Volume 12 Number 21 1984 Nucleic Acids Research

S1-hypersensitive sites in eukaryotic promoter regions

Todd Evans, Eric Schon, Grazyna Gora-Maslak, Jennifer Patterson and Argiris Efstratiadis

Department of Human Genetics and Development, Columbia University, 701 West 168th Street, New York, NY 10032, USA

Received 8 August 1984; Revised and Accepted 4 October 1984

ABSTRACT

We have examined by fine mapping the S1 nuclease-hypersensitivity of the 5' flanking regions of the human β-globin and rat preproinsulin II genes and of the SV40 origin/enhancer region. In all cases S1-hypersensitive sites are located in known or presumed promoter/regulatory regions. Though a consensus DNA sequence is not evident, all of these sites reside in predominantly homopurine-homopyrimidine stretches. The alternate (non-B) DNA structure which is revealed by the enzymatic probe is a sequence-dependent feature of a short stretch of DNA, which is retained upon transplantation into a foreign environment. The alternate structure exhibits S1-nicking patterns uniquely different from those associated with the presence of Z-DNA.

INTRODUCTION

The notion that there might be a relationship between superhelicity and gene expression and that the secondary structure of DNA in the promoter regions of genes might be involved in their transcription is currently drawing increasing attention (1-3). In particular, experiments demonstrating characteristic DNase I and S1 nuclease hypersensitivities often mapping to the 5' flanking regions of eukaryotic genes in active (transcribing), but not in inactive, chromatin domains have been interpreted as reflecting an altered DNA secondary structure (ref.3 and other references therein).

Recently, we developed an in vitro approach to map at the nucleotide level S1-hypersensitive sites (SHS) in artificially generated supercoiled circles of naked DNA (4). By applying this method using the adult chicken β-globin gene, which had previously been examined by coarser in vivo mapping (5,6), we showed that the fine mapping was in excellent agreement with the in vivo results. Interestingly, the two major SHS we observed mapped to homopurine-homopyrimidine tracts in the immediate 5' flanking region of the gene.

In order to assess the generality of these results and to investigate further the molecular nature of the SHS, we have applied our mapping methodology to a series of promoter DNA elements. Specifically, we have examined a

different globin gene (the human adult β-globin gene), a non-globin gene (the rat preproinsulin II gene), and the DNA segment from SV40 which contains known regulatory elements, including the 72 bp enhancer. In addition, we have studied the nicking patterns of B-DNA/Z-DNA junctions and of SHS transplanted into foreign DNA.

MATERIALS AND METHODS

Enzymes

Restriction enzymes were from New England Biolabs, BRL, and Boehringer-Mannheim; S1 nuclease and T4 polynucleotide kinase were from BRL; T4 DNA polymerase was from P-L Biochemicals; exonuclease III, T4 DNA ligase, and Eco RI linkers were from New England Biolabs; reverse transcriptase was from Life Sciences; bacterial alkaline phosphatase was from Worthington; α-^{32}P-dNTPs (700 Ci/mmole) and γ-^{32}P-ATP (3000 Ci/mmole) were from New England Nuclear.

Mapping of SHS

SHS were mapped as previously described (4). Briefly, a DNA fragment of interest is circularized, by ligation in the presence of ethidium bromide (EB). Upon EB removal, negatively supercoiled circles are formed which are the substrate for nicking by S1 nuclease (usually for 1 min at 37^0). Following this treatment, the now relaxed nicked circles are linearized by cutting at a suitable restriction site, labeled at either their 5' or 3' ends, and then cut at a second site on the other side of the postulated nick to yield molecules labeled at only one end. Nicks are mapped by electrophoresis of the molecules on a denaturing urea-polyacrylamide gel alongside chemical sequencing ladders (7) derived from material which was not digested by S1.

Plasmid Constructions

a) Human β-globin. A 342 bp Hinf I fragment, extending between positions -278 and +64 (in relation to the capping site at +1), was isolated from a subclone carrying the human adult β-globin gene and flanking sequences (provided by C. Dobkin). Following end-repair by T4 DNA polymerase and attachment of Eco RI linkers, the resulting 350 bp fragment was inserted into the Eco RI site of pUC9 (8). For circularization and S1 nicking experiments we excised the 350 bp fragment from this subclone with Eco RI. After S1 treatment, the circles were reopened and labeled at the Eco RI site; a Mbo II site at position -131 was used for secondary restriction cutting to generate uniquely labeled 150 and 200 bp fragments (Fig. 1a).

b) Rat preproinsulin II. For S1 nicking analysis we used a 582 bp fragment subcloned into the Eco RI site of pUC9. This fragment extends from the Mst II

site (converted to an Eco RI site with linkers) at position -200 of the rat preproinsulin II gene (9, and unpublished sequencing data from our laboratory) to the natural Eco RI site at position +382. After Eco RI digestion and labeling, a Bam HI site at position +190 was used for secondary restriction cutting (Fig. 1b).

c) SV40. The 1118 bp Hind III fragment of SV40 (10), containing the origin of replication and the late region, was subcloned into the Hind III site of pUC9. This subclone was digested with Rsa I and Hinc II to release a 380 bp fragment extending from the Kpn I/Rsa I site at position 295 to the Hinc II site in the pUC9 polylinker. This fragment, containing 369 bp of SV40 sequence (including the origin of replication, the three 21 bp repeats, and the two 72 bp repeats) and 11 bp of pUC9 polylinker sequence (i.e. Hind III to Hinc II), was ligated to Eco RI linkers and subcloned into the Eco RI site of pUC9. For experiments we used this subcloned 388 bp Eco RI fragment. Following S1 treatment, primary cutting and labeling was at the Eco RI site; both Bgl I and Pvu II (with recognition sequences at positions 1 and 270, respectively) were used for separate secondary restriction cutting (Fig. 1c). For some experiments a 345 bp circle was also used, extending from the natural Hind III site at position 5171 to the Pvu II site at position 270 to which a Hind III linker was attached; primary cutting and labeling was at the Hind III site and secondary cutting was at the Bgl I site.

d) "Portable Nicking Box". A major SHS in the 5' flanking region of the chicken adult β-globin gene (4) extends between positions -49 and -59 ("-55 box"). This region was excised with Hpa II, which cuts at positions -42 and -60 and releases an 18 bp fragment (Fig. 1d). Following subcloning of this fragment into the Acc I site of pUC9 and digestion with Eco RI and Hind III, we isolated a 48 bp fragment containing the 18 bp chicken Hpa II element embedded in 30 bp of flanking pUC9 polylinker DNA. This 48 bp fragment was then inserted into the Eco RI and Hind III sites of pBR322. For S1 analysis we used a 384 bp fragment released from the construction by Bam HI digestion. It extends from the Bam HI site in the pUC9 polylinker, immediately upstream of the chicken 18 bp Hpa II element, to the natural Bam HI site in pBR322 at position 375 (11). In some cases circularized fragments were labeled at the Taq I site at pBR322 position 339 and cut secondarily at the Bst NI site at position 130. In other cases the primary cut and label was at the Bst NI site and the secondary cut was at the Hgi AI site at position 276 (Fig. 1e). A "control" construction was also prepared, in which the 30 bp Eco RI-Hind III polylinker region of pUC9 was inserted into the Eco RI and Hind III sites of

pBR322. A 366 bp control fragment was released from this subclone by Bam HI digestion and used for circularization and S1 nicking (map not shown). The DNA circles were then cut and labeled at the Bst NI site (position 130) and subsequently cut at the Hae II site (position 232) to release uniquely labeled fragments.

e) Z-DNA. As a substrate for S1 nicking we used plasmid pLP42 (12) which contains 42 bp of alternating dC-dG inserted into the Bam HI site of pBR322 through the use of Bam HI linkers (provided by J. Wang). The plasmids were cut and labeled at the Taq I site at position 339, and cut secondarily at the Sph I site at position 562 (Fig. 1f).

RESULTS

Human Adult β-globin Gene

We have mapped SHS in the human adult β-globin gene in order to compare their nicking patterns to those of the chicken adult β-globin gene which we reported previously (4), It was important to determine whether these genes, which are developmentally regulated in a similar manner in two diverse organisms, show by the criterion of an anzymatic probe common structural features not obvious from the primary sequence alone. This is because the DNA sequence of the regions recognized by S1 in the chicken gene does not exhibit obvious homology to the sequence at the corresponding regions in the human gene. For example, a $(G/C)_{18}$ element around position -190 (the G-string) which is a major SHS in the chicken, does not appear in the human gene. However, a number of purine-rich stretches are present in this region.

After S1 treatment of the human β-globin 350 bp supercoiled circles under standard conditions and secondary restriction cutting, we consistently noted on our preparative gels an unexpected 80 bp fragment in addition to the predicted 150 and 200 bp uniquely labeled fragments (Fig. 1a). This fragment, which was sequenced (not shown), arises as a result of rapid cleavage by S1 nuclease in the -200 region (Fig. 2a, lane L). Digestion with S1 for only 10 sec at 0^0 allows for the isolation of nicked circles, but still results in the appearance of significant amounts of the linearized material. Using these nicked circles, we showed that the nicks map to the region -199 to -207, which contains 10 purines out of 13 residues. Since we have only mapped the nicks on the bottom strand in this experiment, we do not know whether S1 nicks this region unilaterally, as in the case of the chicken G-string (4).

A second weaker nicking box (lower hierarchical order) was detected in a subpopulation of the same molecules under standard S1 digestion conditions.

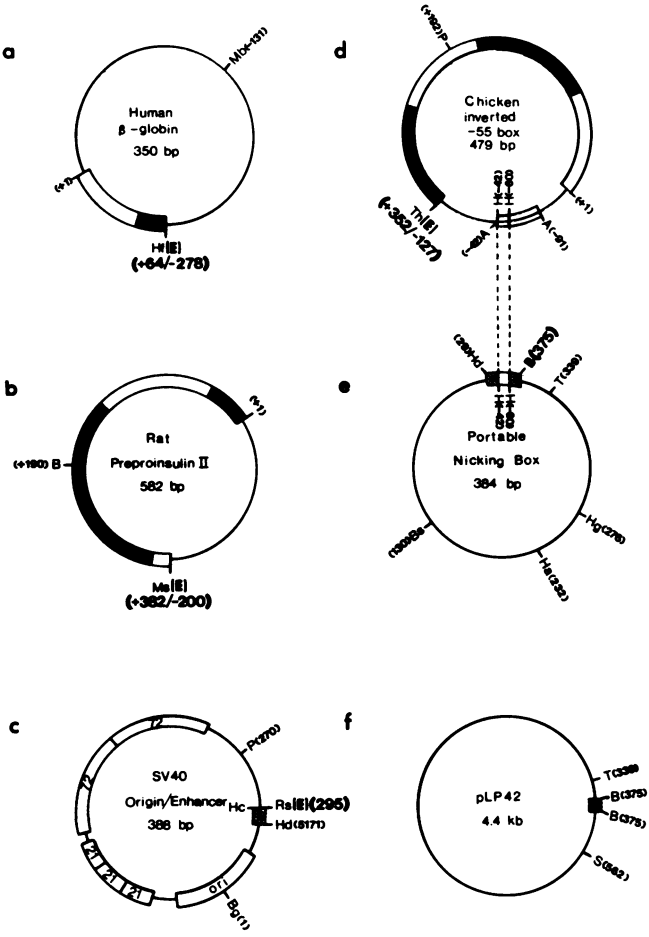


Fig. 1. Maps of supercoiled circles used for SHS analyses. Circles in a-e represent gene sequences purified from plasmid vectors and circularized, as described in the text. a) Human adult β-globin. b) Rat preproinsulin II. c) SV40 origin/enhancer region; open boxes represent the origin, the 21 bp repeats, and the 72 bp repeats; the hatched box represents 11 bp of pUC9 polylinker; numbering is according to ref. 10. d) Chicken adult β-globin with the 49 bp Apa I fragment (cutting sites at -42 and -91) inverted (4). e) "Portable nicking box", consisting of the chicken 18 bp Hpa II fragment (dashed lines connecting d and e) transplanted into pBR322 (see text); the hatched areas represent flanking pUC9 polylinker sequences; the numbering is according to ref. 11. In a, b and d, filled boxes represent exon sequences; open boxes represent intron and 5' noncoding sequences; numbering is relative to the capping site at +1. The circles in a-e were ligated at the restriction sites shown in boldface; those sites containing Eco RI linkers are indicated with brackets. f) pLP42; the shaded box represents the Z-DNA element; numbering is according to ref. 11. Restriction sites are: A, Apa I; B, Bam HI; Bg, Bgl I; Bs, Bst NI; E, Eco RI; H, Hpa II; Ha, Hae II; Hc, Hinc II; Hd, Hind III; Hf, Hinf I; Hg, HgiA I; Mb, Mbo II; Ms, Mst II; P, Pvu II; Rs, Rsa I; S, Sph I; and T, Taq I.

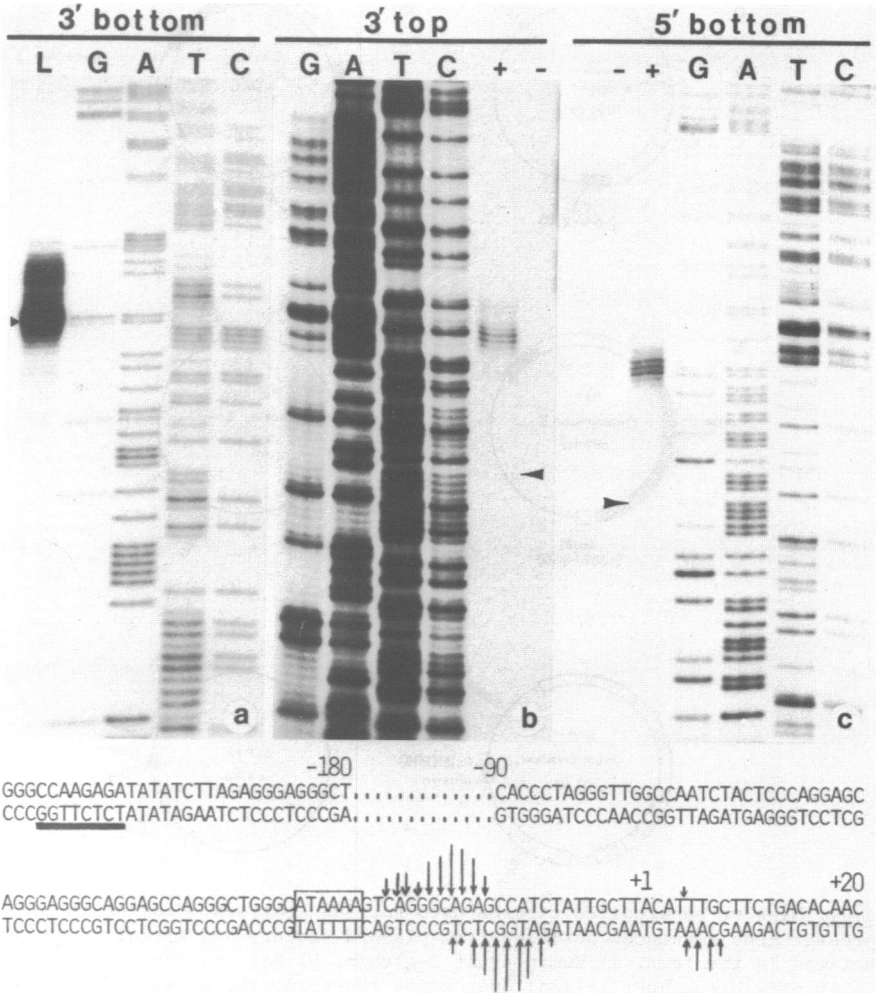


Fig. 2. Nicking patterns in the human adult β-globin promoter region. Autoradiograms of 6% urea-polyacrylamide gels. Lanes G,A,T and C, represent chemical sequencing reactions (7) of untreated material labeled and cut in a similar manner as the S1-treated material. a) Lane L displays cleaved molecules derived from linearization of S1-treated supercoiled circles. Migration of cleaved molecules is slightly faster than that of the corresponding nicked (uncleaved) molecules (not shown), due to nibbling by S1 after cleavage. The arrowhead indicates the location of the major nick in the population of the corresponding uncleaved molecules. b) and c) Nicking patterns of the "-15 box", displaying SHS on the top and bottom strand, respectively. The label is at the 5' or 3' end, as indicated. Arrowheads indicate additional light nicking clearly visible on the original autoradiograms. The (+) lanes contain molecules nicked by S1; the (-) lanes contain control molecules treated in the identical manner as the (+)S1 samples,

but without exposure to the enzyme. The data are summarized on the DNA sequence of the region (bottom). The solid line indicates the SHS around position -200. The intensity of the nicks (corresponding to the percentage of the molecules in the population nicked between particular bases) is represented by the magnitude of the arrows. The intensities in the nicking pattern of each strand were derived separately by densitometric scanning of the autoradiograms and normalization of peak heights to that of the most intense nick (100%). Thus, the patterns cannot be compared between strands (different specific activities).

This elements is hit by S1 on both strands. It extends between positions -14 and -24 in regard to the top strand, and between positions -8 and -18 in regard to the bottom strand (Figs. 2b and c). In other words, the overall nicking pattern of this box in the population of molecules has a staggered appearance (the nicks are displaced by 6 bases between strands). The nicking profile of this SHS (which we call the "-15 box") is strikingly similar to the pattern of nicking found in the chicken gene -55 box (Fig. 5a): the nicks have a skewed distribution, their intensities vary, and they form a W- or M-like pattern. This suggests that a similar altered structure is being recognized by S1 in the two genes. When the sequences of the top strands of the human -15 and the chicken -55 nicking boxes are aligned they show a homology of 8 out of 10 residues ($C^{A}_{G}GGG^{C}_{A}AGAG$).

Rat Preproinsulin II Gene

We examined the 5' flanking sequence of the rat preproinsulin II gene to determine whether a non-globin eukaryotic promoter region contains SHS, and if so, in what position. S1 digestion of the 582 bp supercoiled circles (Fig. 1b) revealed a nicking box around position -50 (Fig. 3). This element resides in a predominant (19/24) homopurine-homopyrimidine region and is hit on both strands. It corresponds in position, but not in sequence, to the chicken β-globin -55 box. No further upstream nicking was detected, although sequences further upstream from position -200 were not investigated. Two other nicking boxes, both hit unilaterally, are found in the 582 bp supercoiled circles, which also reside in homopurine-homopyrimidine stretches (data not shown). They map to sequences within the structural gene [positions +206 to +224 (15/19 pyrimidines), and positions +344 to +355 (10/12 purines)]. In the first box the homopyrimidine stretch is the one which is hit, as in the case of the chicken G-string. However, the homopurine strand is the one nicked in the second box.

SV40

The 388 bp and 345 bp SV40 supercoiled circles (Fig. 1c) contain four regions of S1 hypersensitivity, all located in the origin/enhancer segment

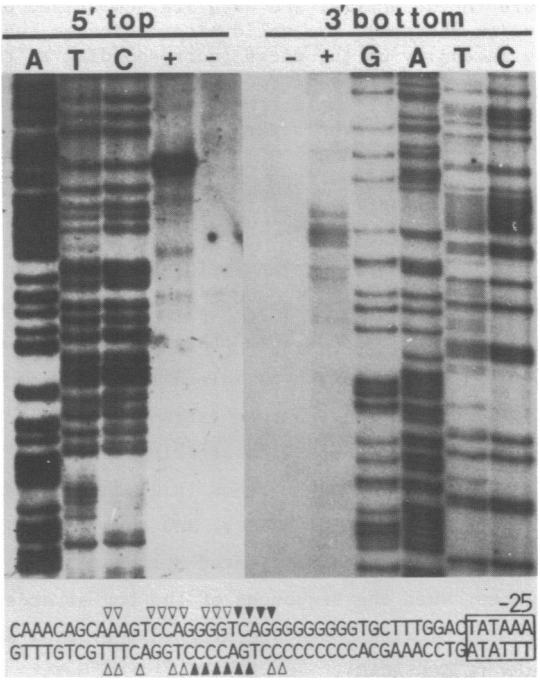


Fig. 3. Nicking pattern in the rat preproinsulin II gene promoter region. The data are summarized on the DNA sequence (bottom). The location of each nick is indicated by an arrowhead. Filled symbols indicate predominant nicks in the population of molecules.

(Fig. 4), which can be detected simultaneously in a single nicking experiment.

The nicking patterns of these multiple and dispersed SHS (which were independently reproduced six times) were difficult to visualize clearly in any single experiment. This might be due to the overlapping character of the various functional elements in this relatively short DNA region. Thus, Fig. 4 is a composite of a number of different experiments highlighting each of the four SHS. The nicking at one of these sites (Fig. 4a) often results in cleavage of the strands (Fig. 4c, lane L). These nicks map to the A/T-rich stretch of the origin of replication, between positions 15 and 27; they might arise from digestion of a melted duplex.

In the direction towards the trascriptional start-sites of the late genes, the location of the other three SHS is as follows:

The first (and strongest) bilateral nicking box is found between positions 5212 and 5222 in the region of the origin of replication (Figs. 4a and c). The sequence which is hit (CACTACTTCTG), on the top strand, is part of a predomi-

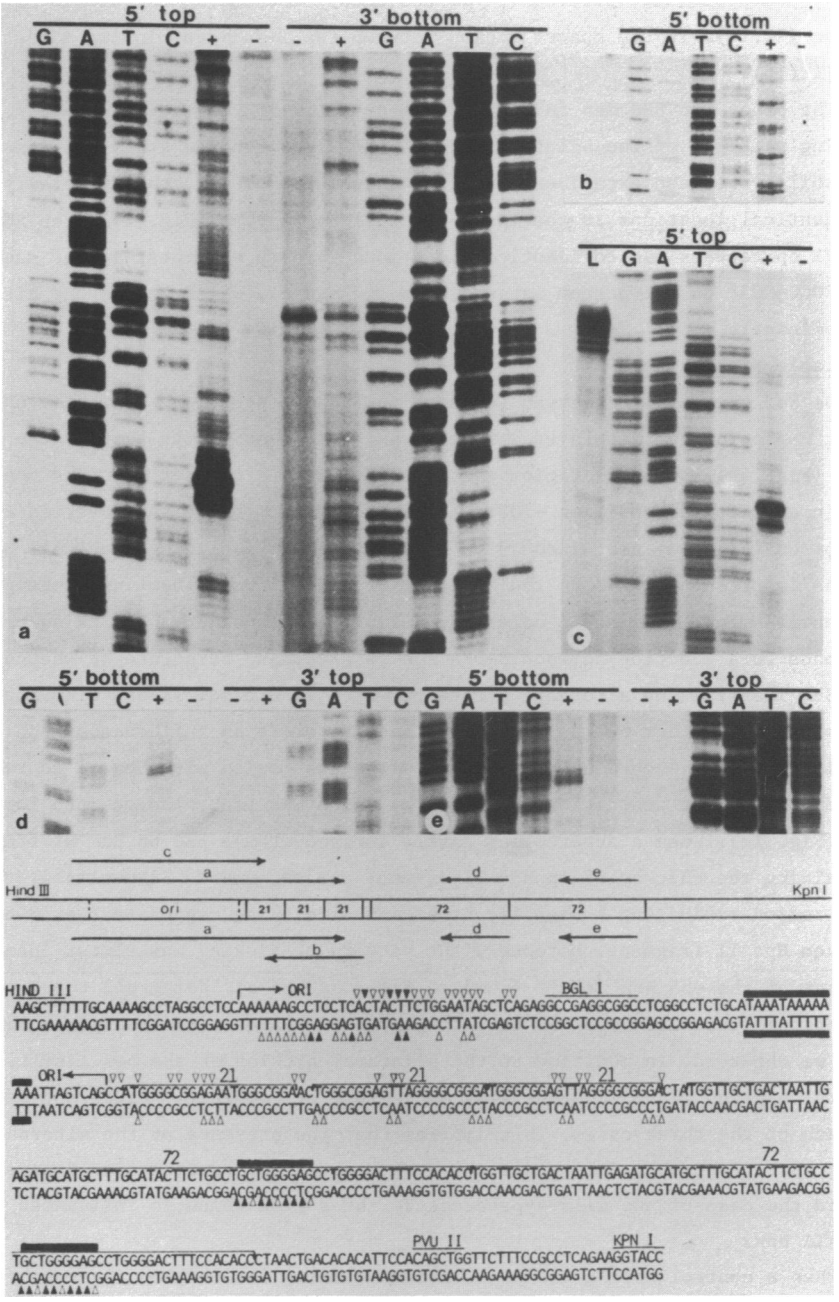
nantly homopyrimidine stretch (14/17 residues), located at the origin of replication (13,14), in the spacer region between the T-antigen binding sites I and II (15).

The other two SHS map in the 21 bp and the 72 bp repetitive elements. The nicking patterns of the bilateral SHS of the 21 bp repeats exhibited some variability between experiments (Figs. 4a and b). However, they generally map to identical locations in the two perfect 21 bp repeats (Fig. 4b). The SHS in the 72 bp repeats map to identical locations in both elements, at the sequence 5'CTCCCCAGC3' on the bottom strand (Figs. 4d and e), which is the one hit predominantly.

"Portable Nicking Box"

We had previously observed (4) that the SHS at position -55 in the chicken adult β-globin gene displayed a nicking pattern identical to that of the wild-type (Fig. 5a) when this region was inverted, with a new set of nicks appearing in addition at position -100 (Fig. 5b). However, since the inverted construction (Fig. 1d) contained 49 bp of flanking DNA,including the CCAAT box, it was important to examine the behavior of the -55 box alone in a foreign DNA environment. This would determine definitively whether the nicking corresponds to a junction between B-DNA and an alternate structure or whether the SHS itself is an alternate structure which is nicked. It was also important to determine whether nearby flanking sequences exert an influence on the nicking pattern. Accordingly, we excised the -55 region with Hpa II to release an 18 bp fragment containing the -55 box, which was transplanted into foreign DNA (Fig. 1e). When a supercoiled circle derived from a 384 bp Bam HI fragment containing the chicken 18 bp Hpa II element was exposed to S1 nuclease, the only region exhibiting a significant amount of nicking mapped to the 18 bp chicken Hpa II fragment. Moreover, the pattern of nicking was almost identical to that of the -55 box itself (compare Figs. 5a and c). Using all three constructions (the wild-type -55 box, the inverted -55 box, and the portable -55 box) we observed, in addition to the bilateral nicking of the box itself, unilateral nicking 17 to 20 bases away in regions of completely different sequence in each of the three cases. This implies that the presence of the alternate DNA structure somehow influences the structure of its environment. Interestingly, in the case of the wild-type sequence the region which is influenced is the ATA box.

When a control circle of 366 bp not containing the chicken fragment (see Materials and Methods) was treated in the same manner as the portable nicking box construction, no nicks appeared in the region of the Acc I site (where the


5' top
3' bottom
5' bottom
5' top
3' top
5' bottom
3' top
G A T C + -
a
b
c
d
e
L
Hind III
Kpn I
ori
21
72
ORI
BGL I
PVU II
KPN I

18 bp Hpa II fragment resides in the 384 bp circle); rather, a new set of nicks arose elsewhere in the circle, around position 340 of pBR322 (data not shown), a result consistent with the hierarchical behavior of SHS. We conclude from these experiments that a SHS is a sequence-dependent feature of only a small stretch of DNA.

Z-DNA

Since there is speculation that the SHS might be in a left-handed non-Z conformation (16), we decided to examine for comparison the nicking around a region of classical Z-DNA by fine mapping. Coarser mapping had indicated (17, 18) that a short region of Z-DNA embedded in a supercoiled circle of B-DNA generates S1 hypersensitivity, with cleavage sites mapping to the approximate locations of the B-Z junctions. Using our procedure, we examined the nicking pattern of plasmid pLP42, which is pBR322 containing 21 dC-dG pairs inserted into the Bam HI site (Fig. 1f). As shown in Fig. 6, pLP42 contains two regions of S1 hypersensitivity which map exactly to the Bam HI borders of the Z-DNA insert, while the Z-DNA itself is S1-insensitive. However, the nicks at the two B-Z borders do not display equal intensities. The left-side border exhibits at least tenfold greater sensitivity to S1 than the right-side border. This may be explained by the fact that the sequence of the Z-DNA insert of pLP42 is actually GGATC$(CG)_{21}$GCGCGGATCC, so that the regularity of the Z-DNA sequence is broken just prior to the right-side border. Recent results (18) showed that a 64 bp Z-DNA element of dT-dG/dA-dC in plasmid pRW777, which is cleaved by S1 at the B-Z junctions, also exhibits a marked preference for cleavage at one junction. This Z-DNA insert is also imperfect, with the fourth GC base pair from the weak junction substituted by an AT.

Our results indicate that if the SHS are left-handed DNA, their structure is very different from that of Z-DNA, because the former are themselves the nicking substrates, while their borders with normal B-DNA are insensitive to S1 nuclease. We note that antibodies to Z-DNA do not recognize the chicken -55 SHS (data not shown).

Fig. 4. Nicking patterns in the SV40 origin/enhancer region. The areas shown in panels a-e are indicated by correspondingly labeled arrows in the diagram of the region. The direction of each arrow corresponds to the reading of a sequencing ladder from bottom to top of an autoradiogram. The data are summarized on the DNA sequence. Solid lines, arrowheads and lane headings are as in Figs. 2 and 3. The nicking patterns shown for the 21 bp repeats are based on a consensus from six independent, but not identical, sets of data. Nicking on the top strands of the 72 bp repeats (solid lines) was observed only occasionally.

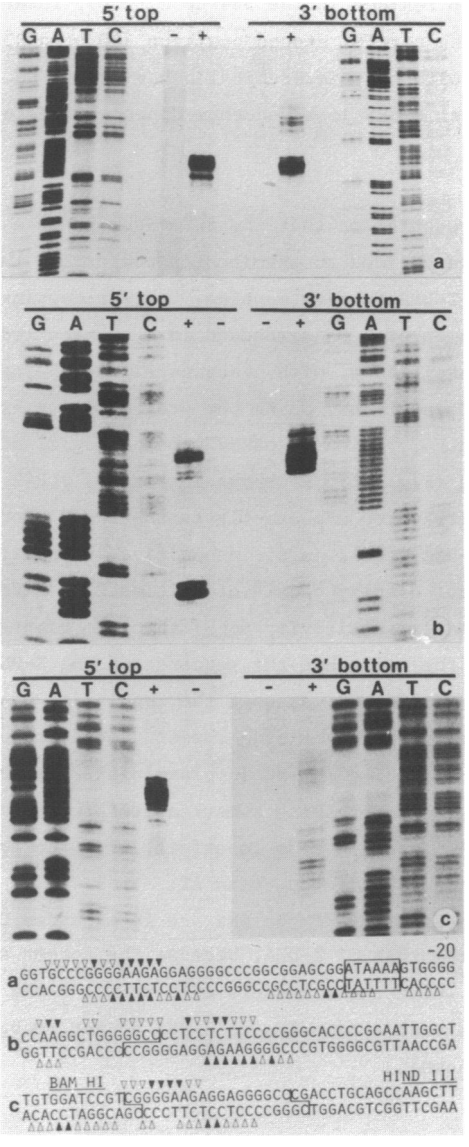


Fig. 5. Nicking patterns of the wild-type (a), inverted (b), and transplanted (c) chicken β-globin gene -55 SHS. The DNA sequences a, b, and c (bottom) correspond to the panels. The inverted construction is shown in Fig. 1d. One of the two Apa I sites (staggered line) delineating the boundaries of the inversion is shown in sequence b. In sequence c, the staggered lines bracket the 18 bp of the SHS inserted into foreign plasmid sequences (see Fig.1e and text).

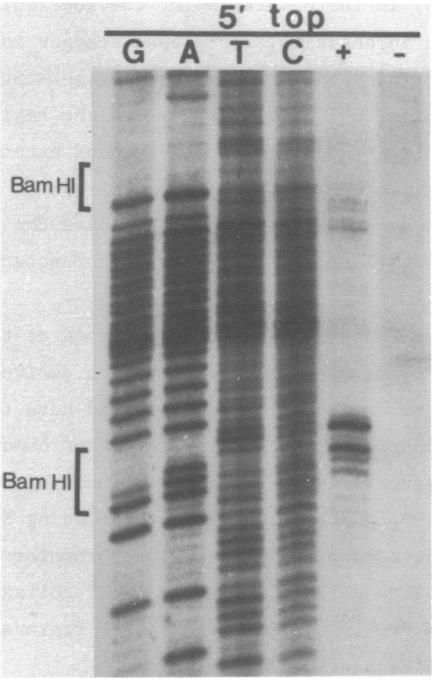


Fig. 6. Nicking patterns of a region containing Z-DNA. The Z-DNA element of pLP42 is flanked by two Bam HI sites (brackets). The sequencing ladders are compressed. Essentially identical results (strong and weak nicking at the Bam HI borders of the Z-DNA insert) were obtained upon examination of the bottom strand (not shown).

DISCUSSION

In vitro fine mapping of the SHS of the chicken β-globin gene (4) indicated that there are two major regions of non-B DNA structure in the 5' flanking region: a $(G/C)_{18}$ element, between nucleotide position -184 and -201 upstream from the capping site, and the -55 nicking box (positions -49 to -59). Bilateral nicking of a variant G-string $[(G/C)_{16}]$ was also detected by others (19). A similar but not identical situation exists for the human β-globin gene. An element which might be equivalent to the G-string is present between positions -199 and -207, and a second element, similar in sequence but not in position to the -55 box, is present in the -8 to -24 region. The hierarchical order between the two elements in the chicken and the human genes is the same. However, the chicken G-string is not only of higher order in the hierarchy but also its presence masks completely the appearance of the -55 box; in the case of the human gene the appearance of both of its nicking boxes can be

detected simultaneously in the population of the supercoiled molecules, but with different nicking intensities. The 6 base stagger in the pattern of nicks between the top and bottom strands in the human gene -15 box (Fig. 2) suggests that the region recognized by S1 is duplex. Were the region melted, the pattern would be expected to be both non-staggered and extended into the AT-rich area of the ATA box.

When we examined a non-globin gene, in this case the rat preproinsulin II gene, we again observed bilateral nicking in the promoter area, around position -50.

When we compare the SHS which we have now mapped at the nucleotide level, it is apparent that a consensus sequence cannot be derived. However, it is obvious from the more extensive analysis presented here that our previous observation that S1 hypersensitivity is a feature of homopurine-homopyrimidine stretches can now be generalized without exception. This is consistent with the mapping of other SHS by other techniques, including SHS in the human α-globin gene (20), the adenovirus 2 major late promoter (21), the Drosophila heat-shock genes (22), the chicken and mouse α2(I) collagen genes (23,24), the Drosophila and sea urchin histone gene spacer regions (25,26), and a chicken repetitive DNA sequence (27).

We reasoned that if S1 nuclease is a probe which can reveal functionally important regions, it might nick not only promoter-containing supercoiled circles but also DNA carrying other transcription-related regulatory sequences, such as enhancers. This is indeed the case with the SV40 origin/enhancer region. A major SHS was found at equivalent positions in each of the 72 bp enhancers. It does not map at junctions of the SV40 mini-Z-DNA (28), but lies adjacent to the core sequence of the enhancer element (29). Another SHS maps to a short 10 bp region between the T-antigen binding sites I and II, around position 5212-5222. This element happens to be at the location of four closely spaced mouse primase initiation sites of SV40 replication (13) and the point where continuous and discontinuous replication initiates (14). Finally, nicking was also detected in the 21 bp repeats. It is already known that the 21 bp elements are required for early region transcription (30) and that they bind the specific transcription factor Sp1 (31). Detailed examination of a series of SV40 origin/enhancer region mutants would be necessary to establish a direct relationship between the novel DNA structure(s) revealed by S1 nuclease and biological activity.

Although the presence of SHS in the promoter regions of eukaryotic genes, in combination with the fact that they constitute in vivo a feature of only

active chromatin (5,6), and the correlations mentioned above point to biological significance (which may involve both replication and transcription), the actual functional role of the non-B-DNA structure which the enzymatic probe reveals is unknown. It remains to be seen whether the promoter-specific SHS are DNA signals for binding of regulatory proteins and/or energy sinks related to conformational changes in a chromatin domain when the latter is activated for expression. In this regard, it is striking that a factor stimulating transcription which was recently identified in Drosophila cultured cells footprints at the S1 hypersensitive (25) $(GA/CT)_{11}$ stretch of DNA (32) in the spacer region between the Drosophila histone genes H3 and H4 (Carl Parker, personal communication). We note that this binding, as well as the binding of T-antigen and factor Sp1 on SV40 DNA,has been observed on linear DNA molecules. Thus, the relationship of the structure of the binding signals on linear DNA to SHS observed in supercoiled circles is not clear. However, we have recently observed that both S1 and mung bean nucleases recognize long GA/CT stretches embedded not only in supercoiled circles but in linear molecules as well (in preparation), thus confirming an earlier observation of S1-sensitivity of a linear homopurine-homopyrimidine stretch (26).

Another suggestive piece of evidence is that one of the transcriptional start sites mapping upstream from the normal capping site of the human ε-globin gene, at position -200, is characterized by S1-hypersensitivity (33,34). Moreover, the notion that nuclease hypersensitivity is related to gene expression has been strengthened by the isolation of a tissue- and developmental-stage-specific DNA-binding factor which confers DNase I hypersensitivity to the 5' flanking region of the chicken adult β-globin gene (35). Although S1 nuclease and DNase I hypersensitive regions seem to be different entities, they map in close proximity in the promoter region of this gene (36).

ACKNOWLEDGEMENTS

We thank C. Dobkin and J. Wang for providing materials; Carl Parker for communicating unpublished information; L. Hanau for performing the Z-DNA antibody binding experiments; and Sean Cotter for expert technical assistance. This work was supported by a grant to A.E. from the National Institutes of Health.

REFERENCES

1. Smith, G.R. (1981) Cell 24, 599-600.
2. Wells, R.D., Goodman, T.C., Hillen, W., Horn, G.T., Klein, R.D., Larson, J.E., Muller, V.R., Neuendorf, S.K., Panayotatos, N., and Stirdivant, S.M. (1980) Prog. Nucl. Acids Res. Mol. Biol. 24, 167-267.

3. Elgin, S.C.R. (1984) Nature 309, 213-214.
4. Schon, E., Evans, T., Welsh, J., and Efstratiadis, A. (1983) Cell 35, 837-848.
5. Larsen, A. and Weintraub, H. (1982) Cell 29, 609-622.
6. Weintraub, H. (1983) Cell 32, 1191-1203.
7. Maxam, A.M. and Gilbert, W. (1980) Meth. Enzymol. 65, 499-560.
8. Vieira, J. and Messing, J. (1982) Gene 19, 259-268.
9. Lomedico, P., Rosenthal, N., Efstratiadis, A., Gilbert, W., Kolodner, R., and Tizard, R. (1979) Cell 18, 545-558.
10. Tooze, J., ed. (1981) DNA Tumor Viruses, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
11. Sutcliffe, J.G. (1978) Cold Spr. Harb. Symp. Quant. Biol. 43, 77-90.
12. Peck, L.J., Nordheim, A., Rich, A., and Wang, J.C. (1982) Proc. Nat. Acad. Sci. USA 79, 4560-4564.
13. Tseng, B.Y. and Ahlem, C.N. (1984) Proc. Nat. Acad. Sci. USA 81, 2342-2346.
14. Hay, R.T. and DePamphilis, M.L. (1982) Cell 28, 767-779.
15. Tjian, R. (1981) Cell 26, 1-2.
16. Cantor, C. and Efstratiadis, A. (1984) Accompanying paper.
17. Singleton, C.K., Klysik, J., Stirdivant, S.M., and Wells, R.D. (1982) Nature 299, 312-316.
18. Singleton, C.K., Kilpatrick, M.W., and Wells, R.D. (1984) J. Biol. Chem. 259, 1963-1967.
19. Nickol, J.M. and Felsenfeld, G. (1983) Cell 35, 467-477.
20. Shen, C.-K.J. (1983) Nucl. Acids Res. 11, 7899-7910.
21. Goding, C.R. and Russel, W.C. (1983) Nucl. Acids Res. 11, 21-36.
22. Mace, H.A.F., Pelham, H.R.B., and Travers, A.A. (1983) Nature 304, 555-557.
23. Finer, M.H., Fodor, E.J.B., Boedtker, H., and Doty, P. (1984) Proc. Nat. Acad. Sci. USA 81, 1659-1663.
24. McKeon, C., Schmidt, A., and deCrombrugghe, B. (1984) J. Biol. Chem. 259, 6636-6640.
25. Glikin, G.C., Gargiulo, G., Rena-Descalzi, L., and Worcel, A. (1983) Nature 303, 770-774.
26. Hentschel, C.C. (1982) Nature 295, 714-716.
27. Dybvig, K., Clark, C.D., Aliperti, G., and Schlesinger, M.J. (1983) Nucl. Acids Res. 11, 8495-8508.
28. Nordheim, A. and Rich, A. (1983) Nature 303, 674-678.
29. Weiher, H., Konig, M., and Gruss, P. (1983) Science 219, 626-631.
30. Baty, D., Barrera-Saldana, H.A., Everett, R.D., Vigneron, M., and Chambon, P. (1984) Nucl. Acids Res. 12, 915-932.
31. Dynan, W.S. and Tjian, R. (1983) Cell 35, 79-87.
32. Goldberg, M.L. (1979) Ph.D. thesis, Stanford University.
33. Allan, M., Lanyon, W.G., and Paul (1983) Cell 35, 187-197.
34. Allan, M., Zhu, J.-d., Montague, P., and Paul, J. (1984) Cell, in press.
35. Emerson, B.M. and Felsenfeld, G. (1984) Proc. Nat. Acad. Sci. USA 81, 95-99.
36. McGhee, J.D., Wood, W.I., Dolan, M., Engel, J.D., and Felsenfeld, G. (1981) Cell 27, 45-55.