ZNF76 and ZNF143 Are Two Human Homologs of the Transcriptional Activator Staf*

Evelyne Myslinski, Alain Krol, and Philippe Carbon‡

From the UPR 9002 du CNRS "Structure des Macromolécules Biologiques et Mécanismes de Reconnaissance," IBMC, 15, rue René Descartes, 67084 Strasbourg Cedex, France.

The transcriptional activator Staf, originally identified in Xenopus laevis, is implicated in the enhanced transcription of small nuclear RNA (snRNA) and snRNA-type genes by RNA polymerases II (Pol II) and III (Pol III). This zinc finger protein also possesses the capacity to stimulate expression from a Pol II mRNA promoter. Here, we report a study on two human proteins, ZNF76 and ZNF143, that are 64 and 84% identical to their Xenopus counterpart, respectively. Northern blot analysis revealed that ZNF76 and ZNF143 mRNAs were expressed in all normal adult tissues examined. By using in vivo and in vitro assays, we have analyzed the DNA binding capacities and transcriptional properties of ZNF76 and ZNF143. The binding affinities of ZNF76 and ZNF143 for Staf divergent responsive elements were determined by gel shift assays, which revealed that the two proteins bound a same DNA motif with similar affinities. Also, polypeptide sequences containing the seven zinc fingers of ZNF76 and ZNF143 could efficiently repress in vivo the activated transcription from an snRNA-type promoter. Transfection experiments in Drosophila cells showed that ZNF76 and ZNF143 can activate transcription from an mRNA promoter through the Staf binding site. Finally, chimeric ZNF76 and ZNF143 proteins, carrying a heterologous DNA binding domain, are able to activate a Pol II mRNA promoter and snRNA Pol II and Pol III promoters in Xenopus oocytes, through the heterologous DNA binding site. Taken together, these findings demonstrate that ZNF76 and ZNF143 are two members of a same family of transactivator proteins. ZNF143 constitutes the human ortholog of the Xenopus Staf, and ZNF76 is a novel DNA binding protein related to Staf and ZNF143.

Transcription is a major regulatory point in gene expression and depends largely on the interaction of regulatory proteins with their cognate DNA elements in gene promoters (1, 2). Analysis of promoters in a variety of snRNA1 genes transcribed by either Pol II or Pol III has identified a number of distinct DNA elements required for gene expression. The Pol II and Pol III snRNA gene promoters both contain an essential PSE, which binds the basal transcription factor PTF also called SNAPc (3–5), and a DSE playing a major role in transcription efficiency. The DSE contains an octamer motif that binds the well characterized transcriptional activator Oct-1 (6, 7). In addition to Oct-1, Sp1 has been shown in some instances to be involved in mediating the activation properties of the DSE (8–11). A number of other short transcription units, such as the 7SK, Y, MRP and tRNASec genes, have similar promoter organization and can be classified as snRNA-type genes (6). Recently, we have demonstrated that the zinc finger protein Staf, originally identified in Xenopus laevis as the transcriptional activator of the tRNASec gene (12, 13), is also involved in transcriptional activation of snRNA and snRNA-type genes transcribed by RNA Pol II and Pol III (14). 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, devoted to the specific activation of snRNA-type or mRNA promoters, constitutes the molecular basis of these pleiotropic effects on transcriptional activity. Whereas a 93-amino acid domain, with the presence of four repeated units, is specialized for the transcriptional activation of an mRNA promoter, a segment of only 18 amino acids acts specifically on Pol II and Pol III snRNA and snRNA-type promoters (15).

Here, we report the characterization of two human factors highly homologous to the Xenopus Staf transcriptional activator. These human zinc finger proteins, originally denominated ZNF76 and ZNF143 (16, 17), contain structural domains very similar to those of Staf. In keeping with Staf, our studies also revealed that ZNF76 and ZNF143 are able to transactivate not only Pol II and Pol III snRNA and snRNA-type, but also TATA box-containing mRNA promoters.

EXPERIMENTAL PROCEDURES

Effector Constructs—pBRN3-ZNF76 and pBRN3-ZNF143 were constructed by cloning the coding sequence of ZNF76 and ZNF143 into the EcoRI site of the vector pBR3 (18) between the 5′- and 3′-untranslated regions of the X. laevis β-globin gene. DNA fragments containing the ZNF76 and ZNF143 coding regions were prepared by PCR amplification of the CDM8-ZNF76 and pcDNA3 ZNF143 clones (kindly provided by J. Ragoussis (Human Immunogenetics Laboratory, ICRF Laboratories, London, United Kingdom) and H. Vissing (Danish Center for Human Genome Research, John F. Kennedy Institute, Glostrup, Denmark), respectively). Primers for ZNF76 were: forward, GCAGAAATTCGC- GCCACATGGAGAGCTTGGGGC, complementary to positions 164–179 in the ZNF76 cDNA clone, incorporating an EcoRI site and a Kozak consensus sequence upstream of ATG (positions 164–166); and reverse, CCGGATCTGCTTGTCTAGATGCT, complementary to positions 1734–1750 and incorporating an EcoRI site. Primers for ZNF143 were: forward, CGCGAATTCGCCGCCACCATGACAGAGTT- TTCTTAATCATCCAACCCTG, complementary to positions 38 to 53 of the ZNF143 cDNA, incorporating an EcoRI site and a Kozak consensus sequence upstream of ATG (positions 38–40); and reverse, CCGGAA- TTCTTAAATCATCCAACTCGT, complementary to positions 1902–1918 and incorporating an EcoRI site. The pBRN3-ZNF76-Krox-20 and

* This work was supported by grants from the Université Louis Pasteur in Strasbourg and the Association pour la Recherche sur le Cancer (ARC). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1724 solely to indicate this fact.
‡ To whom correspondence should be addressed. Tel.: 33 3 88 41 70 50; Fax: 33 3 88 60 22 18; E-mail: p.carbon@ibmc.u-strasbg.fr.
1 The abbreviations used are: snRNA, small nuclear RNA; Pol II, RNA polymerase II; Pol III, RNA polymerase III; PSE, proximal sequence element; DSE, distal sequence element; SL2, Schneider line 2; CAT, chloramphenicol acetyltransferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; tRNA Sec, selenocysteine tRNA; ORF, open reading frame; AE, activator element.
pBR3-ZNF143-Krox-20 constructs were obtained as follows. Substitutions in the amino acid positions 161–166 GDRAFR (nucleotide sequence GGAGACAGgatccTCCGC, mutations in lowercase) of ZNF76 and 179–184 GEKAFR (nucleotide sequence GGAGAGAggatccTTCGA, mutations in lowercase) of ZNF143 created a BamHI site. To create an EcoRV site in the resulting constructs, amino acid sequences ELEAT in ZNF76 and DTEPI in ZNF143 (amino acid positions 370–374 and 430–434, respectively, downstream of the seventh zinc finger; see Fig. 1) were changed to ELEIT and DTEII. In the BamHI/EcoRV-cleaved resulting constructs, the zinc finger domains were exchanged with the 263-base pair BamHI/EcoRV fragment (containing the sequence Pro336-His417 of Krox-20; Ref.19) obtained by PCR amplification of pPAC-Krox-20 (20) using forward and reverse primers complementary to positions 937–952 and 1161–1177 of the noncoding and coding strands, respectively. The forward, CGGGATCCTTCCCTGCCCAGCAGAA, and reverse, ACCACTGATATCTGTGTCGTTGTGGATCTTGGTGTGGC, primers incorporated BamHI and EcoRV sites, respectively. The sequence of the distal exchange junction is GDR/ILPC in the ZNF76-Krox-20 and GER/ILPC in the ZNF143-Krox-20 chimeric proteins. That of the proximal junction is TKIH/NDTEITE in ZNF76-Krox-20 and TKIH/NDTEIEE in ZNF143-Krox-20. The pBRN3-ZNF76-DBD and pBRN3-ZNF143-DBD constructs were obtained by cloning into pBRN3 the DNA fragments containing the ZNF76-DBD and ZNF143-DBD, prepared by PCR amplification of the ZNF76 and ZNF143 cDNAs, using forward and reverse primers incorporating an EcoRI site. The forward primer (complementary to positions 628 to 646 and 789 to 807 in the ZNF76 and ZNF143 cDNAs, respectively) contains an ATG initiator codon in the Kozak consensus sequence. The reverse primer (complementary to positions 1271 to 1288 and 1432 to 1439 in ZNF76 and ZNF143 cDNAs, respectively) carries a TAG stop codon. The pSK(2)-ZNF76 and pSK(2)-ZNF143 constructs were obtained by cloning into pBluescript SK(2) the amplification products of the PCR reactions performed on the cDM8-ZNF76 and pcDNA3-ZNF143 constructs. For ZNF76, the forward primer, CGGGATCCGCCGCCACCATFIG.1. Predicted amino acid sequences of ZNF76 and ZNF143 and comparison with that of Staf. Amino acid identity is indicated by a star (*), Gaps (--) have been introduced at several locations to maximize the match. Amino acid homologies are indicated by (:) or (.). The zinc finger domain and the six regions highly conserved (regions I to VI) are underlined. The accession numbers for the proteins included in this analysis are: X84996 (Staf), M91592 (ZNF76), and U09850 (ZNF143).
GGAGAG, complementary to positions 162–171 of the ZNF76 cDNA sequence, incorporated a BamHI site and a Kozak consensus sequence upstream of ATG (position 164–166). The reverse primer, CGGGATCCTTCAGCAGCCACTCTCCG, complementary to positions 1695–1711, incorporated a BamHI site. For ZNF143, the forward primer, CGGGATCTCCGCCGCCACCATGGCAGAGTTTCCTGGAGGAGGG, complementary to positions 38–61 of the ZNF143 cDNA sequence, incorporated a BamHI site and a Kozak consensus sequence upstream of ATG (positions 38–40); the reverse primer, CGGGATCCTTAATCATCCAACCTG, complementary to positions 1902–1918, incorporated a BamHI site. The Drosophila expression vectors pPAC-ZNF76 and pPAC-ZNF143 were made as follows. The ORF of pSK(2)-ZNF76 and pSK(2)-ZNF143 were excised by digestion with BamHI and inserted into the BamHI-cut pPAC vector (21).

**Fig. 2.** Primary structure features of human ZNF76 and ZNF143 in comparison with Xenopus Staf. A, schematic representation of ZNF143, Staf, and ZNF76 with delineation of the various domains. Values for amino acid identities between the full-length proteins and the different domains are indicated. B, protein sequence alignments of the zinc finger domains of Staf, ZNF143, and ZNF76. Amino acid identity is indicated by a bar. Gaps (−) have been introduced at two locations to maximize the match. Cysteines, histidines, and invariant hydrophobic residues are depicted with bold letters. Amino acids at positions +1, +2, +3 and +6 of the α-helix are indicated. C, protein sequence alignments of repeats R1, R2, R3, and R4 in the Staf mRNA activation domain with homologous regions of ZNF76 and ZNF143; the deduced consensus sequence is shown. D, protein sequence alignments of the Staf snRNA activation domain with homologous regions of ZNF76 and ZNF143; the deduced consensus sequence is shown.

GGAGAG, complementary to positions 162–171 of the ZNF76 cDNA sequence, incorporated a BamHI site and a Kozak consensus sequence upstream of ATG (position 164–166). The reverse primer, CGGGATCTTCACGCAGCCACTCTCCG, complementary to positions 1695–1711, incorporated a BamHI site. For ZNF143, the forward primer, CGGGATCTCCGCCGCCACCATGGCAGAGTTTCCTGGAGGAGGG, complementary to positions 38–61 of the ZNF143 cDNA sequence, incorporated a BamHI site and a Kozak consensus sequence upstream of ATG (positions 38–40); the reverse primer, CGGGATCTCCGTTAATCATCCAACCTG, complementary to positions 1902–1918, incorporated a BamHI site. The Drosophila expression vectors pPAC-ZNF76 and pPAC-ZNF143 were made as follows. The ORF of pSK(2)-ZNF76 and pSK(2)-ZNF143 were excised by digestion with BamHI and inserted into the BamHI-cut pPAC vector (21).

**Reporter Constructs**—p6E.tKCAT and 3E.tRNA<sup>sec</sup> are described in Vesque and Charnay (22) and Schuster et al. (13), respectively. tRNA<sup>sec</sup>wt, tRNA<sup>sec</sup>AE and AE.tKCAT are described in (12). 3E.U1 and U1DSE are described in (14).

**Oocyte Microinjections, Nuclear Localization, and DNA Binding Assays**—Capped mRNAs were synthesized in vitro by T3 RNA polymerase as described in Schuster et al. (13) and injected (20 nl, 1 ng) into the cytoplasm of X. laevis oocytes, 20 h before the nuclear injection of 20 nl containing the reporter. Concentrations of the reporters were 300 μg/ml for 6E.tKAT, 25 μg/ml for tRNA<sup>sec</sup> in Fig. 4, 50 μg/ml for U1 and tRNA<sup>sec</sup> in Fig. 5. tRNA<sup>sec</sup> and U1 reporters were injected in the presence of [α-<sup>32</sup>P]GTP (800 Ci/mmol, 0.2 μCi/oocyte) and 5 S RNA maxigenix (5 μg/ml) as an internal control for nuclear injection and RNA recovery. The 6E.tKAT was injected in the presence of pCH110 (300
Human Homologs of Staf

RESULTS

ZN7F6 and ZNF143, Two Human Proteins with High Similarity to Xenopus Staf—The Xenopus Staf sequence (13) was submitted to a computer search with the EBI Data Bank. A significant homology was found between Staf and the human proteins of unknown function ZNF76 and ZNF143 (16, 17). The ZNF76 and ZNF143 cDNA sequences contain a complete open reading frame of 515 and 626 amino acids, respectively. The calculated molecular mass of the ZNF76 and ZNF143 products are 56.3 and 67.6 kDa, respectively. Whereas the predicted amino acid sequences of human ZNF76 and Xenopus Staf are highly conserved (84%), that of human ZNF76 shares only 64% identity with Xenopus Staf, a value equivalent to the 63% identity observed between ZNF76 and ZNF143 (see Figs. 1 and 2A). Outside the zinc finger domain, identity to Staf is 52% for ZNF76 and 81% for ZNF143 in the N-domain, on the one hand, and 43% for ZNF76 and 73% for ZNF143 in the C-domain, on the other (Figs. 1 and 2A). Interestingly, both ZNF76 and ZNF143 also contain domains with characteristic features very similar to the Staf transactivation domains. In deed, both proteins share with Staf the mRNA activation domain with the striking presence of the four repeat units R1 to R4 (Fig. 2C), but also the snRNA activation domain, located 18 (ZNF76) and 41 (ZNF143) amino acids upstream of the zinc finger domain (Figs. 1 and 2D). In addition, in the C-terminal region, 16 of the 24 residues encompassing positions 561–584 in Staf, 469–492 in ZNF76 and 587–610 in ZNF143 are identical, giving rise to 66% of identity (Fig. 1, region VII). Despite the overall similarity, the sequences of the three proteins display notable differences. First, ZNF143 and Staf contain 32 and 77 additional residues at the amino terminus, respectively; these residues are absent in ZNF76. Second, ZNF143 harbors a large sequence insertion (positions 498–551) in the C-terminal domain, absent in ZNF76 and Staf. Based on their close sequence identity, we concluded that ZNF143 is the human ortholog of Xenopus Staf. Instead, ZNF76 being as divergent from ZNF143 as it is from Staf, we propose that ZNF76 is a novel DNA-binding protein, related to Staf and ZNF143.

Expression of ZNF76 and ZNF143 mRNAs—RNA blot analysis showed that the human ZNF76 and ZNF143 mRNAs were detectable in all the various adult tissues examined, supporting a fundamental role for these two proteins (Fig. 3). The sizes of the ZNF76 and ZNF143 mRNAs were approximately 2.9 and 3.5 kilobases, respectively, in all the tissues assessed. It must be noted, in addition, that the relative concentrations of the two mRNAs apparently differed in some tissues, with ZNF76 and ZNF143 being most abundant in testis and ovary, respectively (Fig. 3).

DNA Binding Affinities of ZNF76 and ZNF143—The high degree of sequence conservation in the ZNF76 and ZNF143 zinc finger regions prompted us to ask whether both proteins could recognize the same DNA motif with similar affinities. To answer the question, we determined the $K_d$ (dissociation constants) of ZNF76 and ZNF143 for the Staf responsive elements identified in the Xenopus tRNA$^{Sec}$ (tRNA$^{Sec}$ site) and human U6 snRNA (U6 site) genes (12, 14). The tRNA$^{Sec}$ and hU6 sites (Fig. 4A) were used in this study because, despite divergent sequences, they were efficiently recognized by Staf in our earlier studies (12–14). Analysis of saturation binding curves established that the affinities of ZNF76 and ZNF143 for the tRNA$^{Sec}$ and hU6 sites are high, with $K_d$ values of about 1 ± 0.15 nm and 1.2 ± 0.1 nm for ZNF76 and ZNF143 on the hU6 site, respectively, and 0.5 ± 0.05 nm for ZNF76 and ZNF143 on the tRNA$^{Sec}$ site (Fig. 4B). From this, it appears that ZNF76...
and ZNF143 recognize a same DNA motif with identical affinities.

The DNA Binding Domains of ZNF76 and ZNF143 Can Repress Transcription of the tRNA\textsubscript{Sec} Gene—To determine whether the ZNF76 and ZNF143 DNA binding domains could recognize efficiently Staf responsive elements in vivo, we used a microinjection assay in Xenopus oocytes to test whether the zinc finger domains of ZNF76 (ZNF76-DBD) and ZNF143 (ZNF143-DBD) can repress transcription from an RNA Pol III promoter. The reporter construct used the well characterized promoter of the tRNASec gene. It contains, in addition to the PSE and TATA elements, a Staf responsive element called AE, which plays a major role in transcription efficiency. In the assay, the mRNAs coding for ZNF76-DBD and ZNF143-DBD were transcribed in vitro and microinjected separately into the oocyte cytoplasm. After 20 h of incubation, the tRNA\textsubscript{Sec} reporter gene (Fig. 5A) was injected into oocyte nuclei with [α-\textsuperscript{32}P]GTP and a 5 S RNA maxigene as the internal standard. After a second incubation, the labeled RNAs were extracted, and the level of transcribed tRNA\textsubscript{Sec}, normalized relative to the 5 S RNA maxigene expression, was used to determine the transcriptional capacities of the effector proteins. Injecting increasing amounts of mRNAs encoding ZNF76-DBD and ZNF143-DBD resulted in a progressive reduction of tRNA\textsubscript{Sec} gene expression (Fig. 5B, compare lane 1 without effector to lanes 3–7 for ZNF76-DBD and lanes 9–11 for ZNF143-DBD). At higher mRNA concentrations (lanes 7 and 11), the tRNA\textsubscript{Sec} level decreased dramatically to become similar to that of a control tRNA\textsubscript{Sec} gene devoid of AE (Fig. 5B, lane 2). A control experiment, performed with full-length ZNF143, confirmed that the observed effects were effectively caused by the unproductive binding of the DBDs to the target DNA sequences because expression of the tRNA\textsubscript{Sec} was unaffected by full-length ZNF143 (Fig. 5B, lane 8).

These data suggest that the DNA-binding domains of ZNF76 and ZNF143, alone, are able to efficiently compete for DNA binding sites with the full-length endogenous Staf, therefore reducing the level of transcriptional activation.

Transactivation Properties of ZNF76 and ZNF143 in Xenopus Oocytes—The striking presence in human cells of two Staf-like proteins raised the question of their functional roles. To solve the issue, the transactivation capabilities of ZNF76 and...
TRNAsec reporters were injected into oocyte nuclei, in separate Krox-20 and ZNF143-Krox-20, the 6E-tKCAT, 3E-U1, and 3E-tRNAsec were significantly enhanced (compare lane 1 with lanes 2 and 3 in Fig. 6B; lane 2 with lanes 4 and 6; and lane 8 with lanes 10 and 12 in Fig. 6C), whereas no effect was observed in the absence of the target sequences (compare lane 1 with lanes 3 and 5, lane 7 with lanes 9 and 11 in Fig. 6C). As control experiments, we wished to evaluate the extent of nuclear accumulation of the newly synthesized proteins and their DNA-binding abilities. In the first case, the compartmental distribution of the newly synthesized ZNF76-Krox-20 and ZNF143-Krox-20 proteins in microinjected oocytes was examined. Oocytes were incubated in the presence of [35S]methionine and manually enucleated, and the cytoplasmic and nuclear fractions were analyzed independently (Fig. 6D). Quantitation indicated that about 80% of ZNF76-Krox-20 and 30% of ZNF143-Krox-20 were found in the nuclear fraction. Because the nucleus represents only 1/20 of the cytoplasm volume, it appears that a high concentration of the recombinant proteins accumulated into the nucleus. The DNA-binding capacities of the chimeric proteins were verified by gel retardation assays with nuclear extracts from microinjected oocytes and a 32P-labeled probe containing one E site. Unique complexes were obtained with nuclear extracts containing the chimeric ZNF76 and ZNF143 proteins (data not shown).

Taken together, the data presented clearly established that ZNF76 and ZNF143 are transcriptional activators possessing, like Staf, the ability to stimulate transcription from Pol II and Pol III snRNA and snRNA-type promoters as well as Pol II TATA box-containing mRNA promoters.

ZNF76 and ZNF143 Transcriptional mRNA Activator in Drosophila SL2 Cells—All the mammalian cell lines that we have tested were found to contain endogenous ZNF76 and ZNF143. These endogenous Staf-like proteins obscure the activities mediated by transfected ZNF76 and ZNF143 (data not shown). Therefore, we decided to use Drosophila SL2 cells (Schneider cells) as a host for the analysis of ZNF76 and ZNF143. SL2 cells are particularly suited for this task because they are devoid of endogenous Staf-like activities and have been utilized successfully in co-transfection assays to analyze Staf activity in vivo (13, 15). In such a cell line, only the mRNA promoter can be assayed because it has been shown that transcriptional activation of snRNA promoters in Drosophila does not proceed similarly to vertebrates (23). Expression vectors for ZNF76 and ZNF143 were constructed by fusing the appropriate cDNA fragments to the Drosophila actin 5C promoter. The AE.tKCAT reporter contains three Staf AE binding sites upstream of the tk promoter fused to the CAT reporter gene. Co-transfections of ZNF76 and ZNF143 resulted in efficient transcriptional activation of the CAT gene with a higher level of transactivation of ZNF143 (Fig. 7, compare lanes 1 with lanes 2 and 3). The expression levels of ZNF76 and ZNF143, in SL2 cells after transfection, were assayed by gel retardation assays with nuclear extracts from transfected cells and a 32P-labeled oligodeoxynucleotide probe containing one AE site. Unique complexes, migrating at similar levels, were generated using cell lysates containing ZNF76 and ZNF143 (data not shown).

These experiments demonstrated that ZNF76 and ZNF143 can mediate the transcriptional activation of an mRNA promoter in Drosophila SL2 cells.

**DISCUSSION**

Staf, originally identified in X. laevis, plays a pivotal role in transcriptional activation not only of snRNA and snRNA-type promoters by RNA Pol II and Pol III, but also of mRNA pro-
FIG. 6. ZNF76 and ZNF143 stimulate transcription from the tkCAT, U1 snRNA, and tRNA\textsuperscript{Sec} promoters in X. laevis oocytes. A, schematic diagrams of the effector mRNAs synthesized in vitro and of the various reporter genes used in the Xenopus microinjection assay. B, CAT assay showing transcriptional activation from the 6E.tkCAT promoter by ZNF76-Krox-20 and ZNF143-Krox-20 using extracts from microinjected oocytes. Lane 1: no effector was expressed. C, enhanced transcription of Pol II 3E-U1 and Pol III 3E-tRNA\textsuperscript{Sec} promoters by ZNF76-Krox-20 and ZNF143-Krox-20 effectors using microinjected oocytes. Identities of the effector proteins and reporters are indicated above the lanes. Lanes 1, 2, 7, and 8, no effector was expressed. Positions of the U1, tRNA\textsuperscript{Sec}, and 5 S maxi RNAs (internal standard) are indicated. D, effector expression and nuclear targeting of the ZNF76-Krox-20 and ZNF143-Krox-20 proteins in oocytes. Oocytes were injected with capped mRNAs and incubated with \textsuperscript{35}S]methionine. Oocytes were manually dissected and both the nuclear (N) and cytoplasmic (C) fractions analyzed by SDS-gel electrophoresis. Arrows indicate the positions of the chimeric proteins. Lanes 1, 2, 5, and 6: uninjected oocytes.
motors. This activity is complex, arising from the action of two physically and functionally distinct activation domains acting specifically on snRNA and mRNA promoters (15). In *X. laevis*, only one molecular species of Staf has been characterized (13). Herein, we describe the characterization of the human Staf ortholog, ZNF143 (17), and the identification of a novel DNA binding protein, ZNF76 (16), with Staf-like properties. DNA binding and functional studies in vitro and in vivo showed that ZNF76 and ZNF143 can bind tightly to Staf responsive elements and transactivate Pol II and Pol III snRNA-type and mRNA promoters. These findings confirm that these proteins are two members of the same family.

A Blast search of the mouse EST database (mESTs) with the human ZNF76 and ZNF143 sequences at the National Center for Biotechnology Information revealed the presence of mESTs for both proteins (accession numbers: AA498451, AA75999, and W74814 for ZNF76; and AA153902 for ZNF143). Surprisingly, the mouse ortholog of ZNF76 contains the additional residues observed in the C-domain of human ZNF143, but absent in human ZNF76. From our and other studies, it appears that ZNF76 and ZNF143 are highly conserved in mammals. Additionally, only the Staf/ZNF143 ortholog is expressed in amphibian and bird cells (13, 16).

ZNF76 and ZNF143 contain seven tandemly repeated zinc fingers of the C2-H2 type, similarly to Staf. The structure of the Staf DNA binding domain is still unknown, but the x-ray structure and NMR structures of other zinc finger-containing DNA-protein complexes, such as Zif268, GLI, Tramtrack, GAGA, YY1, and TFIIIA, have been solved (24–30). These structural data identified amino acids governing DNA-binding specificity at positions $-1, +2, +3$, and $+6$ of the $\alpha$-helix of one zinc finger. The divergent residues between the homologous zinc fingers of Staf, ZNF76, and ZNF143 are essentially located in the two antiparallel $\beta$-strands and in the loop connecting them. Based on the structural data evoked above, it follows that the amino acids putatively involved in DNA recognition are fully conserved between Staf, ZNF76, and ZNF143, with the exception of the amino acid at position $+6$ in zinc finger 6 which is Asn in ZNF76 and Lys in Staf and ZNF143. The sequence of zinc finger 6 in ZNF76 does not match the consensus sequence of the C2-H2 type zinc finger: $\Phi-X-C-X_2-Y-C-X_2-Y-X_5-\Phi-X_2-H-X_2-\Phi-X_2-Y_5-H$ ($\Phi$ being a hydrophobic amino acid and $X$ a nonconserved amino acid). This zinc finger contains only two amino acids between the two invariant His and three residues between the conserved hydrophobic Leu residue and the invariant distal His. However, our results showed that ZNF76 and ZNF143 have comparable affinities for the Staf responsive element contained in the *X. laevis* tRNA$^{\text{Sec}}$ and human U6 promoters, revealing the neutral character of the amino acid changes in the sequences of the ZNF76 and ZNF143 DNA binding domains. This is further confirmed by sequence analysis of the mouse ortholog of ZNF76 (mEST AA475991) that revealed the presence of a K residue at position +6 in the $\alpha$-helix of zinc finger 6, as ZNF143.

Six regions are highly conserved from *Xenopus* Staf to human ZNF76 and ZNF143, outside of the zinc finger domain (referred to as regions I to VI). Regions I to IV, located in the N-terminal part of the proteins, partly encompass the mRNA transactivation domain and contain the four repeated units, a characteristic feature of this domain. The consensus sequence V/LXLGDS/TXAY/F/I/V/H/Q ($X$ stands for any amino acid), derived from comparison of the repeated units in Staf, ZNF76, and ZNF143, contains the leucine and aspartic acid residues previously identified as essential for the Staf transactivation domain (15). The distance between the repeats represents 10–16 amino acids and only the sequence motif L/FEDG, located in front of repeat R3, is conserved in the inter-repeats. Conserved region V, also lying in the N-domain of the proteins, encompasses the Staf snRNA activation domain and possesses the amino acids found essential for that function. Although no specific biological function has been attributed yet to conserved region VI in the C-domain of Staf, its evolutionary conservation definitely points to an important activity.

Previous work from our laboratory identified the human U2 snRNA, U4B snRNA, U4C snRNA, U6 snRNA, U11 snRNA, Y4 RNA, 7SK RNA, and tRNA$^{\text{Sec}}$ genes as direct targets of the transcriptional activator Staf (14). The present study extended this work by demonstrating that the human proteins ZNF76 and ZNF143 are highly identical and functionally equivalent to the *Xenopus* Staf. From this, it appears that the transcriptional activation of the human U2 snRNA, U4B snRNA, U4C snRNA, U6 snRNA, U11 snRNA, Y4 RNA, 7SK RNA, and tRNA$^{\text{Sec}}$ genes is dependent on ZNF76 and ZNF143. It is very likely that these proteins are also involved in the expression of many other genes, perhaps performing particular functions depending on the context of specific promoters. In this respect, we had previously shown that the Staf and Oct-1 transactivators can synergistically stimulate transcription from snRNA and snRNA-type promoters, from two adjacent specific binding sites (31). This raises the possibility that ZNF76 and ZNF143 can function differentially in their combinatorial action with Oct-1.

In conclusion, our results imply that the presence of Staf responsive elements in a number of distal sequence elements of snRNA and snRNA-type genes does not necessarily mean that Staf is the unique factor capable of recognizing these elements to activate transcription. We have shown here that in human cells, at least another homologous factor, ZNF76, that has highly conserved Staf/ZNF143 regions also binds to Staf responsive elements with identical affinities and acts as a transcriptional activator as well. This finding reveals that Staf responsive elements are recognized by a family of transcription factors, in much the same way as other control elements like the Sp1 and octamer sites (32–34). Therefore, the discovery of ZNF76 and ZNF143 as Staf homologs introduces a novel complexity in the transcriptional regulation exerted through Staf responsive elements. Further functional investigations will be necessary to understand the interplay between ZNF76 and ZNF143.

After completion of this manuscript, the characterization of the mouse Staf has been reported by others (35). It appears that the identified mouse Staf is the ortholog of human ZNF143. Surprisingly, the authors described that, in *Drosophila* cells, a truncated mouse Staf containing the snRNA activation domain is unable to activate the mouse Pol III tRNA$^{\text{Sec}}$ reporter (35). It should be noted, however, that the mouse PSE.
sequence contained in this promoter is highly divergent from the *Drosophila* counterpart (36), therefore leading this promoter to be inactive in *Drosophila* cells. From this, it is likely that the mouse tRNA Sec Pol III promoter used in their study was TATA box-dependent only, therefore functioning as an RNA polymerase II mRNA promoter.

Acknowledgments—We are grateful to J. Ragoussis, J. Trowsdale, and H. Vissing for the generous gifts of the ZNF76 and ZNF143 cDNA clones. We also thank G. de Murcia for the GAPDH cDNA clone, J. Hoffmann and C. Kappler for the *Drosophila* cell culture facilities, A. Hoeft for oligonucleotide synthesis, and C. Loegler for technical assistance. O. Poch and C. Schuster are acknowledged for help in sequence comparisons.

REFERENCES

1. Carey, M. (1991) *Curr. Opin. Cell Biol.* 3, 452–460
2. Johnson, P. F., Sterneck, E., and Williams, S. C. (1993) *J. Nutr. Biochem.* 4, 386–398
3. Murphy, S., Yoon, J.-B., Gerster, T., and Roeder, R. G. (1992) *Mol. Cell. Biol.* 12, 3247–3261
4. Sadowski, C. L., Henry, R. W., Lobo, S. M., and Hernandez, N. (1993) *Genes Dev.* 7, 1535–1548
5. Henry, R. W., Sadowski, C. L., Kobayashi, R., and Hernandez, N. (1995) *Nature* 374, 653–657
6. Hernandez, N. (1992) in *Transcriptional Regulation* (MacKnight, S. L., and Yamamoto, K. R., eds) Vol. 1, pp. 281–313, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
7. Herr, W. (1992) in *Transcriptional Regulation* (MacKnight, S. L., and Yamamoto, K. R., eds) Vol. 1, pp. 1103–1135, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
8. Ares, M., Jr., Chung, J.-S., Giglio, L., and Weiner, A. M. (1987) *Genes Dev.* 1, 808–817
9. Janson, L., Bark, C., and Petterson, U. (1987) *Nucleic Acids Res.* 15, 1122–1132
10. Tebb, G., and Mattaj, I. W. (1989) *Mol. Cell. Biol.* 9, 1031–1043
11. Yasukawa, Y., Usheva, A., Shenk, T., and Burley, S. K. (1996) *Proc. Natl. Acad. Sci. U. S. A.* 93, 13577–13582
12. Omichinski, J. G., Pedone, P. V., Felsenfeld, G., Gronenborn, A. M., and Clore, G. M. (1997) *Nat. Struct. Biol.* 4, 122–132
13. Wuttke, D. S., Foster, M. P., Case, D. A., Gottesfeld, J. M., and Wright, P. E. (1997) *J. Mol. Biol.* 273, 183–206
14. Musilinski, E., Krol, A., and Carbon, P. (1992) *Nucleic Acids Res.* 20, 321–318
15. Huang, S. F., Foster, M. P., Case, D. A., Gottesfeld, J. M., and Wright, P. E. (1997) *J. Mol. Biol.* 273, 183–206
16. Wang, Y., and Stumph, W. (1998) *Mol. Cell. Biol.* 18, 1570–1579