Altered DNA conformations in the gene regulatory region of torsionally-stressed SV40 DNA

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ABSTRACT

We used mung bean nuclease to probe the SV40 genome for DNA unwinding and unpairing. Cleavage occurred at a limited number of specific sites in supercoiled, but not relaxed DNA. The number and location of cleavage sites depended upon Mg^{2+} concentration. Without Mg^{2+} , cutting occurred mainly in one early denaturation region located 3' to the t antigen gene and within the T antigen gene intron. With Mg^{2+} , cleavage occurred at a number of alternative sites in the genome. Certain Mg^{2+} concentrations favored cleavage in the gene regulatory region. These cleavages were mapped at single nucleotide resolution and occurred in both transcriptional enhancers and upstream from the start of major late gene transcription. The cleavages occurred between 5 bp inverted repeat sequences, consistent with the recognition of unusually small cruciform structures.

INTRODUCTION

DNA supercoils occur in chromatin in two physically distinguishable states. In one state the supercoils are restrained by wrapping around histones to form nucleosomes (1). In the alternate state the supercoils are not restrained and behave like naked supercoils in that the DNA is torsionally stressed (2,3).

DNA torsional stress has been correlated with transcriptional activity in chromatin (2-4), and may have other biological functions. The mechanism by which torsional stress affects biological function is uncertain. One possibility is that torsional stress leads to DNA untwisting or unpairing in regulatory regions. Consistent with this possibility is the observation that certain eukaryotic genes react with single-strand-specific reagents in 5' flanking regions in both naked supercoiled DNA and chromatin (5,6). These reactive sites map to DNase I hypersensitive regions in active chromatin and are presumed to play a regulatory role in transcription. Simian virus 40 (SV40) DNA has a well characterized gene regulatory region which is also DNase I hypersensitive in minichromosomes. However, despite independent evidence for torsional stress in SV40 minichromosomes (7), the gene regulatory region

is not cleaved by S1 nuclease in naked supercoiled DNA (8,9). Instead, other sites in the genome are preferred.

We were interested in probing the DNA conformation of the torsionally stressed SV40 genome under solution conditions which are compatible with transcription and replication in vitro. Previous studies used S1 nuclease at acid pH. We used single-strand-specific mung bean nuclease to probe for DNA unwinding and unpairing since the enzyme functions at neutral pH (10). We were also interested in the effects of Mg^{2+} on SV40 DNA conformation since Mg^{2+} is required for transcription and replication in vitro. Previous studies using S1 nuclease employed Zn^{2+} or Mn^{2+} (8,9). We report here that mung bean nuclease recognizes the SV40 gene regulatory region in the presence of Mg^{2+} . Prominent recognition occurs in the transcriptional enhancer sequences and upstream from the start of major late gene transcription. Mung bean nuclease cleavages occur between 5 bp inverted repeat sequences, consistent with the recognition of unusually small cruciform structures.

MATERIALS AND METHODS

Enzymes

Mung bean nuclease was isolated and purified to homogeneity as described by Kowalski et al (11). Venom phosphodiesterase was a gift from Dr. H. Takamatsu and was purified as described (12). Restriction enzymes were obtained from New England Biolabs, Bovine Alkali Phosphatase was obtained from Worthington Biochemicals, and Polynucleotide Kinase was purchased from P-L Biochemicals.

DNA

Supercoiled SV40 DNA was prepared as follows: SV40 strain 776 was used to infect nearly confluent RSC-1 cells in 100 mm tissue culture dishes at 5-10 pfu/cell. Virus infection was carried out in Minimum Essential Medium supplemented with 2% Fetal Bovine Serum (Gibco). Infection was terminated after 48 hrs by aspirating off media, and lysing cells with 2.5 mls of 0.6% SDS, 10 mM EDTA. SV40 DNA was separated from cellular DNA as described by Firt (13). Supercoiled SV40 DNA was purified by equilibrium sedimentation in cesium chloride/ethidium bromide gradients. Ethidium bromide was removed from supercoiled DNA using 4 isopropanol extractions. Supercoiled DNA was then precipitated by adding 4 volumes of Robs buffer (10 mM Tris HCl, 100 mM NaCl, 5 mM EDTA, pH 7.2) followed by an ethanol precipitation. Supercoiled DNA was resuspended and dialyzed in 10 mM Tris HCl, pH 7.0. The titratable

superhelical density of SV40 DNA was -0.05 , determined using the method of Deleys and Jackson (14).

Restriction Endonuclease Mapping of Mung Bean Nuclease Nicks

Mung bean nuclease reaction mixtures contained $1.6 \mu\text{g}$ of SV40 DNA, 10 mM Tris HCl (pH 7.0) and varying amounts of Mg^{2+} as described in Figure legends, in a $16 \mu\text{l}$ volume. After pre-incubation for 15 minutes at 37°C $4 \mu\text{l}$ of mung bean nuclease ($0.02 \text{ units}/\mu\text{l}$, units defined in ref. 11) was added, and the reaction was incubated for 1.5 hrs at 37°C . These conditions lead to cleavage of $>90\%$ of the supercoiled molecules. Mung bean nuclease digestion was stopped as described (15). Cleavage opposite the mung bean nuclease nick with venom phosphodiesterase was carried out as described (15) except that Mg^{2+} was added to a final concentration of 1.0 mM and the incubation time was 14 minutes. Solution conditions were adjusted for restriction endonuclease digestions of the linearized DNA. DNA was electrophoresed through 22 cm long, horizontal slabs of 1.4% agarose prepared and submerged in 36 mM Tris, 30 mM $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 1 mM Na_2EDTA , pH 7.8. Electrophoresis was initiated at 50 volts for 10 minutes and continued at 25 volts for 20–24 hrs. Staining of DNA in gels and photography of the fluorescence was performed as described (15). The number of base pairs in DNA fragments were read from a graph of log molecular weight versus mobility of DNA size markers.

DNA Sequencing Around Mung Bean Nuclease Nicks

The above mung bean nuclease reaction was scaled up 3 fold in volume. When Mg^{2+} was present the reaction mixture contained 1 mM Mg^{2+} . After 1.5 hrs incubation at 37°C , the reaction was stopped and cooled to 0°C . The enzyme was removed by phenol (saturated with 10 mM Tris HCl pH 7.4, 1 mM EDTA) extraction. The DNA was precipitated by adding 1–10th volume 3 M Sodium Acetate (pH 6.0) and 2 volumes of ethanol (-70°C for 40 minutes), pelleted at 10K for 35 minutes, and dried under vacuum. DNA was redissolved in the appropriate restriction endonuclease buffers. After restriction nuclease digestion, DNA fragments were $5'$ - ^{32}P labelled, separated by polyacrylamide gel electrophoresis, and isolated as described (16). Singly end-labelled restriction fragments were generated by cleaving end-labelled DNA fragments with a second restriction enzyme, and were isolated. DNA fragments containing mung bean nuclease nicks were denatured and electrophoresed along side the products of Maxam-Gilbert sequencing reactions (17) performed on fragments obtained from supercoiled DNA, on 10% or 15% polyacrylamide-8 M urea slab gels (0.4 mm thick, 18 cm wide and 40 cm long). The locations of the nicks within the nucleotide sequence were determined as previously described (16).

RESULTS**Mg²⁺ Affects The Structures Recognized by Mung Bean Nuclease in Supercoiled SV40 DNA**

Supercoiled SV40 DNA was reacted with mung bean nuclease at neutral pH and in the presence or absence of varying concentrations of Mg²⁺. Predominantly nicked-circular DNA and a small proportion of linear DNA were produced. Linearization of the nicked-circular DNA with venom phosphodiesterase (15) produced unit length linear SV40 DNA (5243 bp) indicating the presence of mainly one mung bean nuclease nick per molecule. The site specificity of nicks was determined by cleaving linearized DNA at the BamHI site (nucleotide 2534) and separating the products by agarose gel electrophoresis. It is known that Mg²⁺ affects the number and locations of mung bean nuclease sites in prokaryotic DNA (15, 18). As shown in Figure 1A, the SV40 DNA cleavage pattern seen in the absence of Mg²⁺ (lane 2) is significantly altered by 25 μ M Mg²⁺ (lane 3). Increasing Mg²⁺ concentration to 75 μ M (lane 4) and 200 μ M (lane 5) lead to further changes in the pattern. At 1 mM Mg²⁺ (lane 6) or 4 mM Mg²⁺ (lane 7) minimal additional changes in the cut pattern are observed.

The cleavage patterns shown in Figure 1A are seen after a 15 minute preincubation prior to mung bean nuclease addition. The cleavage patterns obtained in the presence and absence of 1 mM Mg²⁺ are not affected by shorter (5 minutes) or extended (4 hours) preincubations. Thus, the different structural patterns detected in the presence and absence of Mg²⁺ are established rapidly and are stable with time.

Figure 1. Effects of Varying Mg²⁺ Concentration on Mung Bean Nuclease Specificity. Supercoiled SV40 DNA was nicked with mung bean nuclease, cleaved opposite the nicks with venom phosphodiesterase, cut at either the BamHI site (A) or the EcoRI site (B), and electrophoresed through 1.4% agarose gels. Additions or deletions to the mung bean nuclease reaction conditions corresponding to gel lanes were as follows; (A) 1) No mung bean nuclease. 2) No Mg²⁺. 3) 25 μ M Mg²⁺. 4) 75 μ M Mg²⁺. 5) 200 μ M Mg²⁺. 6) 1.0 mM Mg²⁺. 7) 4 mM Mg²⁺. 8) Size markers. (B) 1) No mung bean nuclease. 2) No Mg²⁺. 3) 1 mM Mg²⁺. Marker DNA sizes in base pairs are shown to the right of panel A. Numbers to the left of the figures are fractional genome lengths (fragment length/5243 bp). Solid arrows mark positions of band pairs consistent with mung bean nuclease cleavage of the SV40 regulatory region. (C) SV40 genomic map with mung bean nuclease cleavage sites indicated by the filled triangles (cleavage sites in the presence of 1 mM Mg²⁺) or open triangles (cleavage site in the absence of Mg²⁺). The overlined numbers indicate the average position of the cleavage ($\pm$ 40 bp). The single lines inside the circular genomic map indicate the protein coding sequences for the 5 SV40 protein products.

Mung bean nuclease recognition of altered DNA conformations in SV40 DNA is dependent on DNA topology. SV40 DNA relaxed by topoisomerase I is not cleaved under conditions where the reaction with supercoiled DNA is complete.

Relaxed SV40 DNA can be cleaved by mung bean nuclease at elevated enzyme concentrations and extended incubation times. In contrast to the limited number of discrete sites seen in supercoiled DNA, locations throughout the genome are cleaved, as previously shown with other DNAs (15,18). The locations of the cleavages in relaxed SV40 DNA and other relaxed DNAs (15,18) are not affected by Mg^{2+} . This result demonstrates that Mg^{2+} does not change the way the enzyme recognizes DNA. Other findings support that the Mg^{2+} effect is on the conformation of supercoiled DNA and not on the enzyme (15). Thus, with supercoiled SV40 DNA, the Mg^{2+} effect on the nuclease cleavage pattern can be simply interpreted as a change in DNA conformation. This interpretation has been confirmed for mung bean nuclease sensitive sites in pBR322 DNA (18) in independent studies using a chemical probe for altered DNA conformations (36).

Restriction Endonuclease Mapping of Mung Bean Nuclease Sites in SV40 DNA

Mung bean nuclease cleavage in the SV40 regulatory region followed by BamHI cleavage would produce a pair of bands approximately 0.56 and 0.44 SV40 length. Three such band pairs are seen when the mung bean nuclease reaction is performed in 75 μM to 4 mM Mg^{2+} (Fig. 1A, lanes 4-7, solid arrows) but are not seen in the absence of Mg^{2+} (lane 2). In addition to these band pairs, other band pairs are seen which map outside the SV40 regulatory region (bands above the top arrow and below the bottom arrow).

The locations of the mung bean nuclease sites were unambiguously determined using EcoRI (nucleotide 1783). Mung bean nuclease cleavage in the SV40 regulatory region followed by EcoRI cleavage would produce a pair of bands approximately 0.70 and 0.30 SV40 length. A set of 4 pairs of prominent bands is seen around 0.70 and 0.30 SV40 length (Fig. 1B) when the mung bean nuclease reaction is performed in the presence of 1 mM Mg^{2+} (lane 3) but not in the absence of Mg^{2+} (lane 2). Combined mapping data place 3 of these sites in the gene regulatory region at nucleotides 144, 229, and 315 (all $\pm$ 40 bp). Similar mapping analyses were carried out for six other cleavage sites located outside the regulatory region and detected in the presence of 1 mM Mg^{2+} (Fig. 1A, Lane 6, Fig. 1B, Lane 3). The locations of the sites in the SV40 genome are shown in Figure 1C (filled triangles). The sites outside the non-coding regulatory region fall in protein coding sequences which essentially saturate the remainder of the genome.

The relative frequency of nuclease recognition at different mung bean nuclease sites can be assessed by comparing the relative ethidium staining of band pairs corresponding to different sites. Inspection of the gel in Figure 1A shows that sites outside the regulatory region (bands above the top arrow and below the bottom arrow) are preferred over those in the regulatory region (arrows) at Mg^{2+} concentrations of 0, 25 and 75 μM (lanes 2, 3 and 4). At much higher Mg^{2+} concentrations, however, the preference is reversed. Sites which map to the regulatory region (arrows) are major mung bean nuclease cut sites at 1 mM Mg^{2+} (lane 6) and are preferred cut sites at 4 mM Mg^{2+} (lane 7), compared to those which map outside the regulatory region.

Preferential mung bean nuclease cleavage of gene regulatory regions in prokaryotic DNA requires torsional stress (15,18). Torsional stress is also required for preferential mung bean nuclease recognition of the SV40 regulatory region since relaxed, closed-circular SV40 DNA is slowly cleaved at many sites throughout the genome (data not shown).

In the absence of Mg^{2+} (Fig. 1A and B, lanes 2), SV40 DNA is almost exclusively cleaved at average nucleotide position 4600 (± 40 bp). This site maps with one of the two early denaturation regions (19) recognized by S1 nuclease (8,9). Mung bean nuclease also cleaves a single early denaturation region within a broad A+T-rich sequence in PM2 and pBR322 DNAs under identical reaction conditions (16,18). The altered conformation in SV40 DNA maps 3' to the tAg gene and within the intron of the TAg gene (Fig. 1C, open triangle).

Localization and Nucleotide Sequences of Mung Bean Nuclease Sites in the Regulatory Region of SV40 DNA

We were interested in localizing the mung bean nuclease sites in the SV40 regulatory region. We analyzed the regulatory region and some attached portions of early and late gene coding regions for mung bean nuclease nicks at higher resolution. The regions analyzed are present in the 470 bp sequence from the *Ava*II site (nucleotide 5119) to the *Msp*I site (nucleotide 347) (Fig. 2). Fine mapping (± 10 bp) of the nicks by polyacrylamide gel electrophoresis (16) revealed that there are 3 mung bean nuclease sites within this region and that the nicks occur in both DNA strands (autoradiogram not shown).

We determined the nucleotide sequence around the nicks introduced into supercoiled DNA by isolating singly end-labelled restriction fragments containing mung bean nuclease nicks and analyzing them on DNA sequencing gels as previously described (16). One site is located at nucleotides 316 and 317 with a major and minor cleavage (Fig. 3, A and B). This site is close to the

Figure 2. A map of the SV40 regulatory region. The 470 bp segment of SV40 DNA shown contains the sequences that have been examined at the nucleotide level for major mung bean nuclease nicks. Open boxes represent respectively from left to right, the perfect 27 bp palindrome centered over the origin of replication, a 17 bp A+T sequence, three 21 bp repeats (transcriptional promoter), and two 72 bp repeats (transcriptional enhancers). The late gene major transcriptional start site is labelled with a solid arrow oriented in the direction of transcription at nucleotide 325. DNase I hypersensitive sites (± 10 bp) are indicated below the map by the half-filled triangles (31), and the 3 major mung bean nuclease sites are indicated by the filled triangles.

start site of the major late mRNA (Fig. 2). The other two sites are located at nucleotides 135 and 207 (Fig. 3C). These sites are located in the transcriptional enhancers (Fig. 2). Enzyme recognition in the opposite strand of the enhancers reveals a major and minor cleavage located at nucleotides 135 and 134 in the first enhancer, and 207 and 206 in the second enhancer (autoradiogram not shown). The number and locations of the mung bean nuclease sites at nucleotide sequence resolution are in excellent agreement with the data obtained at agarose gel resolution (Fig. 1C). The 3 mung bean nuclease sites occur between 3 short inverted repeated sequences. Cleavage between an inverted repeat sequence is consistent with nuclease recognition of the non-base-paired loop of a cruciform structure (Fig. 4, A and B).

DISCUSSION

Our results show that altered DNA conformations in the SV40 regulatory region are recognized by mung bean nuclease in the presence of torsional stress and certain Mg^{2+} concentrations. Preferential recognition of the regulatory region versus sites outside the regulatory region is seen at 4 mM Mg^{2+} . Our findings demonstrate that the DNA conformation of the SV40 regulatory region is significantly different from normal B-DNA at a pH, Mg^{2+} concentration and temperature compatible with DNA replication and transcription *in vitro*.

The regulatory region of the SV40 genome is not recognized by mung bean nuclease or S1 nuclease (8,9) in the absence of Mg^{2+} . Instead, mung bean

nuclease preferentially cleaves a single site located 3' to the tAg gene and within the intron of the TAg gene. This site maps with the same early denaturation region recognized by S1 nuclease under different conditions (8,9). Previously we showed that the site specificity of single-strand-specific nucleases on other supercoiled DNAs is extremely sensitive to Mg^{2+} concentration and other solution conditions such as NaCl concentration and temperature (15,18). The effect of Mg^{2+} and other solution conditions on supercoiled DNA conformation provides a likely explanation for differences in mung bean nuclease and S1 nuclease specificity on the SV40 genome. Under the same conditions, mung bean nuclease and S1 nuclease recognize the same sites in pBR322 DNA (D. Natale, unpublished results).

Mung bean nuclease is known to cleave in or near four types of sequences in supercoiled DNA: 1) inverted repeat (16,18), 2) alternating purine-pyrimidine (20), 3) A+T-rich (16, 18) and 4) polypurine-polypyrimidine (21). We find that the enzyme cleaves the SV40 regulatory region between inverted repeat sequences in the presence of 1 mM Mg^{2+} (Fig. 3, A and B). The other sites which mapped outside the regulatory region and were detected under the same conditions occur within mapping error (± 40 bp) of inverted repeat sequences. Under the same solution conditions, mung bean nuclease cleaves pBR322 DNA at inverted repeat sequences and at no other sequences (18). Inverted repeats are also the only sites cleaved in certain yeast DNA sequences (R. Umek, unpublished results). It appears that these solution conditions favor formation of a mung bean nuclease sensitive structure exclusively at inverted repeat sequences.

Single-strand-specific nuclease cleavage between an inverted repeat sequence has proven to be a reliable indicator of a cruciform structure (22,23,39,40,42). The existence of cruciform structures in certain inverted repeat sequences recognized by mung bean nuclease (18) is supported by T4 endonuclease VII cleavage at the base of the suspected hairpin stem (23). The potential cruciforms recognized by mung bean nuclease in SV40 DNA (Fig. 4, A and B) are unusual in that their stems are smaller (5 bp) than those detected in pBR322 DNA (6-11 bp) (18) or in other DNAs using S1 nuclease under different conditions (9-13 bp) (24,25). Cruciforms with such short stems have not been detected by T4 endonuclease VII (23) and are not easily detected by DNA unwinding experiments (26,27). However, mutational analysis of an inverted repeat sequence recognized by mung bean nuclease in pBR322 DNA has confirmed the existence of cruciforms with stems as small as 6 bp (unpublished results). Mutational analysis of the potential cruciforms detected in SV40

DNA will be required to confirm the nature of the altered DNA structure.

Cruciforms with 5 bp stems and 3-5 bp loops are not expected to be stable in supercoiled DNA. Thermodynamic considerations (26,27,40,41) suggest that the free energy cost to form such cruciforms exceeds the free energy return provided by relaxation of supercoils. Consistent with this expectation, the potential cruciforms are not recognized by mung bean nuclease at 37°C in 10 mM Tris HCL (pH 7.0). Detection of the potential cruciforms requires Mg^{2+} . Mg^{2+} lowers the free energy required to form certain cruciforms with much longer stems (38,39). Mg^{2+} may aid cruciform formation by complexing with the single-stranded loops (38) and stabilizing interactions within and between loops. Mg^{2+} complexes occur at all loops of t-RNA^{Phe} hairpins (32,43,44). Mg^{2+} stabilizes base stacking within t-RNA loops and

Figure 3. Nucleotide sequence analysis around mung bean nuclease nicks in the regulatory region of the SV40 genome. Shown are autoradiograms after electrophoresis of ^{32}P -DNA in 15% (A and B) or 10% (C) Polyacrylamide - 8 M Urea gels. Supercoiled DNA was nicked by mung bean nuclease in the presence (+) or absence (-) of 1 mM Mg^{2+} . DNA fragments containing a single $5' \text{--}^{32}\text{P}$ label at a restriction enzyme site were prepared, denatured and electrophoresed along side the products of Maxam-Gilbert sequencing reactions performed on fragments obtained from supercoiled DNA (lanes A (A > C), G, C and T (T + C)). (A) $5' \text{--}^{32}\text{P}$ at DdeI site (nucleotide 288) in the top strand (sense strand of late mRNA) and $3' \text{--OH}$ at MspI site (nucleotide 346). (B) $5' \text{--}^{32}\text{P}$ at MspI site (nucleotide 348) in the bottom strand (anti-sense strand of late mRNA) and $3' \text{--OH}$ at BglII site (nucleotide 5239). (C) $5' \text{--}^{32}\text{P}$ at MspI site (nucleotide 348) in the bottom strand and $3' \text{--OH}$ at BglII site (nucleotide 5239). Numbers along side autoradiograms are nucleotide positions at the $3' \text{--OH}$ of mung bean nuclease nicks and were determined as previously described (16). Nucleotides are numbered according to Tooze (35). N, the renatured form of the unit length fragment. The light band just above N in the (+) and (-) lanes is a nicking artifact of certain restriction enzymes used and is present in minus mung bean nuclease controls (data not shown).

Figure 4. Nucleotide sequence around mung bean nuclease nicks and the proposed altered secondary structures responsible for recognition. The mung bean nuclease reaction was performed in the presence of 1 mM Mg^{2+} (Fig. 3). Inverted repeat sequences are drawn as base-paired stems of a cruciform structure. The arrows represent the nuclease nicks. Longer stemmed arrows represent the major cuts while short stemmed arrows represent the minor cuts. (A) Sequences within the transcriptional enhancer (second 72 bp repeat). The first 72 bp repeat is cleaved in the same sequence and in the same manner. (B) Sequences 5' to the major late mRNA cap site (position 325).

hydrogen bonding interactions between the loops. Further investigation is required to determine how Mg^{2+} interacts with DNA cruciforms and whether it affects their tertiary structure.

The 27 bp palindrome at the BglI site in the SV40 regulatory region is expected to form a thermodynamically stable cruciform but it is not cleaved by mung bean nuclease. This sequence may behave like those of longer palindromes for which cruciform formation is extremely slow (26,27). It is known that the DNA sequence of the palindrome can affect the rate of cruciform extrusion (37).

Z-DNA has been suspected to form in the SV40 enhancer sequences based on indirect evidence using anti-Z-DNA antibody binding (28). However, no direct evidence for Z-DNA in the enhancer sequences has been obtained using chemical probes (29). Mung bean nuclease cuts to one side of the potential Z-DNA site in the enhancers (Fig. 4A, position 198-205). If Z-DNA were present, we would expect single-strand-specific nuclease cleavage on both sides of the alternating Pu-Py sequence (20,30 and unpublished results). The presumably preferred Z-DNA sequence in the SV40 regulatory region (position 257-266) (28) is not recognized by mung bean nuclease. Thus, under our conditions, mung bean nuclease does not appear to recognize Z-DNA in the SV40 regulatory region.

Altered DNA conformations have been detected by S1 nuclease when the SV40 regulatory region is separated from the remainder of the genome, circularized and artificially supercoiled to a density of about two fold greater than that obtained when SV40 DNA is extracted from mammalian cells (30). No cleavages occurred in the inverted repeat sequences in the enhancers or upstream from the major late mRNA cap site as we see when probing the entire SV40 genome at extractable superhelical density (-0.05). It is not yet clear what levels of superhelicity, what supercoiled domain size or what environmental conditions selected in vitro are relevant to the altered DNA conformations which might occur in vivo.

Prominent altered DNA conformations detected by mung bean nuclease in torsionally-stressed SV40 DNA occur in close proximity to known DNase I hypersensitive sites in SV40 minichromosomes (31) (Fig. 2). DNase I hypersensitive SV40 minichromosomes possess torsional stress (7). It is possible that altered DNA conformations dependent on torsional stress play a role in the altered chromatin structure of the SV40 regulatory region. For example, in the case of cruciforms, it is known that DNA stem-loop structures bind poorly to histone octamers (33,34). Thus, it seems likely that the presence of altered DNA conformations in torsionally-stressed SV40 DNA would affect the binding of histones and could affect the binding of trans-acting transcription factors and other proteins.

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REFERENCES

1. Sinden, R.R., Carlson, J.O. and Pettijohn, D.E. (1980) Cell 21, 773-783
2. Luchnik, A.N., Bakayev, V.V., Zbarsky, I.B. and Georgiev, G.P. (1982) Embo J. 1, 1353-1358 (1982)
3. Ryoji, M. and Worcel, A. (1984) Cell 37, 21-32
4. Han, S., Udvardy, A. and Schedl, P. (1985) J. Mol. Biol. 183, 13-29
5. Larsen, A. and Weintraub, H. (1982) Cell 29, 609-622
6. Kohwi-Shigematsu, T., Gelinas, R. and Weintraub, H. (1983) Proc. Natl. Acad. Sci. U.S.A. 80, 4389-4393
7. Luchnik, A.N., Bakayev, V.V., Yugai, A.A., Zbarsky, I.B. and Georgiev, G.P. (1985) Nucleic Acids Res. 13, 1135-1149
8. Beard, P., Morrow, J.F. and Berg, P. (1973) Journal of Virology 12, 1303-1313

9. Shishido, K. (1979) *Agric. Biol. Chem.* 43, 1093-1102
10. Kowalski, D. and Sanford, J.P. (1982) *J. Biol. Chem.* 257, 7820-7825
11. Kowalski, D., Kroecker, W.D. and Laskowski, M., Sr. (1976) *Biochemistry* 15, 4457-4463
12. Laskowski, M., Sr., (1980) *Meth. Enzymol.* 65, 276-284
13. Hirt, R. (1967) *J. Mol. Biol.* 26, 365-369
14. Deleys, R.J. and Jackson, D.A. (1976) *Biochem. Biophys. Res. Commun.* 69, 446-454
15. Kowalski, D. (1984) *Nucleic Acids Res.* 12, 7071-7086
16. Sheflin, L.G. and Kowalski, D. (1984) *Nucleic Acids Res.* 12, 7087-7104
17. Maxam, A.M. and Gilbert, W. (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74, 560-564
18. Sheflin, L.G. and Kowalski, D. (1985) *Nucleic Acids Res.* 13, 6137-6154
19. Funnell, R.E. and Inman, R.B. (1979) *J. Mol. Biol.* 131, 331-340
20. Singleton, C.K., Kilpatrick, M.W. and Wells, R.D. (1984) *J. Biol. Chem.* 259, 1963-1967
21. Pulleyblank, D.E., Haniford, D.B. and Morgan, A.R. (1985) *Cell* 42, 271-280
22. Lilley, D.M.J. (1983) *Nucleic Acids Res.* 11, 3097-3112
23. Lilley, D.M.J. and Kemper, B. (1984) *Cell* 36, 413-422
24. Panayotatos, N. and Wells, R.D. (1981) *Nature* 289, 466-470
25. Lilley, D.M.J. (1980) *Proc. Natl. Acad. Sci. U.S.A.* 77, 6468-6472
26. Courey, A.J. and Wang, J.C. (1983) *Cell* 33, 817-829
27. Gellert, M., O'Dea, M.F. and Mizuuchi, K. (1983) *Proc. Natl. Acad. Sci. U.S.A.* 80, 5545-5549
28. Nordheim, A. and Rich, A. (1983) *Nature* 303, 674-679
29. Herr, W. (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82, 8009-8013
30. Evans, T., Schon, E., Gora-Maslak, G., Patterson, J. and Efstratiadis, A. (1984) *Nucleic Acids Res.* 12, 8043-8058
31. Cereghini, S., Herbomel, P., Jouanneau, J., Saragosti, S., Katinka, M., Bourachot, B., DeCrombrughe, B. and Yaniv, M. (1983) *Cold Spring Harb. Symp. Quant. Biol.* 47, 935-944
32. Teeter, M.M., Ouigley, G.J. and Rich, A. (1980) *Nucleic Acid-Metal Ion Interactions*, ed. Spiro, T.G., Wiley Interscience, New York, 145-177
33. Nickol, J. and Martin, R.G. (1983) *Proc. Natl. Acad. Sci. U.S.A.* 80, 4669-4673
34. Weintraub, H. (1983) *Cell* 32, 1191-1203
35. Tooze, J. ed. (1981) *DNA Tumor Viruses*, 2nd edition. Cold Spring Harb. Labor., Cold Spring Harbor, New York
36. Sawadaishi, K., Miura, K., Ohtsuka, E., Ueda, T., Shinriki, N. and Ishizaki, K. (1986) *Nucleic Acids Res.* 14, 1159-1169
37. Lilley, D.M.J. (1985) *Nucleic Acids Res.* 13, 1443-1465
38. Singleton, C.K. (1983) *J. Biol. Chem.* 258, 7661-7668
39. Haniford, D.B. and Pulleyblank, D.E. (1985) *Nucleic Acids Res.* 13, 4343-4363
40. Greaves, D.R., Patient, R.K. and Lilley, D.M.J. (1985) *J. Mol. Biol.* 185, 461-478
41. Hsieh, T.-S. and Wang, J.C. (1975) *Biochemistry* 14, 527-535
42. Lyamichev, V.I., Panyutin, I.G. and Frank-Kamenetskii, M.D. (1983) *FERS Lett.* 153, 298-302
43. Holbrook, S.R., Sussman, J.L., Warrant, R.W., Church, G.M. and Kim, S.-H. (1977) *Nucleic Acids Res.* 4, 2811-2820
44. Jack, A., Ladner, J.E., Rhodes, D., Brown, R.S. and Klug, A. (1977) *J. Mol. Biol.* 111, 315-328