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Identification of sequence elements contributing to the intrinsic curvature of the mouse satellite DNA repeat

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ABSTRACT

In this paper, the contribution of different sequence elements to the intrinsic curvature of the mouse satellite DNA repeat was investigated. This DNA fragment contains nineteen groups of three or more consecutive adenines which are only poorly phased with respect to the helical repeat. The mouse satellite DNA repeat shows a sinusoidal pattern of cleavage by the hydroxyl radical; the waves of reactivity are phased with respect to the A-tracts. Some interesting observations arise from a detailed analysis of these cleavage patterns: a) the maxima of hydroxyl radical cleavage are more periodically spaced along the DNA sequence than the A-tracts themselves. As a consequence, the position of each maximum with respect to the A-tract is variable; b) the sequence 5′ TGGAATAT$^{G}/_{A}$A 3′ shows a sinusoidal pattern of hydroxyl radical cleavage. This sequence shows a retarded migration in polyacrylamide gels indicating that it is actually intrinsically curved. These results are discussed in view of the current models for DNA curvature.

INTRODUCTION

Much attention has been devoted recently to the question of DNA curvature (see 1–3, for reviews). Elements of curved DNA are nearly ubiquitous and examples of naturally occurring curved DNA molecules have been reported in a wide variety of biological systems (3). In many cases these elements are localised in regions of biological significance, suggesting that curvature may be important for the functionallity of the DNA molecule. In particular, it has been shown that the repeating unit of the mouse satellite DNA is intrinsically curved (4,5). Other satellite DNAs also share this structural characteristic (5).

DNA curvature is generally associated with the presence of runs of adenine residues spaced periodically along the DNA sequence (3). Apparently, at least three adenines are required to introduce a significant deflection on the DNA helical axis (6). Several models have been proposed to explain the structural basis of DNA curvature. They mainly fall into two categories. A first class of models suggests that curvature is the result of nearest-neighbour interactions along the DNA molecule (7–9). In these models, deflection of the helical axis arises from the periodic distribution of particular dinucleotide steps. A second class of models invoke that the conformation of the sequence elements inducing curvature differs from that of the flanking DNA (6, 10–14). Deflection in these cases is associated with structural distortions occurring at the interfaces of these conformationally distinct helixes. In particular, a periodic change in the conformational parameters, such as the roll, of successive base pairs can result into curvature of the DNA molecule (26). It has been proved to be quite difficult to distinguish experimentally between these types of models.

A fundamental limitation of the available results arises from the fact that they mostly come from studies of the behaviour shown by DNA molecules obtained by oligomerization of short chemically synthesized oligonucleotides. As a consequence, these DNA fragments are highly repetitive in nature, allowing the evaluation of just a limited set of parameters. Therefore, it might be enlightening to study DNA fragments of higher complexity.

In this paper, the identification of sequence elements contributing to the intrinsic curvature of the mouse satellite DNA repeat has been undertaken. A fundamental difficulty here arises from the absence of experimental probes which will differentially detect curved DNA on a known structural basis. Curved DNA molecules show a differential susceptibility to cleavage by the hydroxyl radical. Straight B-DNA is cleaved by the hydroxyl radical nearly equally at each base-step (15). Instead, several curved DNA molecules have been reported to show a characteristic sinusoidal pattern of hydroxyl radical cleavage that was first observed in the kinetoplast DNA (16,17). In general, the waves of reactivity are phased with respect to the adenine tracts and the frequency of cleavage decreases in the 5′ to 3′ direction at each adenine tract. The structural basis of such differential behaviour is not completely understood, placing a serious limitation on the identification of curved DNA elements on the sole basis of their hydroxyl radical cleavage patterns. Here, the hydroxyl radical reaction was used to analyze the structure

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of the intrinsically curved mouse satellite DNA repeat. This DNA molecule shows a sinusoidal pattern of cleavage. The cutting pattern is phased with respect to the adenine tracts but it shows some peculiar features. First of all, the maxima of reactivity are more periodically spaced along the DNA molecule than the adenine tracts themselves. As a consequence, the position of each maximum in relation to the adenine tract is fairly variable. Secondly, the sequence 5′ TGGAATAT$^{G}/_{A}$A 3′ shows a sinusoidal pattern of cleavage though it does not contain any of the elements previously identified as inducers of curvature. Oligomers of this sequence show an anomalous electrophoretic behaviour consistent with being intrinsically curved. These results are discussed in view of the current models for DNA curvature.

MATERIALS AND METHODS

Autocorrelation analysis

To determine the periodicity of the distribution of A-tracts along the mouse satellite DNA repeat the following correlation function was computed:

$$\text{Corr } (X,X)_i = (\Sigma_n X_{n+i} X_n)/N$$

where N, refers to the length of the sequence (234 bp. for the mouse satellite DNA repeat); n, refers to the nucleotide position in the sequence and i, to the lag. Because the repeat of the AA or TT cluster was analyzed, the following code was used. X_n was set to 0 if the dinucleotide sequence at the nth position is not found or if is isolated. If the sequence was in a cluster of three or more nucleotides, then X_n was the number of nucleotides which separates the sequence from the closest nucleotide different from those in the cluster.

Hydroxyl radical cleavage

For the hydroxyl radical cleavage reaction, the mouse satellite DNA repeat was liberated from pUC19 (5) as a 285 bp. EcoRI+HindIII restriction fragment which was labelled with [α-^{32}P]-dATP and the Klenow fragment of the DNA polymerase I, at either the EcoRI site or the HindIII site previously to cleavage with the second restriction endonuclease. The labelled fragment was purified by gel electrophoresis and then subjected to the reaction with the hydroxyl radical essentially as described in (18). About 0.2−0.3 μg of labelled fragment were dissolved in 10μl of 10mM Tris-HCl, 10mM NaCl (pH 7.4). The reaction was carried out at room temperature for 90 seconds and in the presence of 1μg of *E.coli* DNA. Cleavage was stopped by addition of 20μl of 0.1M thiourea. DNA was then precipitated with ethanol and analysed on a 6% polyacrylamide-7M urea denaturing gel. Autoradiographs were recorded on Hyperfilm (Amersham) and scanned on a Joyce-Loebl densitometer.

Polyacrylamide gel electrophoretic analysis

The chemically synthesised oligonucleotides shown in Table I, were purchased from Isogen Bioscience (Amsterdam) and purified by polyacrylamide gel electrophoresis. Annealing was performed by treatment at 90°C for three minutes and then slow cooling down to 35°C. Duplexes were then 5′-end labelled with [γ-^{32}P]-dATP and T4 polynucleotide kinase, and self-ligated with T4 DNA ligase. Ligation was allowed to proceed at 20°C for 4 hours at an enzyme/DNA ratio of 200 units(NEB)/μg in a final volume of 10μl. Ligated products were then run on non-denaturing 8% polyacrylamide gels (44.5 mM Tris-borate, 1.25mM EDTA, pH 8.3). Electrophoresis were performed on a water-jacketed apparatus at either 4°C or 40°C. After electrophoresis gels were dried and autoradiographs were recorded on Hyperfilm (Amersham). For quantitative analysis of the results, autoradiographs were scanned with a Joyce-Loebl densitometer.

RESULTS

The mouse satellite DNA repeat contains several A-tracts which are only poorly phased with respect to the helical repeat

It was shown earlier (4, 5), that the mouse satellite DNA repeat is intrinsically curved as judged by its anomalous electrophoretic behaviour in polyacrylamide gels, as well as its appearance under the electron microscope. In general, DNA curvature is associated with the presence of runs of three or more consecutive adenine residues spaced periodically along the DNA molecule, phasing the helical repeat (3). The mouse satellite DNA is enriched in runs of adenine residues and, nineteen groups of three or more adenines are found in the 234 bp. long repeating unit. These adenine tracts are periodically spaced along the DNA molecule. The autocorrelation analysis of the distribution of A-tracts shown in Figure 1, reflects this periodicity. If the function repeats at given intervals, this will appear as maxima for the corresponding lags. The number and sharpness of the maxima are a measure of the periodicity of the distribution. The more periodic the distribution is, more and sharper maxima are detected. As it is shown in Figure 1, only a limited number of strong maxima are observed, indicating a poor though periodic distribution of A-tracts along the sequence. It is also evident from Figure 1, that four internal regions of correlation are detected which reflect the four internal subrepeats of the mouse satellite repeating unit (19). Similar results are obtained when the same type of analysis is carried out for each individual subrepeat (data not shown). Only the last subrepeat, spanning from position 170 to position 230, shows a good autocorrelation function. These results indicate that, although the mouse satellite DNA repeat contain several runs of three or more adenines, their distribution along the sequence is not strictly periodic, being only poorly phased with respect to the DNA helical repeat.

The mouse satellite DNA repeat shows a sinusoidal pattern of hydroxyl radical cleavage

Cleavage by the hydroxyl radical has been used to study the structure of several curved DNA fragments (16, 17). As shown

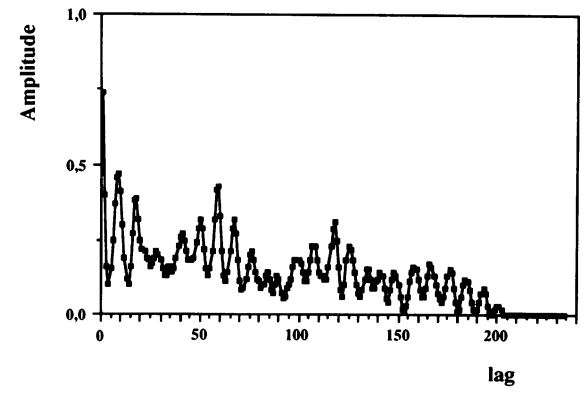

Figure 1. Autocorrelation plot of AA or TT occurrences of the 234 bp. long mouse satellite DNA repeat.

in Figure 2, the A-rich strand of the mouse satellite DNA repeat shows a sinusoidal pattern of hydroxyl radical cutting which is characteristic of a curved DNA molecule. Figure 3, summarises the results obtained from this study. Three regions showing a sine-wave like cutting pattern are observed. These regions (spanning from positions 1−102, 120−160 and 179−218, respectively), are spaced by DNA sequences, about 20 bp. long, that are cut by the hydroxyl radical quite uniformly. Similarly, sequences from the cloning vector flanking the mouse satellite repeat also exhibit a fairly even cleavage pattern. The waves of reactivity are phased with respect to the adenine tracts. The frequency of cleavage decreases along each tract in the 5′ to 3′ direction. All minima in the pattern coincide with the last adenine of the tracts except for the tract starting at position 133 where it occurs one adenine before the last. The T-rich strand also shows a similar sinusoidal pattern of cleavage which is offset by one to three nucleotides compared to the cutting pattern of the A-rich strand (Figure 3, dotted lines). The waves of reactivity observed for the T-rich strand are less pronounced than those detected on the A-rich strand. Therefore it is more difficult to precisely determine the position of the maxima of reactivity. Actually, the T_3 group which occurs at position 50 on the A-rich strand also shows a less pronounced decrease in cutting frequency than any of the A-tracts of the A-rich strand. Similar results have been reported by others (16).

Though the overall pattern of hydroxyl radical cleavage conforms to what is expected for a curved DNA molecule containing A-tracts, some features of it are noteworthy. First of all, the maxima of hydroxyl radical reactivity are more periodically spaced along the DNA sequence than the runs of adenines themselves. In many cases, the position at which the maximum occurs could be assigned to a single nucleotide. However, as it is indicated in Figure 3, in several cases this assignment was impossible since more than one adjacent nucleotide showed a similar degree of cleavage or the position of the maximum was shifted by one nucleotide in different experiments. In any case, the maxima of hydroxyl radical reactivity are principally spaced either 9 bp. or 10 bp. (Figure 4,

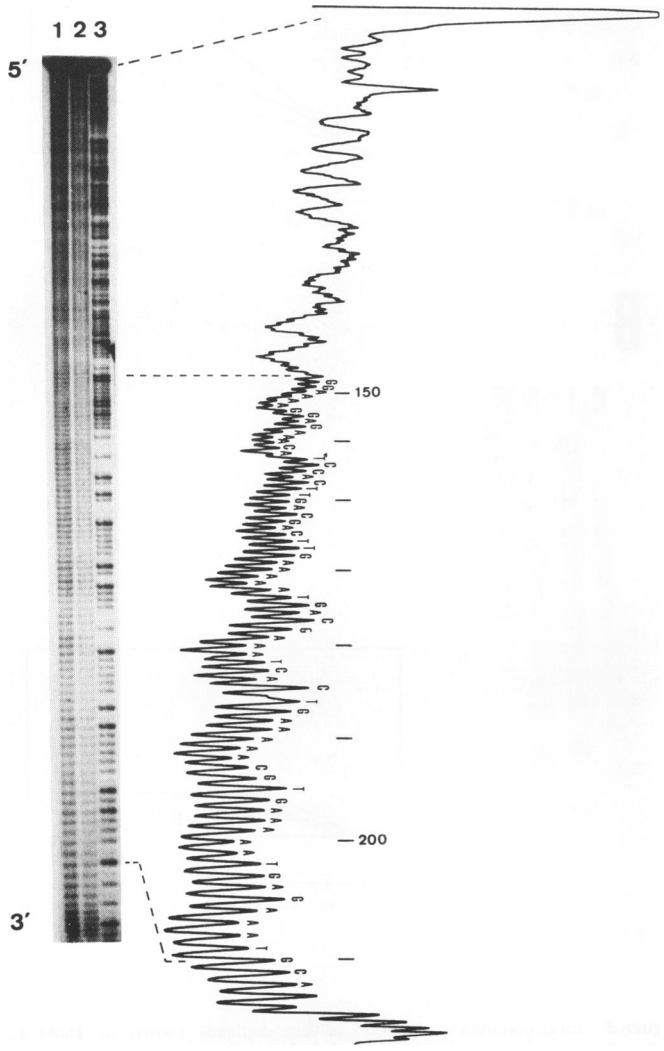

Figure 2. Hydroxyl radical cleavage reaction of the mouse satellite DNA repeat. A 6% polyacrylamide −7M urea gel electrophoresis of the products of the cleavage after a 1 minute reaction (Lane 2) and 3 minutes reaction (Lane 1), is shown on the left. Lane 3, shows a G+A sequencing ladder of the corresponding DNA fragment. The densitometer scan of lane 1 is shown on the right. Nucleotides from position 148 to 212 are indicated.

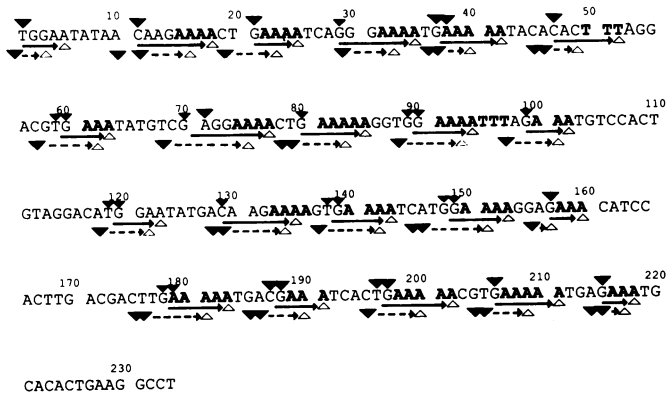

Figure 3. Hydroxyl radical cleavage pattern of the A-rich strand of the mouse satellite DNA repeat. The sequence of the A-rich strand of the cloned satellite repeat is shown. Adenine tracts are indicated in boldface characters. Arrows underneath the sequence indicate regions showing a progressive decrease (on the direction of the arrow) on the frequency of hydroxyl radical cleavage of the A-rich strand (full lines) and T-rich strand (dotted lines). Black and open triangles indicate the position of the maxima and minima of cleavage, respectively.

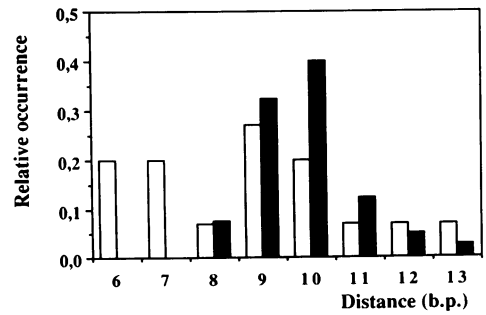

Figure 4. Distribution of the distance, in bp., between consecutive maxima of hydroxyl radical cleavage (closed columns) and the first adenine residue of consecutive A-tracts (open columns), of the mouse satellite DNA repeat. Only the data corresponding to cleavage of the A-rich strand has been considered. Similar results are obtained when the data corresponding to cleavage of the T-rich strand are also included.

close columns). On the other hand, the A-tracts show a much less periodic distribution, as reflected by the autocorrelation analysis shown in Figure 1. The actual distance between the first adenine of consecutive tracts oscillates between 6 and 13 nucleotides (Figure 4, open columns). As a consequence, the position of each maximum in relation to the adenine tract is variable. Generally, it occurs either one or two bases upstream from the tract but, it can occur as far as four bases upstream (maxima at positions 11, 70 and 129) or at the first adenine of the tract (maxima at positions 37 and 179). Maxima occurring at positions 11 and 129 correspond to a citosine residue that is followed by an interrupted A-tract: 5′ CAAGAAAA 3′. In these cases, the decrease in the frequency of hydroxyl radical cleavage starts at the citosine residue but the guanine which interrupts the tract shows a slightly higher reactivity that the preceding nucleotide. A similar situation is found at the sequence element 5′ GAGGAAAA 3′ which occurs at position 70, though in this case the position of the maxima cannot be unambiguously determined and it can occur either three or four bases upstream from the A-tract.

On the other hand, the sequence element, 5′ TGGAATA-$T^{G}/_{A}$A 3′ shows an unexpected sinusoidal cleavage pattern. This sequence occurs twice in the mouse satellite DNA repeat, at nucleotide position 1−10 and 119−128, always 5′ of an A-tract located about 10 bp. away. In both cases, the frequency of hydroxyl radical cleavage decreases in the 5′ to 3′ direction being minimum at the second adenine residue. The maximum of reactivity can occur either at the first T or G. This sequence element does not contain any tract of three or more adenines. Moreover, it contains one or two 5′ TpA 3′ steps which have been shown to abolish DNA curvature (27). In spite of this, it shows a pattern of hydroxyl radical cleavage as if it was intrinsically curved.

The sequence element 5′ TGGAATATGA 3′ is intrinsically curved

The contribution of the sequence element 5′ TGGAATATGA 3′ to the intrinsic curvature of the mouse satellite DNA repeat was analysed through the determination of the electrophoretic behaviour of DNA fragments obtained by ligation of chemically synthesized oligonucleotides containing the sequence indicated above. The different DNA duplexes used in these experiments are indicated in Table I. Duplex I, contains the sequence under investigation spaced one helical turn from an A_5-tract. Duplex III, contains the same A_5-tract as in duplex I, but the sequence under investigation was substituted by a DNA sequence containing no adenine residues so that, it basically contains an A_5-tract spaced every two helical turns. Duplex II, contains the sequence under investigation by itself. Duplex C, is an 18-mer which was used as molecular weight marker.

Table I. Duplexes used in these experiments

	SEQUENCE
DUPLEX I	TGGAATATGACAAAAACGGC
	CTTATACTGTTTTTGCCGAC
DUPLEX II	TGGAATATGATGGAATATGA
	CTTATACTACCTTATACTAC
DUPLEX III	GTGGCGCCACCAAAAACGGC
	CCGCGGTGGTTTTTGCCGCA
DUPLEX C	TTAGGGTTAGGGTTAGGG
	TCCCAATCCCAATCCCAA

Figure 5, shows the results obtained from this study. When electrophoresis was carried out at 4°C, all three duplexes showed a retarded migration for fragments larger than 40 bp. in length (Figure 5,A). The R_L values (apparent length/actual length) increase progressively for fragments between 40 bp. and

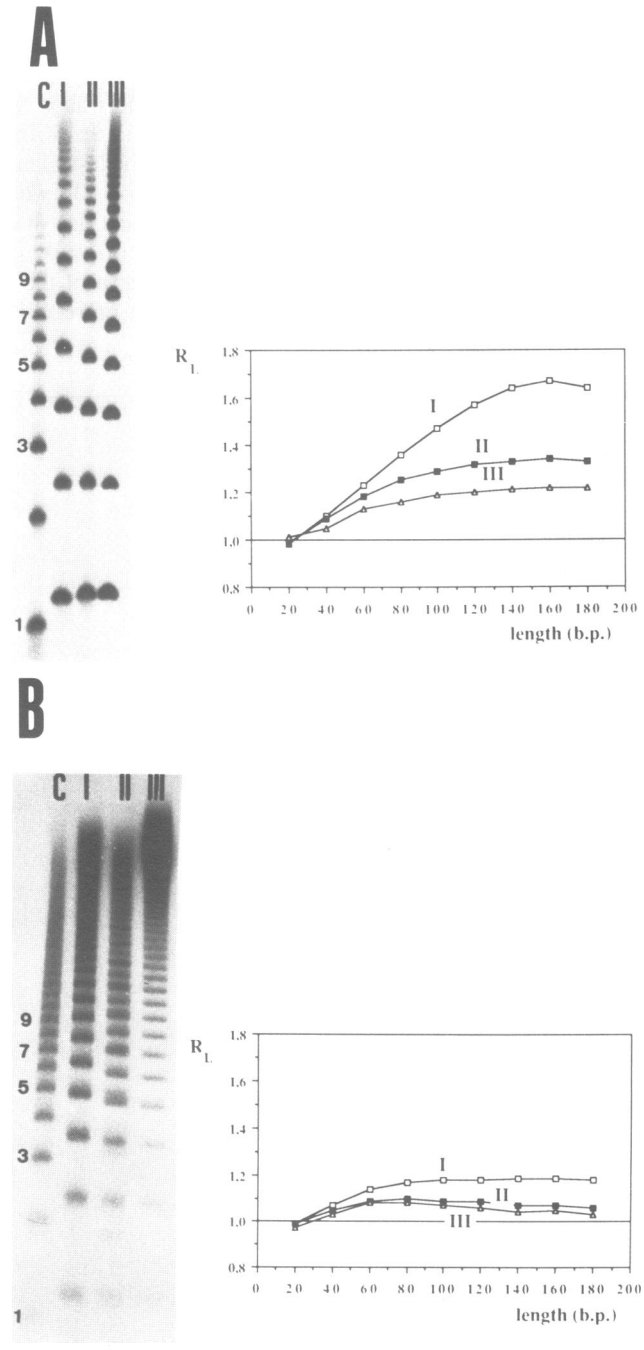

Figure 5. Electrophoretic behaviour of the duplexes shown in Table I. Autoradiograms of the multimers electrophoresed on a non-denaturing 8% polyacrylamide gel run at 4°C (panel A) or 40°C (panel B), are shown on the left. Lanes I to III correspond to duplexes I to III. Lane C corresponds to duplex C. Numbers indicate the position of the corresponding n-mer of duplex C. Quantitative analysis of these results is shown on the right. The R_L values, as a function of multimer length, are shown for each duplex when electrophoresed at 4°C (panel A) or at 40°C (panel B). Values correspond to the average of two independent experiments.

100−120 bp. in length. Similar results have been reported by others for fragments which are also a repetition of a 20 bp. long DNA fragment (6). Duplex III showed an R_L value of a magnitude similar to that reported by others for fragments also containing A-tracts spaced about two helical repeats (11). The electrophoretic anomaly of duplex I is significantly higher than that of duplex III, indicating that the sequence 5′ TGGAATATG-A 3′ reinforces the curvature introduced by the A_5-tract. Moreover duplex II, which only contains the 5′ TGGAATATG-A 3′ sequence, shows also a retarded migration. The R_L value of duplex II is higher than that of duplex III, though lower than that of duplex I.

It is known that the electrophoretic anomaly of curved DNA fragments decreases as the temperature at which the electrophoresis is carried out increases (20). When electrophoresis was carried out at 40°C (Figure 5, B), duplexes II and III showed R_L values very close to 1.0, indicating that under these electrophoretic conditions they are mainly straight. On the other hand, duplex I still shows a retarded migration at this temperature, though its R_L value is much lower than at 4°C.

These results indicate that the sequence element 5′ TGG-AATATGA 3′ is intrinsically curved. Oligomers of this sequence show an anomalous electrophoretic behaviour which is abolished at high temperature. The curvature induced by this sequence adds to that introduced by a phased A-tract, as is shown by the electrophoretic behaviour of duplex I. Therefore, this sequence is likely to deflect the helical axis in the same direction as a phased A-tract.

DISCUSSION

In this paper, the structure of the intrinsically curved mouse satellite DNA repeat was investigated. This DNA fragment, which contains nineteen groups of three or more consecutive adenines, shows a sinusoidal pattern of cleavage by the hydroxyl radical. The waves of hydroxyl radical reactivity are phased with respect to the A-tracts. Similar patterns of cleavage have been reported for several curved DNA molecules (16, 17). Two features of the hydroxyl radical cleavage pattern of the mouse satellite DNA repeat are particularly interesting: a) the maxima of hydroxyl radical cleavage are more periodically spaced along the DNA sequence than the A-tracts themselves. The waves of reactivity phase better the helical repeat than the A-tracts. As a consequence, the position of each maximum with respect to the A-tract is variable. The maxima occur anywhere between four and zero base pairs upstream from the first adenine of the tract. b) the sequence 5′ TGGAATAT$^{G}/_{A}$A 3′ shows a sinusoidal pattern of hydroxyl radical cleavage as if it was curved. This sequence shows a retarded migration in polyacrylamide gels indicating that it is actually intrinsically curved.

Why does the sequence 5′ TGGAATATGA 3′ behave as intrinsically curved? Most likely, it is the central A+T-rich part of the sequence which is responsible for its intrinsic curvature. DNA curvature is generally associated to the presence of runs of three or more consecutive adenine residues. It has also been shown, that the dinucleotide AA by itself can contribute significantly to the curvature introduced by longer A-tracts (28). Other sequences, containing guanine and citosine residues, have been reported to influence DNA curvature (29). Finally, tracts containing combinations of adenines and thymines might also be intrinsically curved (21−23, 27). In these cases, at least four A.T base pairs are required to produce a significant deflection of the helical axis. These A.T pairs might include a 5′ ApT 3′ step but not a 5′ TpA 3′ step. The sequence element indicated above does not contain any run of three or more adenines and, furthermore, it does contain a 5′ TpA 3′ step. Nevertheless, it is intrinsically curved. Preliminary results indicate that the intrinsic curvature of the 5′ TGGAATATGA 3′ sequence is associated to the precise primary structure of the central A+T-rich part. Permutation of the position of the AA dinucleotide significantly affects the curvature of the corresponding DNA fragments which, at some positions, is completely abolished (Carrera and Azorín, in preparation). As a first approximation, one should expect that the internal A+T-rich part of the sequence 5′ TGGAATATGA 3′ would have a narrower minor groove than the flanking regions. This structural feature is also shared by most intrinsically curved DNA molecules (3). Might narrowing of the minor groove be sufficient by itself to produce deflection of the helical axis? In principle, the answer to this question must be no. A more or less straight molecule could accommodate significant changes in minor groove width. Presence of a narrow minor groove results into curvature at A-tracts containing fragments but not at AT-tracts containing fragments (24, 25). Whether or not curvature is induced will basically depend on how these minor groove width changes will affect to the local helical parameters that determine the trajectory of the helical axis.

What sort and where on the DNA molecule do such distortions occur? Our results strongly suggest that the structural distortions resulting in the curvature of the mouse satellite DNA repeat occur mainly at the regions flanking the A-tracts. From the results reported here and elsewhere (16, 17), it becomes evident that, whatever the hydroxyl radical cleavage pattern actually reflects, the waves of hydroxyl radical reactivity are defining the regions of the molecule where curvature is manifested. In the case of the mouse satellite DNA repeat, these waves of reactivity are principally phased with respect to the A-tracts but the maxima of reactivity are not precisely positioned with respect to the tract. They can occur anywhere from four to zero base pairs upstream from the tract. These results indicate that the presence of an A-tract significantly affects the conformation of the adjacent nucleotides, and that this effect can propagate along the DNA sequence to a variable distance. The peaks of hydroxyl radical reactivity are more in phase with respect to the helical repeat than the A-tracts themselves, suggesting that it is precisely the changes in conformation at the flanking regions what is important in determining curvature. Apparently, the structural conformation of the nucleotides flanking the A-tracts is adjusted so to conform with the overall curvature of the fragment, that is with the minor groove pointing in at the A+T-rich regions.

From these results it is not possible to determine the precise type of distortions that occurs at the flanking regions. In other words, there is not a clear answer to the question of what is the hydroxyl radical differentially detecting in a curved DNA molecule. The one to three base pairs stagger of the cutting patterns of both strands, indicates that the hydroxyl radical recognises and cuts the DNA molecule through its minor groove. However, from the available data it is not possible to ascertain the precise minor groove feature that the hydroxyl radical is sensing. It is not just the narrowing of the groove, since DNA fragments such as those of the $(NT_4A_4N)n$ series, show an uniform pattern of cleavage regardless of their narrow minor groove (17). A.T base pairs show a high propeller twist when embeded in an A-tract (13, 14). It is unlikely that the hydroxyl radical will be detecting a peculiar sugar configuration associated

with high propeller twisting of the bases, since the sequence 5′ TGGAATATGA 3′ is most likely lacking this structural feature. Interestingly, the distribution of preferred roll along the 5′ TGGAATATGA 3′ sequence, determined according to (26), shows a periodicity which is very similar to what is obtained for an A-tract containing fragment; low preferred roll at the central A+T-rich part of the fragment, and high preferred roll at the flanking regions. On the other hand, from the results reported here we cannot exclude the possibility that the hydroxyl radical will just differentially detect the inside versus the outside of a curved DNA fragment.

In summary, our results strongly suggest that the intrinsic curvature of the mouse satellite DNA repeat originates from a periodic narrowing of the minor groove, occurring every helical repeat along the DNA sequence. Narrowing of the minor groove creates structural distortions at the flanking regions which can propagate along the DNA molecule to a variable distance. Deflection of the helical axis is likely to occur principally at these regions. Knowledge of the structural basis of the differential recognition of a curved molecule by the hydroxyl radical might facilitate the determination of the structural characteristics of these regions.

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