Conformational flexibility of junctions between contiguous B- and Z-DNAs in supercoiled plasmids

(left-handed DNA/protein recognition/DNA structure/effect of (dC-dG)_n length/S1 nuclease)

CHARLES K. SINGLETON, JAN KLYSIK, AND ROBERT D. WELLS

Department of Biochemistry, Schools of Medicine and Dentistry, University of Alabama in Birmingham, Birmingham, Alabama 35294

Communicated by Bruno H. Zimm, January 6, 1983

ABSTRACT The junctions between contiguous right- and left-handed helical DNA segments in supercoiled plasmids are shown to exhibit both sequence- and superhelical-dependent conformational flexibility. The nonalternating (purine-pyrimidine) tetranucleotide d(G-A-T-C), which is imbedded within a (dC-dG) tract and exists as a junction at low-density values, may be adopting a left-handed helix under the influence of higher negative supercoiling. The effect of supercoiling on the transition from a right- to a left-handed state for (dC-dG)_n inserts, where *n* = 16, 13, and 5, also was evaluated. Only moderate amounts of superhelicity (−0.03 to −0.075) are required for formation and stabilization of left-handed DNA even for the (dC-dG) region of 10 dC-dG base pairs. Finally, we demonstrate that junction formation within a *Bam*HI restriction site causes a drastic inhibition (>80%) of cleavage by this restriction endonuclease.

The torsional strain of negative supercoiling can cause the right- to left-handed helical transition in alternating (dC-dG)_n sequences contained within recombinant plasmids (1–3). This recent finding of supercoil-stabilized left-handed DNA is important because of the biological significance of superhelicity (4–6). Contiguous right- and left-handed DNA sequences must have a junction region between them. Physical, topological, and enzymatic studies suggest that the junction region is small, is partially single stranded, and exists within the nonalternating (dC-dG) sequences (1–3, 7, 8). We have demonstrated that S1 nuclease recognizes and cleaves within the junction region (1); thus, S1 nuclease serves as a convenient probe for junctions and consequently for left-handed DNA imbedded within right-handed DNA.

Herein, we examine several features of the supercoil-induced left-handed state of (dC-dG)_n regions and the concomitant junction regions. (dC-dG)_n length effects are studied (*n* = 16, 13, and 5), and we show that junction formation within a *Bam*HI restriction site effectively inhibits cleavage by *Bam*HI endonuclease. Also, by utilizing a plasmid containing (dC-dG)_n inserts separated by only four nonalternating base pairs, it is demonstrated that the junction regions are conformationally flexible.

MATERIALS AND METHODS

Plasmid DNA was isolated as described (9). The construction and characterization of pRW751, pRW756, pRW755, and pRW757 have been described (10). Plasmid topoisomers were obtained essentially as detailed (11). *Bam*HI was a gift of T. O'Connor and was purified as in ref. 12. *Ava* II was obtained from Bethesda Research Laboratories, and *Hinf*I was from Boehringer Mannheim. S1 nuclease preparation and charac-

terization was as described (13). S1 nuclease reactions, subsequent restriction endonuclease digests, electrophoresis, gel staining, and microdensitometric tracings were as described (1).

***Bam*HI Cleavage.** *Bam*HI reactions with pRW751 and pRW451 (7) were as follows: 1 μg of plasmid DNA in 50 μl of 15 mM Tris chloride/6 mM NaCl/13 mM MgCl₂/2.5 mM dithiothreitol, pH 7.7, was incubated at 37°C for 15 min in the presence of *Bam*HI (sufficient for complete digestion within 2 hr); the reactions were terminated by adding 2 μl of 0.5 M EDTA.

Construction of pRW759. pRW759 was constructed with the *Eco*RI-*Bam*HI inserts of pRW755 and pRW756 (10). Equal amounts of the 125-base-pair (bp) pRW755 and 131-bp pRW756 inserts [purified by gel electrophoresis (10)] were ligated in the presence of *Eco*RI-linearized pBR322. Transformation and screening were as described (10). pRW759 contained the 256-bp insert (see Fig. 1a). *Eco*RI/*Bam*HI double digestion of pRW759 released the expected 125- and 131-bp fragments. Digestion with *Eco*RI alone showed that about 5% of the plasmid molecules in this preparation possessed a deletion of ≈10 bp. Double digestion with *Bam*HI/*Eco*RI demonstrated that the deletion was centered around the *Bam*HI site. This is consistent with previous findings which showed that (dC-dG)_n sequences longer than ≈40 bp were unstable and suffered deletions (7, 10).

RESULTS

S1 Nuclease Probing of Right- to Left-Hand DNA Junctions. Fig. 1a outlines the *Ava* II restriction fragments that span the (dC-dG)_n inserts of each plasmid used in this study. We have demonstrated (1) that S1 nuclease can recognize and cleave within the junction region existing between contiguous right- and left-handed DNA sequences. This cleavage is illustrated in Fig. 1b for each of the plasmids. S1 nuclease cleavage was followed by *Ava* II digestion, and the resulting DNA fragments were separated by gel electrophoresis. The characteristic pattern of S1 nuclease-specific bands, which indicates the existence of left-handed DNA regions within a given plasmid, is sets of doublet or triplet bands (enclosed by brackets) corresponding to S1 nuclease cleavage at the ends (junctions) of the (dC-dG)_n inserts. Thus, for pRW756, for example, two sets of doublet bands were seen; the larger band of each doublet represented cleavage near the boundary of the (dC-dG)₁₆ block distal to an *Ava* II site, whereas the smaller band resulted from cleavage near the proximal boundary (1). For pRW759, triplet bands were found, indicating that the *Bam*HI site (G-G-A-T-C-C) interrupting the two (dC-dG)_n blocks was being recognized as a junction. For each plasmid, the doublet or triplet bands were mapped to within 3–15 bp of the first alternating residue of the (dC-dG) region and were derived from the (dC-dG)_n-containing *Ava* II frag-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: bp, base pair(s).

FIG. 1. (dC-dG)_n-containing recombinant plasmids used in this study. (a) The *Ava* II fragments containing the various (dC-dG)_n inserts are diagrammed for each plasmid. The number of base pairs from the *Ava* II sites to the first alternating residues are cited. Hatched areas denote the *E. coli* *lac* 95-bp region (10). No attempt has been made to keep the various lengths to scale. (b) Plasmids were treated with S1 nuclease followed by *Ava* II restriction digestion. DNA fragments were separated by 2% agarose gel electrophoresis. The lengths (bp) of the *Ava* II fragments are indicated. The (dC-dG)_n-containing *Ava* II fragment lengths (second band from top) for pRW756 (lane 1), pRW755 (lane 2), pRW757 (lane 3), pRW759 (lane 4), and pRW751 (lane 5) are 1,184, 1,178, 1,162, 1,684, and 1,585 bp, respectively. The brackets denote S1 nuclease-specific doublet or triplet bands that have an *Ava* II terminus and an S1 nuclease end. Cf, fragment that results from S1 nuclease cleavage at the major inverted repeat of pBR322 (14, 15) (the other cruciform-specific fragment migrates between the lower doublet bands of all the plasmids except pRW759).

ment (second from the top for each plasmid).

Effect of Length of (dC-dG) Regions on Supercoil-Induced Left-Handed DNA. We have utilized the S1 nuclease sensitivity of the junction regions as a probe for left-handed DNA formation within the (dC-dG)_n blocks of pRW756, pRW755, and pRW757 (*n* = 16, 13, and 5, respectively). Topoisomeric samples of each plasmid [consisting of a Gaussian population of about eight topoisomers centered around a mean density (11)] were treated with S1 nuclease, cleaved with *Ava* II, and electrophoresed to separate the resulting fragments. Fig. 2a shows the resulting cleavage pattern obtained for pRW757; similar patterns were found for the other two plasmids. The negative superhelical density of each initial plasmid sample increased from left to right. At low negative densities, no S1 nuclease-specific doublet bands appeared. However, as the negative density was increased, the characteristic doublet bands corresponding to cleavage at the (dC-dG)₅ boundaries became apparent and indicated a supercoil-induced transition from the right- to left-handed state.

FIG. 2. Supercoil-induced left-handed DNA formation in pRW756, pRW755, and pRW757 as determined by S1 nuclease cleavage. Topoisomeric samples for each plasmid were generated and characterized as described (11). (a) Agarose (2%) gel electrophoresis on negatively supercoiled pRW757 samples after S1 nuclease cleavage followed by *Ava* II digestion. The lengths (bp) of the *Ava* II fragments are indicated. Brackets denote the S1 nuclease-specific doublet bands representing recognition and cleavage by the nuclease at the right- to left-handed junctions. Cf, S1 nuclease-specific cleavage at the major inverted repeat of pBR322 (the other *Ava* II/S1 nuclease cruciform cleavage product comigrates with the upper band of the lower doublet). The mean negative superhelical density (under S1 nuclease reaction conditions) of the sample in each lane is as follows: 1, 0.031; 2, 0.042; 3, 0.048; 4, 0.057; 5, 0.064; 6, 0.068; 7, 0.074; 8, 0.077; 9, 0.081; 10, 0.091; 11, 0.103. (b) Percentage of specific cleavage by S1 nuclease versus the mean negative superhelical density ($-\sigma$) under S1 nuclease reaction conditions. A photographic negative of a (as well as a similar analysis of topoisomeric pRW756 and pRW757 samples) was traced by using a Joyce-Loebl microdensitometer. Specific cleavage represents the ratio of the area under the upper S1 nuclease-specific doublet to the area of the 345-bp band divided by the molar ratio (representing 100% cleavage) of these bands based on their calculated lengths. $\circ$, pRW756; $\bullet$, pRW755; Δ , pRW757.

Fig. 2b is a plot of the percentage of specific cleavage versus the mean negative titratable superhelicity of each sample for pRW757 as well as for pRW756 and pRW755. Specific cleavage is defined as the relative appearance of the doublet bands and represents the transition from right- to left-handed helical DNA within the (dC-dG)_n blocks (1). The plateauing of S1 nuclease cleavage represents a complete stabilization of the left-handed state over the right-handed state, as demonstrated (1). Under the conditions of S1 nuclease cleavage, this occurred at a superhelical density of about -0.055 for pRW756 and pRW755 and -0.075 for pRW757.

The conversion of the helical sense for the (dC-dG)_n blocks of pRW756 and pRW755 was determined also by relaxation of supercoil turns as revealed by gel electrophoresis (1-3) (data

FIG. 3. Total free energy contributed to left-handed DNA formation from loss of negative supercoils is plotted against the length (bp) of the (dC-dG)_n block undergoing the transition.

not shown). Following the relaxation of supercoils can define the exact end point (± 1 turn) of the transition because individual topoisomers can be analyzed as opposed to topoisomer populations. This analysis revealed that the transition was complete for pRW756 at a density of -0.048 , whereas that for pRW755 was complete at -0.051 . This difference, although slight, was nonetheless significant and reproducible. The transition for pRW757 could not be analyzed in this manner because it occurred in a density range where individual topoisomers are not resolved by gel electrophoresis.

Energetics of Supercoil-Stabilized Left-Handed DNA. The driving force behind the supercoil-induced left-handed helical state is the loss of negative supercoils (about two turns lost per turn of converted helix). This loss of negative supercoils contributes a favorable free-energy input to the helix transition. This follows from the fact that the formation of negative supercoils is energetically unfavorable, and this unfavorable free

energy of formation increases quadratically as the negative supercoiling increases (16, 17).

The gel electrophoresis results indicated that the entire (dC-dG)_n regions for pRW756 and pRW755 were converted to the left-handed state, and we assume this is also true for pRW757. Thus, a total of 5.7, 4.7, and 1.8 supercoil turns (for pRW756, pRW755, and pRW757, respectively) (2) were lost upon completion of the transition. Using these values, the transition end points, and estimates of the free energy of superhelix formation (16, 17), we calculated the total favorable free energy contributed to the formation and stabilization of the left-handed state, which is derived from relaxation of negative supercoils. The values obtained are plotted in Fig. 3 against the number of G-C bp which are left-handed. A linear relationship was obtained which is reasonable if it is assumed that the lengths of the (dC-dG)_n sequences under investigation are short relative to the co-operative unit defined by the polymeric (dC-dG)_n transition (18).

Junction Formation Within BamHI Restriction Sites. pRW751 contains two (dC-dG)_n regions (with the 95-bp *lac* operator in between) inserted into the *Bam*HI site of pBR322 (ref. 10; Fig. 1a). The *Bam*HI sites were regenerated and about the (dC-dG)_n blocks so that the last C of the *Bam*HI site (G-G-A-T-C-C) is the first C of the alternating (dC-dG)_n sequence. Thus, it was of interest to determine if cleavage by *Bam*HI is affected when the (dC-dG)_n blocks of pRW751 are in a supercoil-stabilized left-handed state. As a control plasmid for these studies, we used pRW451, which contains a 178-bp *Bam*HI insert (possessing no G-C regions) in pBR322 (7).

Fig. 4a shows the electrophoretic separation of *Bam*HI-cleaved pRW751 and pRW451 at a mean superhelical density of either -0.030 or -0.086 . At the low negative superhelical density value (two left lanes), at which left-handed DNA within the (dC-dG)_n blocks is not stable, *Bam*HI cleavage resulted in the loss of about 40% of the initially supercoiled substrate for both pRW751 and the control, pRW451. However, a difference in *Bam*HI susceptibility was found at the higher negative density (two right lanes), a value at which left-handed DNA does exist within the

FIG. 4. Inhibition of *Bam*HI cleavage by left-handed DNA formation. (a) Agarose (1%) gel electrophoresis after *Bam*HI digestion of pRW451 (lanes 1 and 3) and pRW751 (lanes 2 and 4). The mean negative superhelical density $-\bar{\sigma} = 0.03$ (lanes 1 and 2) and 0.086 (lanes 3 and 4) under *Bam*HI reaction conditions. R, relaxed plasmid; L₁, linearized plasmid; L₂, linearized plasmid minus the *Bam*HI insert (157 bp for pRW751 and 178 bp for pRW451); and Sc, supercoiled plasmid. (b) Percentage inhibition of *Bam*HI cleavage for pRW751 versus the mean negative superhelical density ($-\bar{\sigma}$) under *Bam*HI reaction conditions. Inhibition of cleavage (○) was calculated by determining the percentage of supercoiled DNA cleaved by *Bam*HI for pRW751 and pRW451 at each superhelical density value shown. *Bam*HI cleavage for the pRW451 control plasmid increased smoothly from 40% at a density of -0.03 to 63% at -0.045 . Further increases in negative supercoiling resulted in no change in the level of cleavage. Thus, it was important to compare cleavage levels of each plasmid at the same density values to compensate for the supercoil-induced change in the amount of cleavage. The percentage of cleavage for pRW751 was subtracted from this value for pRW451 at identical superhelicity for each, and the result was divided by the pRW451 value. ---, Supercoil-induced right- to left-handed transition for the (dC-dG)_n blocks of pRW751 as revealed by S1 nuclease cleavage; the density values are those under S1 nuclease digestion conditions (1).

(dC-dG)_n blocks of pRW751. About 33% of the initially supercoiled pRW451 remained after cleavage, whereas 84% remained for pRW751.

A similar analysis of *Bam*HI susceptibility for pRW751 and pRW451 was carried out at several different density values. The percentage of inhibition of *Bam*HI cleavage for pRW751 relative to pRW451 was determined and is plotted against the mean negative superhelical density of the starting samples in Fig. 4b. Cleavage of both plasmids at identical levels of supercoiling was done so as to compensate for any supercoil-induced changes in the *Bam*HI cleavage rate. The dashed line represents the right-to left-handed transition of the two (dC-dG)_n blocks of pRW751 as revealed by S1 nuclease cleavage (1). The inhibition of *Bam*HI cleavage closely paralleled this transition; thus, we attribute the inhibition to formation of left-handed DNA within the (dC-dG)_n blocks. *Bam*HI digestion of the samples shown in Fig. 4a for longer periods of time resulted in complete loss of supercoiled DNA within 2 hr (i) for pRW451 at both the high and low density values but (ii) for pRW751 at only the lower density. For pRW751 at the higher density, only 30% of the supercoiled plasmid was cleaved.

Conformational Flexibility of Right- to Left-Handed DNA Junctions. pRW759 was constructed to investigate several features of the junctions between contiguous right- and left-handed DNA regions. This plasmid contains the (dC-dG)₁₆ and (dC-dG)₁₃

blocks separated by the nonalternating sequence d(G-A-T-C); thus, the two blocks are joined by a *Bam*HI site. Fig. 1b showed that S1 nuclease cleavage of pRW759 produced sets of triplet bands, implying that the intervening d(G-A-T-C) sequence is a junction when the two flanking (dC-dG)_n blocks are in the left-handed state. However, when topoisomeric pRW759 samples were treated with S1 nuclease, the S1 cleavage at this site relative to the two outer junctions revealed conformational flexibility of this "junction."

Fig. 5a shows the electrophoretic separation of the bands produced when topoisomeric samples of pRW759 were treated first with S1 nuclease and then with *Hinf*I. The triplet bands corresponding to cleavage at the junctions are indicated by brackets. In lanes 1 and 2, only the bands corresponding to left-handedness in the (dC-dG)₁₆ block were seen, consistent with previous results (1, 2) which showed that this block undergoes the transition before the (dC-dG)₁₃ block does. At intermediate densities, all three bands were observed (Fig. 5a, lanes 3–5), demonstrating left-handedness in both (dC-dG) blocks. Cleavage at the outer junction of the (dC-dG)₁₃ block increased relative to that of the (dC-dG)₁₆ block until the transition in both blocks was complete and a ratio of the two was reached consistent with the ratio observed for pRW751 (1). Finally, as the titratable negative density was increased further (lanes 6–9), the middle junction corresponding to cleavage at the d(G-A-T-C) interruption became progressively less recognizable by S1 nuclease. These features are shown as a plot of the frequency of cleavage versus supercoiling in Fig. 5b.

DISCUSSION

We defined the relationship between the formation and stabilization of left-handed DNA and the negative superhelical density for plasmids containing (dC-dG)_n inserts for *n* = 16, 13, and 5. The transitions revealed by S1 nuclease-specific cleavage at the junctions represent the equilibrium between the right- and left-handed states because the conversion of helix sense within a (dC-dG) block is an all-or-none transition (1, 2). Even for the short (dC-dG)₅ sequence, only moderate amounts of negative supercoil density (0.05–0.075) were needed to stabilize the left-handed state. This result is especially significant because high NaCl concentrations did not cause the formation of a left-handed helix in the (dC-dG)₅ block (2) and, thus, further demonstrates the effectiveness of supercoiling on this conformational transition.

It should be emphasized that the superhelical density values used herein are the classical titratable superhelical densities based on the total amount of unwinding of a sample relative to the completely relaxed state (1, 4, 11). Thus, the stated values do not account for supercoil turns lost by left-handed DNA formation. Compensation was made for changes in superhelical density brought about by differences in salt concentration and temperature (4) between the various stated conditions.

An interesting feature of the study of supercoil-induced left-handed DNA for the plasmids containing a single (dC-dG) block compared to those containing two blocks is the apparent "competition" between the two blocks. Previous data (1) and the gel electrophoresis analyses herein showed that the left-handed transition began at lower negative superhelical densities for the (dC-dG)₁₆ block than for the (dC-dG)₁₃ block. For pRW751 or pRW759, this means that the transition for the (dC-dG)₁₃ block will be delayed (require more supercoiling) relative to the transition for this block in pRW755 because formation of the left-handed state within the (dC-dG)₁₆ block reduces the negative supercoiling by the density equivalent of about 5.7 turns. Thus, the "effective" density and, therefore, torsional strain that can contribute to the (dC-dG)₁₃ transition is reduced. Our results

FIG. 5. S1 nuclease cleavage of topoisomeric pRW759 samples followed by *Hinf*I digestion. (a) Agarose (2%) gel electrophoretic separation of the resulting fragments. Lengths (bp) are indicated for the *Hinf*I fragments. Brackets designate S1 nuclease-specific bands. The mean negative superhelical density (under S1 nuclease reaction conditions) for the sample in each lane is as follows: 1, 0.026; 2, 0.031; 3, 0.041; 4, 0.052; 5, 0.061; 6, 0.071; 7, 0.081; 8, 0.094; 9, 0.103. (b) Frequency of cleavage at each individual junction is plotted against the mean negative superhelical density. The area of each peak of the lower triplet set was determined for each sample and was divided by the area of the *Hinf*I 396-bp peak of that sample to account for any differences in amount of loaded DNA. Δ, Middle junction; ○, top junction; ●, bottom junction.

suggest that the left-handed state for the (dC-dG)₁₃ block of pRW751 should not be stabilized completely until a density equivalent to 26 or 27 negative supercoils is reached. This agrees well with the value of 28 to 29 obtained previously (1). The competitive nature of such transitions is seen also in the delayed appearance of the major cruciform of pBR322 in the (dC-dG)_n-containing pBR322 derivatives relative to pBR322 itself (unpublished observations). The "extra" negative density required to bring about formation of this cruciform corresponds well to the amount of relaxation expected upon left-handed DNA formation for pRW751, pRW756, and pRW755. Such competition illustrates that a structural transition within one region of DNA can have a significant influence on other transitions within entirely different regions.

The plot of total free energy gained from relaxation of negative supercoils upon formation of the left-handed state versus the number of G-C base pairs undergoing the conversion should provide useful predictive information. By assuming the complete (dC-dG)_n sequence is in the left-handed state (as demonstrated here and in refs. 1 and 2), it is possible to calculate back to the amount of supercoiling that should be required to stabilize the left-handed state. For example, for six G-C base pairs, we estimate that a superhelical density of about -0.105 will be needed. Similar estimates can be made for other lengths of (dC-dG)_n.

The free-energy contribution from loss of supercoils must overcome two unfavorable energetic aspects of the left-handed state: junction formation and the free-energy difference between the right- and left-handed states under the conditions used. Thus, extrapolating to zero G-C base pairs should give an estimate of the free energy costs of the junction regions. The value so obtained from Fig. 3 is about 10 kcal/mol of (dC-dG)_n or about 5 kcal/mol of junction. This value is in excellent agreement with estimates of 3-5 kcal/mol of junction determined by other methods (19).

Our results concerning the inhibition of *Bam*HI cleavage for pRW751 when the (dC-dG)_n blocks undergo left-handed DNA formation suggest that the conformation of the *Bam*HI sites abutting the left-handed DNA segments is drastically altered, in agreement with the S1 nuclease sensitivity of this region. The >80% inhibition observed after the right- to left-handed transition is complete demonstrates that left-handed DNA and the junctions between it and right-handed DNA can significantly alter the interaction between nucleic acid-metabolizing proteins and the DNA.

S1 nuclease sensitivity of the three junctions of pRW759 demonstrates the conformational flexibility of such regions. As we have observed before, the junction between the *lac* sequence and the (dC-dG)₁₃ block is preferentially cleaved relative to all other junctions in pRW759, pRW751, and pRW755. Comparison of the frequency of cleavage at the various junctions within the plasmids shown in Fig. 1 results in a distinctive hierarchy of susceptibility to S1 nuclease. These observed differences suggest sequence-dependent conformational effects within the junctions. However, an examination of the se-

quences reveals no obvious reasons (A-T-richness, strand bias, etc.) for this observed difference. The relative S1 nuclease sensitivity is also dependent on the level of supercoiling. This is illustrated by pRW759 (Fig. 5b) and by pRW756 (data not shown) for which the difference in relative cleavage of the two junctions progressively decreases from about 2.1:1 to 1.2:1 as the negative supercoiling increases over the range studied, indicating conformational flexibility even after the left-handed state is completely stabilized.

Furthermore, the loss of S1 nuclease sensitivity of the d(G-A-T-C) sequence between the two (dC-dG)_n blocks of pRW759 suggests that this sequence adopts a non-B-DNA structure, which may be fully left-handed at the high negative superhelical density values. This loss occurs only after the two flanking (dC-dG) tracts are stabilized in left-handed states. The high density values required are consistent with overcoming the energy differences between the junction conformation and left-handed DNA in a nonalternating purine-pyrimidine tract flanked by (dC-dG) regions. These and other results (refs. 1-3 and 19; unpublished data) demonstrate the power of supercoiling in inducing formation of left-handed helices.

This work was supported by grants from the National Institutes of Health (CA20279) and the National Science Foundation (PCM8002622). C.K.S. was supported, in part, by the American Cancer Society (PF-1904).

1. Singleton, C. K., Klysik, J., Stirdivant, S. M. & Wells, R. D. (1982) *Nature (London)* **299**, 312-316.
2. Stirdivant, S. M., Klysik, J. & Wells, R. D. (1982) *J. Biol. Chem.* **257**, 10159-10165.
3. Peck, L. J., Nordheim, A., Rich, A. & Wang, J. C. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 4560-4564.
4. Bauer, W. R. (1978) *Annu. Rev. Biophys. Bioeng.* **7**, 287-313.
5. Wang, J. C. (1980) *Trends Biochem. Sci.* **5**, 219-221.
6. Gellert, M. (1981) *Annu. Rev. Biochem.* **50**, 879-910.
7. Klysik, J., Stirdivant, S. M., Larson, J. E., Hart, P. A. & Wells, R. D. (1981) *Nature (London)* **290**, 672-677.
8. Wartell, R. M., Klysik, J., Hillen, W. & Wells, R. D. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 2549-2553.
9. Hardies, S. C., Patient, R. K., Klein, R. D., Ho, F., Reznikoff, W. S. & Wells, R. D. (1979) *J. Biol. Chem.* **254**, 5527-5534.
10. Klysik, J., Stirdivant, S. M. & Wells, R. D. (1982) *J. Biol. Chem.* **257**, 10152-10158.
11. Singleton, C. K. & Wells, R. D. (1982) *Anal. Biochem.* **122**, 253-257.
12. Greene, P. J., Heyneker, H. L., Bolivar, F., Rodriguez, R. L., Betlach, M. C., Covarrubias, A. A., Backman, K., Russel, D. J., Tait, R. & Boyer, H. W. (1978) *Nucleic Acids Res.* **5**, 2372-2380.
13. Dodgson, J. B. & Wells, R. D. (1977) *Biochemistry* **16**, 2374-2379.
14. Panayotatos, N. & Wells, R. D. (1981) *Nature (London)* **289**, 466-470.
15. Lilley, D. M. J. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 6468-6472.
16. Pulleyblank, D. E., Shure, M., Tang, D., Vinograd, J. & Vosberg, H.-P. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4280-4284.
17. Depew, R. E. & Wang, J. C. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4275-4279.
18. Ivanov, V. I. & Minyat, E. (1981) *Nucleic Acids Res.* **9**, 4783-4798.
19. Klysik, J., Stirdivant, S. M., Singleton, C. K., Zacharias, W. & Wells, R. D. (1983) *J. Mol. Biol.*, in press.