Locations of Contacts between Individual Zinc Fingers of *Xenopus laevis* Transcription Factor IIIA and the Internal Control Region of a 5S RNA Gene†

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ABSTRACT: A set of mutants of transcription factor IIIA (TFIIIA) have been prepared in which successive zinc-finger domains have been deleted from the carboxyl terminus of the protein. These have been analyzed by hydroxyl radical footprinting to map the location of contacts to DNA by individual zinc-finger domains of TFIIIA. The results suggest that the nine zinc fingers of TFIIIA are organized into three DNA-binding domains of three fingers each. The spatial relationship between zinc-finger contacts to 5S DNA suggests that the two domains which interact with either end of the DNA-binding site of TFIIIA (fingers 1–3 and 7–9) have a compact conformation, similar to that exemplified by the zif268 cocrystal structure [Pavletich, N. P., & Pabo, C. O. (1991) *Science 252*, 809–817]. However, the central domain (fingers 4–6) has a much more extended conformation, following a path nearly parallel to the helix axis and contacting over 20 base pairs of DNA in the center of the binding site of TFIIIA. These results strongly support two recently proposed and radically new models for the TFIIIA/5S DNA complex [Hayes, J. J., & Tullius, T. D. (1992) *J. Mol. Biol. 227*, 407–417; Clemens, K. R., Liao, X., Wolf, V., Wright, P. E., & Gottesfeld, J. M. (1992) *Proc. Natl. Acad. Sci. U.S.A.* (in press)].

Xenopus transcription factor IIIA (TFIIIA) is a 38-kDa protein that binds specifically to the internal promoter of 5S RNA genes and, with at least two general class III transcription factors (TFIIIB and TFIIIC), promotes the subsequent formation of a complex required for accurate polymerase III initiation of transcription (Engelke et al., 1980; Wolffe & Brown, 1988). TFIIIA exhibits several unusual structural features. The protein is highly asymmetric when free in solution (Bieker & Roeder, 1984) and when bound to DNA (Sakonju & Brown, 1982; Smith et al., 1984). The entire amino-terminal three-fourths of the protein comprises nine tandemly repeated zinc-binding motifs (Zn fingers) which constitute the DNA-binding domain of TFIIIA (Miller et al., 1985). These domains are arrayed roughly consecutively along the 45 bp DNA binding site for TFIIIA (Smith et al., 1984; Vrana et al., 1988). The structure of several individual TFIIIA-like Zn-finger domains has been determined by NMR spectroscopic analysis (Parraga et al., 1988; Lee et al., 1989; Omichinski et al., 1990). A recent X-ray crystal structure of the complex of the three-finger protein zif268 with DNA (Pavletich & Pabo, 1991) shows at least one way in which multiple Zn-finger domains may be combined into a single DNA-binding domain. In this complex each Zn finger makes structurally equivalent contacts in the major groove of the DNA.

However, it is unlikely that all nine Zn fingers in the DNA-binding domain of TFIIIA can be modeled by a simple extrapolation of the zif268 structure. For example, fingers near the N-terminal part of TFIIIA are absolutely required for binding to DNA, while specific binding is retained when several carboxy-terminal fingers are deleted (Smith et al., 1984; Vrana et al., 1988; Clemens et al., 1992). The binding site contains on average about 5 bp of DNA per finger unit (Fairall et al., 1986; Rhodes & Klug, 1986), not 3 as might be predicted on the basis of the zif268 structure. Further, the hydroxyl radical footprint of the TFIIIA/5S DNA complex contains details that are inconsistent with a structure containing nine equivalent finger domains (Churchill et al., 1990).

Several detailed models for the structure of the TFIIIA/5S DNA complex have been proposed. A modeling study of a consensus sequence for the stretch of amino acid residues comprising the linkers between individual finger domains in TFIIIA supports a zif268-related structure (Berg, 1990). In this model all of the fingers of TFIIIA are envisioned to wrap around the DNA in the major groove except finger 6. This finger has severely shortened linkers and is proposed to cross the minor groove in the center of the complex (Berg, 1990). Detailed analysis of the hydroxyl radical footprint of TFIIIA led to a model with a repeating unit consisting of *two* contiguous Zn fingers in the major groove of DNA (Churchill et al., 1990). These units are connected by linkers which cross over the minor groove of DNA such that the protein lies entirely on one side of the helix. Experimental attempts to locate contacts between TFIIIA and DNA have found that the interactions are generally clustered into three regions that coincide with important DNA sequence elements within the internal promoter as identified by mutagenesis (Pieler et al., 1987; Vrana et al., 1988; Hayes & Tullius, 1992; Clemens et al., 1992). Recent models based on these results have an overall tripartite structure in which three Zn-finger domains at either end of the complex are envisioned to wrap around the DNA, following in the major groove of the helix, while the three central finger domains are proposed to lie on one side of the DNA helix, in a manner similar to prokaryotic repressors, in the very center of the complex (Hayes & Tullius, 1992; Clemens et al., 1992).

A knowledge of the precise base pairs of 5S DNA contacted by the individual zinc-finger domains of TFIIIA would be

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extremely useful in the evaluation of proposed models for this protein. To this end, a set of bacterially expressed carboxy-terminal deletion mutants of TFIIIA (containing fingers 1–*N*, where *N* = 3, 4, 5, 6, 8, and 9) were prepared (Clemens et al., 1992). These mutants are precisely truncated after each finger domain such that the terminal Zn finger is left intact. Purified preparations of these mutants in quantities suitable for biophysical studies were obtained. These proteins have been analyzed for specific DNA-binding affinity and by DNase I footprinting (Clemens et al., 1992). Hydroxyl radical footprinting has a demonstrated ability to reveal structural details about protein/DNA complexes not seen with DNase I footprinting (Tullius & Dombroski, 1986; Vrana et al., 1988; Hayes et al., 1990; Churchill et al., 1990). Further, DNase I can actually displace portions of TFIIIA deletion mutants with reduced DNA-binding affinity, whereas hydroxyl radical can detect the presence of protein–DNA interactions in these cases (Vrana et al., 1988). We report here results of high-resolution hydroxyl radical footprinting of these deletion mutants. Features in the DNA cleavage pattern of the mutants are correlated with features in the footprint of the native protein. These experiments afford an accurate picture of the location of contacts to DNA by each of the Zn-finger domains of TFIIIA and place stringent requirements on models of this complex.

MATERIALS AND METHODS

Mutant proteins were prepared as described (Clemens et al., 1992). Plasmid DNA was prepared according to standard methods and stored as a precipitate in ethanol or as a frozen solution in TE buffer [10 mM Tris-HCl (pH 8.0), 1 mM EDTA] at −20 °C. A uniquely end-labeled DNA fragment containing the *Xenopus borealis* 5S RNA gene was obtained by digestion of plasmid pXbs201 (Bogenhagen et al., 1980) with the restriction endonuclease *Hin*dIII. The 5′ terminus of this DNA was then labeled at the *Hin*dIII site located 50 bp upstream from the beginning of the 5S gene with ^{32}P according to standard methods. A 246 bp long 5S DNA fragment, specifically radiolabeled on the noncoding strand, was obtained by subsequent restriction at the *Bam*HI site located at position +195. Labeled 5S DNA was specifically complexed with TFIIIA or deletion mutants as described (Clemens et al., 1992). Hydroxyl radcial footpringing was performed as described (Hayes & Tullius, 1992) in standard TFIIIA binding buffer lacking glycerol (Vrana et al., 1988) and containing 1 mM Mg^{2+} (Liao et al., 1992). Typically, 0.1 pmol of labeled DNA fragment (about 10 ng) was incubated for 10 min at 25 °C with 200 ng of nonspecific competitor DNA and with the following amounts of each of the TFIIIA deletion mutants: zf1–3, 2 pmol; zf1–4, 14 pmol; zf1–5; 10 pmol; zf1–6; 10 pmol; zf1–8; 20 pmol; zf1–9; 5 or 2 pmol of native TFIIIA. Products of the cleavage reaction were analyzed by denaturing gel electrophoresis and densitometry as described (Hayes & Tullius, 1992).

RESULTS

The hydroxyl radical footprint of TFIIIA bound to a 5S RNA gene reveals detailed information about the complicated protein–DNA contacts in this complex (Figure 1) (Vrana et al., 1988; Hayes et al., 1989; Churchill et al., 1990). Although TFIIIA yields a slightly stronger footprint with the noncoding strand, the protection patterns on either strand are quite similar (Churchill et al., 1990). Thus, for simplicity, only experiments in which the noncoding strand is labeled will be shown in this work and all sequence positions noted for 5S DNA will refer

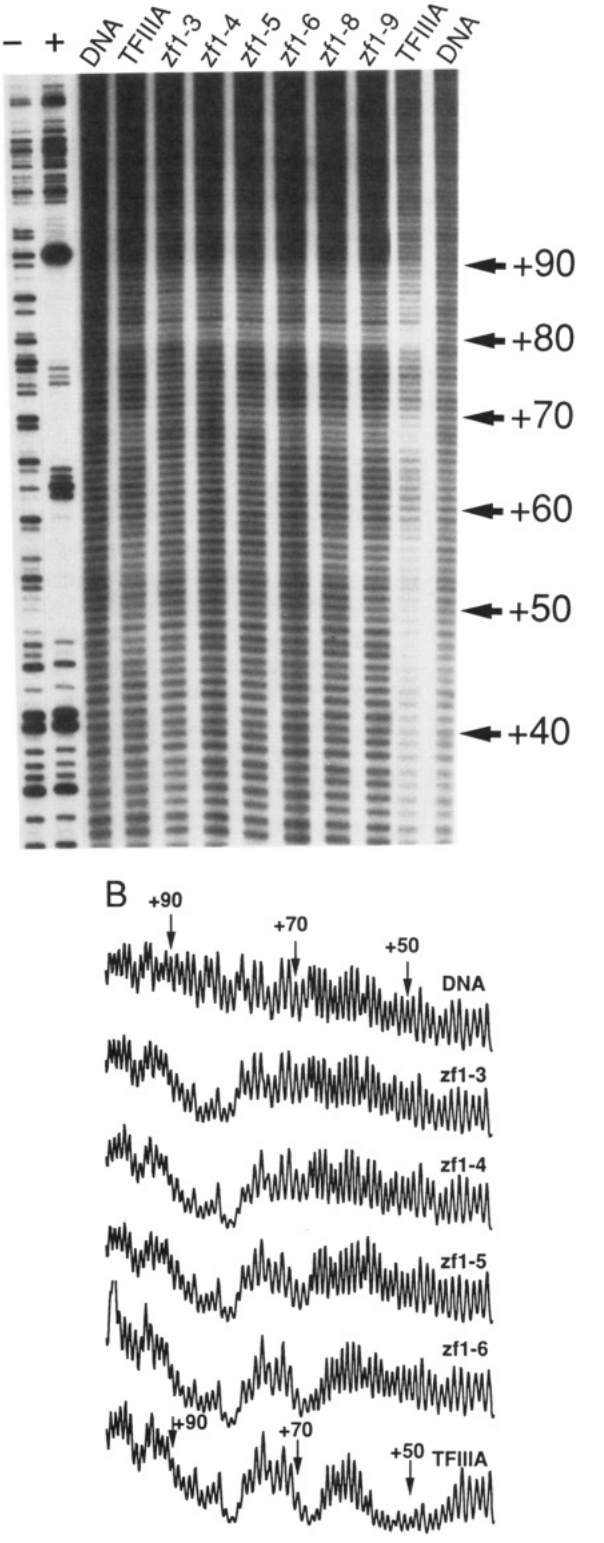

FIGURE 1: (A) Hydroxyl radical footprinting of TFIIIA deletion mutants. Products of the hydroxyl radical footprinting of each deletion mutant were separated by denaturing gel electrophoresis. (Lane 1) Naked DNA; (lane 2) native TFIIIA; (lane 3) zf1–3; (lane 4) zf1–4; (lane 5) zf1–5; (lane 6) zf1–6; (lane 7) zf1–8; (lane 8) zf1–9; (lanes 10 and 11) DNase I footprint of TFIIIA and naked DNA, respectively. (B) Densitometric analysis of the autoradiography shown in (A). Scans of lanes 7 and 8 are shown in Figure 5. Arrows indicate positions +90, +70, and +50 from left to right.

only to this strand. A comparison of the cleavage pattern of this complex with that of the DNA alone reveals two broad (~10 bp each) and relatively even regions of protection at either end of the complex in which cleavage by hydroxyl radical is reduced about 50–75% (Figure 1A, lanes 3 and 4; Figure

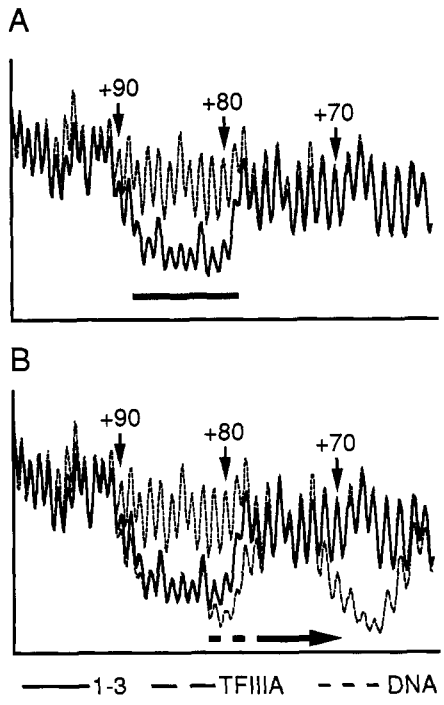

FIGURE 2: Hydroxyl radical footprint of zf1–3. (A) Comparison of the zf1–3 footprint and the naked DNA cleavage patterns. (B) Same as in (A) but also with a comparison to the native TFIIIA footprint. Only the downstream portion of the cleavage pattern is shown for clarity. Numbers and arrows indicate positions on the noncoding strand of the 5S gene (see Materials and Methods). Horizontal bar highlights the region of DNA protected by bound zf1–3, and the horizontal arrow indicates the portion of the native TFIIIA footprint not included in the zf1–3 pattern.

1B). This suggests that TFIIIA makes similar contacts to all of the base pairs in an entire turn of the DNA helix in these regions and is consistent with portions of TFIIIA lying in the major groove of DNA (Sakonju et al., 1984; Vinson et al., 1989; Hayes & Tullius, 1992). Conversely, the center of the footprint of TFIIIA exhibits regions in which the cleavage is about normal (+62; +73) and regions with almost complete protection (+80; +67) (Figure 1B). This pattern is reminiscent of the footprints of proteins that contact only one side of a DNA helix, periodically covering the minor groove (Tullius & Dombroski, 1986). However, in these previous studies it was impossible to determine with certainty which of the nine fingers of TFIIIA are responsible for each of the features in the hydroxyl radical footprint of TFIIIA. Previous studies with deletion mutants of TFIIIA have shown that fingers 1–3 bind as a unit which makes the most energetically important contacts to DNA in the entire TFIIIA/DNA complex (Vrana et al., 1988; Liao et al., 1992). Polypeptides containing only fingers 1 and 2 or 2–4 do not bind DNA specifically. However, a polypeptide containing fingers 1–3 (zf1–3) binds strongly and specifically to 5S DNA sequences between +80 and +92 (Liao et al., 1992). The DNase I footprint of a peptide containing these fingers extends from +96 to +77 (Christensen et al., 1991; Liao et al., 1992). A densitometric analysis of the hydroxyl radical footprint of a polypeptide containing zinc fingers 1–3 (zf1–3) is shown in Figure 2. The footprint of zf1–3 is identical to the footprint of native TFIIIA from the downstream-most edge to about position +81 (Figure 2). The most prominent feature of the footprint of TFIIIA in this region, the broad zone of protection between +90 and +79, is clearly present in the zf1–3 footprint (Figure 2B). Thus, the protection over these 10–11 bases is due to a DNA-binding domain comprising zinc fingers 1–3.

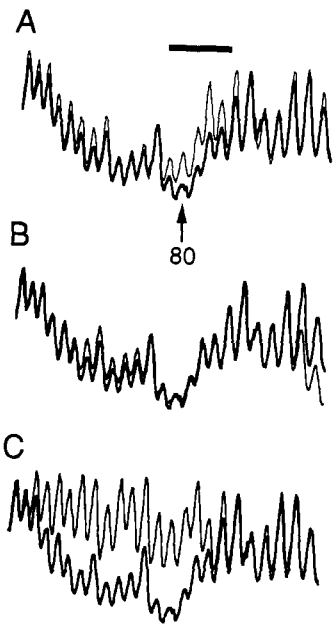

FIGURE 3: Hydroxyl radical footprint of zf1–4. The zf1–4 footprint (bold line in all cases) is shown with (A) the zf1–3 footprint, (B) the native TFIIIA footprint, and (C) the naked DNA pattern. Position +80 on the noncoding strand is indicated, and the horizontal bar indicates the region protected by finger 4.

The hydroxyl radical footprint of zf1–4 is nearly identical to that of zf1–3 downstream of +80 (Figure 3A). However, an important difference between the two patterns is found near position +80. In the footprint of native TFIIIA, a drastic diminution in hydroxyl radical cleavage is found around positions +80 and +79 on the noncoding strand of 5S DNA (Figure 1; Churchill et al., 1990). This feature is not found in the zf1–3 footprint (Figure 2A) but is found in the zf1–4 footprint (Figure 3). Since cleavage of DNA by hydroxyl radical occurs by direct modification of the backbone in the minor groove of DNA, proteins that cover this groove will afford almost complete protection from cleavage (Tullius & Dombroski, 1986; Tullius, 1987). Thus, the data suggest that finger 4 directly covers the minor groove near position +80 on the noncoding strand of 5S DNA.

A comparison of the zf1–4 and zf1–5 footprints clearly indicates the position of finger 5 in the complex (Figure 4A). The footprint of zf1–5 resembles that of zf1–4 but is extended further upstream, with additional protection of positions +71 to +68. Thus, finger 5 is responsible for about half of the deep well of protection near position +67 in the footprint of native TFIIIA. The pattern of zf1–6 is, likewise, similar to that of zf1–5 except for additional protections at positions +69 to +66, filling out the remainder of this deep well of protection (Figure 4B).

These results indicate that the end of finger 5 and finger 6 comprise the portion of TFIIIA that crosses the minor groove of 5S DNA near positions +69 to +67 (Churchill et al., 1990; Berg, 1990; Hayes & Tullius, 1992). Note that these positions are on the same face of the DNA helix and one helical turn away from where finger 4 also crosses the minor groove (Figure 3). Close inspection of Figure 4B reveals another smaller but reproducible difference between the zf1–5 and zf1–6 footprints. Cleavage at positions +58, +57, and +56 is clearly reduced by the additional presence of finger 6. These positions are located one helical turn away and on the same face of the DNA helix as the main finger 6 footprint at +69 to +66 and *two* helical turns away from finger 4. These results suggest that fingers 4–6 contact only one side of the central 20 base

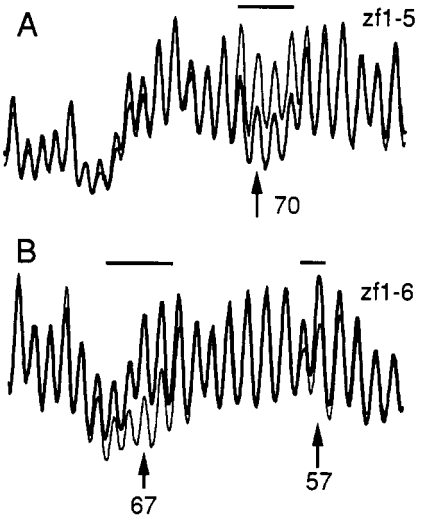

FIGURE 4: (A) Hydroxyl radical footprint of zf1–5 (bold line) shown with the zf1–4 footprint (thin line). Peaks between +85 and +62 from the complete hydroxyl radical footprints (Figure 1B) are shown. Horizontal bars indicate the bases protected by finger 5. (B) Hydroxyl radical footprint of zf1–6 (thin line) shown with the zf1–5 footprint (bold line). Peaks between +75 and +53 from the complete hydroxyl radical footprints (Figure 1B) are shown. Horizontal bars indicate the bases protected by finger 6.

pairs of the binding site for TFIIIA and that these fingers bind DNA in a manner which is different from that of fingers 1–3.

The deletion mutant of TFIIIA containing fingers 1–7 could not be obtained in our bacterial expression system (Clemens et al., 1992). However, zf1–8 yields a hydroxyl radical footprint extending up to about position +52 (Figure 1A). Although the general degree of protection in the 5′ part of the footprint is not as strong (see below), the pattern of protection in the 3′ part of the footprint is similar to that of native TFIIIA (Figure 5A). A comparison of the zf1–6 and zf1–8 footprints indicates that fingers 7 and 8 contact bases between +57 and +52 on the noncoding strand of 5S DNA (Figure 5A). The location of finger 9 on the DNA is clearly indicated by a comparison of the hydroxyl radical footprints of zf1–8 and zf1–9 (Figure 5A,B). The patterns are virtually identical except for a difference in cleavage at three contiguous bases. In the peptide lacking finger 9, the DNA is left more exposed at positions +51, +50, and +49 (Figure 5B).

Limited proteolysis of TFIIIA yields a 30-kDa fragment that contains all nine fingers and has been shown to have the same hydroxyl radical footprint as the intact protein (Smith et al., 1984; Hayes et al., 1989). However, the exact location of the edge of finger 9 cannot be deduced from these experiments since an undetermined number of residues beyond finger 9 were still present in the 30-kDa fragment which may contribute to the footprint. A comparison of the hydroxyl radical cleavage pattern of the 1–9 peptide (in which all residues after the ninth finger domain are deleted) and the native TFIIIA and naked DNA scans is shown in Figure 6. The 1–9 peptide footprint is very similar to that of native TFIIIA from the 3′ end to position +49 and matches the naked DNA pattern from +48 to the 5′ end of the scan (Figure 6). However, the footprint of the native protein extends to about position +44. Thus, the protection observed in the region +48 to +44 is likely due to residues to the COOH side of finger 9. Moreover, this comparison indicates that the edge of finger 9 is in contact with the base at position +49—in exact agreement with the position indicated by the zf1–8/zf1–9 comparison (Figure 5B).

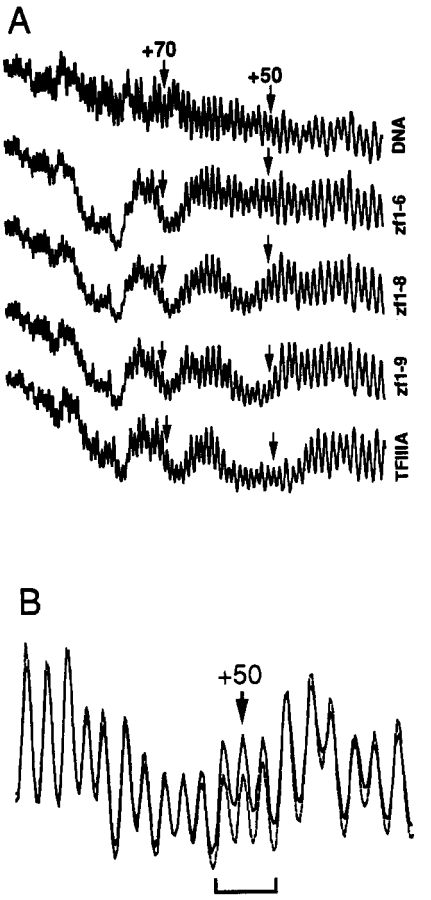

FIGURE 5: (A) Densitometer scans of the hydroxyl radical footprints of zf1–8 and zf1–9 are shown with those of zf1–6, TFIIIA, and the naked DNA pattern for comparison. (B) zf1–8 footprint (solid line) compared with the zf1–9 footprint (dashed line). Horizontal bracket indicates the bases protected by finger 9.

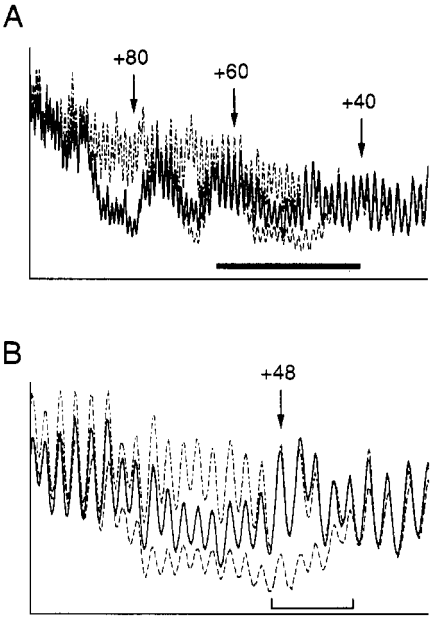

FIGURE 6: Hydroxyl radical footprint of zf1–9. (A) Comparison of the zf1–9 (solid line), naked DNA (dash-dot line), and native TFIIIA (dashed line) cleavage patterns. Horizontal line indicates the region expanded in (B). Horizontal bar in (B) indicates the region not protected by zf1–9 that is footprinted by native TFIIIA.

Another interesting feature of the complex is revealed in Figure 6A. Although clearly protected from cleavage, the footprint of the 1–9 mutant is not as strong as the footprint

of the native protein in the region +69 to +45. However, the degrees of protection found in the rest of the pattern are virtually identical between the two. Thus, the binding of the protein domains of TFIIIA which interact with the +69 to +45 region may be destabilized by the loss of residues to the COOH of finger 9. Thus, these nonfinger residues not only contribute to the hydroxyl radical footprint but also make energetically important interactions with DNA. Alternatively, the loss of these residues could in some way affect the conformational stability of fingers 7–9. This result highlights the modular nature of the TFIIIA/DNA complex since partial disruption of the DNA-binding domain comprising fingers 7–9 clearly has little effect of the remainder of the protein/DNA complex.

DISCUSSION

Our results confirm and extend a previous footprinting study using a series of deletion mutants of TFIIIA which allowed approximate locations of contacts by general regions of the protein to be identified (Vrana et al., 1988). However, this study was hampered by the random location of the site of truncation and the small amounts of these deletion mutants which could be generated, necessitating footprinting directly in a translation extract. The precisely engineered and bacterially expressed set of deletion mutants used in the present work allows the exact location of the zinc fingers in the TFIIIA/5S DNA complex to be determined.

Truncated versions of TFIIIA specifically bind to DNA as long as the critical region including fingers 1–3 is present (Vrana et al., 1988; Liao et al., 1992; Clemens et al., 1992). Interference experiments indicate that positions +88 to +79 make the strongest contacts to TFIIIA (Sakonju & Brown, 1982; Hayes & Tullius, 1992). The zf1–3 polypeptide strongly protects about 10–11 DNA backbone positions between +90 and +79 from hydroxyl radical cleavage (Figure 3). Our results support models in which the first three fingers of TFIIIA bind to these bases in the major groove of 5S DNA perhaps in a manner analogous to that exemplified by the zif268 cocrystal structure (Pavletich & Pabo, 1991; Hayes & Tullius, 1992; Clemens et al., 1992).

Features in the center of the hydroxyl radical footprint of native TFIIIA have been interpreted as being due to protein contacting only one side of the DNA helix in this region (Churchill, 1987; Berg, 1990; Hayes & Tullius, 1992; Clemens et al., 1992). Our results show that this central region is contacted by fingers 4–6 which form an extended structural domain clearly different from that of fingers 1–3. It has been shown that finger 5 contacts the major groove of 5S DNA near positions +70 and +71 (Clemens et al., 1992). We find that finger 4 crosses that minor groove near positions +79 and part of finger 5 and finger 6 cross the minor groove near position +68 (Figures 3 and 4). Moreover, some interactions are also detected between finger 6 and the DNA near position +58 (Figure 4), and it has been shown that this finger makes energetically important contacts with position +59 (Clemens et al., 1992). Taken together, these results indicate that the entire central domain of TFIIIA lies along one side of the DNA, contacting both grooves of 5S DNA for more than two turns of DNA in the center of the binding site for TFIIIA (see below).

Our results suggest that the remaining three fingers of TFIIIA (7–9) are primarily responsible for the protection from hydroxyl radical cleavage found in the region +59 to +47 (Figure 5A). The pattern of protection in this region is qualitatively similar to the protection caused by fingers 1–3 in the +90 to +80 region. Other experiments have also suggested a structural similarity between these two outer portions of the TFIIIA DNA-binding domain (Hayes & Tullius, 1992; Clemens et al., 1992). Indeed, our results are consistent with the proposal that fingers 7–9 are part of a three-finger structural unit which wraps around the DNA in a manner similar to that of the zif268 protein and fingers 1–3 (Hayes & Tullius, 1992). As mentioned above, in the zif268 structure each finger is found to contact three base pairs of DNA (Pavletich & Pabo, 1991). Interestingly, we find that finger 9 contacts exactly three bases at positions +51, +50, and +49, and thus contacts by the entire three-finger unit extend from about position +57 to position +49 (Figure 5B). Further, the results show that finger 8 contact extends to position +52 and that fingers 7 and 8 together contact the six base positions between +57 and +52. This arrangement also would place the downstream edge of finger 7 exactly where the upstream-most contacts between finger 6 and the DNA were detected (+58, +57; Figure 4B).

Our results indicate that TFIIIA has a tripartite structure in which fingers 1–3 and 7–9 form two independent three-finger DNA-binding domains which contact about 10 base pairs of DNA each and may have conformations similar to the zif268 three-finger protein (Pavletich & Pabo, 1991). The 22 or so bases between the outer domains are spanned by the central three fingers of the proteins (fingers 4–6) and the linkers connecting them. A single Zn-finger domain is roughly 20 Å in length (excluding linkers) (Pavletich & Pabo, 1991). If fingers 4–6 lie with their long axes parallel to the DNA helical axis, then about 60 Å of the approximately 70-Å distance spanned by these fingers can be accounted for. The extra distance may be an indication of bending of the DNA by TFIIIA in this central region (Schroth et al., 1990; Berg, 1990). Indeed, DNase I sensitive sites are found at positions 60–62, exactly opposite where we have determined finger 6 to be located. Bending of the DNA near finger 6 also has been proposed on the basis of the divergent nature of the linker sequences adjacent to this finger domain (Berg, 1990). In light of the results presented in this paper, a comparison of the DNA bending properties of these deletion mutants might be useful in locating the exact site of DNA deformation in the TFIIIA/5S DNA complex.

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