Transcriptional Repression by Zinc Finger Peptides

EXPLORING THE POTENTIAL FOR APPLICATIONS IN GENE THERAPY*

Jin-Soo Kim and Carl O. Pabo‡

From the Howard Hughes Medical Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

A series of studies were performed to determine whether zinc finger peptides could efficiently repress transcription from RNA polymerase II promoters in vivo and to determine how such repression might depend on the position of the zinc finger binding site with respect to those of the TATA box or the initiator element. Promoter constructs were prepared with Zif268 binding sites inserted at various positions, and the activity of a reporter gene was measured in transfection studies. We found that the peptide containing the three zinc fingers of Zif268 could efficiently repress activated transcription when bound to a site near the TATA box (19-fold repression) or when bound to a site near the initiator element (18-fold repression). Repression was even more effective when the zinc finger peptide was bound to both of these sites (63-fold repression). Novel zinc finger peptides that had been selected via phage display also served as repressors of activated transcription, but repression with these proteins was somewhat less efficient than with the Zif268 peptide.

Designer DNA-binding proteins may eventually provide novel reagents for gene therapy and for the regulation of gene expression in transgenic organisms. In the past several years, there has been remarkable progress in the design and selection of novel zinc finger proteins (1–8) and in the structure-based design of hybrid proteins that contain zinc fingers fused to other site-specific DNA-binding domains (9, 10). A zinc finger-homeodomain fusion already is being tested for potential applications in gene therapy (11). Recent phage display experiments suggest that it may be possible to select zinc finger peptides that will recognize almost any desired target site on duplex DNA (8). Despite this rapid progress in design, there has been relatively little information about how such novel proteins might be used to regulate transcription or about the differential effects of targeting the zinc finger peptides to various promoter regions. In this study, we begin exploring these issues to develop a more systematic basis for the application of zinc finger proteins in gene therapy and in the regulation of transgenic organisms. We systematically scan the promoter region to find sites where zinc fingers are most effective as repressors. We also show that novel zinc finger peptides selected via phage display can function effectively as repressors in vivo.

EXPERIMENTAL PROCEDURES

Plasmid Construction and Mutagenesis—Reporter plasmids were constructed by subcloning and subsequent mutagenesis. A 260-bp DNA fragment containing 5 GAL4 binding sites, the TATA box from the adenovirus major late promoter, and the initiator element from the murine terminal deoxynucleotidyltransferase promoter was generated by digesting B111T (kindly provided by S. T. Smale) with HpaI and BamHI. This 260-bp fragment was inserted into pG63-Basic (Promega), which had been digested with NheI and extended with Klenow, and digested with BglII. The resulting plasmid (pGL3-TATA/Inr) served as a template for site-directed mutagenesis, and the QuikChange™ kit (Stratagene) was then used to make various reporter plasmids. Zinc finger expression plasmids were constructed by polymerase chain reaction amplifying DNA fragments encoding three fingers of Zif268 (12) or of the Zif268 variants selected by phage display (8). These zinc finger-coding regions were inserted into the HpaI and BamHI sites of pCFN, which had been constructed by subcloning an annealed oligonucleotide duplex (5′-AGCTTCCACCTCCCAAAAAGAAAAGTTAACG-3′ + 5′-ATTTCGTAACCTTCTTTCTTTTTGGGAGGTGGA-3′) into the EcoRI and HindIII sites of pFLAG-CMV2 (Eastman Kodak Co.). These expression plasmids were designed to produce zinc finger peptides that would have both the FLAG peptide and the nuclear localization signal from SV40 large T-antigen (13) at their N terminus. (A typical sequence reads NH2-MDVKKDDDKLPPKKRRRKVEPRYAC... , where the nuclear localization signal is underlined and the start of the zinc finger sequence is shown in boldface.) The DNA sequences of the reporter and expression plasmids were confirmed by dyeoxy sequencing.

Transient Cotransfection—The 293 cells were transfected by calcium phosphate precipitation with a glycerol shock (14). Transfection experiments typically used cells at 10–30% confluency in monolayer cultures (100-mm diameter plates) and the following plasmids: 1 µg of the empty expression plasmid (pCFN) or of expression plasmids encoding zinc finger peptides; 1 µg of a reporter plasmid; 5 µg of activator plasmid (GAL-VP16; kindly provided by S. T. Smale); 0.5 µg of β-galactosidase expression plasmid (pCMVβ; CLONTECH); and 12.5 µg of carrier plasmid (pUC19). In a parallel assay of basal transcription, GAL4-VP16 was omitted, and the amount of carrier plasmid was increased by 5 µg. Plasmids used in this assay were purified using the QIAGEN Maxi kit (QIAGEN), and the luciferase activity in the transfected cells was measured as described (14).

RESULTS

Transcriptional Repression by Zinc Finger Peptides; Effects of Binding Site Placement within the Promoter Region—We used transient cotransfection studies, with various promoter constructs coupled to a luciferase reporter, to test whether zinc finger peptides can repress transcriptional initiation and elongation from RNA polymerase II promoters in vivo. Our reporter constructs used a well characterized synthetic promoter that contains the adenovirus major late TATA box (5′-TATAAAAAAAG-3′) and the murine terminal deoxynucleotidyltransferase initiator (5′-CCTCATTTCT-3′) (15, 16). A set of five GAL4 binding sites was included upstream of the promoter so that the effects of the zinc finger peptides on activated transcription could be studied (by using a GAL4-VP16 fusion). A three-finger peptide

* This research was supported by the Howard Hughes Medical Institute. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed. Tel.: 617-253-8865; Fax: 617-253-8728.

1 The abbreviations used are: bp, base pair(s); NRE, nuclear hormone responsive element; TBP, TATA-binding protein.
from Zif268 was tested as a repressor, and the optimal binding site for this zinc finger peptide (5'-GGGGTGGGCGg-3') was incorporated at various positions between the TATA box and the initiator element (Figs. 1 and 2A). We also tested Zif binding sites at two positions downstream of the initiator element (Figs. 1 and 2A). (Note that the numbering scheme used for these constructs (−22, −19, and so forth) indicates the relationship between the 5' G of the inserted Zif268 binding site and the transcriptional start point (the A, which is labeled as +1 in Fig. 1.) The spacing between the TATA box, the initiator element, and the start codon of the luciferase reporter gene was kept constant in all reporter constructs. Promoters were tested via transient cotransfection in the 293 human cell line.

We first tested whether the Zif268 peptide could repress activated transcription from these promoters, and thus the GAL4-VP16 expression plasmid was added during these cotransfection assays (Fig. 2). Testing promoters with Zif binding sites at various positions gave the following levels of repression: 19-fold for a site at base pair position −22 with respect to the start of transcription, 6.2-fold for a site at position −19, 5.4-fold for a site at position −16, and 18-fold for a site at position +18. No repression was observed for a construct containing a Zif binding site at position +49. The protein concentration did not seem to be limiting in this set of experiments, since increasing the amount of the zinc finger expression plasmid in the cotransfection assays (up to 5-fold) gave no significant difference in the observed levels of repression. Further experiments revealed that higher levels of repression could be achieved by including multiple zinc finger binding sites within a single promoter construct. Thus Zif268 gave 63-fold repression of activated transcription when binding sites were included at both positions −22 and +18 (i.e. near the TATA box and the initiator). (Note: The TATA/Inr promoter fragment to cover a potential binding site for Zif268, 5'-GGGGTGGGCGG-3', beginning at bp position −22 with respect to the transcription start site (Fig. 1). However, gel shift assays (17) have indicated that this sequence was a very poor binding site for Zif268, and the Zif268 peptide showed no repression from this promoter in our cotransfection assays (Fig. 2B.).

A parallel set of experiments was done to determine whether Zif268 could repress basal transcription from these promoters. No repression was observed with the initial TATA/Inr promoter construct, while moderate repression levels (typically 2–5-fold) were obtained with promoters containing the Zif268 binding site. (The right panel of Fig. 2B gives specific repression levels observed with each of the constructs.) Repression of basal transcription shows that Zif268 can act directly on the general transcription machinery (either by blocking the assembly of the preinitiation complex or by preventing subsequent steps such as isomerization or elongation) (18). However, at most sites, we find that repression of activated transcription is considerably stronger than repression of basal transcription. The only exception involved a promoter construct with the Zif site at position +49, which gave 2-fold repression of basal transcription but no repression of activated transcription. When binding to this site at +49, it seems unlikely that Zif268 could affect the assembly of the preinitiation complex, but it may weakly inhibit elongation during basal transcription.

Transcriptional Repression from Promoters Lacking a TATA Box or an Initiator Element—Since many promoters lack either a TATA box or an initiator element, we tested whether Zif268 could inhibit VP16-activated transcription from promoters containing only one of these elements (Figs. 1 and 3). The Zif268 binding site was tested at position −22 and position +18, since these had been most effective with the original promoter construct. In these experiments, Zif268 gave about 4-fold repression of activated transcription from the promoters lacking the TATA box. Zif268 gave 9–10-fold repression of activated transcription from promoters lacking the initiator. The degree of repression from these promoters was comparable to that from a promoter containing both the TATA box and the initiator (where Zif268 at corresponding positions had given 18–19-fold repression), but the zinc finger peptides still give significant repression of activated transcription from these promoters.

**Repression with Novel Zinc Finger Peptides Selected via Phage Display**—One of the central motivations for using zinc fingers in these studies is that the DNA binding specificities of zinc finger peptides can be systematically altered by phage display methods (3–8). This should eventually allow us to target endogenous promoters by selecting zinc finger peptides that bind to appropriate sites in the wild type promoters. (For example, one might use phage display to select zinc finger peptides that would recognize the wild type sequence starting at
positions corresponding to base pairs -22 and +18 of our current promoter constructs.) As a step toward this goal of selecting zinc finger proteins for novel sites, Greisman and Pabo (8) had selected novel three-finger peptides that recognize important regulatory elements, such as the P53 binding site, the nuclear hormone responsive element (NRE), and the TATA box of the adenovirus major late promoter. These peptides (termed P53, NRE, and TATA peptide, respectively) can bind to their cognate DNA sites with specificity and affinity comparable to those of the three-finger Zif268 peptide. (Gel shift studies had shown that all of these complexes have dissociation constants of $1 \times 10^{-9}$ M.)

We tested these novel zinc finger peptides in transfection studies, using the same transcriptional repression assay we had used with Zif268 (Fig. 4). Twofold repression was observed with the NRE peptide and 5-fold repression was observed with the P53 peptide when they were used to block activated transcription. (These promoter constructs were designed so that the NRE or p53 binding sites are located near the TATA box and the initiator.) No repression was obtained when the promoters lacked the appropriate zinc finger binding sites. Although these “first generation” peptides are not as effective as Zif268 in vivo, our results clearly demonstrate that novel zinc finger peptides selected by phage display systems can bind to their target sites in human cells and repress transcription. Improvements in selection protocols may give even more effective repressors for future studies.

The measured DNA-binding affinities of the new peptides (8) show a general correlation with their effectiveness as repressors. Thus gel shift studies indicate that the Zif268 peptide binds slightly better than the P53 peptide, and the p53 peptide binds somewhat better than the NRE peptide. This is the same rank order as observed for their effectiveness as repressors. Immunoblotting experiments showed comparable expression levels for the Zif, p53, and NRE peptides (data not shown), and thus differences in the degree of repression (19-, 5-, and 2-fold, respectively) do not appear to result from differences in the amount of peptide expressed. We also note that increasing the amount of transfected expression plasmids in these experiments gave no significant difference in the level of repression. Finally, we note that the TATA peptide did not repress activated transcription from the TATA/Inr promoter. The appropriate recognition site is already present in this construct (the peptide had been selected to recognize the adenovirus major late TATA box), but the TATA box may already be occupied in vivo by TFIIID (19). In vitro binding data from our laboratory (8) also show that the TATA peptide binds somewhat less tightly...

---

**Fig. 2.** Transcriptional repression by Zif268 at various locations within the promoter region. A, schematic summary of the structure of reporter promoters. The Zif268 binding site (symbolized here by GCG) is boxed and the numbering scheme corresponds to that used in Fig. 1. B, results of transient cotransfection assays. Human 293 cells were transfected as described (14), using the calcium phosphate precipitation method. Luciferase and β-galactosidase activities were measured 48 h later. Total luciferase activities were divided by corresponding β-galactosidase activities to yield the relative luciferase activities. The relative luciferase activity from the cells transfected with empty expression plasmid (open bar) was then set to an arbitrary value of 100 units, and activity from cells transfected with the Zif268 expression plasmid (dark bar) was expressed on this same scale. The GAL4-VP16 expression plasmid was included for transfection studies of activated transcription, and this gave luciferase activities about 1,000-fold higher than those obtained via basal transcription. Reported activity levels represent an average obtained from three independent experiments, and the standard error of the mean is shown.
Transcriptional Repression by Zinc Finger Peptides

**FIG. 3.** Repression of activated transcription from promoters lacking a TATA box or an initiator element. The structure of the promoters and the results of the transfection assays are shown. Mutated TATA box or initiator regions are represented as crossed boxes, and their DNA sequences are shown in Fig. 1. The GAL4-VP16 activator plasmid was included in all of these transfections. Relative luciferase activities were calculated as before (Fig. 2). The relative activity from the cells transfected with the empty expression plasmid and the TATA/Inr/Z(-22) reporter plasmid was set to an arbitrary value of 100. Reported activity levels represent an average obtained from three independent experiments, and the standard error of the mean is shown.

| promoter | luciferase activity with empty vector | Zif268 | fold repression |
|----------|--------------------------------------|--------|----------------|
| TATA/Inr: Z(-22) | 100 +/-4 | 5.1 +/-0.5 | 19 |
| TATA/Inr: Z(+18) | 105 +/-7 | 5.8 +/-0.1 | 18 |
| -/Inr: Z(-22) | 8.7 +/-0.5 | 2.5 +/-0.2 | 3.5 |
| -/Inr: Z(+18) | 3.5 +/-0.4 | 0.90 +/-0.06 | 3.9 |
| TATA/-: Z(-22) | 29 +/-2 | 3.4 +/-0.3 | 8.5 |
| TATA/-: Z(+18) | 21 +/-2 | 2.2 +/-0.2 | 9.5 |

**FIG. 4.** Transcriptional repression with novel zinc finger peptides that had been selected by phage display. A, the structure of the promoters, showing location of the zinc finger binding sites. B, results of the transfection assay. The relative luciferase activities from cells transfected with the empty expression plasmid are shown in open bars, those with the NRE peptide expression plasmid in gray bars, and those with the P53 peptide expression plasmid in black bars. The GAL4-VP16 activator plasmid was included in all of these transfections. Reported activity levels represent an average obtained from three independent experiments, and the standard error of the mean is shown.

**FIG. 5.** Computer modeling of the structure of Zif268 bound to TBP-TFIIB-TATA complex. The cocrystal structures of Zif268-DNA (21, 22) and of the TBP-TFIIB-TATA complex (23) were aligned by superimposing phosphates such that Zif268 binds to its site at the TATA/Inr/Z(-22) promoter, whose DNA sequence around the TATA box is 5’-TATAAAAGNNNNGCGTGGGCG-3’. This arrangement leads to a collision between Zif268 and TFIIB, and this collision is marked with a circle. TBP is shown in red, TFIIB in green, Zif268 in yellow, and the DNA in blue.

and specifically than the other peptides, and this may limit its effectiveness in vivo.

**Plausible Mechanism of Transcriptional Repression by the Zinc Finger Peptides**—In most of our promoter constructs, the zinc finger binding sites are close to the TATA box and to the initiator (Fig. 1), and it seems likely that zinc finger peptides bound at these positions would block assembly of the preinitiation complex. Specifically, computer modeling studies (and the known location of the TFIIB footprint) (20) suggest that binding of the Zif peptide at positions -22, -19, or -16 may interfere with normal binding of TFIIB to the promoter. In Fig. 5, the Zif268-DNA complex (21, 22) is juxtaposed with the TBP-TFIIB-TATA box complex (23) in the arrangement expected when there is a Zif site at position -22. The protein-DNA complexes were aligned by superimposing sets of phosphates, and there is no obvious steric interference between TBP and Zif268 at this promoter. However, this arrangement causes the first finger at the N terminus of Zif268 to collide with TFIIB.

2 S. Wolfe and C. O. Pabo, unpublished results.
(Fig. 5, collision marked with circle). This suggests that Zif268 and TFIIB cannot bind simultaneously (at least not in their normal binding modes) when the Zif site is at position −22, and modeling indicates that similar problems may occur with Zif sites at positions −19 and −16.

**DISCUSSION**

Studies with novel zinc finger proteins may help elucidate transcriptional regulatory mechanisms (earlier studies using zinc fingers to block RNA polymerase III transcription helped delineate key regulatory sites) (24), and these studies also may provide a basis for using zinc finger proteins in gene therapy. (As mentioned in the Introduction, a designer zinc finger/homodomain fusion (9) already is being tested for applications in gene therapy (11.).) In the studies reported here, we have shown that zinc finger peptides can repress both basal and VP16-activated transcription when bound close to the TATA box or the initiator element. Computer modeling studies suggest that the Zif268 zinc fingers, when bound to DNA sites between the TATA box and the initiator element, may interfere with proper binding of TBP and TFIIB to the promoter. The Zif268 peptide also functioned effectively as a repressor when bound to DNA at position +18 (but not at position +49). It seems plausible that binding of the Zif268 peptide at position +18 may interfere with the binding of TBP-associated factors. (Drosophila TAF150 binds around the initiator and the footprint extends to position +33 (25.).) We find, with several different promoter constructs, that positions −22 and +18 give essentially equivalent levels of repression (Fig. 3). When both a TATA box and an initiator element were present, the Zif peptide gave 19-fold (position −22) and 18-fold (position +18) repression. When only the initiator element was present, each site gave about 4-fold repression. When only the TATA box was present, these sites gave 9-fold (position −22) and 10-fold (position +18) repression. Agreement of these repression levels may be fortuitous, but since 1) other proteins are tightly associated with TBP in vivo to form the multiprotein TFIID complex, and since 2) the TFIID footprint covers both the −22 and +18 sites, it seems plausible that binding of the Zif peptide at these positions may actually block the same step (i.e. binding of TFIID to the promoter), explaining why similar levels of repression are observed for these two positions.

Phage display systems (3–8) and design strategies (1, 2) have been successfully used to change the DNA-binding specificities of zinc finger proteins. Recent results suggest that it may be possible to select zinc fingers that will specifically recognize almost any desired site (8). This should allow the design of transcription factors that target critical sites in the promoters of viral genes and of oncogenes. Such novel zinc finger peptides may be used alone (as tested here), or possibly as fusions with repression domains (26) to down-regulate target genes in a sequence-specific manner. Specificity of the DNA binding proteins may be especially critical in gene therapy, since it will be vitally important to minimize adventitious repression of endogenous genes. (Affecting regulation of other genes might lead to dangerous side effects in a clinical application.) In this regard, we note that it may be possible to improve the effectiveness and specificity of transcriptional repression by independently targeting multiple sites in a promoter region. We found that the Zif268 peptide, when bound to two critical sites, gave much stronger repression than when bound to a single site. Thus, when repressing activated transcription from a promoter containing a TATA box and an initiator element, binding to position −22 gave 19-fold repression, binding to position +18 gave 18-fold repression, whereas binding to both positions gave 63-fold repression (Fig. 2). These results suggest that it may be desirable to select several zinc finger peptides, recognizing critical but nonoverlapping regions in a given promoter, and to combine these peptides for target-specific repression. This strategy has interesting parallels with the mechanisms of “combinatorial control” that are so common in eukaryotic gene regulation.

The strategies and results reported in this study should provide a good background for developing zinc finger peptides as repressors for use in gene therapy and in the regulation of gene expression in transgenic organisms. We find that zinc finger peptides can effectively repress transcription when they bind to key regions of the promoter, and phage display methods should allow selection of zinc finger peptides that recognize the corresponding regions of endogenous promoters.

Previous studies (24) had shown that zinc fingers could efficiently block RNA polymerase III transcription in vitro (repressing up to 17-fold with optimal placement of the binding sites). We show that comparable levels can be obtained in vivo at RNA polymerase II promoters. However, our results raise interesting questions about two other studies that have tested designer zinc finger proteins as repressors. A few years ago, Choo et al. (27) designed a zinc finger protein that would recognize a 9-bp site within the coding region of the targeted gene. They reported that this designer protein gave effective repression in vivo, interpreting changes in cell viability as evidence for “transcriptional blockage” of the targeted gene. However, it is important to note that their protein had a relatively modest affinity for the targeted DNA site (reported $K_d = 620$ nm, which is about 1,000-fold weaker than Zif268), and that the putative binding site for this zinc finger peptide was significantly downstream of the transcription initiation point. (It would need to block transcriptional elongation rather than merely prevent initiation.) The results of Choo et al. appear to be in striking contrast to our own; the Zif268 peptide binds much more tightly than their designed peptide and yet Zif268 has, at best, a modest effect on transcription when binding at position +49. The basis for these differences is not clear but our results certainly raise questions about the general utility of targeting zinc fingers to binding sites within the coding region of a gene.

It also is interesting to compare our results with those reported by Liu et al. (26) who designed proteins containing six zinc fingers (arguing that these might be specifically targeted to unique sites in a complex genome). They also added a 43-amino acid peptide representing the minimal Krüppel-associated box-A repression domain (28). Liu et al. report 14-fold repression for their construct when it binds to a promoter that contains six tandem copies of the binding site for this polyacetylated protein. Thus, they have good evidence for repression of the targeted gene, but many questions remain about the utility and specificity of these six-finger proteins and about the most effective methods of using zinc finger proteins as repressors. For repression of activated transcription, our strategy seems at least as efficient; we can obtain equal or greater levels of repression with a three-finger protein and a single binding site. Selection of zinc finger proteins recognizing endogenous (gene-specific) promoter regions should allow further testing and the potential for broad application of our strategy.

**Acknowledgments**—We thank H. A. Greisman and S. T. Smale for kindly providing plasmids used in these studies, and P. A. Sharp for generously allowing us to use his tissue culture facilities in the Center for Cancer Research at MIT.

**REFERENCES**

1. Desjarlais, J. R., and Berg, J. M. (1992) Proteins Struct. Funct. Genet. 12, 101–104
2. Desjarlais, J. R., and Berg, J. M. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 2256–2260
3. Rebar, E. J., and Pabo, C. O. (1994) Science 263, 671–673
4. Jamieson, A. C., Kim, S.-H., and Wells, J. A. (1994) Biochemistry 33,
Transcriptional Repression by Zinc Finger Peptides

5. Choo, Y., and Klug, A. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11163–11167
6. Wu, H., Yang, W.-P., and Barbas, C. F., III (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 344–348
7. Jamieson, A. C., Wang, H., and Kim, S.-H. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 12834–12839
8. Greisman, H. A., and Pabo, C. O. (1997) Science 275, 657–661
9. Pomerantzev, J. L., Sharp, P. A., and Pabo, C. O. (1995) Science 267, 93–96
10. Kim, J.-S., Kim, J., Cepek, K. L., Sharp, P. A., and Pabo, C. O. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 3616–3620
11. Rivera, V. M., Clackson, T., Natesan, S., Pollock, R., Amara, J. F., Keenan, T., Magari, S. R., Phillips, T., Courage, N. L., Cerasoli, F., Jr., Holt, D. A., and Gilman, M. (1996) Nat. Med. 2, 1028–1032
12. Christy, B. A., Lau, L. F., and Nathans, D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 7857–7861
13. Kalderon, D., Roberts, B. L., Richardson, W. D., and Smith, A. E. (1984) Cell 39, 499–509
14. Cepek, K. L., Chasman, D. I., and Sharp, P. A. (1996) Genes Dev. 10, 2079–2088
15. Smale, S. T., and Baltimore, D. (1989) Cell 57, 103–113
16. Emami, K. H., Navarre, W. W., and Smale, S. T. (1995) Mol. Cell. Biol. 15, 5906–5916
17. Swirnoff, A. H., and Milbrandt, J. (1995) Mol. Cell. Biol. 15, 2277–2287
18. Johnson, A. D. (1995) Cell 81, 655–658
19. Chen, J., Ding, M., and Pederson, D. S. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 11909–11913
20. Lee, S., and Hahn, S. (1995) Nature 376, 609–612
21. Pavletich, N. P., and Pabo, C. O. (1991) Science 252, 809–817
22. Elrod-Erickson, M., Rould, M. A., Nekludova, L., and Pabo, C. O. (1996) Structure 4, 1171–1180
23. Nikolov, D. B., Chen, H., Halay, E. D., Ushava, A. A., Hisatake, K., Lee, D. K., Roeder, R. G., and Burley, S. K. (1995) Nature 377, 119–128
24. McBryant, S. J., Kassavetis, G. A., and Gottesfeld, J. M. (1995) J. Mol. Biol. 250, 315–326
25. Verrijzer, C. P., Yokomori, K., Chen, J.-L., and Tjian, R. (1994) Science 264, 935–941
26. Liu, Q., Segal, D. J., Ghiaera, J. B., and Barbas, C. F., III (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5525–5530
27. Choo, Y., Sanchez-Garcia, I., and Klug, A. (1994) Nature 372, 642–645
28. Margolin, J. F., Friedman, J. B., Meyer, W. K.-H., Vissing, H., Thiesen, H.-J., and Rauscher, F. J., III (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 4509–4513