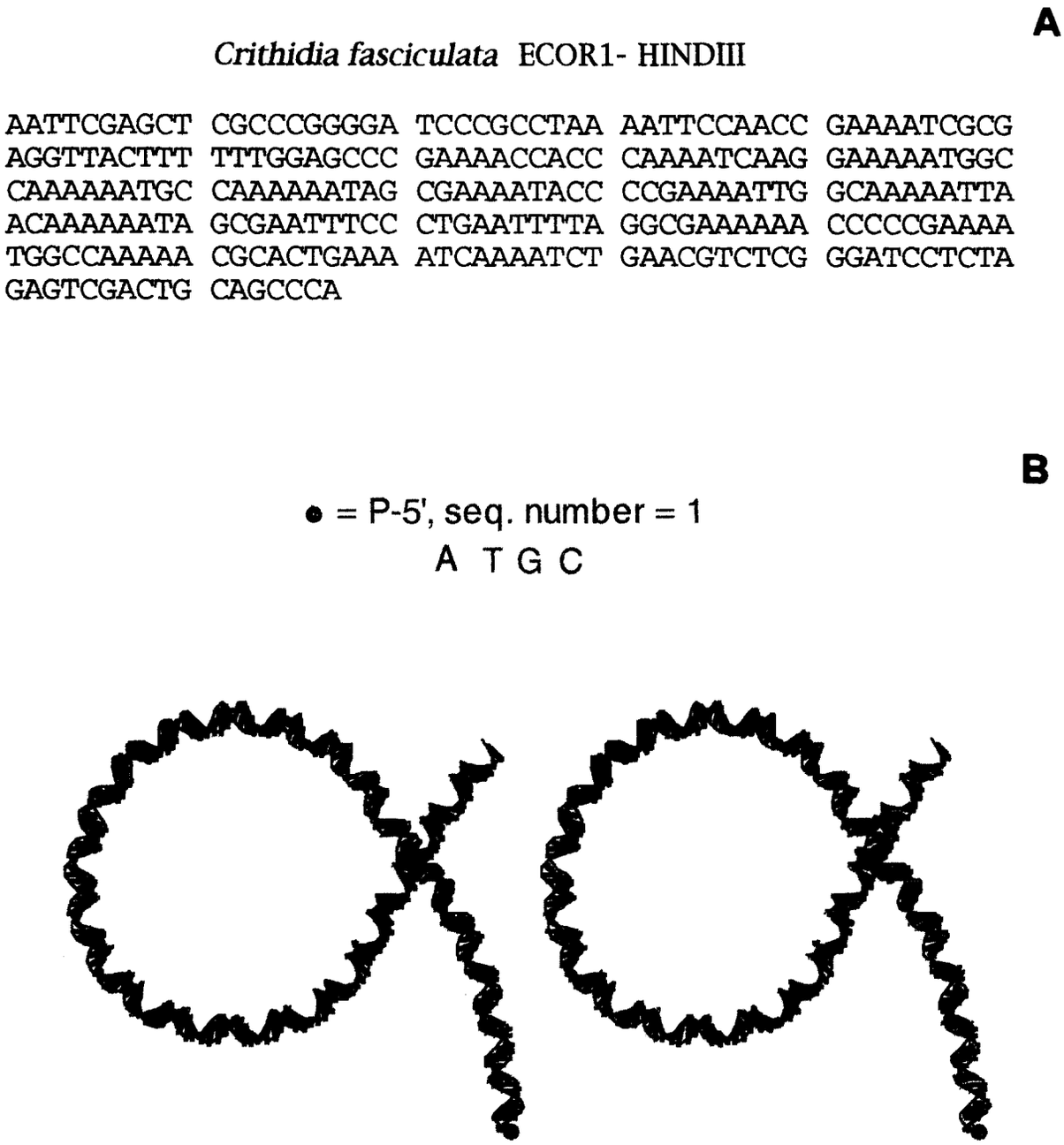


Fig. 1. (A) Sequence of 268 bp *Crithidia* DNA fragment. (B) Stereoviews of the 268 bp *Crithidia* DNA fragment superstructure

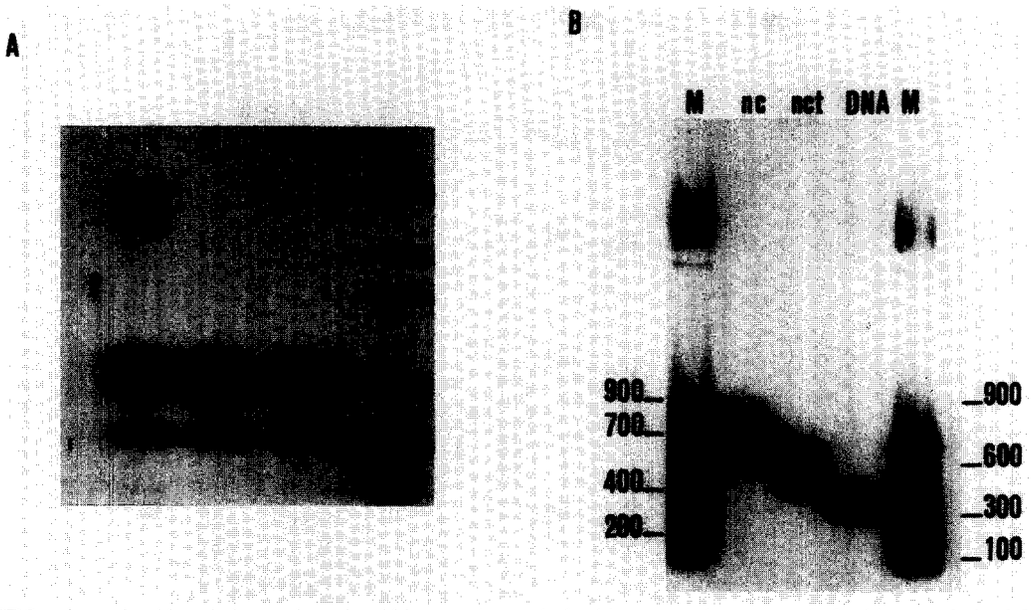


Fig. 2. (A) 18% SDS-polyacrylamide gel electrophoresis of histones: h stands for histone marker; n stands for histones derived from nucleosomes and nt for histones derived from nucleosomes previously selectively digested with trypsin. (B) 0.8% agarose gel electrophoresis of the 268 bp curved DNA fragment reconstituted with normal nucleosomes (nc) or with selectively trypsinized nucleosomes (nct): the naked 268 bp *Crithidia* curved DNA fragment is marked as DNA and M is *Hpa*II digest of pUC18.

trypsinized nucleosome (nct) and the 268 bp DNA fragment containing the highly curved sequence of *Crithidia fasciculata* was obtained by gel electrophoresis, taking advantage of the reduced nucleosome mobility on account of its higher molecular weight and reduced charge with respect to naked DNA. As shown in Fig. 2B the fragment either reconstituted by exchange with normal nucleosome or with selectively trypsinized nucleosomes shows a reduced electrophoretic mobility with respect to that of naked DNA, the reduction being larger in the case of nc. The enhanced electrophoretic mobility of nct with respect to that of nc can be accounted mainly by the increase in the net negative charge of the particle as a consequence of the removing of the highly positively charged histone tails [17].

3.2. *Exo III digestion of the complexes between normal or trypsinized histone octamer and the curved DNA fragment from Crithidia allows to obtain nucleosome borders by the enzyme stops*

The localisation of nucleosome borders was obtained by Exo III digestion; in fact this enzyme, digesting from the 3′ end, will have its progress impeded by the presence of a nucleosome. Prominent pauses in the time course of Exo III digestion should therefore signal the presence of major nucleosome positions [19].

Fig. 3A shows Exo III digests of the naked DNA and the reconstituted nc or nct on the *Crithidia* fragment 5′-end labelled on either strand; experimental details are reported in the figure legend. Since the naked *Crithidia* DNA shows some bands due probably to sequence specific pausing of the enzyme (see Fig. 3A, lane 2) care was taken to carry out Exo III digestion in the absence of the naked DNA , as shown in Fig. 2B.

The band patterns of the reconstituted nc or nct, as shown in Fig. 3A, appear complex, indicating that nucleosome in both cases have more than one preferential locations on the considered sequence and that a number of differently populated sets of molecules with one well positioned nucleosome are present.

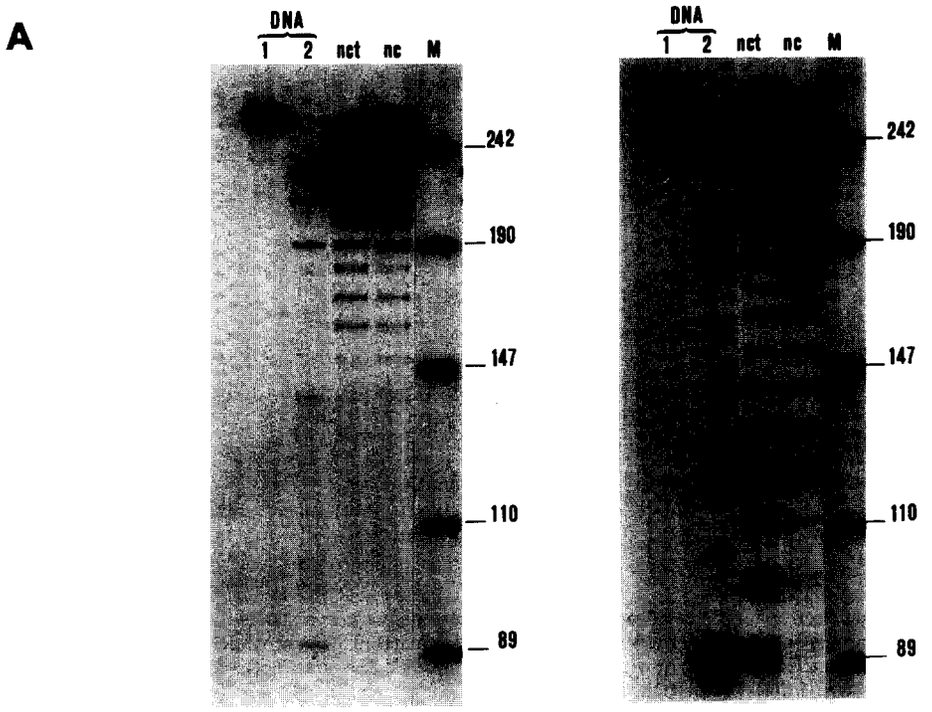
To localize nucleosome stops with respect to the DNA sequence, the band patterns have been analyzed as densitometric tracings as reported in Fig. 3B. Twelve main positions, assigned taking in consideration Exo III digestion from both directions, are detectable on nc or nct reconstituted on the *Crithidia* fragment. It is worth noting that Exo III stops are considered and numbered if their distance on the two DNA strands is equal to 146 ± 4 bp, corresponding to the nucleosomal DNA length.

From densitometric analysis, the patterns of normal and trypsinized nucleosome positioning appear very similar, although some differences are evident in the heights of densitometric profiles; these differences could be attributed to different nucleosome frequencies on a number of positions, as discussed in the next section.

The Exo III stops indexed with asterisks see Fig. 3B (a) and (c), correspond to DNA fragments less than 146 bp long, deriving probably from histone tetramer complexes; these were not considered in our analysis.

4. Discussion

The comparison between the normal and selectively trypsinized nucleosome positioning as well as the correlation between experimental and theoretical nucleosome positioning can be carried out by deriving , from gel electrophoresis densitometric tracings, the preferential locations of nucleosomes dyad axis along the sequence. To this end , we have considered densitometric tracings from a number of different experiments, each consisting of two densitometric profiles derived from the Exo III digestion of the same fragment labelled on the two 5′-ends. With this approach it is possible to derive the dyad location from two independent measurements of the same experiment.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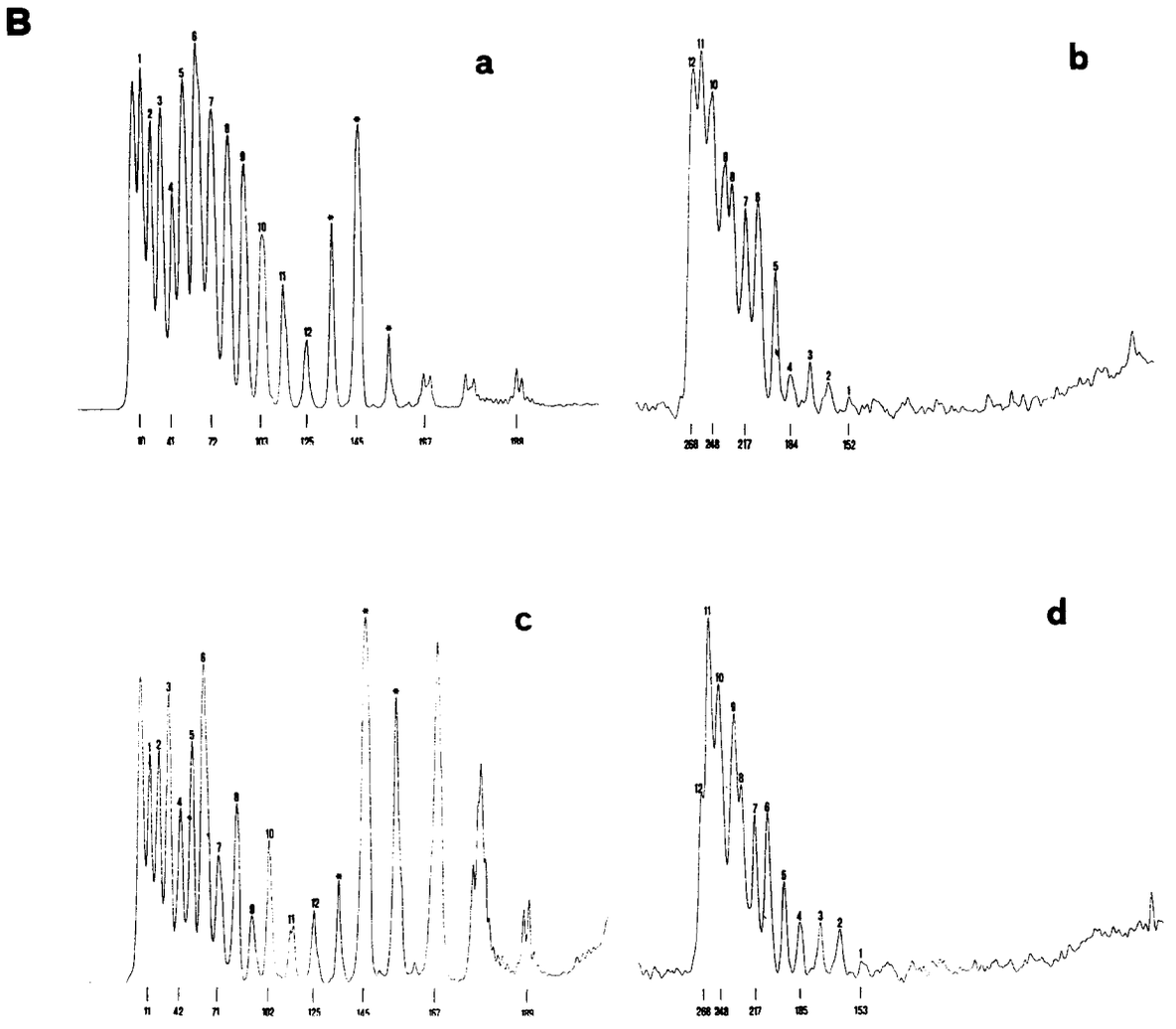
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b
c
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Fig. 3. (A) Autoradiographs of Exo III digestion patterns of nc or nct reconstituted on the 268 bp *Crithidia* curved fragment. The DNA was 5′-end labelled at the *Eco*RI (left panel) or *Hind*III (right panel) extremity. Lanes marked as DNA are respectively the naked *Crithidia* DNA fragment (1) and the same fragment digested with Exo III (2). Lanes nc and nct are respectively nucleosomes and selectively trypsinized nucleosomes reconstituted on the *Crithidia* DNA fragment, digested with Exo III for 30 min at 30°C. M are molecular weight markers obtained by Hpa II digestion of pUC 18 DNA. (B) Densitometric profiles of the autoradiographs lanes reported in 3A. (a) and (c) are respectively nc and nct 5′-end labelled with *Hind*III; (b) and (d) are respectively nc and nct 5′-end labelled with *Eco*RI.
←

Furthermore, considering a number of densitometric profiles from different experiments, we have averaged nucleosome locations frequency.

Fig. 4A and B show the comparison between experimental mapping by Exo III analysis of normal and selectively trypsinized nucleosomes. A similar nucleosome multiple positioning with the same rotational phase (the multiple dyad axis positions differ of 10–11 bp) is evident in the two cases. This result shows that the ability of normal and selectively trypsinized histone octamer to organize nucleosome along the DNA sequence is equal whereas if we consider the two positioning in details, namely the frequency of nucleosome population for different positions of the dyad axis, same differences are evident. It is tempting to suggest that local specific interactions between histone side chains and DNA bases could be involved; this topic, however, deserves further investigations.

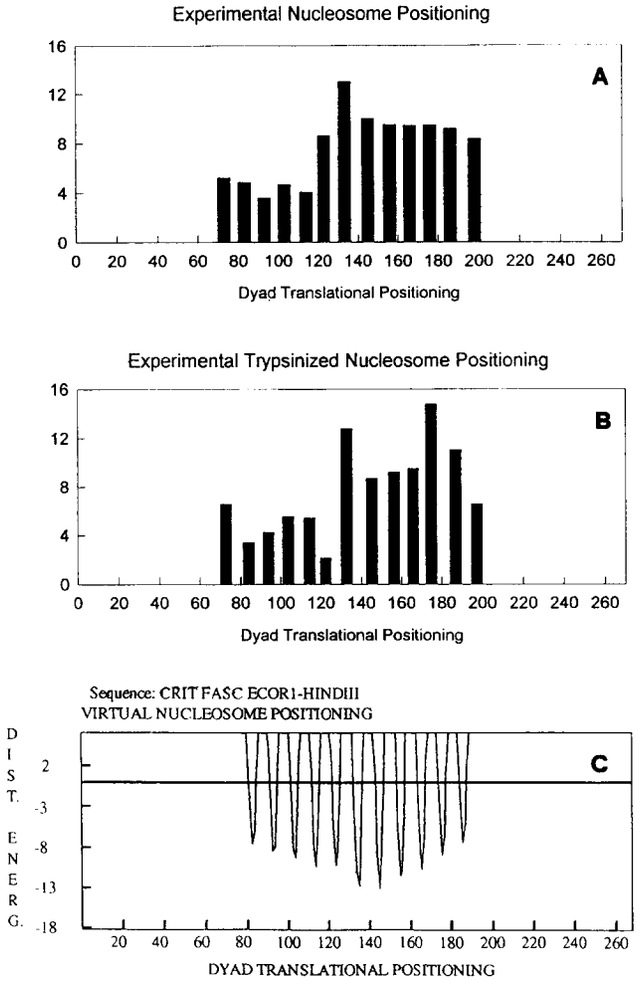

Fig. 4. (A) Experimental nucleosome positioning along the *Crithidia* DNA sequence. On the horizontal axis, the positions of the dyad axis are indicated. On the vertical axis, the percent frequency of nucleosome diverse positions from Exo III mapping. (B) The same than in A except that selectively trypsinized nucleosome was considered. (C) The distortion energy (kcal/mol of nucleosome) of recurrent 145 bp DNA tracts, with respect to that of an equivalent tract of a straight DNA represented by the zero line, is reported for each of the possible positions of nucleosome dyad axis. Each minimum represents a virtual position of a nucleosome spanning a DNA tract 72 bp on both sides. The average distance between the minima is 10.3, as evaluated via Fourier Transform.

Fig. 4A and C allow us to compare experimental nucleosome mapping and theoretical distortion energy profiles. The agreement between theoretical and experimental nucleosome positioning is very satisfactory also in this selective system, where is possible to test an extensive multiple positioning; this demonstrates that DNA intrinsic bendability has a dominant role with respect to histone modifications. Considering the nucleosome theoretical distribution of dyad axis, it is interesting to note that whilst energy differences in the nucleosome rotational positioning are of the order of 20–25 RT about the minima of the energy diagrams, changes in the translational positioning nearby the energy minima require an energy cost of the order of RT. This explains why nucleosome multiple positioning with the same rotational phase can be considered characteristic of many investigated systems in vitro and in vivo; in this last case it is intriguing to suggest its possible influence on chromatin dynamics.

Acknowledgements: Thanks are due to P. De Santis for many useful discussions. R. Gargamelli contributed with photographic aid and M. Arceci with preparation of plasmid pPK201/CAT.

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