Maximization of Selenocysteine tRNA and U6 Small Nuclear RNA Transcriptional Activation Achieved by Flexible Utilization of a Staf Zinc Finger*

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Transcriptional activators Staf and Oct-1 play critical roles in the activation of small nuclear RNA (snRNA) and snRNA-type gene transcription. Recently, we established that Staf binding to the human U6 snRNA (hU6) and Xenopus selenocysteine tRNA (xtRNA Sec) genes requires different sets of the seven C2–H2 zinc fingers. In this work, using a combination of oocyte microinjection, electrophoretic mobility shift assays, and missing nucleotide experiments with wild-type and mutant promoters, we demonstrate that the hU6 gene requires zinc fingers 2–7 for Staf binding and Oct-1 for maximal transcriptional activity. In contrast, the xtRNA Sec gene needs the binding of the seven Staf zinc fingers, but not Oct-1, for optimal transcriptional capacity. Mutation in the binding site for Staf zinc finger 1 in the tRNA Sec promoter reduced both Staf binding and transcriptional activity. Conversely, introduction of a zinc finger 1 binding site in the hU6 promoter increased Staf binding but interfered with the simultaneous Staf and Oct-1 binding, thus reducing transcriptional activity. Collectively, these results show that the differential utilization of Staf zinc finger 1 represents a new, critical determinant of the transcriptional activation mechanism for the Xenopus tRNA Sec and human U6 snRNA genes.

Formation of a higher order transcription complex requires the interplay between specific DNA sequences and DNA-binding transcription factors. Protein-protein interactions are also involved between additional components and the DNA-protein complexes. Protein motifs such as zinc fingers of the C2–H2 type, basic helix-loop-helix, basic leucine zippers, and POU/homeodomains are shared by DNA-binding proteins and serve as an interface for DNA protein recognition (for review, see Ref. 1). Genes for vertebrate small nuclear RNAs (snRNAs) are transcribed by either RNA polymerase II (Pol II) or RNA polymerase III (Pol III), depending on the type of promoters they harbor (for review, see Ref. 2). In the Pol II snRNA promoters, basal transcription is afforded by a single element, the proximal sequence element. The basal Pol III-dependent promoters possess, additionally, a TATA box that acts as a major determinant for Pol III specificity (3, 4). The proximal sequence elements of RNA Pol II and Pol III promoters are interchangeable and recruit a stable protein complex containing five subunits, known as SNAPc or PTF (5–7). A number of other short transcription units, such as the 7SK, Y, MRP, selenocysteine tRNA (tRNA Sec), and H1 RNA genes, have similar basal promoter elements and can be classified as snRNA-type genes. These will be referred to as snRNA-type promoters. Activated transcriptions of snRNA and snRNA-type promoters are provided by the distal sequence element (DSE) (2). The DSEs are composed of several functional sub motifs, two of which are the octamer and the Staf motifs (2, 8–10). The fact that Staf contains two physically and functionally distinct activation domains constitutes the molecular basis for this dichotomous transcriptional activity (13). The sub motif composition of the DSE is characteristic for each type of promoter, containing either both the octamer and Staf motifs or only one of these. For example, optimal transcription of the human U6 snRNA promoter (hU6) depends on the simultaneous presence of the octamer and Staf motifs (10, 15). In contrast, that of the Xenopus laevis tRNA Sec promoter (xtRNA Sec) relies on the Staf motif only (8). Seven contiguous zinc fingers of the C2–H2 type, located in the central part of the protein, contain the Staf DNA binding domain (9). In a recent study, we demonstrated that not all of the seven zinc fingers are required for Staf by binding to the Staf DNA motifs. In particular, the zinc finger 1 requirement is flexible, because it does contact the DNA at the xtRNA Sec but not at the hU6 Staf motifs (16). The objective of the work described here was to delineate the functional significance of the flexible utilization of zinc finger 1 for the binding of Staf to the hU6 and xtRNA Sec promoters. Our results show that this flexibility promotes maximization of transcriptional activation from both promoters. They also indicate that the nonutilization of zinc finger 1 at the hU6 DSE enables the simultaneous binding of Staf and Oct-1 to their cognate DNA motifs. Formation of the resulting ternary complex correlates downstream to maximal transcriptional activity from the human U6 basal promoter.

EXPERIMENTAL PROCEDURES

Plasmid Constructions—pSK(−)Staf, pGST-ZF1–7 and pGST-ZF2–7 have been described (16). Mutants of Xenopus tRNA Sec (17) and human U6 genes were obtained by site-directed mutagenesis of the parental vector. Constructs were verified by DNA sequencing.
Staf and Oct-1 Protein Preparation—Crude bacterial extracts were prepared from Escherichia coli TG2 strain containing pSK(−)Staf, essentially as previously described (9). The wild-type (ZI 1–7) and truncated (ZI 2–7) zinc finger domains were produced using the glutathione S-transferase (GST) gene fusion system as previously described (10, 16). Oct-1 was synthesized by in vitro coupled transcription-translation with the TnT system (Promega) programmed with pSK(−)Oct-1 (18). 6 μl of programmed lysate were used for the experiments described in the legends to Figs. 4, 6, and 7.

Generation of Anti-peptide Antibodies—Synthetic peptides KDGLK-IEGQVIQLED and EQQSLEEAIRIASRIQQGE derived from the Staf Binding Site of Xenopus tRNA Sec and human U6 snRNA, respectively (9), were coupled to keyhole limpet hemocyanin and injected into rabbits to generate polyclonal anti-peptide antibodies.

DNA Binding Assays—Probes were prepared as follows. The non-template strand of human U6 (positions 272 to 193) and Xenopus tRNA Sec (positions 242 to 159) were 5′-end labeled by polymerase chain reaction amplification of the corresponding gene using distal DNA-binding site sequences in the Staf Binding Site of Xenopus tRNA Sec and human U6 snRNA (hU6 site) DSEs (10).

Fig. 1. Sequence comparisons between the Staf and Staf consensus binding sites (16) in the X. laevis tRNA Sec (xtRNA Sec site) and human U6 snRNA (hU6 site) DSEs (10).

RESULTS
Opposite Effects on Transcription Provoked by a GCG Sequence in the Staf Binding Site of Xenopus tRNA Sec and Human U6 Promoters—Sequence comparisons of xtRNA Sec and hU6 Staf binding sites with the consensus sequence derived from binding site selection revealed that the GCG sequence located in the 3′ part of the xtRNA Sec Staf binding site and in the consensus was not found in the hU6 site (positions 18–20 in Fig. 1) (16). Moreover, we discovered that zinc finger 1 is involved in contacting the DNA at the xtRNA Sec but not the hU6 promoter (16). To investigate whether the flexible utilization of zinc finger 1 had functional significance, the mutant tRNA Sec and U6 templates shown in Fig. 2, A and B, were constructed. Constructs 1 are the wild-type xtRNA Sec and hU6 templates from which the others were derived. In construct 2 (Fig. 2A), the ACCA sequence in the Staf binding site of xtRNA Sec was replaced by CAAC (mut ACCA). In the hU6 Staf binding site, the CCACA was replaced by AACG to create mut CCCA (Fig. 2B, construct 2).

The Deleterious Effect of a GCG Sequence Adjacent to the hU6 Staf Binding Site Is Linked to the Presence of an Octamer Motif—It is known that full activation of the hU6 promoter also requires an octamer motif of sequence ATGCAAAT located in the reverse orientation at positions −215 to −222 (Fig. 2B) and at close proximity to the Staf binding site (15). To determine whether the deleterious effect of the CAT to GCG substitution 3′ to the hU6 Staf binding site is linked to the presence of the octamer sequence, we constructed U6 templates in which the octamer was disabled by the double point mutation ATGCAAAT to ATGCAGCT either alone (mut oct in Fig. 2B, construct 4) or in combination with the CAT to GCG substitution (mut CAT/oct in Fig. 2B, construct 5). Mutation of the octamer motif resulted in a severe drop of transcriptional efficiency to about 10% of the wt level (Fig. 2D, compare lanes 1 and 4). Surprisingly, when the octamer motif was mutated in combination with the CAT to GCG substitution, the transcriptional signal was 4-fold higher than with the octamer motif alone (Fig. 2D, compare lanes 4 and 5). Hence, the CAT to GCG substitution enhances the transcriptional capacity of the hU6 DSE if this mutation is associated with a mutant octamer motif. Finally, another hU6 template was constructed in which the spacing between the octamer motif and the CAT to GCG mutation was incremented by the CTAT 4-bp insertion (mut CAT + 4 in Fig. 2B, construct 6). This template was efficiently transcribed to 85% of the wt level.

From these results, it appears that the deleterious effect of the CAT to GCG mutation on hU6 transcription is linked to the presence of the octamer motif adjacent to the Staf binding site. This effect can be partially relieved by disabling the octamer motif or increasing the spacing between the mutated Staf binding site and the octamer sequence.

Although Deleterious to Transcription in Vivo, the CAT to GCG Substitution in the hU6 DSE Augments the Efficiency of Staf Binding in Vitro—Templates that exhibit a reduced transcriptional activity because of mutations in the Staf binding site and octamer motif would be expected to have a reduced affinity for the binding of Staf and Oct-1, respectively. Therefore, electrophoretic mobility shift assays were carried out using Staf or Oct-1 and DNA fragments encompassing the DSEs of wild-type and mutant xtRNA Sec and hU6. As established in a previous work, when the wild-type xtRNA Sec or hU6 DSEs...
were incubated with bacterial extracts expressing Staf and then analyzed on polyacrylamide gels, two major (C1, C2) and a minor (C3) binary complexes were obtained (Fig. 3, \( A \) and \( B \), lanes 2) (9). The C2 and C3 complexes arose from proteolytic cleavages of Staf that could not be prevented by inclusion of any inhibitor (9). The C1–C3 complexes are specific because they are competed by an excess of unlabeled Staf consensus binding site (Fig. 3, \( A \) and \( B \), compare lanes 2 and 3) but not by an excess of mutated xtRNASec binding site (Fig. 3, \( A \) and \( B \), compare lanes 2 and 4). The addition of preimmune sera had no effects on the C1–C3 complexes (Fig. 3, \( A \) and \( B \), compare lanes 2 and 6). In contrast, Staf antisera led to the disappearance of complexes C1–C3 and affected to a large extent the intensity of complex C2 (Fig. 3, \( A \) and \( B \), compare lanes 2 and 5), thus demonstrating that Staf is a component of the retarded complexes.

The relative efficiency with which Staf bound to wild-type and mutant versions of the xtRNASec and hU6 DSEs was estimated by comparing the amount of shifted complexes formed with the different probes. Each xtRNASec or hU6 probe was made using the same tRNASec or hU6 labeled primer, ensuring that a family of probes possesses the same specific activity. Thus, comparison of the shifted complexes produced in the presence of a constant amount of Staf represents an easy way to assess the relative efficiency of Staf binding. A 5-fold decrease in the efficiency of Staf binding in each of the complexes was observed with mut GCG xtRNASec (Fig. 3C, compare lanes 2 and 4). As expected, the substitution (mut oct) in the octamer motif of the hU6 DSE was without effect on the efficiency of Staf binding (Fig. 3D, compare lanes 2 and 8). On the contrary, we observed a 2-fold increase in the binding of Staf to the hU6 DSE bearing the CAT to GCG substitution, alone or associated

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**Fig. 2.** Structure and template activities of different Xenopus tRNASec and human U6 genes carrying mutations in the DSEs. A and B, plasmid templates constructed for xtRNASec and hU6 transcription studies. The wild-type sequence of the Xenopus tRNASec and human U6 genes are shown from positions −216 to −187 and −244 to −210, respectively. The Staf binding site and the octamer motif are boxed. Constructs with substitutions in the Staf binding site or octamer motif, with altered spacing between the Staf binding site and the octamer motif, are depicted below the wt sequence. \( \text{Dots} \) indicate identity with the wt sequence. The triangle represents a 4-bp CTAT insertion to alter the spacing between mut CAT and the octamer motif. \( T \), level of transcription. \( C \) and \( D \), effects of DSE mutations on xtRNASec and hU6 template activities. xtRNASec and hU6 mutant templates for which the sequences are shown in A and B were injected into Xenopus oocyte nuclei. Panel C, lanes 1−3, and panel D, lanes 1−6, correspond to the construct numbers given in A and B, respectively. Positions of the xtRNASec, hU6, and 5 S maxi RNAs are indicated.

The identity of the injected template is indicated above each lane.
with either the substitution in the octamer motif (mut CAT/oct) or the insertion (mut CAT+14) between the octamer motif and the mutated Staf binding site (Fig. 3D, compare lanes 2 with lanes 6, 10, and 12).

We next examined the binding of Oct-1 to wild-type and mutant versions of the hU6 DSE. When the wild-type hU6 DSE was incubated with in vitro translated Oct-1 and then analyzed on polyacrylamide gels, a major retarded complex was obtained (Fig. 4, compare lanes 1 and 2). This complex reflects a specific Oct-1 DNA interaction because an excess of the wt but not of the mutant octamer motif could compete for Oct-1 binding (Fig. 4, compare lanes 2, 3, and 4). A complete loss of Oct-1 binding was detected when the mutant versions of the hU6 DSE containing the disabled octamer motif were used as probes (Fig. 4, lanes 10 and 12). As predicted, mutations within the Staf binding site (mut CCCA) as well as the 4-bp insertion between the octamer motif and the mutated Staf binding site (mut CAT+14) had no effect on the efficiency of Oct-1 binding (Fig. 4, lanes 6, 8, and 14). Regarding xRNASec whose transcriptional activation depends on the sole Staf motif, no binding of Oct-1 could be detected on wt or mutant xRNASec DSEs (Fig. 4, lanes 14-18).

Significantly, there was a good correlation between transcription levels and the abilities of wild-type and mutant xRNASec and hU6 templates to be recognized by Staf or Oct-1. However, the increased level of the Staf DNA binary complex formed on the mut CAT hU6 template does not correlate with the diminution of transcription from this template.

The CAT to GCG Substitution in the hU6 DSE Promotes the Binding of Staf Zinc Finger 1—To obtain more information on the interaction of Staf with mut GCG xRNA Sec and mut CAT hU6 DSEs, missing nucleoside experiments with hydroxyl radicals were performed with the entire or truncated Staf zinc finger domains. For the Staf constructs, we used two recombinant polypeptides containing GST fusions to zinc fingers 1–7 (Zf 1–7) and 2–7 (Zf 2–7). The labeled DNA fragments containing the xRNA Sec and hU6 mutant DSEs were subjected to a mild cleavage treatment with hydroxyl radicals so that each
and complex containing Oct-1 (the base pairs of the xtRNASec and hU6 sites being numbered consensus binding site (16) stands as a numbering reference, Xenopus of labeled DSE is indicated above lane 3 DNA fragment was cleaved at no more than one position. The Zf 1–7 and Zf 2–7 proteins were incubated with the modified DNAs and the DNA-bound fragments separated from unbound DNAs by polyacrylamide gel electrophoresis. DNAs eluted from regions of the gel containing the bound and free fragments were electrophoresed on a sequencing gel. In such an assay, a nucleoside that is important for forming the DNA-protein complex yields a weak or missing band 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. The pattern of DNA fragments resulting from these experiments is shown in Fig. 5, A and B. Compilation of the data in 1–14, and 16, 18, 6 μl of in vitro translated Oct-1 protein (+). Incubation was in the absence (lanes 1, 2, and 5–18) or presence of a 1000-fold molar excess of unlabeled wt (octSC, lane 3) or mutant (octNSC, lane 4) octamer motifs. The identity of each labeled DSE is indicated above the lanes. The locations of the free probe and complex containing Oct-1 (C4) are indicated.

DNA fragment was cleaved at no more than one position. The Zf 1–7 and Zf 2–7 proteins were incubated with the modified DNAs and the DNA-bound fragments separated from unbound DNAs by polyacrylamide gel electrophoresis. DNAs eluted from regions of the gel containing the bound and free fragments were electrophoresed on a sequencing gel. In such an assay, a nucleoside that is important for forming the DNA-protein complex yields a weak or missing band 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. The pattern of DNA fragments resulting from these experiments is shown in Fig. 5, A and B. Compilation of the data in comparison with previous results obtained with the wild-type xtRNASec and hU6 DSEs is shown in Fig. 5C; the 21-bp Staf consensus binding site (16) stands as a numbering reference, the base pairs of the xtRNASec and hU6 sites being numbered –1 to 22, starting at the 5’-end of the non-template strand (see Fig. 5C). Obviously, removal of any nucleoside from positions 1 to 15 (xtRNASec) and –1 to 20 (hU6) on the non-template strand of the mutant DSEs strongly interfered with the binding of Zf 1–7 (Fig. 5). These results are quite different from those obtained with the wild-type xtRNASec and hU6 DSEs where the interference pattern in the 3’ part, is extended by 6 nucleosides for the wt xtRNASec site (positions 16–20 and 22) and 5 nucleosides shorter for the wt hU6 site (positions 16–20). Deletion of zinc finger 1 in Zf 2–7 provoked a reduction of the interference pattern of 5 nucleosides on the non-template strand of the mutant hU6 site, and this pattern was identical to that previously observed (16) where Zf 2–7 was added to gapped wild-type hU6 sites (Fig. 5, B and C). In stark contrast, the same zinc finger 1 deletion did not alter the missing nucleoside pattern on the mutant xtRNASec site (Fig. 5, A and C).

Taken together, these results demonstrate that zinc finger 1 contacts the DNA at the 3’ part of the site in the mutant hU6 DSE bearing a CAT to GGC substitution. Conversely, the GGC to CAT substitution in the xtRNASec DSE results in the loss of binding of zinc finger 1 to the mutated site. Thus, these results strongly suggest that the GCG triplet at positions 18–20 on the non-template strand (or GGC on the opposite strand) of the Staf binding site represents a critical determinant for zinc finger 1 binding.

The Transcriptional Activities of wt and Mutant hU6 Templates Correlate with Their Abilities to Form Staf-Oct-1-DNA Ternary Complexes—Because maximal hU6 transcriptional activity is strictly dependent on the integrity of the wild-type Staf and Oct-1 binding sites, we asked whether this property reflected the capabilities of Staf and Oct-1 to bind simultaneously to the DNA and form a Staf-Oct-1-DNA ternary complex. Electrophoretic mobility shift assays showed that, as anticipated, Staf and Oct-1 bound separately to the hU6 DSE to form the binary complexes C1–C3 and C4, respectively (Fig. 6, lanes 1–4). These complexes are specific by the same criteria used previously in Figs. 3 and 4 (Fig. 6, compare lanes 2 and 3 and lanes 4 and 5). When Staf and Oct-1 were added together, three new complexes of lower electrophoretic mobilities were formed, termed C5–C7 (Fig. 6, lane 6). Complexes C5–C7 are specific because their formations were abrogated by an excess of unlabeled Staf consensus (Fig. 6, lane 7) or Oct-1 (lane 9) binding sites but not by an excess of mutated Staf or Oct-1 binding sites (data not shown). As for C1–C3, complexes C5–C7 result from full-length and truncated Staf polypeptides generated by proteolytic cleavages in the bacterial extracts. Additionally, we could show by competition assays that complexes C5–C7 arose from the formation of a ternary complex between Staf, Oct-1, and the probe. Effectively, competition with an oligonucleotide corresponding to a high affinity Staf binding site resulted in their conversion to the Oct-1 complex C4 (Fig. 6, compare lanes 6 and 7), whereas competition with an excess of an oligonucleotide corresponding to the Oct-1 binding site induced the conversion of complexes C5–C7 to the Staf C1–C3 complexes (Fig. 6, compare lanes 6 and 9). The presence of Staf in these complexes was further demonstrated by the reactions with antisera specific for Staf, leading to the disappearance of complexes C5–C7 (Fig. 6, compare lanes 6 and 8). The assumption that the C5–C7 complexes contain both Staf and Oct-1 was confirmed by using hU6 labeled probes containing point mutations either within the Staf (mut CCCA) or the Oct-1 binding sites (mut oct). Staf failed to interact with the mutant Staf binding site (mut CCCA), whereas Oct-1 could still form the same C4 complex as with the wild-type probe (Fig. 6, lane 11). Conversely, using a probe containing the mut oct binding site, the C1–C3 complexes did form with Staf, but the C4 complex with Oct-1 was not detected (Fig. 6, lane 13). Thus, formation of the slowest migrating complexes requires the intact Staf binding site and octamer motif. Taken together, these results strongly argue that complexes C5–C7 are generated by Staf and Oct-1 binding simultaneously to the hU6 DSE to form a ternary complex. In the presence of the Oct-1-specific competitor DNA, about 90% of the wild-type hU6 probe was found in complexes C1–C3 (Fig. 6, lane 9), whereas about 25% of the probe was found in complex C4 in the presence of the Staf-specific competitor DNA (Fig. 6, lane 7). In an attempt to analyze the fashion with which Staf and Oct-1 bind the DNA, we measured the total intensity in the bands corresponding to complexes C5–C7 (Fig. 6, lane 6) and found 20% of the input probe. If Staf and Oct-1 interact cooperatively with the hU6 DSE, one would expect to find more than 22% (90 × 25%) in the complexes C5–C7 in the absence of any competitor instead of the actual 20%. Conspicuously, this finding implies that Staf and Oct-1 do not cooperate in binding to the hU6 DSE under the assay conditions.

We next examined whether the mut CAT, mut CAT/oct, or
FIG. 5. Hydroxyl radicals interference pattern with the Staf zinc finger polypeptides 1–7 and 2–7 on the mutant \textit{Xenopus} tRNA\textsuperscript{Sec} and human U6 Staf binding sites. The 5' end-labeled non-template strands of xtRNA\textsuperscript{Sec} and hU6 harboring the GCG to CAC and CAC to GCG substitutions, respectively, were subjected to hydroxyl radical cleavages as described under “Experimental Procedures.” Gapped DNAs were incubated separately with Zf 1–7 and Zf 2–7 fused to GST. Complexed and free DNA fragments were separated by native polyacrylamide gel electrophoresis. The bound and free DNA fragments were excised from the gel and further electrophoresed on a sequencing gel. A and B, missing nucleoside interference patterns on the mutant xtRNA\textsuperscript{Sec} and hU6 DSEs. The nature of the proteins is 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 DNAs, and bound DNAs, respectively. C, schematic representation of the results for Zf 1–7 and Zf 2–7 on the mutant xtRNA\textsuperscript{Sec} and hU6 DSEs in comparison with previous results obtained with the wt tRNA\textsuperscript{Sec} and hU6 DSEs. Regions of interference are boxed: filled boxes, strongest interference; hatched boxes, moderate interference; open boxes, weakest interference. The base pairs in the Staf binding site are numbered 1 to 22, starting at the 5'-end of the non-template strand with reference to the consensus binding site derived by \textit{in vitro} selection (16).
mut CAT + I4 mutant versions of the hU6 DSE can lead to ternary complex formation. When Oct-1 was mixed with the quantity of Staf allowing formation of the same amount of C1–C3 complexes (as in Fig. 7A, lanes 2, 4, 6, and 8), the ternary complex formation was profoundly impaired with mut CAT (Fig. 7B, compare lanes 2 and 4) but restored with mut CAT + I4 (Fig. 7B, compare lanes 2 and 6). As expected, no ternary complex was observed with mut CAT/oct containing mut CAT associated with the crippled octamer (Fig. 7B, compare lanes 2 and 8). In conclusion, these experiments conclusively demonstrate that the acquisition of the zinc finger 1 binding site in the hU6 Staf binding site is strongly inhibitory to the formation of the Staf-Oct-1-DNA ternary complex.

DISCUSSION

A large number of DSEs in vertebrate snRNA-type promoters are composed of the octamer and Staf motifs constituting the binding sites for Oct-1 and Staf, respectively (8–12). These factors activate transcription through interactions with the DSE. Staf contains seven contiguous zinc fingers of the C2–H2 type. Such zinc finger motifs have been shown to carry many key functions. They were first recognized as DNA binding motifs. Indeed, structural studies of the Zif 268-DNA complex showed that each of the C2–H2 finger represents a structurally and functionally independent domain contributing to the recognition of three consecutive nucleotides in the DNA major groove (20, 21). Later, solving the crystal structure of the five zinc finger GLI-DNA complex established that the different zinc fingers can play distinct roles. Zinc finger 1 in the GLI-DNA complex does not contact the DNA, but rather it makes extensive protein-protein interactions with adjacent zinc fingers (22). Finally, an additional role has been uncovered for C2–H2 zinc fingers in the formation of an intermolecular protein-protein interaction (23). In the study presented here, we have shown that the alternative binding of Staf zinc finger 1 to DNA profoundly influences the transcription activity of snRNA-type promoters. In fact, binding of this zinc finger to the Xenopus tRNA$^{Sec}$ promoter maximizes transcriptional activation, whereas in the case of the human U6 snRNA promoter, it is precisely the lack of zinc finger 1 binding that leads to maximization of transcription. Thus, our data suggest that the alternative usage of a same zinc finger motif represents a new critical determinant for the mechanism of transcriptional activation.

To our knowledge, this is the first report describing that the same transcription factor can achieve maximization of transcriptional activation, from two different promoter contexts, by
the alternative binding of the same zinc finger. Conceivably, this finding introduces a new dimension to the functional versatility of zinc finger motifs.

We have found that substitution of the wt hU6 DSE CAT sequence by GCG, or that of the wt tRNA\textsubscript{Sec} DSE GCG sequence by CAT, at the same positions 18–20 on the non-template strand of the Staf binding sites was sufficient to promote the binding of zinc finger 1 to the hU6 DSE or instead to abolish it in the tRNA\textsubscript{Sec} counterpart. In a previous work (16), we established that Staf associates more closely with the non-template than with the template strand of the Staf binding site. From this work, it looks as though the GCG triplet is critical to zinc finger 1 binding. With regard to the CAT sequence, binding site selection experiments performed with Staf showed that the selected sequences never contained a CA motif at positions 18–19 (16). If a CA sequence without being strictly necessary were simply acceptable for the binding of Staf, it would have appeared in the selection, even at a low rate. Obviously, this was not the case. Therefore, we propose that the CA motif in the hU6 DSE CAT sequence is not only unable to bind zinc finger 1 but in all likelihood constitutes a sequence bearing repulsive determinants for binding.

The addition of the transcription levels afforded by the human U6 mutant promoters containing either the Staf or the octamer motif yielded a much weaker value than that of the wild-type U6 promoter containing both the Staf and octamer motifs. Obviously, this interesting finding can be interpreted to mean that Staf and Oct-1 act synergistically to activate transcription from the human U6 promoter. The possibility for this synergistic role in transcriptional activation from other snRNA promoters was already predictable from our and other studies (24, 25), but the underlying mechanism has not yet been understood. Nevertheless, bandshift experiments and microinjection assays in this work provided evidence for a role of the Staf-Oct-1-DNA ternary complex in the maximization of hU6 transcriptional activation, suggesting that the synergistic activation is mediated by this complex. Knowing that the nonbinding of zinc finger 1 to DNA is mandatory for formation of the ternary complex, two possibilities can be entertained to explain its formation on the human U6 promoter. In the first one, zinc finger 1 sterically blocks access to the Oct-1 homeodomain at the octamer motif, resulting in an impairment of complex formation and subsequent repression of transcriptional activation. In the second scenario, formation of the StafOct-1-DNA ternary complex involves an interaction between the DNA-unbound zinc finger 1 and Oct-1. However, this second possibility seems improbable because formation of the ternary complex was still obtained with the hU6 mutant DSE containing a 4-bp insertion between the octamer and the Staf motifs and a mutation actually promoting the binding of zinc finger 1 to the DNA.

Bandshift experiments attested to the lack of cooperative binding between Staf and Oct-1 to the human U6 DSE. Therefore, if cooperativity of binding is not involved in the synergistic activation, two other possibilities can be invoked to explain this fact: (i) the simultaneous presence of Staf and Oct-1 creates a unique surface for interaction with a coactivator(s) of the basal transcriptional activation; or (ii) Staf and Oct-1 each interacts with a distinct coactivator or protein surface of the basal transcriptional complex. However, the case of the Xenopus tRNA\textsubscript{Sec} pro-
moter, where the DSE function is mediated only by Staf, suggests that this transcription factor possesses per se the capacity to contact alone, or via a coactivator, the basal transcription complex. In conclusion, the results presented in this paper demonstrate that the Staf zinc finger 1 can influence the binding of another transcription factor, Oct-1, thus unraveling a mechanism by which Staf can modulate transcription levels from snRNA-type promoters.

Acknowledgments—We are grateful to W. Herr for the gift of the Oct-1 vector, S. Lodmell for reading the manuscript, and C. Loegler for excellent technical assistance.

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