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Identification of transcriptionally induced Z-DNA segments in the human c-*myc* gene

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Abstract

Using monoclonal antibodies against Z-DNA three *Alu*I restriction fragments of the human c-myc gene were previously found to form Z-DNA in agarose-embedded, metabolically active permeabilized nuclei. The formation of Z-DNA in these fragments was dependent on negative supercoiling generated by transcription of the gene. Here we show which sequence elements of these three *Alu*I restriction fragments adopt the Z conformation upon negative supercoiling. The three fragments (Z1, Z2 and Z3) were inserted in a suitable plasmid vector. Z-DNA forming elements were detected by comparing DEPC reactivity in relaxed circular and supercoiled plasmid DNA. Z1 and Z3 each contained one major Z-DNA forming region 20–25 nucleotides long, whereas Z2 contained two discrete regions 90 nucleotides apart one about 35 nucleotides the other about 20 nucleotides long.

Keywords: Negative supercoiling; Diethyl pyrocarbonate reactivity; Potassium permanganate reactivity; Non B-DNA conformation

1. Introduction

It is now generally recognized that in the genome of cells DNA can exist in many different conformations. Some of these conformational changes are clearly associated with different physiological states. Among the more profound conformational changes is the conversion of the right-handed DNA duplex into a left-handed helix (Z-DNA). Z-DNA exists as a higher energy state of the normal right-handed B-DNA duplex [1,2]. Z-DNA can be stabilized by many conditions in vitro. However, in vivo, its stabilization is associated largely with negative supercoiling [3,4]. This supercoiling stores an energy which is proportional to the square of the number of negative superhelical turns. Relaxing torsionally-strained DNA the formation of left-handed Z-DNA can absorb some of the energy. An important feature bearing on Z-DNA formation is the DNA sequence. The formation of Z-DNA is favored by alternating purine and pyrimidine sequences, especially alternating CG sequences [1].

The binding of monoclonal antibodies against Z-DNA in metabolically active permeabilized nuclei has been used as a measure of Z-DNA formation [5]. The amount that is bound is related to the negative torsional strain of the DNA molecule. Under appropriate conditions it was shown that addition of the antibody did not disturb the B-Z equilibrium [5]. Topoisomerase I relaxes negatively supercoiled DNA and decreases the amount of Z-DNA [5]. Transcription leads to the formation of negative supercoils upstream of the RNA polymerase [6]. Stimulation of transcription increases the amount of Z-DNA formation in metabolically active permeabilized human nuclei. In these experiments, gradually increasing levels of RNA synthesis were associated with a corresponding increase in Z-DNA formation [7].

An experimental protocol was developed that allows us to measure Z-DNA formation in individual genes, using permeabilized nuclei. Biotin labeled monoclonal antibodies against Z-DNA are diffused into these nuclei. A 10 nanosecond laser pulse at 260 nm crosslinks the bound antibody to the Z-DNA segments. After restriction diges-

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tion, restriction fragments diffuse out of the nuclei, some crosslinked to antibodies containing the biotin label. These fragments are then separated using streptavidin-coated magnetic particles. Release of the DNA from the magnetic particle through proteolytic digestion of the antibodies yields the restriction fragments that form Z-DNA. An experiment of this type was carried out on U-937 human leukemic cells, and the Z-DNA forming restriction fragments were probed with segments of the human c-*myc* gene [8]. Three *Alu*I restriction fragments (Z1, Z2 and Z3) were identified as forming Z-DNA when c-*myc* is transcribed. When c-*myc* is down-regulated and no longer transcribed, these *Alu*I fragments are no longer bound to the antibody. This work clearly demonstrated that Z-DNA formation in the three fragments is coupled to the transcriptional activity in the gene.

In our previous work a computer program surveying nucleotide sequence was used to identify those segments of the c-*myc* gene that were most likely to form Z-DNA under negative torsional strain [8]. The three most probable nucleotide sequences were found to reside in the three *Alu*I fragments that had been isolated bound to the streptavidin-coated magnetic particles. Thus, we presumed that these sequences were the most likely candidates for Z-DNA formation. In the present communication, we determine which nucleotide sequences in these three *Alu*I restriction fragments actually form Z-DNA when subjected to negative torsional strain. To make this measurement, chemical modification studies were carried out. It is well established that the chemical reactivity of Z-DNA differs in a distinctive way from that of right-handed B-DNA. The results reported here demonstrate that there is reasonable agreement between the predictions made by the computer program and the experimental observations regarding the formation of Z-DNA in these three *Alu*I restriction fragments. This is among the first delineations of Z-DNA forming sequences in naturally occurring genes without repetitive DNA sequences.

2. Materials and methods

2.1. Subcloning of Z-DNA forming human c-myc fragments

ZI and Z3 *Alu*I fragments of the human c-myc gene were inserted into the *Sma*I site of pBluescript by blunt end ligation. Subsequently the fragments were cloned in the vector pDPL6 (Pulleyblank [9]) using the *Bam*HI and *Hin*dIII restriction sites. The Z3 insert used here was cut at 145 bp to allow for better analysis.

Z2 was cloned in pBluescript in two steps: first cloning of a *Pvu*II c-*myc* fragment into the *Sma*I site, then subcloning of the *Nsi*I/*Hin*dIII fragment of this construct into the *Pst*I and *Hin*dIII sites of pBluescript and finally introducing the insert into pDPL6 using the *Bam*HI and *Hin*dIII restriction sites. This clone does not contain 14 bp from the 5′ end of the 336 bp long Z2 *Alu*I fragment. Part of the sequences of *Bam*HI/*Hin*dIII fragments are displayed in Fig. 3.

2.2. Preparation of plasmids with various degrees of negative super coiling

Plasmids were prepared using CsCl banding or Qiagen columns. The superhelicity of plasmids prepared with Qiagen columns was comparable to plasmids purified by CsCl banding, if the growth of the bacterial culture was monitored carefully. Overgrowing of the bacterial culture led to an increase of nicked plasmid DNA. Plasmids with various degrees of negative supercoiling were generated by topoisomerase I treatment in the presence of ethidium bromide. 3.5 μg of DNA were treated with 10 U of topoisomerase I (Topogen, Columbus, OH) in 50 mM Tris-HCl pH 7.9, 50 mM NaCl and 2 mM $MgCl_2$ in the presence of 0; 2; 4 and 8 μg/ml ethidium bromide for 6 h. The degree of superhelicity was derived from the ethidium bromide concentration present in the topoisomerase I reaction and monitored on agarose gels [10]. Samples were extracted three times with phenol/$CHCl_3$ and ethanol precipitated. Plasmids were resuspended in 25 μl of H_2O (about 0.1 μg/μl). Negative supercoiling was monitored on agarose gels.

2.3. DEPC treatment of supercoiled plasmid DNA

5 μl (0.5 μg) of plasmid DNA were adjusted to 80 μl in 50 mM cacodylate, 1 mM EDTA buffer pH 7.1. 3 μl of DEPC were added, the reaction was carried out at 20°C for 15 min and stopped by adding 22 μl of 1.5 M sodium acetate, pH 5.2. 1 μg tRNA and 300 μl ethanol were added for precipitation. Pellets were resuspended in 100 μl 0.3 M sodium acetate (pH 5.2) and again precipitated with 300 μl of ethanol. Pellets were washed once with 70% ethanol and resuspended in buffer for restriction enzyme digestion [11,12].

2.4. $KMnO_4$ treatment

5 μl (0.5 μg) of plasmid DNA were adjusted to 50 μl in 10 mM Tris-HCl, 1 mM EDTA buffer pH 7.5. $KMnO_4$ was added to a concentration of 10 mM, the reaction was carried out at 37°C for 2 min and stopped by adding 5 μl β-mercaptoethanol and 5 μl 2 M sodium acetate, pH 5.2. 1 μg tRNA and 180 μl ethanol were added for precipitation. Pellets were washed once with 70% ethanol and resuspended in buffer for restriction enzyme digestion [13].

2.5. Endlabeling, sequencing reactions, gel electrophoreses

Plasmids were cut with *Bam*HI and *Hin*dIII. To label *Bam*HI sites, fill in reactions were carried out with [α-^{32}P]dGTP; to label *Hin*dIII sites fill in reactions were

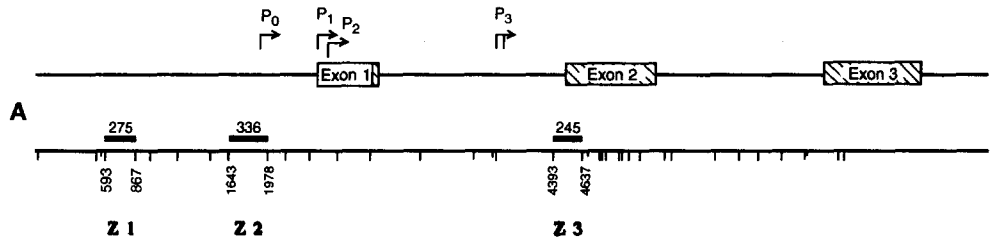

Fig. 1. A diagram illustrating the human c-*myc* gene and various points of interest. The c-*myc* gene is shown at the top together with its three exons and four promoters, P0, P1, P2, P3. The crosshatched areas on exons 1, 2 and 3 represent the structural gene for the 67 kDa protein. The 8 kb *Hin*dIII-*Eco*RI segment is shown together with vertical lines that illustrate the sites of AluI restriction enzyme cleavage. The numbers indicate the nucleotide positions flanking the three *Alu*I fragments that contain Z-DNA forming sequences, Z1, Z2 and Z3. The numbers above them are the length of the fragments in nucleotides.

carried out with [α-^{32}P]dATP and Klenow fragment in restriction buffer. Unique label was obtained by second digestion, or by gel purification of the fragment of interest. Strand breaks were generated by treatment with 1 M piperidine at 90°C for 30 min (β-elimination). Piperidine was removed by lyophilization and ethanol precipitation. Pellets were resuspended in 2 μl of H_2O and 8 μl of formamide gel loading dye. G&A and T&C sequencing

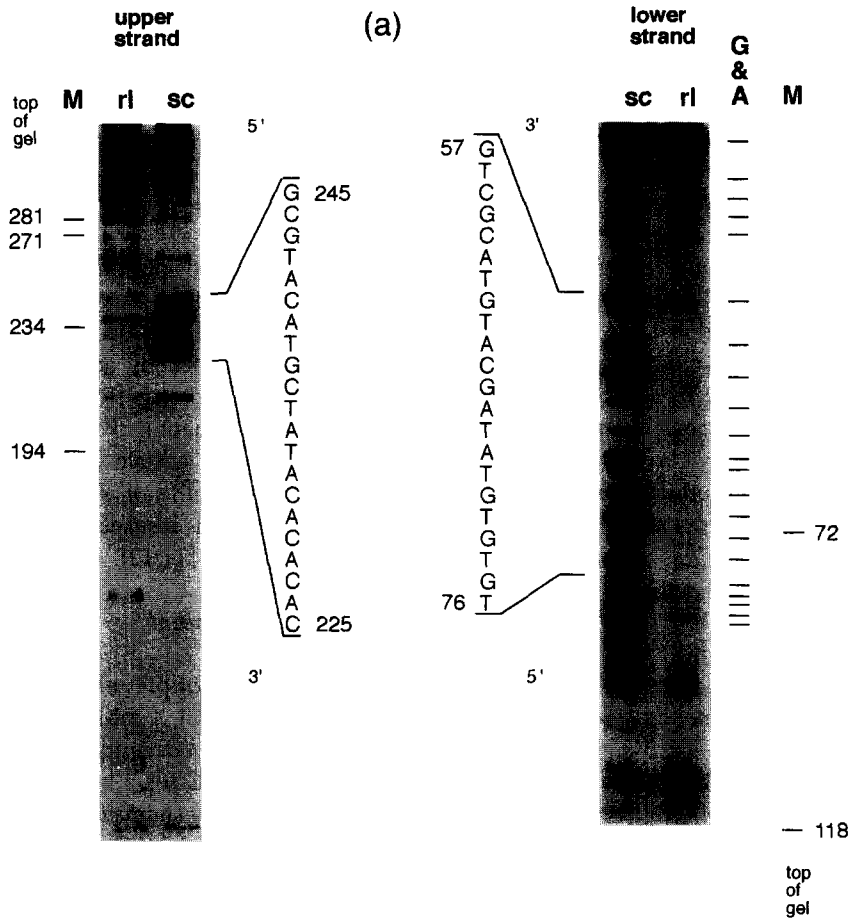

Fig. 2. DEPC reactivity of the restriction fragments Z1, Z2 and Z3 of the human c-*myc* gene. (a) shows pME.Z1; (b) pME.Z2 and (c) pME.Z3. Upper strand refers to the DNA sequence that corresponds to the mRNA sequence. Lower strand refers to the template strand used by the RNA polymerase. The orientation of the panels for the lower strands is oriented with the top of gel down to allow for easier comparison of the two antiparallel strands. Lanes rl: relaxed circular plasmids; lanes sc: supercoiled plasmids (mean superhelical density −0.12); lanes M: size marker; lanes G&A and T&C sequence references. The sequencing lane in Fig. 2a was overexposed so that the bands could be seen clearly only in transmitted light. Accordingly, horizontal lines are drawn at the band positions. Base sequences of hyperreactive areas are displayed in brackets that align the sequence with its position in the gel. Numbers refer to the distance of a base to the radioactive label at the 3'-end of the analyzed fragments. Dashed lines indicate hyperreactive regions that are less intense than neighboring areas.

reactions were carried out as described [14]. Gel electrophoreses were done in 8% polyacrylamide, 8 M urea sequencing gels.

3. Results

3.1. Detection of Z-DNA in three AluI restriction fragments of the human c-myc gene with DEPC

In the experiments in which biotin labeled monoclonal antibodies against Z-DNA were diffused into permeabilized human leukemic nuclei, three *Alu*I restriction fragments of the human c-*myc* gene were identified through their crosslinking to the antibody [8]. These fragments are shown in Fig. 1 which shows the organization of the human c-*myc* gene. The c-*myc* gene has four transcription start sites labeled P0–P3. The cross-hatched area covering part of exon 1, as well as exons 2 and 3 represent the coding sequence for the 67 kDa c-*myc* protein. The lower line in Fig. 1 has a series of vertical markings which represent the *Alu*I cleavage sites. The three Z-DNA forming segments are marked with heavy horizontal lines. Two of them, Z1 and Z2 are upstream of exon 1, while Z3 covers part of the first intron and the beginning of exon 2 fairly close to the transcription start site P3.

Z-DNA formation in these three *Alu*I restriction fragments was determined by measuring reactivity with diethyl

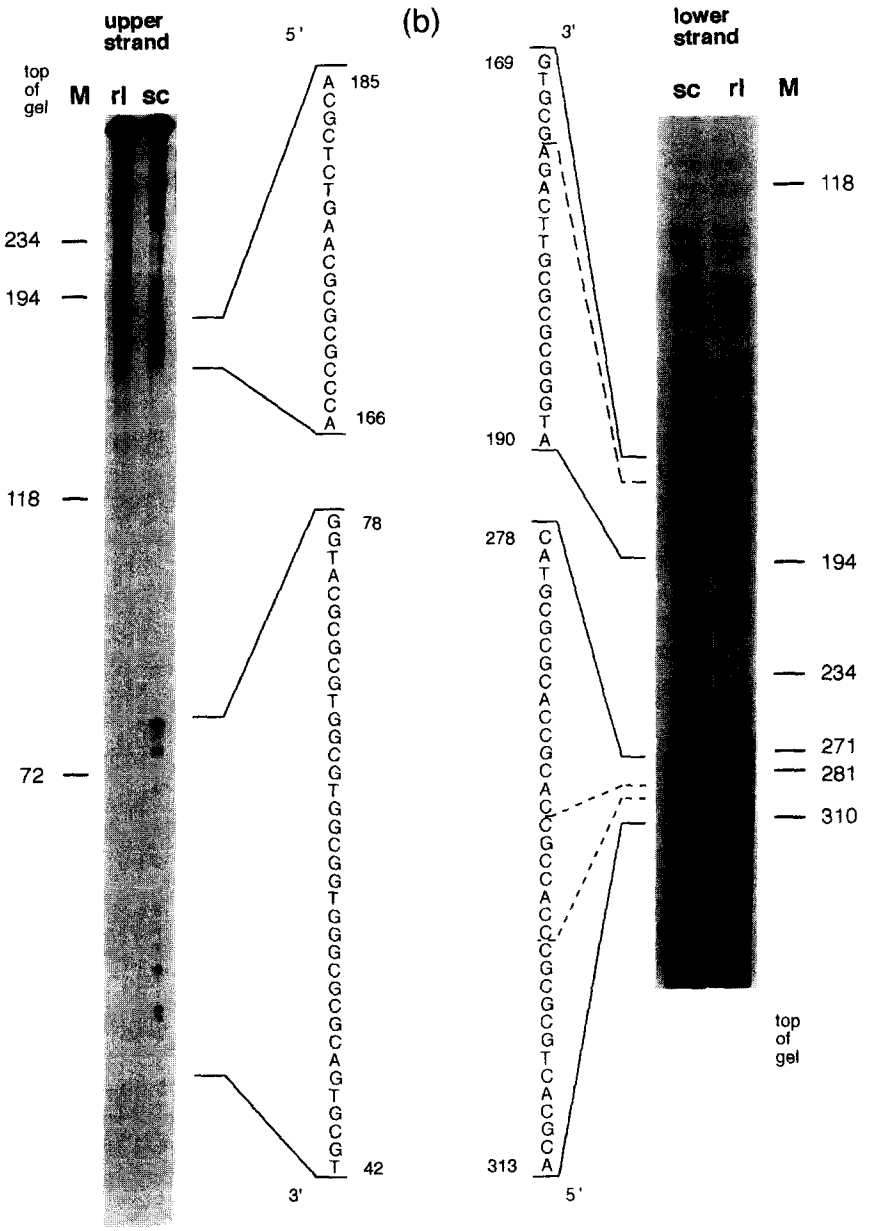

Fig. 2 (continued).

pyrocarbonate [11,12]. Diethyl pyrocarbonate (DEPC) is known to react at the N7 position of both guanine and adenine. Z-DNA is favored in sequences with alternations of purines and pyrimidines. The Z-DNA purines are generally in the *syn* conformation. This has the effect of pushing the imidazole ring of the purine away from the center of the molecule so that it is readily accessible to the solvent and to chemical reagents. In contrast, because they are in the *anti* conformation, the N7 position of adenine and guanine in B-DNA is found at the bottom of the major groove, and it is largely inaccessible to these reagents. Thus, reactivity with DEPC has been used to define Z-DNA formation [11,12,15]. It should be noted that most mapping of Z-DNA formation in DNA has been carried out in artificial constructs, usually containing inserts of alternating purine-pyrimidine segments. There have only been few characterizations of Z-DNA formation in naturally occurring sequences, most of them uninterrupted stretches of GC or AC/GT repeats [16,17].

The three Z-DNA forming *Alu*I fragments of the human c-myc gene were inserted into the plasmid pDPL6 [9], and the plasmid was then negatively supercoiled using topoisomerase I treatment in the presence of ethidium bromide [10]. This vector was chosen because it contains fewer endogenous elements that are likely to undergo conformational changes upon increase of negative supercoiling than other pBR322 derived plasmid vectors [9]. Different degrees of negative supercoiling were produced in the plasmids and then both strands of the *Alu*I inserts were studied for chemical reactivity.

In the following discussion, upper strand refers to the DNA sequence that in the transcribed area corresponds to the mRNA sequence, while lower strand corresponds to the template strand used by the RNA polymerase. Fig. 2a shows the DEPC reactivity of the Z1 fragment. The two strands are in antiparallel orientation, the gel of the lower strand is oriented with the top of the gel down, to facilitate the comparison of the two strands. Lanes (rl) show the

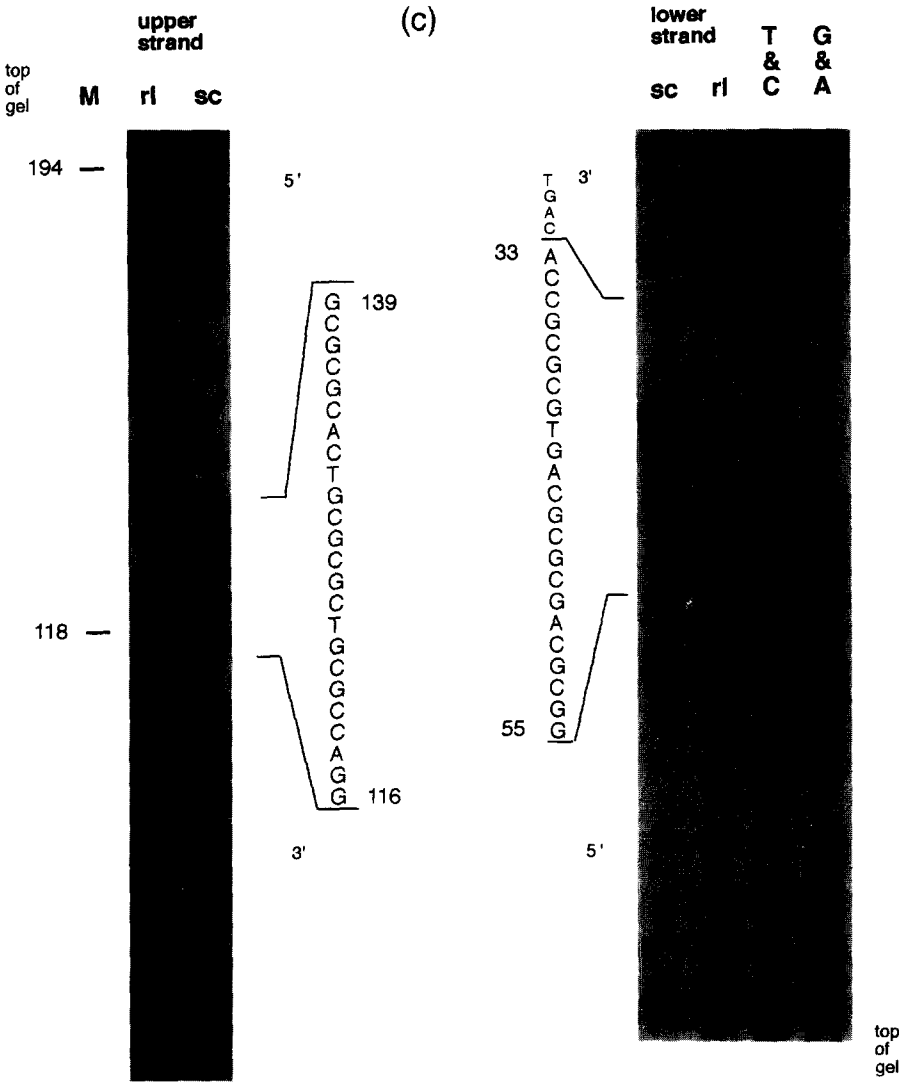

Fig. 2 (continued).

DEPC reactivity of relaxed circular plasmid. The reactivity of supercoiled plasmid is shown in lanes (sc). The mean superhelical density in lanes (sc) shown is about −0.12. An area of DEPC hyperreactivity that is only present in the supercoiled plasmid is indicated by brackets with the corresponding sequence indicated. The numbering at the sequence corresponds to the length of the end labeled fragment. The guanosine in the center of the hyperreactive area in the upper strand shows enhanced reactivity even in the relaxed plasmid. A singular site of enhanced reactivity in the supercoiled lane is found about 10 base pairs away from the brackets in the 3′ direction. This band corresponds to purine residues flanking a long pyrimidine stretch. The lower strand shows DEPC hyperreactivity in the supercoiled plasmid in the corresponding area. The resolution of the gel shows hyperreactivity of individual bases. Several purine residues in the bracketed area show a very high reactivity, including the two flanking guanosines at positions 57 and 75. These bands are somewhat overexposed. The long stretch of purines 5′ of the bracketed area is somewhat more reactive in the supercoiled plasmid than in the relaxed plasmid. This, as well as the slightly increased reactivity of purines in the 3′ flanking could be due to some structural variations and might be influenced by the Z-DNA formation. Differences in reactivity clearly limit significant structural changes to the marked area. The DEPC reactivity of both upper and lower strand is found in the same sequence element.

The DEPC experiments of the *Alu*I fragment Z2 are shown in Fig. 2b. As above, the mean superhelical density of the supercoiled lane (sc) is −0.12. In the relaxed lane (rl) of the upper strand a high background signal is found towards the top of the gel. In the supercoiled lane enhanced reactivity is found in the two marked areas. One area without single base resolution lies between fragment length 185 to 166 and one with single base resolution lies

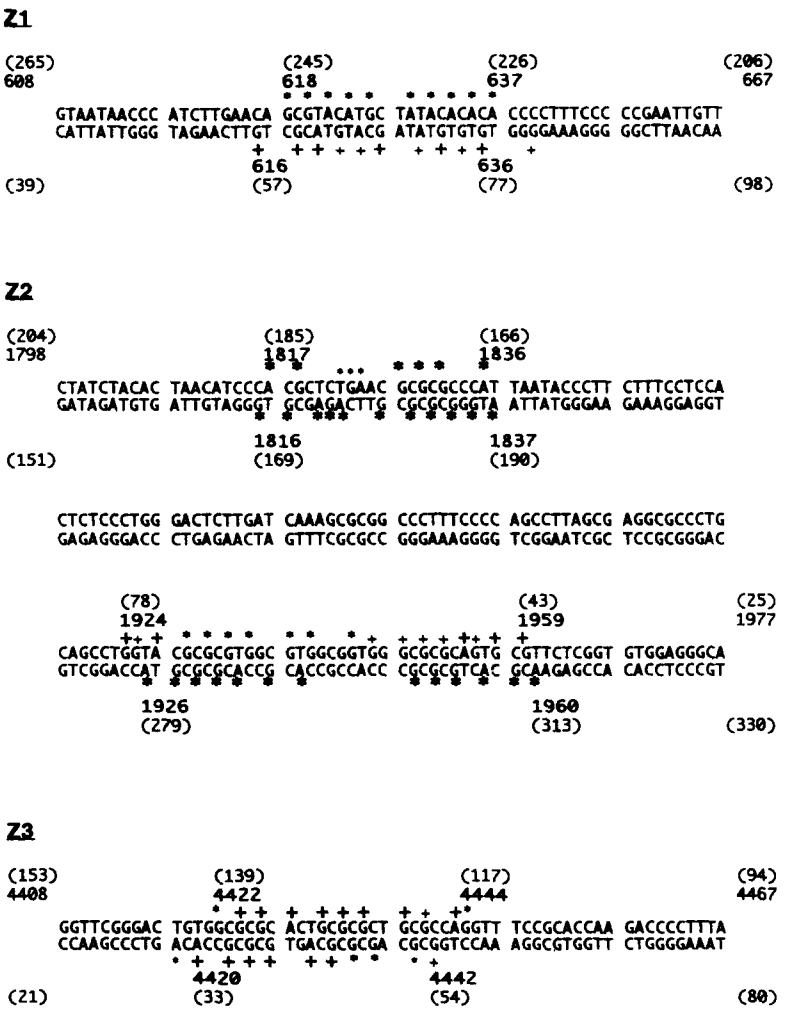

Fig. 3. Sequences of the c-*myc* inserts in pME.Z1, pME.Z2 and pME.Z3, containing the DEPC hyperreactive sites. Numbers in brackets indicate the distance to 3′-labels at *Hin*dIII or *Bam*HI restriction sites respectively. Numbers in plain text refer to positions in the *Hin*dIII-*Eco*RI segment of the human c-*myc* gene, this numbers correspond to the numbering in Fig. 1. DEPC hyperreactive areas are marked with (+) at positions with single base resolution, and (*) at purines in hyperreactive areas without single base resolution.

between guanosine-78 and guanosine-43. Here again hyperreactivity is not uniform throughout the marked area. The lower strand shows enhanced reactivity in the corresponding areas from '169 to 190' and from '278 to 313'. Dashed bracket-lines indicate separate areas with less DEPC reactivity within the hyperreactive areas. It is interesting that a clear hyperreactive site in the relaxed lane of the lower strand can be seen in a short purine stretch (5'-AGAG-3') inside the area from '169 to 190'.

The two panels in Fig. 2c show the DEPC treatment of the *Alu*I fragment Z3. Clear hyperreactivity is found between bases 139 and 116 in the supercoiled lane of the upper strand. G-137 is also hyperreactive in the relaxed circular plasmid. In the lower strand enhanced reactivity is found in the corresponding area. In both, upper and lower strand, hyperreactivity is more pronounced in the two $(GC)_3$ repeats. In the lower strand sequence compression prevents resolution of the central $(GC)_3$ repeat. It is characteristic in these naturally occurring Z-DNA sequences that the degree of DEPC hyperreactivity appears to vary somewhat.

The above results are summarized in Fig. 3 which shows the sequence of the DEPC hyperreactive sites of Z1, Z2 and Z3 together with flanking sequences. The position relative to the 3'-end label of the analyzed plasmid fragments is indicated by numbers in brackets. The positions in respect to the 8 kb *Hin*dIII-*Eco*RI c-myc fragment (Fig. 1) are indicated below. Stars indicate purine positions in DEPC hyperreactive sites without clear single base resolution and (+) indicates single purine bases with supercoiling dependent enhanced DEPC reactivity.

All supercoiling dependent hyperreactive areas contain alternating purine-pyrimidine stretches. This includes all sequence variations from GC through GT/AC to AT. Z1 contains one clear Z–Z junction (253/254; upper strand), that is, a region in which there are two Z-DNA forming segments out of phase with each other. This occurs at the position where two purines are next to each other on one strand and two pyrimidines on the other. Hyperreactivity at guanosine (57; lower strand) indicates a second Z–Z junction or is part of a B–Z junction. Similarly Z3 contains two clear internal Z–Z junctions (132/131 and 125/124; upper strand). The hyperreactivity at the boundaries (118; upper and 33; lower strand) might mark two more Z–Z junctions or extended B–Z junctions. In Z2 the two hyperreactive areas are 90 nucleotides apart. One region 37 nucleotides long contains five possible Z–Z junctions, three separated by only three nucleotides. The second is 20 nucleotides long containing a short $(GC)_3$ core element and an element of 5 alternating nucleotides.

3.2. Detection of B–Z junctions in the Z2 AluI restriction fragment

Another type of chemical reactivity is found at B–Z junctions. Several reagents, including potassium permanganate have been used to define this junction point [13]. Permanganate reacts with the C5, C6 portion of pyrimidine residues at the B–Z junction, and as such it is useful for defining the limit of Z-DNA formation. Potassium perman-

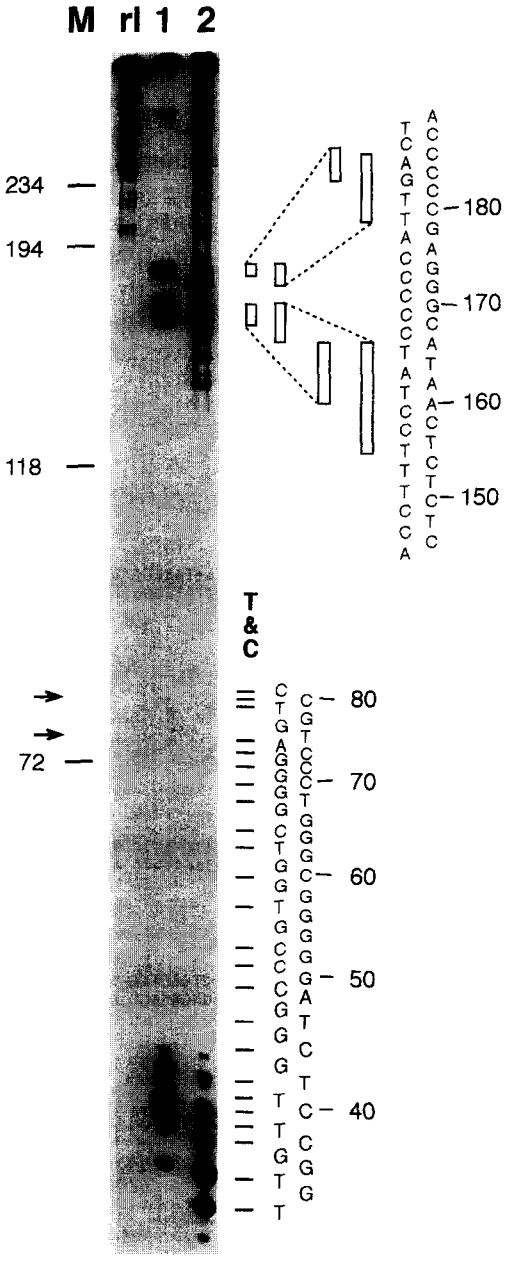

Fig. 4. $KMnO_4$ treatment of the upper strand of pME.Z2. Lane rl: relaxed circular plasmid; lane 1: supercoiled plasmid, mean superhelical density −0.08; lane 2: supercoiled plasmid, mean superhelical density −0.12. T&C shows a graphic representation of pyrimidine positions in the lower part of the gel. The sequence is shown in two parallel lanes to be followed in zigzag going from one lane to the other. Numbers indicate the distance to the ^{32}P label at the 3'-end of the DNA fragment. Upper part: to facilitate correlation of the sequence and the hyperreactive area in lanes 1 and 2, corresponding segments in lanes 1 and 2, and in the sequence are marked by vertical open bars. Lower part: the weaker $KMnO_4$ reactivity at the upper end of the Z-DNA stretch is indicated by horizontal arrows.

ganate was used to characterize the Z-DNA formation in the Z2 *Alu*I fragment because of the more complex character of the DEPC reactions, showing two hyperreactive elements. Fig. 4 shows the reactivity with potassium permanganate in relaxed plasmid and in two supercoiled plasmids (mean superhelical density −0.08 and −0.12). In both lanes containing supercoiled plasmid potassium permanganate reactivity can be seen, corresponding to the boundaries of the DEPC hyperreactive stretches. The signal at the thymine residue (79) is much weaker. The other boundaries show hyperreactivity at more than one position indicating longer stretches of single strandedness or more likely variations of the B–Z junction position in the plasmid population. An increase in superhelical density from −0.08 to −0.12 leads to a spreading of the permanganate reactivity. Indicating an extension of the Z-DNA formation or other changes at the B–Z boundary. The two prominent hyperreactive bands in lane 2 just below the boundaries of the upper Z-element are within a long stretches of pyrimidines interrupted twice by purine bases.

4. Discussion

The potential of forming Z-DNA at given positions within the genome is an important prerequisite to analyzing functions of Z-DNA in vivo. Using metabolically active permeabilized nuclei, we showed that three different restriction fragments in the human c-myc gene reacted with monoclonal antibodies against Z-DNA diffused into the nuclei [8]. The present experiment was designed to uncover the nucleotides in these fragments that converted to Z-DNA. When subjected to negative torsional strain, all three fragments yielded specific areas that reacted with DEPC, which has been used to identify segments of Z-DNA formation. The three different segments have elements in common. All have alternating purine pyrimidine stretches. The content of alternating G and C ranges from stretches of 8 to as little as two bases. All regions of hyperreactivity include other residues as well. In addition, they generally have Z–Z junctions, that is, segments of alternating purines and pyrimidines in which there is a change of phase so that in moving along one chain the sequence goes purine-pyrimidine-purine-pyrimidine-pyrimidine-purine-pyrimidine-purine.... These Z–Z junctions have been studied extensively, and require only a relatively small amount of energy for stabilization [18].

Compared to other Z-DNA forming elements studied by DEPC reactivity, the sequences analyzed here contain quite a large number of base pairs not ideal for Z-DNA formation, including alternating A and T and bases out of alternation. Thus, Z-DNA formation is not as stable as in simple GC or GT/AC repeats. Negative superhelical density of eukaryotic DNA is estimated to be about −0.06, but in cell nuclei most of this torsional stress is masked in the chromatin [19]. Transcription and replication influence the level of supercoiling in vivo. However, the local level of torsional stress actually present in vivo is difficult to analyze. The amount of negative supercoiling required to detect Z-DNA formation in the fragments studied here is above a superhelical density of −0.04. At −0.04 no Z-DNA specific DEPC reactivity was found. Reactivity with DEPC was observed at bacterial superhelical density and increased somewhat at higher superhelical densities (−0.08 and −0.12) (data not shown.) In Z3, which consists of two long stretches of alternating GC, no Z-DNA specific reactivity is found at a superhelical density of −0.04, but strong DEPC reactivity could be readily observed at bacterial superhelical density [20]. This indicates a slightly different kinetic profile for Z-DNA formation depending on the sequence composition. Formation of Z-DNA in this element in vivo, therefore, requires free negative superhelical densities above the transition level which is between −0.04 and −0.06. This amount is higher than negative torsional tension of −0.02 to −0.03 reported for the promoter area of the dihydrofolate reductase gene that was deduced from in vivo psoralen photocrosslinking experiments [21]. The value of the psoralen experiment relates to a 2 kb large segment. The negative supercoiling that is required to stabilize Z-DNA can be confined to a small area just around the Z-DNA forming element. Thus, Z-DNA formation in naturally occurring sequence elements can be used to assay local levels of negative supercoiling in vivo.

Theoretical calculations about the sequences most likely to form Z-DNA in the c-*myc* gene pointed to three segments in these three restriction fragments. The three segments that were predicted were those found in Z1 and Z3, plus the larger region found in Z2. It was quite surprising that we observed another segment in Z2 90 nucleotides away. Because of this, we used potassium permanganate to map the B–Z junctions. These reactions show that both DEPC reactive areas are flanked by positions accessible for potassium permanganate indicating that there are two separate Z-DNA forming elements. It is interesting that increases in negative superhelicity shifts the positions of $KMnO_4$ reactivity and that most of the B–Z junctions extend over several bases. Higher negative supercoiling may stabilize part of the boundary sequence in the Z-form giving rise to a larger Z-DNA stretch including less favorable sequences. The large number of reactive bases either shows extended B–Z junctions or rapid fluctuations of the junction positions, making all possible junction positions accessible to potassium permanganate. Alternatively, there could also be different junction positions in the plasmid population.

There have been comparatively few regions of naturally occurring Z-DNA in which possible Z-forming segments have been identified at the nucleotide level [16,17,22]. In the human c-*myc* gene three regions have been shown to form Z-DNA in metabolically active nuclei, two in the upstream promoter region and the third in a region at the

junction of the first intron and the second exon. In the later case the actual sequence that forms Z-DNA is in the intron itself, 60 nucleotides away from the intron/exon boundary.

In all three elements Z-DNA formation is detected by antibody binding only when the gene is actively transcribed. When the U-937 cells are induced to go down a differentiation pathway toward becoming a macrophage, the c-*myc* gene is down-regulated and within a few hours no reactivity with these antibodies is observed. In short, Z-DNA formation appears to be associated with an actively transcribed gene rather than a quiescent gene. The mechanism for this formation is believed to be associated with the negative torsional strain induced upstream of the moving RNA polymerase [6]. This leads to increased levels of negative supercoiling upstream of the gene but also within the transcribed gene. Thus, transcription is sufficient to stabilize Z-DNA in all three elements. When the polymerase is no longer moving, this torsional strain is released by the action of topoisomerase I fairly quickly.

The material presented here are examples of naturally occurring Z-DNA sequences elements that do not consist entirely of repetitive GC or AC/GT. The observed changes in reactivity with the chemical reagents used to map the Z-DNA sequences might be due to some structural deviations from the Z-DNA structures seen with simple repeats. They are likely to be sequence related. As variations in the reactivity in the relaxed plasmid. Different from other studies we used relaxed circular plasmids instead of linear DNA for control reactions. This results underline the possibility, that a large number of naturally occurring sequences may be prone to form Z-DNA in vivo.

These experiments have served to define the precise nucleotide sequences that form Z-DNA in the c-*myc* gene. They illustrate the extent to which DNA is conformationally labile in that it can assume different conformations, depending upon the physiological state of the gene. These different conformational states are governed by the detailed nucleotide sequence found in the regions of the gene. The physiological consequences of these conformational changes are currently being investigated, and the results will be reported elsewhere.

Acknowledgements

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