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Definition of the binding sites of individual zinc fingers in the transcription factor IIIA–5S RNA gene complex

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ABSTRACT **A series of polypeptides containing increasing numbers of zinc fingers of *Xenopus* transcription factor IIIA has been generated and binding to the 5S RNA gene internal control region has been studied in order to elucidate the mode of interaction of the individual fingers with DNA. By using a combination of DNase I footprinting, methylation interference, and differential binding to mixtures of DNA fragments differing in length by single base pairs, the binding sites for individual fingers have been defined. These results have led to a model for the interaction of transcription factor IIIA with the internal control region in which fingers 1–3 bind in the major groove of the promoter C block, fingers 7–9 bind in the major groove of the A block, and finger 5 binds in the major groove of the intermediate element. Fingers 4 and 6 each bind across the minor groove, spanning these promoter elements.**

Transcription factor IIIA (TFIIIA), which was first isolated from *Xenopus* oocytes, contains nine zinc finger domains (1, 2) and is required for transcription of the 5S RNA gene by RNA polymerase III (3). The protein binds specifically within the internal control region (ICR) of the 5S RNA gene (3) and to the 5S RNA product (4, 5). A number of studies of the TFIIIA–DNA interaction have been published (3, 6–11), as have models for the binding interaction (7, 12, 13). In one model, the entire protein lies along one face of the DNA helix with pairs of fingers contacting bases in the major groove and with every second linker crossing the minor groove (7). A further model (12) predicts that fingers 1–5 and 7–9 wrap around the major groove as modular units, with finger 6 crossing the minor groove at the DNA bend (14, 15). Finally, it has been proposed, on the basis of sequence similarity to linkers found in the male sex-factor protein ZFY, that the linker between fingers 3 and 4 crosses the minor groove (13). In the current study, the DNA-binding properties of recombinant fragments of TFIIIA were studied and binding sites were identified for individual fingers. The data cannot be reconciled with the structural features of the models proposed to date for binding of TFIIIA to the ICR of the 5S RNA gene. A radically different model for DNA binding is proposed which accounts for all of the current results as well as previously published data.

MATERIALS AND METHODS

Zinc Finger Proteins. A series of seven proteins was created by the expression cassette PCR method (11, 16, 17) from the cDNA clone for the full-length protein [gift of Lawrence Korn and Jay T'so (18)]. Beginning with the first three fingers of TFIIIA (zf1–3), each protein contained one additional zinc finger, through zf1–9. The initiating oligonucleotide for the native N terminus, 5'-GTTGCTGAAGGACATATGG-GAGAGAAG-3', contained an *Nde* I restriction site of which the last three bases formed the start codon (underlined). Terminating oligonucleotides contained an *Eco*RI restriction site 5' to the stop anticodon (underlined): zf1–3, 5'-CACACAGGAATTCATTACTTGATGTTATGG-3'; zf1–4, 5'-CGCCCGGGAATTCTTACTGCTGTGTGTGACT-GAACTGATGAACC-3'; zf1–5, 5'-CAACTATTGAAT-TCATTAGCCTGCATGGACTTTTTC-3'; zf1–6, 5'-CAACTATTGAATTCATTAGTCCTGATGGCATTC-TGC-3'; zf1–7, 5'-CAACTATTGAATTCATTACTCTT-TTTCGTGAGTTTTC-3'; zf1–8, 5'-CAACTATTGAAT-TCATTACTGTTCCTCATGAAATGATTG-3'; zf1–9, 5'-CAACTATTGAATTCATTACTCTGGATCATGTA-CAACTG-3'. Site-directed mutagenesis (19) of the zf1–3 coding sequence in pTZ19R (Bio-Rad) was used to generate ΔNzf1–3, which has a deletion from Gly^2 to Tyr^{10} and Ser in place of Cys^{35}. The mutagenic oligonucleotides were 5'-GCGCATCTGAGCAAACACACAGG-3', for Ser substitution, and 5'-GCCGGTGGCATATGAAGCGGTACATC-3', for truncation of the N terminus. The coding sequences were ligated into plasmid pRK172 (20) as described (11). Plasmids were amplified in *Escherichia coli* strain DH5α and shuttled to *E. coli* strain BL21(DE3) for protein expression (21). Plasmids reisolated from BL21(DE3) were sequenced (Applied Biosystems).

Cells were grown, harvested, and lysed as described in the purification scheme for zf1–3 (11). All other proteins were isolated from inclusion bodies as follows. Pellets of lysed cells were solubilized in buffered 6 M urea or 8 M guanidine hydrochloride (50 mM phosphate, pH 7.0/100 mM NaCl/10 mM dithiothreitol/50 μM $ZnCl_2$). After centrifugation (17,000 rpm, 15 min, in a Beckman JA-21 rotor), the supernatant was diluted 1:1 by addition of buffer without denaturant and loaded onto a heparin-Sepharose column. The column was washed thoroughly and the protein was eluted with a 0.1–1.0 M NaCl gradient. Fractions containing the protein of interest (analyzed by silver staining after SDS/PAGE) were pooled, concentrated by ultrafiltration, and stored at −20°C. The protein yield was generally 5–10 mg/liter of culture (Bradford's reagent, Bio-Rad). The 23-kDa trypsin fragment of TFIIIA was prepared from 7S ribonucleoprotein particles (22) and was purified on a heparin-agarose column as described for the recombinant zinc finger proteins. The purity of each protein preparation (75% to near homogeneity) was analyzed by SDS/PAGE followed by staining, either with Coomassie blue or with silver, and by Western blotting (11) using anti-TFIIIA antibody (23). Protein concentrations and amounts quoted have been corrected to exclude the percentage of impurities. Additional binding data, including dissociation constants for each of the proteins, will be published elsewhere.

DNase I Footprinting. DNase I protection analysis was carried out on both the coding and the noncoding strand of DNA fragments containing either the somatic-type or oocyte-type 5S RNA gene (see refs. 3 and 24–27). Reaction mixtures

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Abbreviations: TFIIIA, transcription factor IIIA; ICR, internal control region.

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contained 500 ng of poly(dI)·poly(dC) and 180 fmol of labeled DNA, and reactions were carried out as protein titrations with molar ratios of protein to DNA from 2:1 up to 125:1, as required (3, 27).

Primer Extension Ladders. The primer extension method of Liu-Johnson *et al.* (28) was used to produce DNA fragments corresponding in sequence to the 5S gene ICR. A 72-base noncoding-strand template (11) was used with a 23-base primer (bases 75–97) for zf1–9 binding or a 14-base primer (bases 79–92) for zf1–4 binding experiments. For binding of zf1–6, a 45-base template (bases 49–93, noncoding strand) was employed with the 14-mer primer. Synthetic oligonucleotides were purified by gel electrophoresis. Labeled primers were hybridized to a 2.5-molar excess of the unlabeled template. Annealed DNA (3 pmol) was used in the primer extension reaction (15 min at 37°C, final volume of 20 μl) containing 0.1 mM each dNTP, 0.01 mM each ddNTP, and 10 units of Sequenase (United States Biochemical). Blunt ends were created with mung bean nuclease for all primer extension experiments, and the DNA was purified by phenol extraction and ethanol precipitation. For some experiments with zf1–4, the primer extension ladder was digested with the restriction enzyme *Sau*96I (Stratagene) and the 3′ recessed ends were filled in with dGTP and dCTP (1 mM) so that the longest fragment ended at base pair (bp) 65.

Each binding experiment consisted of multiple reactions with increasing, but limiting, amounts of protein such that <10% of the DNA was bound. Bound and free oligonucleotides were separated by immunoprecipitation (11) and the fractions were analyzed in a 10% or 12% sequencing gel. Autoradiographs were scanned with a densitometer and probability analysis was performed (7) such that P/P_{total} is the ratio of intensities of the bound and input (total) DNA for each fragment. Base pairs 5′ to the endpoint of the DNase I footprint were normalized to zero to account for inequality of the amounts of DNA loaded on the gel. The standard deviation (σ) for each base position was calculated for reactions from a single experiment; the mean standard deviation (σ_{av}) was calculated across all bases within an experiment. The larger deviation was used to determine the significance of the effect of a base pair on binding. The variance of the endpoints determined in this manner is ±1 bp.

Binding of zf1–5 and zf1–6 to Restriction Fragments. The labeled 72-mer coding-strand oligonucleotide (11) was hybridized to a 1.5-molar excess of the unlabeled noncoding strand. The duplex 72-mer was digested in separate reactions with restriction enzymes (see Fig. 4 legend) and the 3′ recessed ends were filled in with 1 mM dNTPs as appropriate. Equal amounts of each fragment were pooled for binding experiments. Bound and unbound restriction fragments were separated by gel mobility shift (29), eluted from the gel, and analyzed in a sequencing gel.

Methylation Interference. Methylation interference experiments were carried out according to established procedures (11), using the 130-bp *Eco*RI–*Dra* I fragment of the synthetic oocyte-type gene (26). This gene has the oocyte-specific sequence over the 5′ "A-block" region but has the somatic-specific cytosine (C) at position 79. Binding reactions were performed with 0.2–0.5 mol of TFIIIA per mol of DNA.

RESULTS

Cloning and Purification of Zinc Finger Proteins. Fragments of TFIIIA were constructed by termination of the protein either two or three amino acids after the last histidine of fingers 3–9 (Fig. 1). An additional construct of zf1–3 was made (ΔNzf1–3) in which residues 2–10 were deleted in order to facilitate NMR structural studies. DNA sequencing indicated the expected amino acid sequence (1, 18) for ΔNzf1–3 through zf1–6 and for zf1–8. In zf1–9, $G^{810} \rightarrow T$ causes the mutation of Ala^{257} to Ser. Residue 257 is in the loop between the two Cys residues of finger 9 and would not be expected to contact the DNA (30) or otherwise interfere with binding. Affinity measurements (K.R.C., X.L., P.E.W., and J.M.G., unpublished work) bear out this expectation. A zf1–7 clone selected on the basis of the level of protein expression contained two point mutations ($A^{404} \rightarrow G$; $C^{568} \rightarrow T$) which alter the protein sequence near the tips of fingers 4 and 6 ($Gln^{121} \rightarrow Arg$; $Thr^{176} \rightarrow Ile$). Screening of numerous *E. coli* colonies failed to detect any clone which produced the seven-fingered protein of the wild-type sequence. We therefore resorted to the 23-kDa trypsin fragment of TFIIIA (22), which contains fingers 1–7 (31), for binding studies.

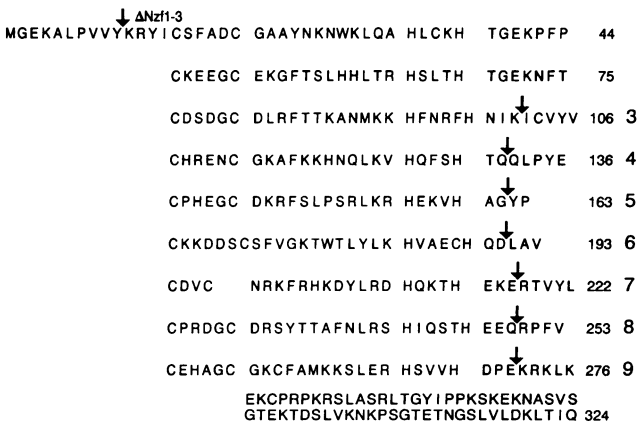

FIG. 1. Amino acid sequence of TFIIIA (1, 18). Position of each arrow indicates the last amino acid of the recombinant protein containing fingers 1–*n*; *n* is in large font at right. Spaces within the sequence define the elements of the zinc finger domain, the loop between coordinating Cys ligands, the finger region, the His–His loop, and the linker.

DNase I Footprints. Footprinting data (Fig. 2) show that the protected region increases substantially only with the addition of multiple fingers; however, some weak interactions are detectable with the addition of single fingers. Footprints for zf1–3 and ΔNzf1–3 are identical, with protection of the

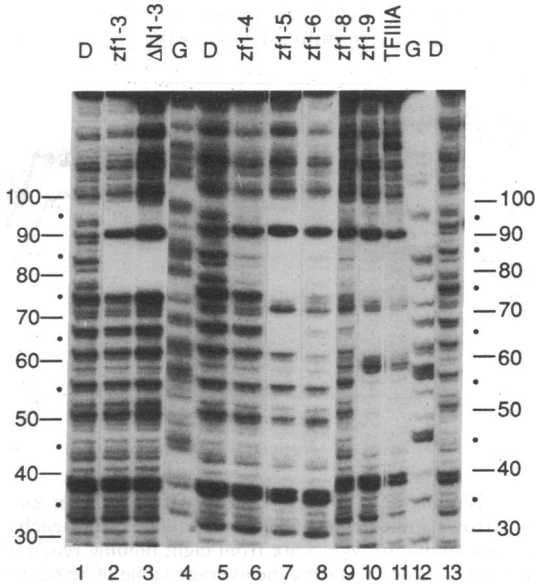

FIG. 2. DNase I footprints of the noncoding strand. A single lane is shown for each of the zinc finger proteins. Lanes 1, 5, and 13 contained naked DNA (D); lanes 4 and 12 contained guanine-specific markers (G). Molar ratios of protein to DNA were as follows: zf1–3, 39; ΔNzf1–3, 49; zf1–4, 30; zf1–5, 30; zf1–6, 106; zf1–8, 57; zf1–9, 43; and TFIIIA, 5.2.

backbone between bases 95 and 77 on the noncoding strand and between bases 92 and 74 on the coding strand (data not shown). zfl–4 weakly protects an additional 3 nucleotides on the noncoding strand. (Weak protection is that which could be detected only by quantitative densitometry.) Footprints of zfl–5 are significantly longer, extending from nucleotide 92 to 63 on the coding strand and from 95 to 65 on the noncoding strand. The footprint does not change dramatically with the addition of the next three fingers. zfl–6 weakly protects an additional 2 nucleotides on each of the DNA strands. A footprint is not observed for the weakly binding mutant zfl–7; but the 23-kDa trypsin fragment protects to nucleotide 63 of the noncoding strand (data not shown; see ref. 22). Additional weak protection is seen with zfl–8 (20–50% reduction of cleavage), extending nearly the full length of the TFIIIA footprint, to nucleotide 47 of the coding strand and 52 of the noncoding strand. The possibility that the weak protection is due to a second molecule of protein binding cannot be definitively excluded. zfl–9 yields a strong footprint, identical to that of full-length TFIIIA, from nucleotide 92 to 45 on the coding strand and from 95 to 45 on the noncoding strand. Within the noncoding-strand footprint, cleavage occurs between bases 60-61-62-63-64 and between 74-75-76-77 of both gene types with proteins which were long enough to extend to these regions. With the exception of sites 76-77 and 60-61, the cleavages are enhanced in the complex over the free DNA. Cleavages of the coding strand between bases 67-68-69 and 70-71-72 in the free DNA remain in footprints of TFIIIA or zfl–9. Sites hypersensitive to DNase I cleavage were induced at the 92-93 bond of the noncoding strand of both gene types upon binding of all of the zinc finger proteins. A hypersensitive site was also induced by zfl–9 or intact TFIIIA between bases 52 and 53 on the coding strand of the oocyte-type gene, but not the somatic-type gene.

Binding Sites of Individual Fingers. To distinguish the endpoints of interaction of the C-terminal fingers in the series of proteins, binding experiments were carried out with mixtures of DNA fragments (28) differing in length by a single base pair. In the probability analysis of binding data (Fig. 3), a value of $\ln[P/P_{total}] = 0$ indicates that the base pair is not required for binding; the more negative $\ln[P/P_{total}]$, the greater the binding energy of, or the requirement for, that base pair. The level of significance was taken to be 2σ or $2\sigma_{av}$;

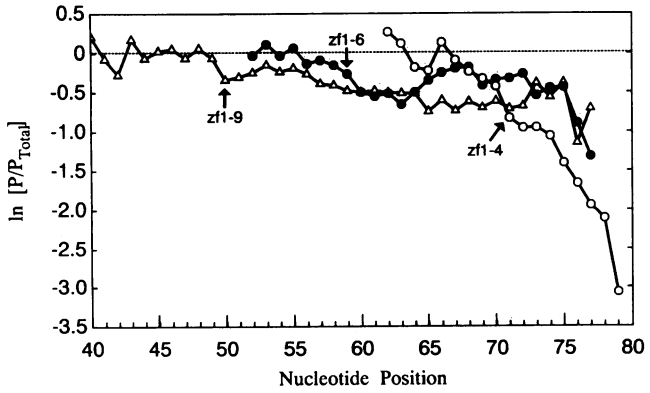

FIG. 3. Binding of zfl–4, zfl–6, and zfl–9 to primer extension ladders. Arrows indicate the endpoint obtained from each of the curves. Data shown for zfl–4 are from eight binding reactions; the data were normalized by setting the average value of bp 62–65 equal to zero for each reaction. zfl–6 binding data are the average of one experiment (three reactions) and were normalized for bases 51–54 for each reaction. The same zfl–6 endpoint was obtained from a second independent experiment. zfl–9 data shown are averaged from a single experiment (three reactions) and were normalized for bases 42–45 for each reaction. In three experiments, the endpoint of zfl–9 was determined twice to be bp 50 and once to be bp 51.

endpoints determined in this manner have a variance of ±1 bp. Note that a base pair may have a significant effect on binding without implying the presence of a direct contact.

The primer extension ladder was used in binding reactions with limiting amounts of zfl–4 or ΔNzfl–3 (control, data not shown). Analysis of the data (Fig. 3) shows that binding of finger 4 in zfl–4 is influenced by base pairs throughout the region from 79 to 71; for bp 71, $\ln[P_{zfl-4}/P_{total}] = -0.830$, $\sigma = 0.361$, $\sigma_{av} = 0.266$. The probability value for bp 70 is just outside of one standard deviation from zero (-0.424; $\sigma = 0.384$), and thus no reliable inference as to the importance of this base pair can be made. As expected, ΔNzfl–3 shows no discrimination in binding the mixture of DNA fragments beyond nucleotide 78; however, the fragment ending at 79 does not compete for ΔNzfl–3.

zfl–5 and zfl–6 were initially studied with a mixture of modified restriction fragments of the 72-mer duplex which terminate within the binding sites. The pool of oligonucleotides was incubated with limiting amounts of zfl–5 or zfl–6. Comparison of the bound and free DNA fractions (Fig. 4) shows discriminatory binding for zfl–5 between bp 69 and 70. When T^{69} on the noncoding strand is paired, the protein binds well; when it is unpaired, zfl–5 does not bind under conditions of limiting protein. Thus, the last base pair required for binding of finger 5 is the A·T at position 69 of the ICR. zfl–6 exhibits discrimination between bp 65 and 67; no fragment terminating at bp 66 was present. Significant binding of zfl–6 is observed only with the longest fragment, ending at bp 65. zfl–3 served as a control and did not exhibit any discrimination among the fragments utilized in the experiment. Binding of zfl–6 was studied in more detail (Fig. 3) by using a primer extension ladder. This experiment shows that finger 6 requires bases extending beyond those presented in the restriction fragment experiments. For optimal binding, finger 6 requires up to bp 59 ($\ln[P_{zfl-6}/P_{total}] = -0.263$, $\sigma = 0.018$, $\sigma_{av} = 0.092$). The probability value for bp 58 (-0.149, $\sigma = 0.073$) is not deemed significant by the $2\sigma_{av}$ criterion.

The primer extension experiment was again used to distinguish the last point of interaction of finger 9 with the DNA. The mixture of extension fragments created with the 72-mer was used in binding reactions with limiting amounts of zfl–9. Densitometric and probability analysis of the gels (Fig. 3) shows bp 50 as the last base pair to deviate significantly from

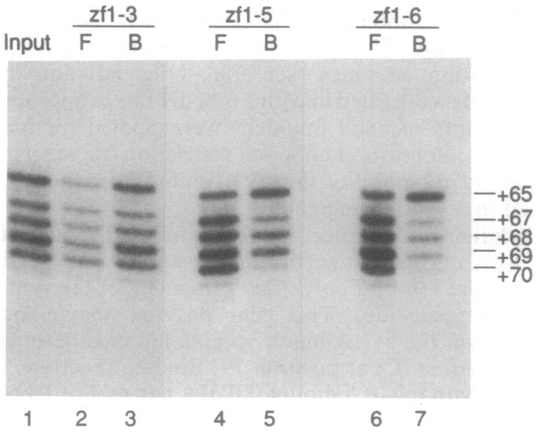

FIG. 4. Binding of zinc finger proteins to restriction fragments. Reaction mixtures (20 μl) contained 40 pmol of total DNA and protein as indicated: zfl–3, 15 pmol; zfl–5, 6.2 pmol; or zfl–6, 24 pmol. *Sau*96I digestion of the 72-mer gave a fragment ending at bp 68 and having a 3-base single-stranded tail on the noncoding strand; a blunt-ended fragment ending at bp 65 was obtained after dCTP and dGTP were used to fill in the *Sau*96I site. *Pal* I gave a fragment ending at bp 67 (blunt); and *Bst*NI digestion produced fragments ending at bp 70 (single-base tail) and bp 69 (blunt after dATP fill-in). Lanes show free (F) and bound (B) DNA fractions.

equal probability of appearing in the bound or free fraction ($\ln[P_{zf1-9}/P_{total}] = -0.339$, $\sigma = 0.051$, $\sigma_{av} = 0.101$); thus, bp 50 appears to be the last base pair required for binding of finger 9.

Methylation Interference. The original methylation interference contacts determined by Sakonju and Brown (6) represent the strong interaction of fingers 1–3 and finger 5 with the major groove of the DNA (guanines at positions 70, 71, 81, 82, 85, 86, 87, and 89 of the noncoding strand and guanine at 91 of the coding strand). To investigate the weaker interactions of the zinc fingers with the DNA, we performed methylation interference experiments under conditions of limiting TFIIIA and excess DNA (≈5% bound). In addition to the contacts listed above, nucleotides G^{51} (53%) of the noncoding strand and G^{52} (45%), G^{57} (40%), G^{79} (35%), and G^{92} (70%) of the coding strand interfere with binding; the percent reduction in binding is given in parentheses. Three of these contacts occur within the 5′ A block, which is the binding site of fingers 7–9. Methylation of G^{70} and G^{71} interfere with binding of zf1–5, as well as with binding of TFIIIA. Methylation of guanine residues on either strand in the regions of binding by finger 4 and finger 6 does not interfere with TFIIIA binding (Fig. 5).

DISCUSSION

It has been shown that fingers 1–3 bind to a minimal site of nucleotides 80–92 in the C block of the ICR, with the requirement that nucleotide 92 be phosphorylated on the coding strand (11); ΔNzf1–3 exhibits the same minimal requirements (data not shown). In the current study, methylation interference observed at base 79 of the oocyte-type gene and lack of binding of ΔNzf1–3 to the primer extension fragment ending at bp 79 suggest that finger 3 also interacts at this nucleotide. Several studies of the interaction of TFIIIA with its recognition sequence show that binding of the N-terminal fingers occurs in the major groove and that the contacts occur primarily on the noncoding strand of the DNA (Fig. 5*A*). The major-groove interaction of fingers 1–3 appears to be similar to the binding mode of transcription factor Zif268 observed in the crystal structure (30), with the exception of the number of base pairs contacted per finger. zf1–3 spans 14 bp (79 to 92), as opposed to 10 bp for the three fingers of Zif268.

Primer extension experiments show finger 4 to be in contact with, or influenced by, base pairs from the end of the finger 3 binding site to bp 71 (Fig. 3). This long region of interaction is inconsistent with continued major-groove binding but is consistent with finger 4 crossing the minor groove. The absence of methylation protection (7) or methylation interference at G^{75} or G^{78} (Fig. 5) also suggests that finger 4 does not bind in the major groove in this region. Further evidence in support of finger 4 crossing the minor groove comes from hydroxyl radical and micrococcal nuclease protection studies (7–9). The backbone is highly protected in the region from nucleotide 74 to 77 on the coding strand and 77 to 81 on the noncoding strand. The offset of three nucleotides in the 3′ direction is indicative of a minor-groove interaction (33, 34). Together, the above results are entirely consistent with finger 4 crossing, or possibly binding in, the minor groove. Bonds between 73-74-75-76-77 on the noncoding strand are highly accessible to nucleases (refs. 7 and 34; this work); this is the side of the helix opposite that contacted by finger 4 (Fig. 5*B*). Docking of Corey–Pauling–Kolton models suggests that the finger and its adjacent linkers are sufficient to span the proposed binding site and that residues in the α-helix of finger 4 are capable of making minor-groove contacts. However, attempts to determine whether minor-groove methylation of adenine bases interferes with binding were inconclusive.

Finger 5 contacts nucleotides 69, 70, and 71 of the promoter intermediate element. Methylation interference experiments with zf1–5 show the interaction at G^{70} and G^{71} to be attributable to finger 5 (data not shown). The possibility that finger 5 might also contact a base pair on either side of the 69-70-71 triplet cannot be ruled out.

The primer extension experiments show that optimal binding of finger 6 requires the presence of bp 68–59 (Fig. 3). In the TFIIIA complex, a few nucleotides centered at position 64 on the coding strand and centered at nucleotide 67 on the

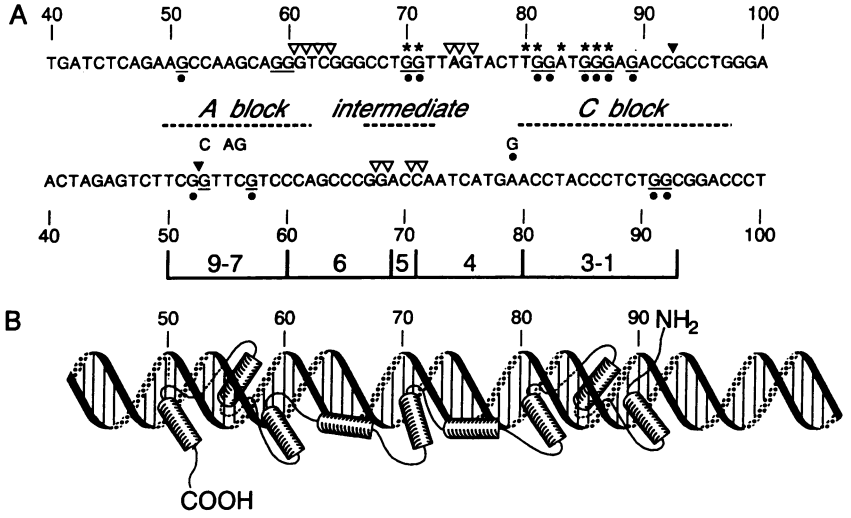

FIG. 5. Model for binding of TFIIIA to the 5S RNA gene. (*A*) Summary of interactions. The nucleotide sequence of the noncoding (upper) and coding (lower) strands of the somatic-type gene are shown; base changes specific to the oocyte-type gene are shown above the coding strand. The promoter elements (32) are indicated by broken lines. Guanine methylation interference sites (ref. 6; this work) are indicated by filled circles, sites of methylation protection (7) are underlined (G^{52} shows enhanced methylation in protection experiments), and backbone ethylation interference (6) is indicated by stars. Open triangles indicate points of cleavages within the footprint; filled triangles indicate hypersensitive sites. (*B*) Schematic representation of the proposed model for TFIIIA binding to the 5S RNA gene ICR. Fingers 1–3 (at right) wrap around the major groove for approximately one turn of helix. Finger 4 crosses the minor groove. Finger 5 binds in the major groove, followed by finger 6, which again crosses the minor groove. Fingers 7–9 wrap around the major groove for approximately one helical turn. This schematic diagram does not attempt to represent structural changes in the DNA upon TFIIIA binding or the precise orientation of fingers spanning the minor groove.

noncoding strand are protected from hydroxyl radical attack (8, 9). Strong protection from attack by hydroxyl radical of the nucleotides required for binding of finger 6 implies that the finger crosses the minor groove. Both the linker between fingers 5 and 6 and the linker between fingers 6 and 7 are too short to span the minor groove; therefore, it must be finger 6 itself which is responsible for the observed hydroxyl radical protection. We propose that, in a manner analogous to finger 4, finger 6 spans nearly one turn of helix from bp 59 to bp 68 (Fig. 5). Within the region from bp 61 to bp 68 there are four guanine residues on the noncoding strand and three on the coding strand, none of which show methylation protection (7) or interference (ref. 6; this work) greater than that observed for regions outside of the binding site. As with finger 4, the face of the helix opposite the proposed contact site of finger 6 is highly accessible to nucleases, with bonds between bases 60-61-62-63-64 on the noncoding strand being cleaved (refs. 7 and 34; this work).

We propose that bp 60 is the beginning of the major-groove interaction of fingers 7, 8, and 9 with the A block of the ICR; others have proposed a major-groove interaction for the C-terminal fingers (8, 12). Major-groove binding of these fingers is implicated by methylation protection and/or interference in the region from G^{51} to G^{60} (Fig. 5). Binding of zf1–9 to the primer extension ladder suggests that the last contact of finger 9 is bp 50 of the ICR. We currently favor a model in which fingers 7–9 interact continuously with the A-block major groove in a fashion similar to fingers 1–3 at the 3′ end of the ICR. However, it is clear that binding at the 5′ end of the ICR is considerably weaker than that at the 3′ end (6, 8, 11).

In our model, approximately one turn of helix at each end of the binding site is protected on all faces of the helix by binding of three zinc fingers in the major groove. Approximately 20 bp in the central region of the ICR are protected along one face by fingers 4, 5, and 6, while the opposite face is exposed in this region. Cleavages observed within DNase I footprints occur within the binding sites of fingers 4 and 6, but on the opposite face of the helix. These cleavages may indicate an altered conformation in the DNA and mark sites of bending (14, 15) in the complex.

In summary, this study has led to the development of a comprehensive model for binding of TFIIIA to the 5S RNA gene ICR. The results show that the fingers interact through both the major and the minor groove. Because of the importance of zinc finger proteins in regulation of eukaryotic gene transcription, and the growing data base of finger proteins, these findings point to the importance of delineating the range of binding modes employed by zinc fingers.

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