Flexible Zinc Finger Requirement for Binding of the Transcriptional Activator Staf to U6 Small Nuclear RNA and tRNA\textsuperscript{Sec} Promoters*  

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The transactivator Staf, which contains seven zinc finger motifs, exerts its effect on gene expression by binding to specific targets in small nuclear RNA (snRNA) and snRNA-type gene promoters. In this work, binding site selection allowed us to identify the 21-base pair ATTACCCAATGCATYGCGG sequence as the high affinity consensus binding site for Staf. It shows a high sequence divergence with Staf-responsive elements in the Xenopus selenocysteine tRNA (tRNA\textsuperscript{Sec}) and human U6 snRNA promoters. By using a combination of approaches, we analyzed the interaction of wild-type and truncated Staf zinc finger domains with the consensus, Xenopus tRNA\textsuperscript{Sec}, and human U6 sites. Two main conclusions emerged from our data. First, the data clearly indicate that zinc finger 7 does not establish base-specific contacts in Staf-DNA complexes. The second conclusion concerns zinc finger 1, which is required for the binding to the Xenopus tRNA\textsuperscript{Sec} site but is dispensable in the case of the human U6 site. Taking into account the sequence differences in the two sites, these findings demonstrate that Staf utilizes zinc finger 1 in a rather flexible manner, illustrating how a protein can interact with DNAs containing targets of different sequences.

Regulation of gene expression is mediated by trans-acting proteins that recognize and bind at specific DNA elements in the regulatory regions of the genes. A common feature of many activating proteins is that DNA binding and activation functions reside in different domains of the protein. The majority of sequence-specific DNA-binding proteins can be classified according to the presence of conserved domains, such as helix-turn-helix, zinc finger, or leucine zipper (for reviews, see Refs. 1 and 2). Recently, we have demonstrated that the zinc finger protein Staf, originally identified in Xenopus laevis as the transcriptional activator of the tRNA\textsuperscript{Sec} \textsuperscript{1} gene (3, 4), is also involved in transcriptional activation of snRNA and snRNA-type genes, some of which are transcribed by RNA polymerase II and others by RNA polymerase III (5). In addition, Staf possesses the capacity to stimulate expression from an RNA polymerase II mRNA promoter. The presence of two physically and functionally distinct activation domains, one specifically activating snRNA-type promoters and the other mRNA promoters, constitutes the molecular basis for this dichotomous transcriptional activity (6). In human cells, ZNF76 and ZNF143 are two human homologs of Staf. ZNF143 is the ortholog, whereas ZNF76 is a DNA-binding protein related to Staf and ZNF143 (7). In its central part, Staf contains seven contiguous zinc fingers of the C2-H2 type (2, 8). The first six are of the CX\textsubscript{2}CX\textsubscript{3}(F/Y)XX\textsubscript{2}LX\textsubscript{2}HX\textsubscript{2}H type (X stands for any amino acid), except that the leucine residue is not found in the fourth and fifth fingers, where it is replaced by arginine and tyrosine residues, respectively. The seventh zinc finger, however, is of the CX\textsubscript{2}CX\textsubscript{3}YX\textsubscript{2}LX\textsubscript{2}HX\textsubscript{2}H type. The sequence linking the last histidine of one zinc finger to the first cysteine of the next one is highly conserved, giving rise to the consensus TG(E/D)(K/R)PYN, but the (E/D)(K/R) sequence is not found between the sixth and seventh finger. Comparative DNase I footprinting analysis performed either with the entire protein or with the zinc finger domain only established that the seven tandemly repeated zinc fingers contain the DNA binding domain of Staf (4). Structural analysis of DNA-zinc finger complexes has demonstrated that each interacting zinc finger contacts 3–5 bp of DNA (9–16). Assuming that all of the seven Staf zinc fingers would contact the DNA, the protein should recognize a binding site composed of at least 21 bp. However, sequence comparisons of known Staf binding sites with the consensus sequence derived from binding site selection revealed a high degree of sequence divergence. This is well illustrated by the Staf-responsive elements that lack the 5′ part of the consensus sequence in the Xenopus tRNA\textsuperscript{Sec} and the 3′ part in the human U6 promoters (5).

To elucidate the mechanism by which Staf can recognize divergent DNA sequences, we have assayed the relative contributions of individual zinc fingers, and sets of zinc fingers, to the binding to an optimal site and Staf-responsive elements that are sequence divergent. In this study, determinations of the relative binding affinities of Staf and recombinant zinc finger polypeptides to the optimal site, Xenopus tRNA\textsuperscript{Sec}, and human U6 Staf-responsive elements were performed, in combination with DNase I footprinting, missing nucleoside assays, and binding site selection. This revealed that zinc finger 7 does not establish base-specific contacts with the DNA and that zinc finger 1 is utilized by Staf in a flexible manner depending on the target site with which it interacts.

EXPERIMENTAL PROCEDURES

Plasmid Constructions—pSK(−)-Staf corresponds to M2 cDNA in pSK(−) (4). pSK(−)-Staf-Zf 1–7 was made by cloning, into the SacI and
**Staf Binding to U6 snRNA and tRNA\textsubscript{Sec} Promoters**

| Position | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | +1 |
|----------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|
| A%       | 36.8 | 61.4 | 12.7 | 6.6 | 71.6 | 27 | 2.7 | 0 | 0 | 0 | 96.5 | 5.4 | 63.4 | 98.3 | 0.9 | 1.7 | 0.0 | 78.0 | 22.3 | 1.6 | 27.5 | 0.0 | 9.7 | 15.4 | 20.0 |
| C%       | 8.8 | 1.2 | 14.9 | 6.6 | 2.7 | 96.4 | 100.0 | 100.0 | 0.0 | 17.8 | 23.6 | 0.0 | 0.0 | 20.7 | 99.1 | 7.3 | 21.4 | 54.1 | 0.0 | 83.8 | 3.2 | 23.1 | 22.0 |
| G%       | 36.3 | 22.9 | 9.2 | 4.4 | 0.0 | 0.0 | 0.0 | 0.0 | 3.5 | 25.0 | 13.0 | 1.7 | 0.0 | 70.7 | 0.0 | 0.0 | 1.0 | 0.0 | 57.5 | 8.1 | 71.0 | 61.5 | 20.0 |
| T%       | 28.1 | 14.5 | 63.2 | 82.4 | 25.7 | 9.9 | 0.0 | 0.0 | 0.0 | 51.8 | 0.0 | 0.0 | 99.1 | 6.9 | 0.9 | 14.7 | 55.3 | 44.3 | 15.0 | 8.1 | 16.1 | 0.0 | 40.0 |

**FIG. 1. Derivation of the Staf consensus DNA binding sequence.** Compilation of the sequences selected by Staf, under stringent conditions, from an oligonucleotide duplex bearing a 17-bp random region. Indicated are the frequencies with which the four bases A, C, G, and T were selected at each position; the most prevalent bases are shown in **boldface**. The data are summarized to give a consensus Staf DNA binding site, with **lowercase letters** indicating bases selected with a frequency of <70%.

**RESULTS**

A 21-bp Consensus Binding Site for Staf—Our previous studies, using binding site selection, identified the 19-bp sequence YY(AT)/CC/AG/N(A)/C/AT/CG/C/G/AC/YYRCR as the consensus for recognition of the zinc finger domain of Staf (5). Within this sequence, position 8 is fully degenerate, and positions 4–6, 10, 11, and 13 are more highly constrained than bases at positions 1–3, 7, 9, 12, and 14–19. In order to identify the base preferences for the 12 degenerate positions, chimeric proteins consisting of glutathione S-transferase fused to the Staf zinc finger domain were used in a new binding site selection experiment from a 57-bp oligonucleotide duplex containing an internal core of 17 random nucleotides. Six cycles of binding were performed with decreasing protein concentrations for the fourth, fifth, and sixth cycles to enhance specificity. 123 independent clones were sequenced. Of the 23 positions tabulated, 21 displayed a very significant higher constraint with respect to base preference (Fig. 1). Compared with the consensus identified in our earlier work, the newly derived 21-bp consensus sequence ATTACCCTATACTGATCCTGGG is extended by an A residue on the 5' side (position 1) and a G residue on the 3' side (position 21). The information that could be extracted from this experiment is as follows. A very high constraint is observed for Cs at positions 5–7 and 14, As at positions 8 and 11, and a T at position 12. A strong preference exists for As at positions 4 and 15, Gs at positions 13 and 20, a T at position 3, and a C at a final volume of 10 µl, with continued incubation for 50 min at room temperature to establish the new equilibrium. The proteins used in these assays were synthesized by *in vitro* coupled transcription-translation with the TntX system (Promega). Reactions were programmed with the pSK(−)Staf and pSK(−)Staf-Zf1–7 constructs. 1.5 and 5 µl of the programmed lysate were used for the experiments described in the legend to Fig. 2, B and C, respectively. Following electrophoresis, the bound probe was quantitated, and the fraction of maximal binding at each competitor concentration was calculated as the ratio of bound probe plus competitor to bound probe with no competitor. A curve was then fitted to the values for the fraction of maximal binding at known competitor DNA concentrations. The IC\textsubscript{50} was defined as the concentration of competitor DNA inhibiting 50% of binding. For the measurement of the apparent dissociation constants (K\textsubscript{d} values), a series of 10-µl gel retardation reactions was prepared using a fixed concentration of purified wild-type or truncated Staf zinc finger domain fused to glutathione S-transferase (0.15 nm of Zf 1–7, 1 nm of Zf 1–6, and 1.5 nm of Zf 2–7), and variable concentrations of 5'–end labeled probes (0.1–26 nm). After the binding reaction reached the equilibrium (50 min at room temperature), the bound and free probes were separated by electrophoresis through a 4% native gel. The concentrations of the labeled probes contained in the bound and free DNAs were determined with reference to standard probes run on the same gel and quantitated with a Fuji BAS 2000 bioimage analyzer. The mass action equation (K\textsubscript{d} = [DNA]/[P]/[DNA.P]) can be rearranged in terms of total protein concentration (P_0) to a form similar to that of the Michaelis-Menten equation: [DNA-P] = [P] ([DNA]/[DNA] + K\textsubscript{d}). Data were analyzed as a plot of bound DNA versus free DNA using the Kaleidograph software, from which K\textsubscript{d} values were derived.
position 19. Lastly, a moderate preference for Ts was observed at positions 2, 9, and 16, for As at positions 1 and 10, and for Gs at positions 18 and 21.

Examination of the data revealed the surprising finding that sequences previously identified as binding sites for Staf in the *X. laevis* tRNA^Sec^ (xtRNA^Sec^ site) and human U6 snRNA (hU6 site) promoters (3–5) were not obtained in the selection experiment (Fig. 2A). As a likely explanation, we hypothesized that the nonselected sites were bound with a lower affinity compared with the selected ones. We tested this possibility using quantitative competitive gel shift assays with three different probes: the nonselected xtRNA^Sec^ and hU6 sites, and the sequence the most frequently selected, TTTACCCAATGCGCATGCG, that we called the optimal site (Fig. 2A). It shows 86% identity to the Staf consensus binding site. The relative capacities of increasing concentrations of the two nonselected sites and the optimal site to compete for binding to Staf and Staf zinc finger domain (amino acids 249–475) with a constant concentration of the labeled optimal site were assessed. It is noteworthy that the protein concentrations of Staf and Staf zinc finger domain were different, not allowing comparisons of the values between Fig. 2B and Fig. 2C. Fig. 2B convincingly demonstrates that the xtRNA^Sec^ and hU6 sites are bound by Staf with a lower affinity than that of the optimal site, but with varying magnitudes. At a competitor concentration inhibiting 50% of the maximal binding (IC50), Staf bound about 30- and 180-fold more tightly the optimal sequence than the xtRNA^Sec^ and hU6 sites, respectively (Fig. 2B). Similarly, Fig. 2C shows that the Staf zinc finger domain possesses a 7- and 60-fold better binding capacity to the optimal sequence, with comparison to the xtRNA^Sec^ and hU6 sites. Taken together, these results indicate that the conditions employed in the binding site selection experiment were highly stringent, resulting in the selection of sites that are bound with a higher affinity than the Staf-responsive elements in the Xenopus tRNA^Sec^ and human U6 promoters.

**DNase I Footprints on the Xenopus tRNA^Sec^ and Human U6 Promoters Show Varied Protection Patterns for the Same Proteins**—The amino acid sequence of the seven zinc fingers of the Staf DNA binding domain is 95% conserved (95% identity, 98.5% similarity) between the Xenopus Staf and ZNF143, its human ortholog (7). In contrast, the sequence of the Staf-responsive elements in the Xenopus tRNA^Sec^ and human U6 promoters are clearly divergent, showing only 47% identity (Fig. 2A). This raises the issue of whether the same set of zinc fingers establishes contacts with DNA regulatory motifs that are divergent in sequences. DNase I footprinting provides a means to investigate stable protein-DNA interactions. Also, differences in the mode of binding of various proteins can often be discerned using this methodology. Footprinting experiments were carried out on DNA fragments derived from positions –280 to –102 in the *X. laevis* tRNA^Sec^ and –357 to –171 in the human U6 promoters. As to the Staf constructs, we generated a series of recombinant polypeptides containing the glutathione S-transferase fused to zinc fingers 2–7 (Zf 2–7), 1–5 (Zf 1–5), 1–6 (Zf 1–6), and 1–7 (Zf 1–7) (Fig. 3). Each polypeptide was purified from *Escherichia coli* cell lysates by affinity chromatography. The DNase I footprints on the Xenopus tRNA^Sec^ promoter are shown in Fig. 4, A and B, with a summary of the data in Fig. 4C. Zf 1–5 protected the phosphodiester backbone from positions –211 to –188 on the nontemplate strand and –214 to –191 on the template strand. Addition of finger 6 in Zf 1–6 increased the length of the footprint beyond that of Zf 1–5, yielding additional protection to positions –214 and –219 on the nontemplate and template strands, respectively. With Zf 1–7, the nontemplate strand is strongly protected from positions –216 to –188 and the template strand from –219 to –191. Thus, addition of the seventh zinc finger in Zf 1–7 led to a significantly longer protection on the nontemplate strand only, compared with the footprint obtained with Zf 1–6. Dele-
Staf-DNA Complexes—It is well known that DNase I exhibits which zinc finger 1 very likely contacts the DNA.

The footprint extends from 2 to 5 of zinc finger 1 in Zf 2–7 led to a proximally shortened protection because the footprint extends from –198 and –195 on the nontemplate and template strands, respectively. Inspection of the carboxyl- and amino-terminal deletion end points helped fix the relative orientations of the protein and DNA: Staf sits along the DNA with the carboxyl terminus oriented toward the 5’-end and the amino terminus toward the 3’-end of the Staf-responsive element.

In contrast to the results with the Xenopus tRNA^Sec promoter, Zf 1–5 failed to yield a footprint on the human U6 promoter at similar or higher protein-DNA ratios. Zf 1–7 and Zf 2–7, however, did lead to DNase I footprints, as shown for the template strand of the human U6 promoter in Fig. 5A. A schematic drawing of the GST fusion proteins used in this study. Numbers in parentheses indicate the end points of the wild-type and truncated zinc finger domains.

Zinc Finger 7 Does Not Establish Base-specific Contacts in Staf-DNA Complexes—It is well known that DNase I exhibits sequence preference, inducing nonrandom cleavages of DNA. Furthermore, it is very likely that not all the nucleotides within a region protected by a protein in a DNA-protein complex are involved in the interaction. Lastly, the bulkiness of DNase I can limit access to the complex. For all these reasons, DNase I footprinting does not provide an unbiased view of DNA-protein complexes. We therefore sought those nucleosides in the Staf-responsive elements that are contacted by the wild-type and truncated Staf DNA binding domains. To this end, missing nucleoside experiments were carried out using hydroxyl radicals (18). This reagent generates random cleavages of the phosphodiester backbone, resulting in a 1-nucleoside gap per DNA molecule. The abilities of GST fusions of the wild-type (Zf 1–7) and truncated zinc finger domains to bind the gapped DNA were subsequently assayed by gel mobility shifts, in which the Staf complexes were separated from the free DNA. Both the complexed and free DNAs were isolated and analyzed on a sequencing gel for the determination of the cleavage pattern in the bound and free samples. In such an assay, a nucleoside that is important to forming the DNA-protein complex yields a weak or missing band, on the sequencing gel, in the lane containing the DNA that was bound to the protein. Conversely, a high intensity band appears in the lane where the free DNA was applied. This approach was used to examine the important contacts made by Zf 1–7 and Zf 1–6 to both strands of the xTN^Sec, hU6 and optimal sites. The pattern of DNA fragments resulting from these experiments is shown in Fig. 6. A, B, and C for the tRNA^Sec, optimal, and hU6 sites, respectively, and a compilation of the data is shown in Fig. 6D; the 21-bp consensus sequence stands as a numbering reference, the base pairs of the three sites being numbered –1 to 22, starting at the 5’-end of the top strand (see Fig. 6D). Densitometry of the autoradiograms revealed the relative importance of the individual bases to the formation or maintenance of a Staf-DNA complex. Although interacting nucleosides were detected on both strands of the DNA, contacts to the nontemplate strand appear to be more important than to the template strand. Missing nucleoside experiments between Zf 1–7 and the three sites revealed extensive interference patterns. Obviously, removal of any nucleoside from positions 1–20 or 22 on the nontemplate strand or 2–22 on the template strand in the tRNA^Sec site (Fig. 6, A and D) and positions 1–20 on the nontemplate strand and 3–20 on the template strand in the optimal site (Fig. 6, B and D) strongly interfered with the binding of Zf 1–7. This result is quite different from that obtained with the hU6 site, where the interference pattern is elongated in the 5’ part of the Staf element and shortened in its 3’ part and involves nucleosides at positions –1 to 15 and 1 to 16 of the nontemplate and template strands, respectively (Fig. 6, C and D). Surprisingly, the same missing nucleoside patterns were observed on both strands, whether Zf 1–7 or Zf 1–6 was added to the gapped tRNA^Sec optimal, and hU6 probes (Fig. 6D). The absence of additional nucleoside requirements in the presence of zinc finger 7 strongly suggests that zinc finger 7 does not establish base-specific contacts with the DNA.

To bolster this interpretation, we next analyzed the recognition properties of Zf 1–6 by using it, under stringent conditions, to select binding sites from an oligonucleotide pool of random sequences. Fifty-one amplified products from the sixth selection cycle were cloned and sequenced. As shown in Fig. 7, the fusion protein containing zinc fingers 1–6 (Zf 1–6) led to a consensus identical to that obtained with the entire seven-zinc finger domain (Zf 1–7; see also Fig. 1). Collectively, these results convincingly demonstrate that zinc finger 7 does not specifically contact the bases in Staf-DNA complexes.
To define the contribution of the amino-terminal zinc finger 1 to the formation of Staf-DNA complexes, we examined the important contacts effected by Zf 2–7 to both strands of the xtRNA^Sec, optimal, and hU6 sites. Again, the missing nucleoside assay was performed. The interference signals observed for the binding of Zf 2–7 to the xtRNA^Sec and optimal sites were very different in their 3' parts from those found for the Zf 1–7 binding. Deletion of zinc finger 1 in Zf 2–7 provoked a reduction of the interference pattern of 8 and 5 nucleosides on the template strands of the xtRNA^Sec and optimal sites, respectively (Fig. 6, A, B, and D). Likewise, on the non-template strand, a reduction of the interference signal of 7 (position 21 being excluded; see Fig. 6D) and 6 nucleosides was observed for the xtRNA^Sec and optimal sites, respectively (Fig. 6, A, B, and D). This demonstrates a loss of protein-DNA contacts at the 3’-end of the sites resulting from the zinc finger deletion and suggests that the binding site for zinc finger 1 resides between base pairs 15–22 for the xtRNA^Sec site and 15–20 for the optimal site. In stark contrast, the same deletion did not alter the Zf 1–7 missing nucleoside pattern to the hU6 site (Fig. 6, C, and D). These observed differential effects strongly argue in favor of zinc finger 1 contacting the DNA in the xtRNA^Sec and optimal sites, but not in the hU6 site.
If base-specific contacts are to be invoked for the binding of zinc finger 1 to the DNA, then the binding sites selected, with Zf2–7, from a pool of mixed oligonucleotides should not contain the zinc finger 1 binding site. The PCR product from the sixth round of amplification was cloned, and 54 representative clones were sequenced. Fig. 7 lists a compilation of the frequencies for each nucleotide at the 21 tabulated positions. It also shows that the fusion protein, containing Zf2–7, gave a 15-bp consensus of sequence ATTACCCATAATGCA, which overlaps positions 1–15 of the 21-bp consensus obtained with the entire zinc finger domain (Zf 1–7). For residues at positions 16–20, the frequency of each nucleotide was completely different from that observed with Zf 1–7. In addition, the sequence TGYGG (positions 16–20) of the Zf 1–7 consensus was not recovered. Based on the model that the amino-terminal domain of Staf points toward the 3′-end of the target site, the results of this binding selection showing a highly degenerate 3′-end brought experimental ev-
the central part of the CCCA core motif, the CC dinucleotide was always selected in our experiments. It is interesting to note that the seven highly constrained nucleotides, CCCA, AT, and C, all reside at the same positions in the vast majority of the natural Staf-responsive elements (5). More importantly, the C residues at positions 6, 7, and 14 are conserved in all the identified Staf-responsive elements. In the light of their strict requirement, it is tempting to speculate that these, or the G residues on the opposite strand, establish strong interactions that are important for the binding affinity and specificity.

The question that can arise is whether the selected consensus binding site constitutes the best Staf-responsive element. In vitro assays to determine the affinities of the zinc finger domain found that the optimal sequence exhibited a higher

**Fig. 6.** Missing nucleoside experiments employing three different Staf binding sites and glutathione S-transferase fused to zinc fingers 1–7, 1–6, and 2–7. The 5’-end-labeled nontemplate and template strands containing the xtRNASec, hU6, and optimal sites were subjected to hydroxyl radical cleavages as described under "Experimental Procedures." Gapped DNAs were incubated with Zf 1–7, Zf 1–6, and Zf 2–7. Complexed and free DNA fragments were isolated and electrophoresed on sequencing gels. A–C, missing nucleoside interference patterns obtained on the xtRNASec, optimal, and hU6 sites. The nature of the strand and protein are indicated above the lanes. In each case, lanes marked G + A, F, and B indicate the products of a G + A-specific sequencing reaction, free DNA, and bound DNA, respectively. D, schematic representation of the results for Zf 1–7, Zf 1–6, and Zf 2–7 on the xtRNASec, optimal, and hU6 sites. Regions of interference are boxed; filled boxes, strongest interference; hatched boxes, moderate interference; open boxes, weakest interference. The base pairs in the Staf-responsive element are numbered 1 to 22, starting at the 5’-end of the top strand with reference to the consensus binding site derived by in vitro selection.
The affinity than the natural Staf-responsive elements in the Xenopus tRNASec and human U6 snRNA promoters. However, the overall affinity of the full size protein for Xenopus tRNASec and human U6 sites was about 2-fold higher than that of its truncated derivative containing only the DNA binding domain (Table I) (7). The reason for the decreased affinity of the zinc finger domain alone remains to be established. Yet it is a well known fact, for transcription factors of the NGFI-A family, that the protein context of the zinc finger domain governs the differences in DNA binding affinities (20). Conceivably, binding of Staf to the DNA may involve other portions of the protein, not belonging to the zinc finger domain but contributing to a minor extent to the $\Delta G^o$ of binding.

We found that Staf is able to bind specifically with a relative high affinity to a number of divergent DNA sequences located in the distal sequence elements of Xenopus, human, and mouse gene promoters (this work and Ref. 5). Although the amino acid sequence is 95% conserved within the seven zinc finger domain between the Xenopus and human Staf (7), the specific DNA sequences recognized by Staf in the Xenopus tRNASec and human U6 sites are clearly divergent, showing only 47% identity. The selection assay indicated that nucleotide positions 1, 2, 9, 10, 16, 18, and 21 were moderately selected. This may explain the sequence variability observed in the natural Staf-responsive elements, where only two of these seven residues are conserved between the Xenopus tRNASec and human U6 sites. As a result, one can ask how identical DNA binding domains can contact such divergent regulatory DNA sequences. The answer arises from the present work, in which we have determined that Staf employs zinc finger 1 in a flexible fashion, in order to adjust to the divergent responsive elements in the Xenopus tRNASec and human U6 promoters. Two sets of experiments were determinant, in this respect. First, determination of the $K_d$ for the polypeptide containing Zf 2–7 showed that removal of zinc finger 1 had a more pronounced effect on the affinity for the Xenopus tRNASec than for the human U6 sites. Second, comparison of the results from the missing nucleoside interference and binding site selection assays revealed that zinc finger 1 is involved in contacting the DNA in the Xenopus tRNASec but not in the human U6 promoter. Indeed, the presence of zinc finger 1 increased the length of the missing nucleoside interference pattern on the Xenopus tRNASec promoter beyond that of Zf 2–7, yielding an additional interference of 7 and 8 residues on the nontemplate and template strands, respectively. It is possible that the 8-bp difference between the interference patterns of Zf 1–7 and Zf 2–7 represents a loss of contact, not only by zinc finger 1 but also by the adjacent zinc finger 2. We propose that the reduced

![Consensus Zf 1-6](image)

![Consensus Zf 1-7](image)

### TABLE I

| Protein | $K_d$* | Relative affinity | $\Delta G^{ab}$ |
|---------|--------|------------------|-----------------|
| Optimal site | | | |
| Zf 1–7 | 0.37 ± 0.11 (5) | 1.00 | −12.73 |
| Zf 1–6 | 1.42 ± 0.60 (2) | 0.26 | −11.94 |
| Zf 2–7 | 1.10 ± 0.30 (6) | 0.53 | −12.09 |
| xRNASec site | | | |
| Zf 1–7 | 1.23 ± 0.23 (3) | 1.00 | −12.02 |
| Zf 1–6 | 5.33 ± 0.60 (2) | 0.23 | −11.16 |
| Zf 2–7 | 5.60 ± 1.00 (4) | 0.22 | −11.13 |
| ZNF143 | 0.50 ± 0.05 | 2.46 | −12.55 |
| hU6 site | | | |
| Zf 1–7 | 2.36 ± 0.50 (3) | 1.00 | −11.64 |
| Zf 1–6 | 10.95 ± 3.25 (4) | 0.21 | −10.74 |
| Zf 2–7 | 3.35 ± 0.70 (5) | 0.70 | −11.43 |
| ZNF143 | 1.20 ± 0.10 | 1.96 | −12.04 |

* $K_d$ ± S.D. from the number of determinations in parentheses. The apparent dissociation constants were determined by DNA titration with the optimal, xRNASec, and hU6 sites.

b The standard Gibbs free energy changes, $\Delta G = -RT \ln(1/K_d)$, were calculated using the average equilibrium constants and $T = 295$ K (22 °C).

c Data from Myslinski et al. (7).

Fig. 7. Identification of a consensus binding sequence for the zinc finger polypeptide 1–6 (Zf 1–6) and 2–7 (Zf 2–7). GST fusions containing Zf 1–6 and Zf 2–7 were produced and used in binding and amplification reactions as described under “Experimental Procedures.” At each position, the frequencies with which the four bases A, C, G, and T were selected are indicated. The consensus is shown for Zf 1–6 and Zf 2–7, and the most prevalent bases are shown in boldface.
interference pattern of Zf 2–7 can be accounted for by the lack of, or loose interaction with, zinc finger 2 and the DNA, in the absence of zinc finger 1.

The missing nucleoside signals suggest that Staf makes a continuous set of contacts along the DNA helix. In particular, the lack of stagger in the missing nucleoside pattern from one strand to the other is consistent with the protein following a helical trajectory similar to that of the DNA. In the crystal structures of the protein-DNA complexes with Zif 268, GLI, and Tramtrack, the linked C2-H2 fingers of these proteins bind in the major groove of the DNA, making contacts essentially with one of the strands (9–12). Thus, it is likely that in the Staf-responsive elements of the human U6 and Xenopus tRNA^Sec promoters, Staf runs along the major groove of the DNA helix. Given the more pronounced interference pattern on the non-template than on the template strand, Staf probably associates more closely with the nontemplate strand of the Staf-responsive elements.

Our results showed that zinc finger 7 is not involved in contacting the bases in the Staf-responsive elements. Surprisingly, however, Zf 1–6 binds to Staf-responsive elements 5 times more weakly than Zf 1–7. This can be interpreted to mean that zinc finger 7 is involved in energetically significant contacts with the phosphates of the Staf binding sites. Alternatively, zinc finger 7 can be involved in an important, conserved biological function that might consist of intramolecular protein-protein interactions. In this respect, the crystal structure of the GLI-DNA complex has established that zinc finger 1 of GLI does not contact the DNA but has extensive protein-protein interactions with the adjacent zinc finger 2 (11).

Strikingly, the binding to the Xenopus tRNA^Sec site utilizes six of the seven zinc fingers, whereas no more than five are necessary for Staf to bind to the human U6 site. It is remarkable that although it displays evolutionary sequence conservation, zinc finger 1 is not constantly utilized with all promoters. Rather, it is precisely owing to this flexible utilization that Staf can bind to divergent sequences within the promoters of snRNA-type genes in different species. Conceivably, this ability to recognize different binding site sequences enables Staf to influence gene transcription from a variety of different promoters.

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