S1 Nuclease Recognizes DNA Conformational Junctions between Left-handed Helical \((dT-dG)_n\)•(dC-dA)_n and Contiguous Right-handed Sequences*

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The ability of negative supercoiling to induce a left-handed helix in the recombinant plasmid pRW777, which contains a tract of 64 base pairs of almost perfect \((dT-dG)_n\)•(dC-dA)_n from the mouse \(\kappa\) immunoglobulin gene, was studied. S1 nuclease recognizes and cleaves within the junction region which must exist adjacent to the \((dT-dG)_n\)•(dC-dA)_n tract when in a left-handed state. The cleavage pattern indicates conformational flexibility and structural differences between the two existing junctions. The 64-base pair alternating copolymer undergoes the supercoil-induced formation of a left-handed state over the superhelical density range of -0.04 to -0.06, indicating that \((dT-dG)_n\)•(dC-dA)_n sequences form a left-handed helix less readily than \((dT-dG)_n\)•(dC-dA)_n sequences of equivalent length. However, these supercoil densities are within the range found in vivo. Supercoil relaxation and antibody binding studies confirmed that the \((dT-dG)_n\)•(dC-dA)_n tract in supercoiled pRW777 was in a left-handed helix.

The B to Z DNA structural transition has been demonstrated with oligonucleotides (reviewed in Refs. 1–3) as well as with restriction fragments and recombinant plasmids which contain a segment of the perfectly alternating C and G sequence (4–10). Previously, studies were performed on \((dT-dG)_n\)•(dC-dA)_n, the polymer containing 5-methyl C, and a restriction fragment containing a naturally occurring tract of 64 bp of dC-dA to evaluate the capacity of such sequences to adopt a left-handed helix (11). This sequence formed a left-handed structure as indicated by CD measurements, but only when the dG residues were fully modified by reaction with N-acetoxy-N-acetyl-2-aminofluorene and the modified DNA is in concentrated salt solutions. Other CD studies (12, 13) as well as fiber X-ray diffraction investigations on the polymer agree with this interpretation (reviewed in Ref. 1).

The presence of a region of left-handed helix within a DNA which is otherwise right-handed (4, 6–10) necessitates a conformational junction. It was found (4, 6, 8–10) that the torsional strain of negative supercoiling was sufficient to cause the formation of left-handed helices in \((dC-dG)_n\)•(dC-dG)_n, \((dC-dA)_n\)•(dC-dA)_n, and a tract under physiological salt concentrations and at negative supercoil densities (0.03–0.07) which are within the range found for the majority of supercoiled DNAs (14). We discovered (4) that the junctions are specifically recognized and cleaved by the single strand specific S1 nuclease and thus have developed an assay for the presence of \((dC-dG)_n\) junctions when the CG region is left-handed (6).

This assay (4) was used herein to investigate the ability of negative supercoiling to induce a left-handed helix in the \((dT-dG)_n\)•(dC-dA)_n regions of pRW777 and to determine if S1 nuclease also cleaves at the junctions which were generated. This plasmid (11) contains an insertion into pBR322 of a 105-bp fragment derived from the \(3\)' side of the mouse \(\kappa\) immunoglobulin gene containing a region of left-handed (14). The fourth dG–dC pair is substituted by a dA–dT pair, thus preserving the alternating purine–pyrimidine sequence. Our results demonstrate that S1 nuclease does recognize and cleave within junction regions between left-handed \((dC-dG)_n\)•(dC-dA)_n, \((dC-pA)_n\) sequences and nonalternating B-DNA sequences. Furthermore, the junctions have different extents of conformational flexibility as measured by nuclease sensitivity.

Preliminary accounts of these results were described (15–17); other workers (18, 19) reached similar conclusions.

MATERIALS AND METHODS

DNA and Enzymes—Plasmid DNA was isolated as described (11). S1 nuclease preparation and characterization was as described (20). Restriction enzymes were from Bethesda Research Laboratories. Topoisomeric samples of pRW777 (11) were generated and characterized essentially as previously reported (21). The mung bean nuclease was isolated and characterized as reported (22).

Nuclease Reactions—S1 nuclease reactions were carried out as follows: 1.5 \(\mu\)g of DNA were incubated at 37°C for 50 min in the presence of 0.08 units of S1 nuclease in 50 \(\mu\)l of 40 mM Na acetate, 50 mM NaCl, 1 mM ZnSO4, pH 4.6. Reactions were terminated by adding 0.2 \(\mu\)l of 0.5 M EDTA, pH 7.7, followed by dialysis against 15 mM Tris–Cl, 6 mM MgCl2, 2.5 mM dithiothreitol, 6 mM NaCl, pH 7.7. After dialysis, 1 unit of either HindIII or HinfI was added and the samples were incubated at 37°C for 4 h.

Samples were electrophoresed in 2% agarose gels (80 mM Tris acetate, 40 mM Na acetate, 2.5 mM EDTA, pH 8.3, for 5 h at 175 volts. Microdensitometric tracing of a photographic negative of the electrophoretic separations has been described (4, 10).

Supercoil Relaxation Studies—Two-dimensional gel electrophoresis was performed essentially as described (23). Electrophoresis in the first dimension was in a 1.5% agarose gel in 80 mM Tris acetate, 40 mM Na acetate, 2.5 mM EDTA, pH 8.3, at 125 V for 55 h. The strip containing the ladder of separated topoisomers was then cut from the gel, soaked for 1 h in the same buffer plus 1.25 \(\mu\)M chloroquine (Sigma), then embedded in a 1.5% agarose gel containing 1.25 \(\mu\)M chloroquine. The second dimension electrophoresis was then performed at right angles to the first dimension in the chloroquine
RESULTS AND DISCUSSION

S1 Nuclease Cleavage of Junctions—S1 nuclease was used to probe for conformational junctions which would exist if the (dT-dG)$_{32}$-(dC-dA)$_{32}$ region of pRW777 were stabilized in a left-handed state by the torsional strain of negative supercoiling. Fig. 1a shows the gel electrophoretic separation of the DNA fragments generated when either pRW777 or the control plasmid pBR322 was treated first with S1 nuclease followed by cleavage with HindII. Both plasmids were at an initial superhelical density of approximately 0.06 under S1 nuclease reaction conditions. For pBR322, two S1 nuclease specific fragments were found at about 870 and 2400 bp. These fragments represent S1 nuclease cleavage at the major inverted repeat of pBR322 (24, 25) which lies within the 3255-bp HindII fragment. Four other S1 nuclease specific fragments ranging from 696 to 513 bp were found for pRW777. These two sets of doublet bands were as expected if the (dT-dG)$_{32}$-(dC-dA)$_{32}$ region was in a supercoil-induced left-handed state. This type of doublet pattern was shown (4) to be characteristic for left-handed (dC-dG)$_a$-(dC-dG)$_b$ sequences abutting right-handed regions. Indeed, the four bands which possess one HindII terminus and one S1 nuclease-cleaved terminus have the expected lengths (±4%) if S1 cleavage occurred at the ends of the (dT-dG)$_a$-(dC-dA)$_b$ sequences (Fig. 1b).

Effect of Supercoil Density—Supercoiling causes a sharp transition from a right to a left-handed helix in (dC-dG) segments of recombinant plasmids (4, 6, 8–10). Topoisomeric samples of pRW777 were generated and characterized as described (21) to evaluate this behavior with (dT-dG)$_a$-(dC-dA)$_b$. Each sample was treated with S1 nuclease followed by HindII digestion and the products were separated (Fig. 2a). The mean negative superhelical density of the starting pRW777 increases from left to right (lanes 1–9); lane 10 is the pBR322 control. Fig. 2a shows that the S1 nuclease specific sets of doublet bands appear in increasing intensity as the negative superhelical density increases. Fig. 2b shows a plot of the percent specific cleavage versus supercoiling. The demonstrated transition represents a supercoil induced shift in the equilibrium between the right and left-handed helical states for the (dT-dG)$_{32}$-(dC-dA)$_{32}$ sequences. Two other assays for left-handed DNA (described below) confirm this interpretation. Formation of left-handed DNA causes a loss (relaxation) of negative supercoils (about two supercoils lost/turn of helix converted from B to Z) which allows a

mapping studies with HindII and with AvoII agree with this interpretation.

FIG. 1. pRW777 treated with S1 nuclease followed by cleavage with restriction endonuclease HindII. a, pRW777 (second) and pBR322 (third) initially possessed mean superhelical densities of −0.058 and −0.060, respectively, before S1 and HindII treatments. The samples were electrophoresed in a 2% agarose gel. The first and fourth contain size markers (from top to bottom): 1133, 960, 880, 610, 472, 448–436, 413, and 366 bp. The lengths of the HindII fragments of pRW777 (3255 and 1216 bp) are indicated in parentheses. The lengths (±4%) found for the S1 specific fragments are also given; sizes were determined by using the markers of known lengths (29). b, the top line represents the 1216-bp HindII fragment of pRW777 which contains the alternating (dC-dA)$_a$-(dT-dG)$_b$ region. The number of bp from the HindII sites to the first residue in the alternating dinucleotide sequence are given. The arrow represents the T-A substitution for C-G at the fourth repeat from the left. The value in parentheses indicates the distance to the (dT-dA) interruption. The lower two lines represent the mapping of the S1 nuclease cleavage sites as determined from a.

FIG. 2. Sensitivity of topoisomeric samples of pRW777 to S1 nuclease. a. S1 nuclease treatment of topoisomeric samples of pRW777 followed by digestion with HindII. The lengths (bp) of the HindII fragments are indicated. The S1 nuclease specific bands are indicated by brackets. The mean negative superhelical density (under S1 nuclease reaction conditions) of each sample is as follows: 0.033, lane 1; 0.037, lane 2; 0.043, lane 3; 0.047, lane 4; 0.052, lane 5; 0.057, lane 6; 0.061, lane 7; 0.068, lane 8; 0.077, lane 9. Lane 10 is the control plasmid, pBR322 (superhelical density of −0.06), treated in an identical manner. b. A plot of specific cleavage by S1 nuclease versus the mean negative superhelical density (−σ) of the pRW777 samples. A photographic negative of a was traced using a Joyce-Loebl microdensitometer (under conditions of linearity). Specific cleavage represents the ratio of the area of the top S1 nuclease specific doublet to the area of the 396-bp band divided by the molar ratio (representing 100% cleavage) of these bands based on their calculated lengths.
decrease in the free energy of the supercoil state. Since the decrease in free energy is quadratically related to increasing negative supercoiling (26, 27), the transition found (Fig. 2b) is as expected (4, 10). A plateau of S1 nuclease cleavage around 25% is consistent with plateau levels (20-33%) for the supercoil-induced left-handed state for (dC-dG)n blocks (4, 10).

Thus, these results demonstrate that the torsional strain of negative supercoiling causes the formation and stabilization of a left-handed helical state in regions of (dT-dG)-(dC-dA). A titratable negative superhelical density of about 0.06 (under S1 nuclease reaction conditions) is required to completely stabilize the (dT-dG)n (dC-dA)n block of pRW777 in a left-handed state (in good agreement with supercoil relaxation studies described below). The precision (± 4%) of our mapping of the S1 nuclease cleavage sites does not reveal if the dA-dT interruption near one end is within the left-handed portion of this region.

Conformational Flexibility of Junctions—S1 nuclease (Figs. 1 and 2) showed a marked preference for cleavage within one junction (the right hand junction of Fig. 1b) as compared to the other (left hand) junction. Moreover, this preference became more pronounced as the negative supercoiling increased (Fig. 2c). A similar sequence and supercoil dependent junctional flexibility has been found for (dC-dG)n (dC-dA)n junctions (4, 10). Thus, conformational flexibility within the junction regions appears to be independent of the type of sequence undergoing the right-handed to left-handed transition and must be related to the neighboring base pair sequence (10). This distinctive hierarchy of S1 nuclease susceptibility indicates sequence-dependent conformational efforts within the junctions. An understanding of this behavior must await further detailed structural studies since evaluation of the sequences at the junctions does not reveal an explanation. However, it should be emphasized that even x-ray crystallographic analysis to atomic resolution on one junction will be only partially informative since each junction seems to respond differently to supercoiling as measured by S1 nuclease sensitivity.

Other Single Strand Specific Nucleases as Probes—The ability of other single strand specific nucleases to interact with junction regions was compared. We have shown that another single strand specific nuclease, the T7 gene three protein (24), will not cleave within junctions flanking (dC-dG)n (dC-dG)n, sequences when in a left-handed state (4). Similarly, this nuclease did not cleave the junctions existing within pRW777, even at very high negative superhelical densities (data not shown). In contrast, mung bean nuclease will recognize and cleave within (dC-dG)n (dC-dG)n, junctions but not within (dT-dG)n (dC-dA)n junctions.2 Thus, the use of several different nucleases further emphasizes the conformational differences which exist between various junctions between different types of right and left-handed DNA. Prior studies (Ref. 28 and reviewed in Refs. 1–3) have thoroughly demonstrated the existence of families of both left-handed and right-handed DNA helices.

The BAL31 nuclease has recently been shown (29) to specifically recognize and cleave the salt-induced junctions between right and left-handed-helices. The advantages of this nuclease are its pH optimum near neutrality and its insensitivity to very high ionic conditions. Unfortunately, the BAL31 enzyme is not a useful probe for the types of junctions in pRW777 since (dT-dG)n (dC-dA)n is only partially left-handed in saturated NaCl solution (11) and the presence of an exonucleolytic activity (29) precludes its use as a high resolution (to within a few base pair) tool.

In summary, S1 nuclease is the most sensitive and generally useful probe for these junctions, and thus for left-handed DNA, as compared to these other nucleases.

Since three single strand specific nucleases, isolated from widely different sources, specifically cleave conformational junctions (albeit with different recognition features), we conclude that the structural aberrations possess elements of non-helical structure as found in random coil polynucleotides.

Supercoil Relaxation of pRW777—Two-dimensional gel electrophoresis studies (23) were performed on a family of topoisomers of pRW777 in order to demonstrate directly the presence of a left-handed segment. This assay is similar conceptually to the one-dimensional electrophoretic assay for the supercoil-induced B to Z transition used previously by this laboratory (4–7, 10, 28, 30) but has the advantage of providing higher resolution, especially for topoisomers with greater supercoil densities. Fig. 3 shows the mobilities in the first dimension of topoisomers of pRW777 and pRW451, a control plasmid, as determined by this technique. For pRW451, a smooth curve of increasing mobility with decreasing linking number is observed. For pRW777, however, a sharp break in this pattern is observed between the topoisomer with 20 negative supercoils (topoisomer -20) and that with 21 negative supercoils (topoisomer -21). Instead of having a greater mobility than topoisomer -20, topoisomer -21 migrates at the same rate as topoisomer -19 in the first dimension. Similarly, topoisomer -22 co-migrates with topoisomer -17 and topoisomers -23, -24, -25, -26, and -27 co-migrate with topoisomer -16 in the first dimension. The presence of chloroquine in the second dimension reduces the degree of supercoiling of the topoisomers. Thus, under the conditions of electrophoresis in the second dimension, the (dT-dG)n (dC-dA)n tract in, for example, topoisomer -27 reverts from a left-handed to a right-handed helical form and 2J. Klysik and R. D. Wells, unpublished results.

Fig. 3. Relative mobility in the first dimension of topoisomers of pRW777 and pRW451 as determined by two-dimensional gel electrophoresis. Mobilities were determined relative to the most relaxed topoisomer. Topoisomer mobilities from different experiments were normalized using the distance between the most relaxed topoisomer and the topoisomer with 10 negative supercoils (topoisomer -10) as a normalization factor. Mobilities were determined from three separate experiments for each plasmid. r = number of supercoils, thus the values to the right of zero represent negative supercoils (i.e. right-handed supercoils). 0, pRW777; X, pRW451, a control plasmid (5) containing the 174/1p HhaI fragment from pBR322 cloned into the filled in BamHI site of pBR322. Other details are described under "Materials and Methods."
its mobility in this dimension is normal (slightly faster than topoisomer -26). It is apparent that the superhelical density of topoisomer -21 is sufficient to initiate the right to left-handed transition in the (dA-dC)32-(dT-dG)32 tract and that the superhelical density of topoisomer -27 is required before the maximum relaxation of 11 supercoils is observed. A relaxation of 11 supercoils agrees well with the 11.6 superhelical turns that would be relaxed if the (dT-dG)32-(dC-dA)32 tract underwent a transition from a right-handed B-helix to a left-handed Z-helix. However, junction regions must exist between the right and left-handed helices, and it is quite possible that this (dT-dG)32-(dC-dA)32 region adopts some other type of left-handed helical conformation. Also, the conclusion of a relaxation of 11 supercoils makes the assumption that a topoisomer with -16 superhelical turns will have the same electrophoretic mobility in the first dimension as a topoisomer with -16 superhelical turns and a region of left-handed helicity, i.e., that the left-handed region does not substantially affect the electrophoretic mobility. This assumption appears to be reasonable, especially since the left-handed region comprises < 1.5% of the plasmid. The superhelical density of the topoisomers over which the relaxation of supercoils is observed (~0.044 to 0.060) is in excellent agreement with the superhelical density range over which the (dT-dG)32-(dC-dA)32 region undergoes the right to left-handed transition as detected by the S1 nuclease cleavage of junction regions (Fig. 2b). Any difference between the two could be due to the different ionic conditions and/or pH at which the two sets of experiments were performed.

For topoisomers -21 through -26, it can be seen that less than the maximum observed number of 11 superhelical turns are relaxed. Three possible interpretations of this are, first, for these topoisomers some, but not all, of the (dT-dG)32-(dC-dA)32 region is in a left-handed conformation. Second, for these topoisomers the (dT-dG)32-(dC-dA)32 region adopts intermediate conformations. Third, these topoisomers represent time averaged equilibria between the right and left-handed state of the (dT-dG)32-(dC-dA)32 region, where the transition between the two states is fast compared to the time of electrophoresis. The fact that S1 cleavage is always observed in the same position on the DNA throughout the range of superhelical density at which the right to left-handed transition occurs (Fig. 2a) favors the third interpretation, or possibly the second interpretation if the intermediate conformations extended throughout the (dT-dG)32-(dC-dA)32 region.

The use of this supercoil relaxation assay to monitor B to Z structural changes, in addition to mapping the junctions with a single strand specific nuclease, is important since other types of supercoil-induced structural changes (i.e. cruciforms, nonpaired loops due to slippage, etc.) have been reported (24, 25, 31-33). Also, this relaxation assay reveals that cruciform formation is unlikely since the extent of relaxation would be approximately half of that found; moreover, there is no apparent reason why this sequence should form a cruciform. Furthermore, the antibody binding studies (described below) confirm our conclusions on the formation of left-handed helices.

Energetics of Transition—As stated, a negative superhelical density of 0.06 was required to complete the right to left-handed helical transition for the (dT-dG)32-(dC-dA)32 block of pH7777. From previous data (10), we can estimate that this level of supercoiling would completely stabilize a (dC-dG)32-(dC-dG)32 tract in a left-handed state when n > 8 (assuming a similar free energy of junction formation for both copolymers). Thus, the (dC-dG) copolymer much more readily adopts the supercoil-induced left-handed state than the (dT-dG)32-(dC-dA)32 polymer.

Along with the free energy of superhelix formation (26, 27) and our previous estimates of about 5 kcal/mol for the junction free energy (10, 30), these data can be used to estimate the free energy difference between the right and left-handed state for the 64-bp copolymer of pRW7777. At the transition midpoint (~0.049), the free energy difference will be zero between this sequence in a right-handed state with the plasmid at this density and the copolymer tract in a left-handed state with the plasmid at a density corresponding to a loss of 11.6 turns (5). Thus, after subtracting the $\Delta G$ of junction formation, the $\Delta G_{plasm}^{\star}$ is simply equal to the negative of the free energy difference between the two supercoiled states of the plasmid. This calculation gives an estimate of $\Delta G_{plasm}^{\star} = 0.70$ kcal/mol of bp for the (dT-dG)32-(dC-dA)32 tract. Consistent with the above comparison, this value is significantly higher than the 0.44 kcal/mol of bp estimate for (dG-dC)32-(dG-dC)32, containing plasmids where n = 10, 28, and 32 (30).

Antibody Binding Studies—The capacity of pRW7777 to bind antibodies, both polyclonal and monoclonal, raised versus brominated (dG-dC)32-(dG-dC)32, was tested by the gel retardation assay of Pohl et al. (34). When pRW7777 was supercoiled at a density of approximately -0.075, substantial binding was observed to polyclonal antibodies (data not shown). However, when the plasmid was relaxed, no binding was found. Identical results were observed for pRW751 which has been demonstrated to contain regions of left-handed helices by a number of techniques (6). When pRW7777 (at supercoil densities of either 0 or -0.075) was tested for its capacity to bind monoclonal antibodies, no gel retardation was found. Other studies (35) have revealed that different monoclonal antibodies interact with various parts of helices and probably recognize differences between types of left-handed helices. Hence, this suggests that the TG-CA tract in pRW7777 is in a left-handed structure which differs from the canonical Z-structure found for short oligomers of (dC-dG) (reviewed in Refs. 1-3).

While this manuscript was in preparation, two papers appeared which extend our previous work (11, 15-17) on the left-handed properties of (T-G)32-(C-A)32 sequences. Polyclonal antibodies were shown (18) to bind to negatively supercoiled, but not relaxed, pAN064 (which is similar to pRW7777 (11)) and supercoiled relaxation studies were reported (19) on pDH14, a pBR322 containing a polylinker derivative containing (dT-dG)32-(dC-dA)32 polymer. The experiments described herein with a different plasmid are in excellent agreement conceptually with those data (18, 19) but utilize the approaches of both papers (as well as the use of monoclonal antibodies) and furthermore evaluate the properties of the junctions between the left and right-handed segments.

Biological Role—The biological role of the 64-bp (dT-dG)32-(dC-dA)32 tract which occurs approximately 300 bp on the downstream side of the mouse R2 V-region (36) is uncertain. Similar sequences which appear to be an evolutionarily conserved repeat family in Xenopus, pigeon, slime mold, yeast, mouse, and human have been found between the human $\delta$ and $\beta$ globin genes (37). This copolymer tract was found also in the spacer of a sea urchin histone gene repeat (32), at boundaries between human fetal globin genes which neighbor short tracts (n = 3) of (dG-dC)32-(dG-dC)32, and in telomeric regions of chromosomes (39). One of the introns of the human cardiac muscle actin gene contains 50 alternating dT

3 C. H. Singleton, M. W. Kilpatrick, and R. D. Wells, unpublished results.
4 D. Zarlino and T. M. Jovin, unpublished data.
Left-handed (dT·dG)$_n$, (dC·dA)$_n$

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REFERENCES

1. Wells, R. D., Goodman, T. C., Hillen, W., Horn, G. T., Klein, R. D., Larson, J. E., Muller, U. R., Neuendorf, S. K., Panayotatos, N., and Stirdvant, S. M. (1980) Prog. Nucleic Acid Res. Mol. Biol. 24, 167–267
2. Zimmerman, S. B. (1982) Annu. Rev. Biochem. 51, 395–427
3. Dickerson, R. E., Drew, H. R., Conner, B. N., Wing, R. M., Fratini, A. V., and Kopka, M. L. (1985) Cold Spring Harbor Symp. Quant. Biol. 216, 475–485
4. Singleton, C. K., Klysik, J., Stirdvant, S. M., and Wells, R. D. (1982) Nature (Lond.) 299, 312–316
5. Klysik, J., Stirdvant, S. M., Larson, J. E., Hart, P. A., and Wells, R. D. (1981) Nature (Lond.) 290, 672–677
6. Wells, R. D., Brennan, R., Chapman, K. A., Goodman, T. C., Hart, P. A., Hillen, W., Kellogg, D. R., Kilpatrick, M. W., Klein, R. D., Klysik, J., Lambert, P. F., Larson, J. E., Miettietta, J. J., Neuendorf, S. K., O’Connor, T. R., Singleton, C. K., Stirdivant, S. M., Veneziale, C. M., Wartell, R. M., and Zacharias, W. (1982) Cold Spring Harbor Symp. Quant. Biol. 47, 77–84
7. Stirdvant, S. M., Klysik, J., and Wells, R. D. (1982) J. Biol. Chem. 257, 10159–10165
8. Peck, L. J., Nordheim, A., Rich, A., and Wang, J. C. (1982) Proc. Natl. Acad. Sci. U. S. A. 79, 4560–4564
9. Nordheim, A., Lafer, E. M., Peck, L. J., Wang, J. C., Stollar, B. D., and Rich, A. (1982) Cell 31, 308–318
10. Singleton, C. K., Klysik, J., and Wells, R. D. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 2447–2451
11. Wells, R. D., Miglietta, J. J., Klysik, J., Larson, J. E., Stirdvant, S. M., and Zacharias, W. (1982) J. Biol. Chem. 257, 10166–10171
12. Vorlickova, M., Kyvr, V., Stokrova, S., and Sponar, J. (1982) Nucleic Acids Res. 10, 1071–1080
13. Zimmer, C., Tymen, S., March, C., and Guschlbauer, W. (1982) Nucleic Acids Res. 10, 1081–1091
14. Bauer, W. R. (1978) Annu. Rev. Biophys. Bioeng. 7, 287–313
15. Wells, R. B., Erlanger, B. F., Gray, H. B., Jr., Hanau, L. H., Jovin, T. M., Kilpatrick, M. W., Klysik, J., Larson, J. E., Martin, J. C., Miglietta, J. J., Singleton, C. K., Stirdivant, S. M., Veneziale, C. M., Wartell, R. M., Wei, C. F., Zacharias, W., and Zarling, D. (1983) UCLA Symp. Mol. Cell. Biol. in press
16. Wells, R. D., Erlanger, B. F., Gray, H. B., Jr., Hanau, L. H., Jovin, T. M., Kilpatrick, M. W., Klysik, J., Larson, J. E., Martin, J. C., Miglietta, J. J., Singleton, C. K., Stirdivant, S. M., Veneziale, C. M., Wartell, R. M., Wei, C. F., Zacharias, W., and Zarling, D. (1983) UCLA Symp. Mol. Cell. Biol. in press
17. O’Connor, T., Kilpatrick, M. W., Klysik, J., Larson, J. E., Martin, J. C., Singleton, C. K., Stirdivant, S. M., Zacharias, W., and Wells, R. D. (1983) Conversations in Biomolecular Stereo-dynamics III (Sarasa, R. H., ed) Adenine Press, Guildenr, NY, in press
18. Nordheim, A., and Rich, A. (1983) Proc. Natl. Acad. Sci. U. S. A. 80, 1821–1825
19. Hanford, D. B., and Pulleyblank, D. E. (1983) Nature (Lond.) 302, 632–634
20. Dodgson, J. B., and Wells, R. D. (1977) Biochemistry 16, 2374–2379
21. Singleton, C. K., and Wells, R. D. (1982) Anal. Biochem. 122, 253–257
22. Chen, H. W., Doig, A., and Wells, R. D. (1977) Biochemistry 16, 2367–2374
23. Wang, J. C., Peck, L. J., and Becherer, K. (1982) Cold Spring Harbor Symp. Quant. Biol. 47, 85–91
24. Panayotatos, N., and Wells, R. D. (1981) Nature (Lond.) 289, 466–470
25. Lilley, D. M. J. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 6468–6472
26. Pulleyblank, D. E., Shure, M., Tang, D., Vinograd, J., and Vosberg, H.-P. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 4280–4284
27. Depew, R. E., and Wang, J. C. (1975) Proc. Natl. Acad. Sci. U. S. A. 72, 4725–4729
28. Zacharias, W., Larson, J. E., Klysik, J., Stirdivant, S. M., and Wells, R. D. (1982) J. Biol. Chem. 257, 2775–2782
29. Kilpatrick, M. W., Wei, C. F., Gray, H. B., and Wells, R. D. (1985) Nucleic Acids Res. 11, 3811–3822
30. Klysik, J., Stirdvant, S. M., Singleton, C. K., Zacharias, W., and Wells, R. D. (1983) J. Mol. Biol. 168, 51–71
31. Singleton, C. K. (1983) J. Biol. Chem. 258, 7661–7668
32. Hentschel, C. C. (1982) Nature (Lond.) 295, 714–716
33. Larsen, A., and Weintraub, H. (1982) Cell 29, 609–622
34. Pohl, F. M., Thomas, R., and DiCapua, E. (1982) Nature (Lond.) 300, 545–546
35. Moller, A., Grabriels, J. E., Lafer, E. M., Nordheim, A., Rich, A., and Stollar, B. D. (1982) J. Biol. Chem. 257, 12081–12085
36. Nishioka, Y., and Leder, P. (1980) J. Biol. Chem. 255, 3691–3694
37. Miesfeld, R., Krystal, M., and Arnheim, N. (1981) Nucleic Acids Res. 9, 5931–5947
38. Slighlom, J. L., Blechel, A. E., and Smithies, O. (1980) Cell 21, 627–638
39. Watsley, R. M., Szostak, J. W., and Petes, T. D. (1983) Nature (Lond.) 302, 84–86
40. Hanada, H., and Kakunaga, T. (1982) Nature (Lond.) 298, 396–398
41. Shil, S., Slighlom, J. L., and Smithies, O. (1981) Cell 26, 191–203
S1 nuclease recognizes DNA conformational junctions between left-handed helical (dT-dG)n, (dC-dA)n and contiguous right-handed sequences.

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