DNA Unwinding Induced by Zinc Finger Protein Binding†

Yigong Shi and Jeremy M. Berg*

Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Received October 5, 1995; Revised Manuscript Received January 17, 1996⊗

ABSTRACT: Zinc finger domains of the Cys_2His_2 type are found in a large number of eukaryotic proteins. Various proteins containing these domains have been shown to bind specifically to DNA, RNA, and DNA−RNA hybrids. Structural studies of zinc finger protein−DNA complexes have revealed that the DNA molecules are underwound relative to canonical B-form. It has not been clear if zinc finger proteins recognize preexisting underwound conformations of DNA or if they induce such conformations upon binding. We report that the DNA binding domains of Sp1 and several designed zinc finger proteins unwind DNA upon binding. The extent of unwinding is consistent with that observed in zinc finger protein−DNA cocrystal structures. These DNA deformations may be important in determining overall binding affinities as well as influencing binding site preferences. Furthermore, changes in DNA conformation upon zinc finger protein binding may affect protein−protein interactions important for transcriptional regulation and other activities of zinc finger proteins.

It has been estimated that as many as 1% of all human proteins include zinc fingers of the Cys_2His_2 type (Hoovers et al., 1992). The observation that transcription factor IIIA, the first zinc finger protein to be characterized, bound both 5S RNA and 5S RNA genes led to speculation that zinc finger proteins might recognize nucleic acids with A-like conformations (Rhodes & Klug, 1986). Direct observations of the structures of several zinc finger protein−DNA complexes revealed that such DNA molecules adopt conformations intermediate between A- and B-forms (Pavletich & Pabo, 1991, 1993; Fairall et al., 1993; Nekludova & Pabo, 1994). For example, in the Zif268−DNA complex, the DNA exhibits a narrower and deeper major groove than that in canonical B-form and is somewhat underwound with an average of 11.2 base pairs per turn (Pavletich & Pabo, 1991; Nekludova & Pabo, 1994). Similar structures are observed in regions of the GLI−DNA (Pavletich & Pabo, 1993) and tramtrack−DNA (Fairall et al., 1993) complexes. More recently, studies have revealed that certain zinc finger proteins have high affinities for specific DNA−RNA hybrids (Shi & Berg, 1995b) which are believed to have conformations intermediate between A- and B-forms (Salazar et al., 1993). Earlier studies of DNA sequences related to zinc finger binding sites had suggested that these sequences adopt conformations intermediate between A- and B-forms in the absence of bound protein (McCall et al., 1986; Rhodes & Klug, 1986; Fairall et al., 1989).

In this paper, we report the results of studies of the conformational changes that occur in DNA upon zinc finger protein binding. These results indicate that substantial DNA unwinding but not bending occurs concomitant with the binding process. Thus, it appears that zinc finger proteins do not recognize preexisting underwound DNA structures but instead induce the conformations seen in the zinc finger−DNA cocrystal structures upon binding.

EXPERIMENTAL PROCEDURES

Construction and Preparation of Plasmids pEMBL-15Sp1 and pEMBL-14QQR. A 93-base single-strand DNA oligonucleotide, 5′-GAAGCTTCTAGAC**GGGGCGGGG**CAAGCTCGTACCCGTTACGCCTGTCC**GGGGCGGGG**CAAGCTCGTACCCGTTACGCCTGTCC**GGGGCGGGG**C-3′, was synthesized. The 93-base template was amplified by polymerase chain reaction (PCR) with a pair of primers, 5′-GCGAGAATTCGGATCCCGGGAAGCTTCTAGACGGGGCG-3′ and 5′-GCGAGAATTCGCCCCGCCCCGGACAG-3′. The resulting PCR product was digested with endonuclease *Eco*RI and subcloned into plasmid vector pEMBL (Dente et al., 1983) to create pEMBL-3Sp1. The same template was subsequently amplified with a second pair of PCR primers, 5′-GCGAAAGCTTGGATCCGAAGCTCCTAGACGGGG-3′ and 5′-GCGAGGATCCAAGCTTTCATCTGAGGCCCCGCCCCGGACAG-3′. This product was digested either by *Hin*dIII or by *Bam*HI. The *Hin*dIII fragment was first subcloned into pEMBL-3Sp1 to generate pEMBL-6Sp1; then the *Bam*HI fragment was ligated into pEMBL-6Sp1 to complete pEMBL-9Sp1. Lastly, the 93-base template was amplified by a third pair of PCR primers, 5′-GCGATCTAGAATTCTAGCCGATGAAGCTCCTAGACGGGG-3′ and 5′-GCGATCTAGAATTCGCCCCGCCCCGGACAG-3′. This product was treated with either *Eco*RI or *Xba*I. The *Eco*RI fragment was subcloned into pEMBL-9Sp1 to generate pEMBL-12Sp1; then the XbaI fragment was ligated into pEMBL-12Sp1 to create the final product, pEMBL-15Sp1. All 15 copies of binding sites in this plasmid were in the same orientation as verified by sequencing. Plasmid pEMBL-14QQR with 14 sites of the sequence 5′-GGGGAAGAA-3′, each separated by over 25 base pairs, was created in a completely analogous manner except one copy of binding site was lost during cloning.

† This work was supported by grants from the National Institutes of Health (GM 46257) and the Lucille P. Markey Charitable Trust.

* To whom correspondence should be addressed. (410)955-7322 (Phone). (410)550-6910 (FAX). jeremy_berg@qmail.bs.jhu.edu (E-mail).

⊗ Abstract published in *Advance ACS Abstracts,* March 1, 1996.

0006-2960/96/0435-3845$12.00/0

Supercoiled plasmids were purified through Qiagen Maxi columns and eluted in TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). Ten micrograms of plasmid was treated with 0.004 unit of FPLC-grade DNase I (Phamacia) at 25 °C for 4 min in 100 μL of reaction buffer containing 25 mM Tris-HCl, pH 8.0, 100 mM KCl, 5 mM $CaCl_2$, 2 mM $MgCl_2$, 50 μg/mL bovine serum albumin (BSA), and 2 mM dithiothreitol (DTT) with 100 μM $ZnCl_2$ and 1 μg/mL poly(dI-dC). Reactions were quenched by the addition of 400 μL of stop solution containing 0.5 M ammonium acetate and 90% (v/v) ethanol. The plasmids were precipitated, dried, and resuspended in 10 mM Tris-HCl, pH 8.0. The nicked plasmids were further purified through 1% low-melting Sea-Plaque agarose gel (FMC Bioproducts). The final products were resuspended and stored in 10 mM Tris-HCl, pH 8.0.

DNA Unwinding Assays. All zinc finger proteins were purified and quantitated as described (Desjarlais & Berg, 1993; Shi & Berg, 1995a,b). The religation reactions were performed at 4 °C for 2 h in 50 μL of buffer containing 50 mM Tris-HCl, pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 10 mM KCl, 25 μg/mL BSA, and 100 μM $ZnCl_2$ with 400 units of T4 DNA ligase and various amounts of the zinc finger proteins. The reactions were stopped by equal volumes of phenol extraction followed by chloroform extraction. The resulting mixtures were mixed with one-tenth volume of 50% glycerol in water and loaded into a 1.2% Sea-Kem agarose gel (FMC Bioproducts) in 1× TBE buffer. The gels were run for 14−22 h at 25 °C under an electric field of 2−3 V/cm.

The gels were stained by soaking in 1× TBE with 1 μg/mL ethidium bromide for 2 h. The UV-induced images were digitally recorded through the use of an Eagle Eye still video system (Stratagene). The images were analyzed with program Image (NIH, version 1.44).

Circular Permutation Assays. A 98-base single-stranded DNA oligonucleotide template, 5′-ACAGCTATGACCATGATTAC**GGATCC**GTGATCGCAGTTGCAGCCGT-**GTCGAC**ACAGCTATGATTGCAGCCGT**TCTAGA**-CCATGATTACGTGATCGCAG-3′, containing restriction sites for *Bam*HI, *Sal*I, and *Xba*I (in boldface) was synthesized. This was amplified with two primers, 5′-GCGAG-**GAATTC**ACAGCTATGACCATGATTA-3′, and 5′-CTGC-GAG**AAGCTT**CTGCGATCACGTAATCATG-3′, digested with *Eco*RI and *Hin*dIII, and cloned into pEMBL. The resulting product is termed pEMBL-CP5. The same 98-base template was also amplified with the same 3′ primer, 5′-GCGAG**GAATTC**ACAGCTATGACCATG-ATTA-3′, and a different 5′ primer, 5′-CTGCGAG**AAGCTT**GGGGCGG-GGC**GAATTC**ACAGCTATGACCATGATTAC-3′, treated with *Hin*dIII, and cloned into *Hin*dIII-digested pEMBL-CP5. The final product, termed pEMBL-CP5-Sp1, was used for circular permutation studies on Sp1 and Sp1C. Similar procedures were employed to produce a plasmid, pEMBL-CP5-QQR, for use with finger protein ZF-QQR.

The plasmid, pEMBL-CP5-Sp1, was digested with one of five different restriction enzymes (*Hin*dIII, *Xba*I, *Sal*I, *Bam*HI, and *Eco*RI), end-labeled with [α-^{32}P]dATP, and gel-purified. Pilot experiments were carried out to determine the appropriate protein concentration. The circular permutation studies were performed on 10% polyacrylamide gels using Tris−glycine buffers. The gels were run at 4 °C with a constant electric field of 10 V/cm.

RESULTS AND DISCUSSION

DNA Unwinding by Sp1. We chose the human transcription factor Sp1 (Kadonaga et al., 1987) for initial studies of DNA structural changes upon protein binding. Although the structure of the Sp1−DNA complex has not yet been determined, it is clearly related to that of Zif268 as the DNA binding site preferences of Sp1 can be easily rationalized based on the interaction observed in the Zif268 co-crystal structure (Berg, 1992). The possibility that DNA conformational changes might be induced by protein binding was examined through studies of changes in plasmid topoisomer distributions (Wang et al., 1977; Gamper & Hearst, 1982; Kolb & Buc, 1982). A supercoiled plasmid (pEMBL-15Sp1) with 15 tandem copies of a binding sequence for Sp1 (5′-GGGGCGGGG-3′), each separated by over 25 base pairs, was lightly nicked with DNase I and purified. This material was incubated with various concentrations of the three zinc finger DNA binding domain from Sp1 (Kadonaga et al., 1987; Desjarlais & Berg, 1992) at 4 °C and religated. After removal of all proteins, the distribution of topoisomers was resolved by agarose gel electrophoresis at room temperature and visualized with ethidium bromide staining (Figure 1). The zinc-bound folded form of Sp1 was required for significant effects. Under conditions where there was only 100 mM $ZnCl_2$ (Figure 1a, lane 1) or zinc-free Sp1 (Figure 1a, lane 2) present, the topoisomer distribution was not detectably different from that for the starting plasmid. However, in the presence of both zinc ions and peptide, the distribution of topoisomers was shifted to lower mobility relative to control samples, indicating that the folded Sp1 is capable of unwinding DNA (Figure 1a, lanes 3−5). The center of the topoisomer distribution in each lane was determined and used to derive the linking number difference (DL_k) relative to the control sample in which ligation was performed in the absence of Sp1 (Figure 1b). Additional experiments were performed on the parent plasmid pEMBL which does not contain any cloned Sp1 binding sites. This plasmid also showed some degree of DNA unwinding which became significant at higher Sp1 concentrations (Figure 1c). The difference in linking number change between the two plasmids reached a maximum value of 0.74 ± 0.1 that remained constant at higher Sp1 concentrations (Figure 2). This linking number change of 0.74, which corresponds to unwinding of 266°, is due to interactions with the 15 copies of Sp1 binding sequence. The average degree of DNA unwinding is thus 17.8° ± 2.4° per Sp1 binding site. The nonsaturable changes in linking number of both plasmids are presumably due to interactions with a large number of lower affinity or nonspecific sites present in both plasmids.

These observations suggest that Sp1 binding sites adopt B-like conformations (in terms of helical repeat) that are underwound upon Sp1 binding. These results are consistent with the observed structure of the DNA binding site for Zif268 (Pavletich & Pabo, 1991). Assuming that canonical B-form DNA has 10.5 base pairs per helical turn, the degree of unwinding we observed would lead to 11.2 base pairs per turn, essentially identical with the crystallographically determined parameters.

Studies of Potential DNA Bending. Since DNA bending may influence the topoisomer distribution, experiments were performed to examine the possibility that the observed

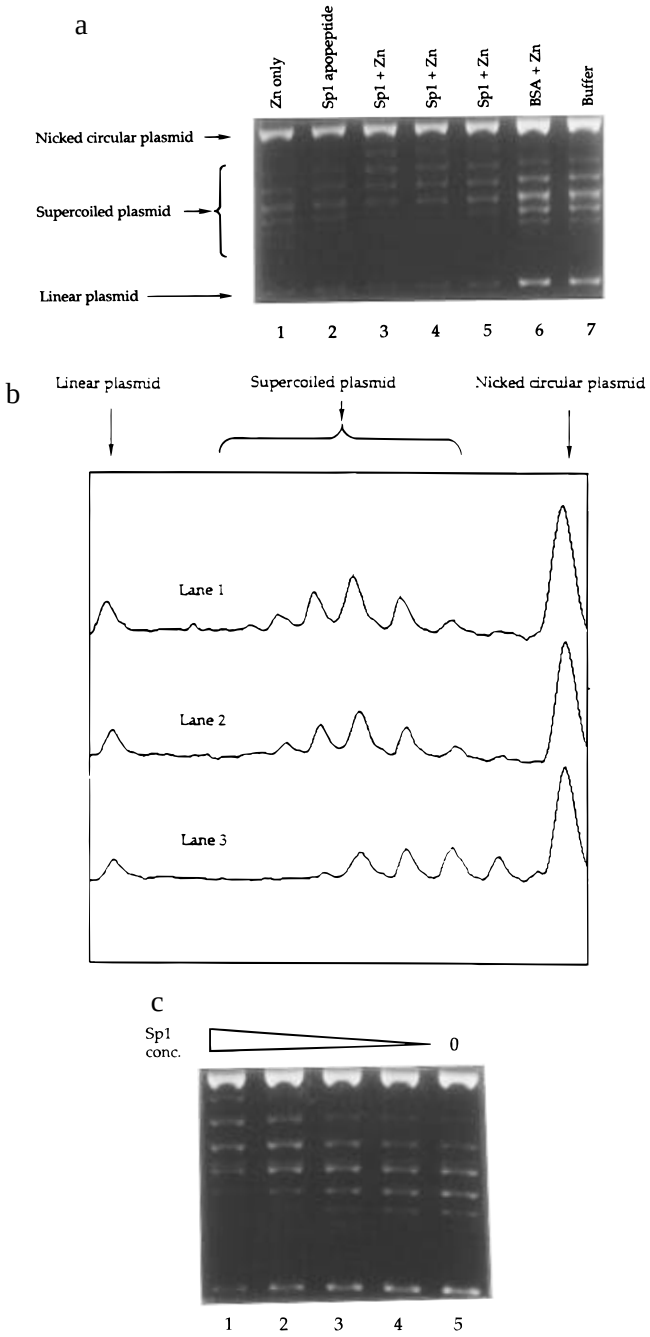

FIGURE 1: DNA unwinding assay performed on Sp1. (a) Plasmid pEMBL-15Sp1 containing 15 isolated Sp1 binding sites was nicked by DNase I and resealed by T4 ligase in the presence of 100 μM $ZnCl_2$ in reaction buffer (lane 1), 4 μM Sp1 in reaction buffer with no zinc (lane 2), 4.0 μM Sp1 + 100 μM $ZnCl_2$ (lane 3), 3.0 μM Sp1 + 100 μM $ZnCl_2$ (lane 4), 1.0 μM Sp1 + 100 μM $ZnCl_2$ (lane 5), 1 mg/mL BSA + 100 μM $ZnCl_2$ (lane 6), and reaction buffer (lane 7). (b) Densitometric traces of lanes 1, 2, and 3 from (a). These traces were generated by Image (NIH version 1.44) and were subsequently used to calculate the center of Gaussian distributions. The difference between the centers of the distributions of lanes 1 and 3 corresponds to a $DL_{k(pEMBL-15Sp1)}$ of 1.5. (c) Nicked circular plasmid pEMBL was resealed in the presence of varying amounts of Sp1 + 100 μM $ZnCl_2$: 12 μM (lane 1), 8 μM (lane 2), 4 μM (lane 3), 1 μM (lane 4), and 0 μM (lane 5).

changes in linking number were due to other DNA structural changes. Circular permutation studies (Wu & Crothers, 1984) were performed to examine possible protein-induced DNA bending. Analysis of the mobilities of 110 bp restriction fragments with Sp1 binding sites at various positions in the presence and absence of bound protein

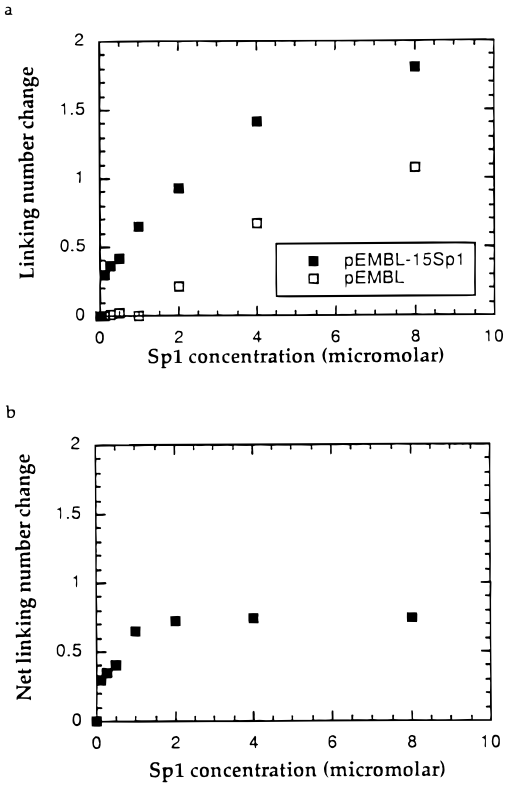

FIGURE 2: Specific and nonspecific unwinding by zinc finger peptide Sp1. (a) Unwinding (DL_k) of plasmid DNA pEMBL-15Sp1 and pEMBL at various concentrations of Sp1. (b) Net DNA unwinding associated with the cloned binding sites. Net linking number changes were calculated by subtracting $DL_{k(pEMBL)}$ from $DL_{k(pEMBL-15Sp1)}$ at the same Sp1 concentration.

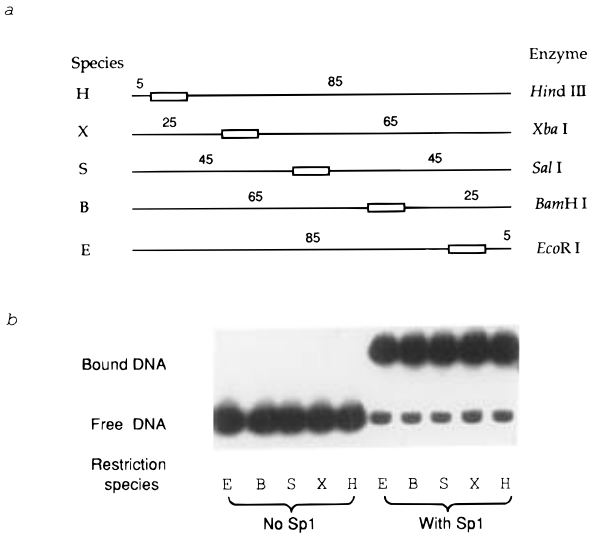

FIGURE 3: Circular permutation studies of zinc finger peptide Sp1. (a) Five 110 bp fragments with an Sp1 binding site at various locations were obtained by digestion of plasmid pEMBL-CP5-Sp1 with five different restriction endonucleases. The spacing between two adjacent restriction sites is 20 bp. The abbreviations are as follows: E, *Eco*RI; B, *Bam*HI; S, *Sal*I; X, *Xma*I; H, *Hin*dIII. (b) Nondenaturing electrophoresis gel showing the mobilities of various DNA fragments with or without bound Sp1. There are no apparent mobility differences among different restriction fragments with or without zinc finger proteins.

indicated that binding of the Sp1 fragment does not induce detectable DNA bending (Figure 3).

DNA Unwinding by Designed Zinc Finger Proteins. To explore the possibility that DNA unwinding is a general

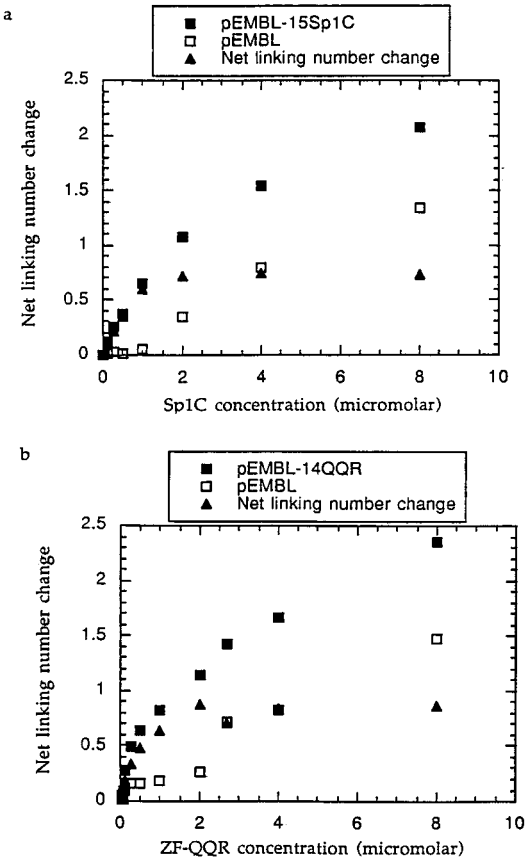

FIGURE 4: DNA unwinding by zinc finger proteins Sp1C and ZF-QQR. Both specific unwinding and nonspecific unwinding are shown for Sp1C (a) and ZF-QQR (b). The plasmid pEMBL-15Sp1 was used with Sp1C, and pEMBL-14QQR was used with ZF-QQR.

Table 1: DNA Unwinding Data for Zinc Finger Proteins

protein	DNA binding sequence	net linking number change	degree of unwinding (per binding site)
Sp1	5′-GGGGCGGGG-3′	0.74 ± 0.1	17.8 ± 2.4
Sp1C	5′-GGGGCGGGG-3′	0.73 ± 0.07	17.5 ± 1.7
ZF-QQR	5′-GGGGAAGAA-3′	0.88 ± 0.11	22.6 ± 2.8

property of zinc finger proteins, we performed unwinding assays on two designed zinc finger proteins, Sp1C (Shi & Berg, 1995a,b) and ZF-QQR (Shi & Berg, 1995a,b) (Figure 4 and Table 1). Both proteins behaved qualitatively like the Sp1 fragment. They unwound DNA to significant degrees and induced nonspecific as well as specific unwinding. Interestingly, Sp1C, which has the same set of contact residues as does Sp1 and recognizes the same binding sites (Shi & Berg, 1995a), exhibited nearly the same degree of specific DNA unwinding (Table 1). A somewhat larger amount of protein-induced DNA unwinding was observed for ZF-QQR. This may reflect different helical parameters of the DNA binding sites either in their protein complexes or in the absence of bound protein since only differences are measured. Circular permutation studies revealed no detectable protein-induced bending for either protein (data not shown).

Conclusions and Biological Implications. The unwinding of the DNA is presumably required to allow proper alignment of the protein residues and the interaction sites on DNA. The free energy cost of this unwinding will reduce the binding affinity of zinc finger proteins relative to the hypothetical case wherein no unwinding was required for proper alignment. These effects may also play a role in determining binding site specificity to the extent that torsional structure and rigidity depend on sequence (Hogan & Austin, 1987). In addition, changes in DNA conformation *in vivo* may also affect zinc finger protein binding (Parvin et al., 1995).

Eukaryotic transcriptional control is mediated through complex interactions between specific DNA binding proteins and DNA regulatory elements as well as interactions between the regulatory factors and RNA polymerase (Mitchell & Tjian, 1989). Protein-induced DNA distortions can change the relative disposition of different factors around the DNA helix and, by so doing, modulate protein−protein interactions. Our observation that unwinding of DNA seems to be a common property for zinc finger proteins suggests that such effects may need to be considered as the roles of zinc finger proteins in gene regulatory and other processes are elucidated.

REFERENCES

Berg, J. M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 11109−11110.
Dente, L., Cesareni, G., & Cortese, R. (1983) *Nucleic Acids Res. 11*, 1645−1655.
Desjarlais, J. R., & Berg, J. M. (1992) *Proc. Natl. Acad. Sci. U.S.A. 89*, 7345−7349.
Desjarlais, J. R., & Berg, J. M. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 2256−2260.
Fairall, L., Martin, S., & Rhodes, D. (1989) *EMBO J. 8*, 1809−1817.
Fairall, L., Schwabe, J. W., Chapman, L., Finch, J. T., & Rhodes, D. (1993) *Nature 366*, 483−487.
Gamper, H. B., & Hearst, J. E. (1982) *Cell 29*, 81−90.
Hogan, M. E., & Austin, R. H. (1987) *Nature 329*, 263−266.
Hoovers, J. M., Mannens, M., John, R., Bliek, J., van Heyningen, V., Porteous, D. J., Leschot, N. J., Westerveld, A., & Little, P. F. (1992) *Genomics 12*, 254−263.
Kadonaga, J. T., Carner, K. R., Masiarz, F. R., & Tjian, R. (1987) *Cell 51*, 1079−1090.
Kolb, A., & Buc, H. (1982) *Nucleic Acids Res. 10*, 473−485.
McCall, M., Brown, T., Hunter, W. N., & Kennard, O. (1986) *Nature 322*, 661−664.
Mitchell, P. J., & Tjian, R. (1989) *Science 245*, 371−378.
Nekludova, L., & Pabo, C. O. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 6948−6952.
Parvin, J. D., McCormick, R. J., Sharp, P. A., & Fisher, D. E. (1995) *Nature 373*, 724−727.
Pavletich, N. P., & Pabo, C. O. (1991) *Science 252*, 809−817.
Pavletich, N. P., & Pabo, C. O. (1993) *Science 261*, 1701−1707.
Rhodes, D., & Klug, A. (1986) *Cell 46*, 123−132.
Salazar, M., Fedoroff, O. Y., Miller, J. M., Ribeiro, N. S., & Reid, B. R. (1993) *Biochemistry 32*, 4207−4215.
Shi, Y., & Berg, J. M. (1995a) *Chem. Biol. 2*, 83−89.
Shi, Y., & Berg, J. M. (1995b) *Science 268*, 282−284.
Wang, J. C., Jacobsen, J. H., & Saucier, J. M. (1977) *Nucleic Acids Res. 4*, 1225−1241.
Wu, H. M., & Crothers, D. M. (1984) *Nature 308*, 509−513.

BI952384P