Molecular Cloning and Characterization of the Murine Staf cDNA Encoding a Transcription Activating Factor for the Selenocysteine tRNA Gene in Mouse Mammary Gland*

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We have isolated and characterized a cDNA encoding a transcription activating factor for the mouse selenocysteine tRNA (tRNA^{sec}) gene from mouse mammary gland. The full-length cDNA, designated m-Staf, has a 1878-base pair open reading frame encoding 626 amino acids. The predicted amino acid sequence of m-Staf is highly homologous to that of Staf, another selenocysteine tRNA gene transcription activating factor of Xenopus laevis. Like Staf, m-Staf contains seven tandemly repeated zinc fingers and four repeated motifs. Gel shift assays indicated that the recombinant m-Staf specifically bound to the activator element region in the mouse tRNA^{sec} gene. Transient co-transfection experiments in Drosophila Schneider cells, which lack endogenous Staf-like binding activity, showed that m-Staf increased the mouse tRNA^{sec} gene transcription about 15-fold, whereas it stimulated Pol II-dependent thymidine kinase promoter only 2-fold. Northern blot analysis detected the presence of a 3.4 kilobase pair m-Staf transcript, which was widely but differentially expressed in various murine tissues. The binding activity of m-Staf in mouse mammary gland was undetectable during virgin and postlactating periods but increased markedly in parallel with the increase of tRNA^{sec} transcript during the periods of pregnancy and lactation, when the gland undergoes growth and development. These results indicate that m-Staf is a transcriptional activator of the mouse tRNA^{sec} gene and that its binding activity in the mammary gland undergoes developmental alterations.

Selenium has been established as a nutritional requirement for the essential trace elements and shown to be indispensable for the biosynthesis of selenoproteins (1). These selenoproteins include type I thyroxine 5'-deiodinase in thyroid hormone metabolism (2), those of the glutathione peroxidase family in an antioxidative system (3, 4), and several other newly found ones, such as selenoprotein P, selenoprotein W, and thioredoxin reductase (5–8). Both type I thyroxine 5'-deiodinase and glutathione peroxidase play an important function in metabolic activities of mammary cells during lactation (9–12).

Selenium is incorporated into selenoproteins in the form of selenocysteine, and its incorporation is directed by a specific UGA codon, which normally functions as a stop codon in both prokaryotes and eucaryotes (13). The selenocysteine tRNA (tRNA^{sec}) serves as a donor of selenocysteine to nascent selenoproteins in response to the specific UGA selenocysteine codons (13, 14). This reaction also requires a selenocysteine insertion sequence, a cis-acting mRNA element, and a specialized elongation factor, the SELB protein (15, 16). It has been shown that transfection of plasmids expressing tRNA^{sec} into human 293 cells, an embryonic kidney cell line, increases the level of 5'-deiodinase activity (17), suggesting that the amount of tRNA^{sec} is critical for the regulation of selenoprotein biosynthesis.

Extensive analysis of the tRNA^{sec} gene promoter has been reported for Xenopus laevis (18–21). Like other tRNA genes, this gene is transcribed by RNA polymerase III (Pol III) (22). Usually, transcription of tRNA genes requires two promoter elements, named A box and B box, situated inside the coding region. However, the tRNA^{sec} gene is atypical in that its transcription is not dependent on the A box, which is naturally depleted in this gene, but requires the B box and three other upstream elements, such as an activator element (AE) located in a distal sequence element, a proximal sequence element, and a TATA motif (18–21). These elements are well conserved among murine and bovine species as well as in X. laevis (23). The factors binding to the proximal sequence element, the TATA motif, and the B box have been well characterized, but the ones to the AE have not (14–17). Recently, a cDNA encoding a DNA-binding protein to the AE has been cloned from X. laevis and named Staf (selenocysteine tRNA gene transcription activating factor) (24). The structure of Staf is characterized by the presence of seven tandemly repeated zinc fingers that facilitate its DNA binding activity (24). Staf has been shown to transactivate tRNA^{sec} gene transcription in a X. laevis oocyte system (24).

We have been studying the regulatory mechanisms involved in developmental and tissue-specific gene expression in the mouse mammary gland (25). During the course of our study to clone and characterize cDNA for mammary transcription factors, we have cloned a cDNA encoding a protein containing zinc finger motifs and named it m-Staf. In this report, we describe the molecular cloning and functional characterization of m-Staf and present evidence indicating that it is a mouse counterpart
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of Staf, which activates transcription of the mammalian tRNAe gene. We also present data suggesting that it is involved in regulating mouse tRNAe gene expression during the development of mouse mammary gland.

EXPERIMENTAL PROCEDURES
cDNA Cloning—A mouse mammary gland cDNA library (Stratagene) was screened using a 32P-labeled probe corresponding to the DNA binding domain of mouse YY1 cDNA (26). Among several clones obtained, one clone, named IBA, was sequenced and analyzed further. The 5′ and 3′ regions of the gene were obtained from the mouse spleen message-ready cDNA (Clontech) by the methods of 5′- and 3′-RACE (rapid amplification of cDNA ends) (27). Using a set of primers corresponding to the 5′ or 3′ non-coding regions, a full-length cDNA was amplified from mouse mammary gland poly(A)+ RNA and ligated to a TA cloning vector (Invitrogen). The resultant plasmid was named pCRfull. The sequence was verified by sequencing three independent clones.

Plasmid Constructions—Plasmids pCRfull, pCR5, pCR3, and pCR53 were prepared in the following way. A part of m-Staf cDNA (position 54–140) was amplified using primers 5′-GGGCGGCGAGCT-TTCAGGAGGTCAATCAGTTCT-3′ and 5′-TCTAATCTGAACCCTG-CGCTAGTGAGCTC-3′ and then digested with HindIII and SacI. The resultant fragment was ligated into a EcoRI-SacI digested pCRBstA19 form pCRfull. The 5′-linker element harboring the initial methionine codon was made by hybridization of oligonucleotides 5′-AGGCTCT-CAGGAGGTCAATCAGTCTTCTGAGGAATGGAGCT-3′ and 5′-ATTCTTACGGACTAAATTTCCACCTGAGA-3′. The resultant fragment was ligated into the HindIII-Staf-digested pCRSTaf to form pCR5. For construction of pCR3 and pCR53, pCRfull and pCR53 were digested with SacII and EcoRI to remove a portion of the cDNA (positions 1696–1941) and ligated with the 3′-linker element having the termination codon. The 3′- linker element was made by hybridization of oligonucleotides 5′-GATGTAAGGGCCTGATG-3′ and 5′-ATTCTACGGCCCTACTAGC-3′.

For transfection experiments using Drosophila Schneider cells, ADH-pEXPfull, ADH-pEXP53, and ADH-pEXP3 were prepared as follows. A Drosophila alcohol dehydrogenase promoter (−351/36) (Promega) was ligated into the XhoI-SalI site of PBS11 (Stratagene) to form ADH-0. ADH-pEXPfull was formed from pCRfull by using the SalI-EcoRI-digested ADH-0. Two plasmids expressing truncated variants of m-Staf, ADH-pEXP53 and ADH-pEXP3, were prepared by treating pCR53 and pCR3, respectively. ADH-βgal was prepared as follows. The HindIII-XbaI fragment of PSV-βgal (Promega) was ligated into the HindIII-BamHI-digested PBS11. This construct was treated with SalI-BamHI and inserted into the SalI-BamHI-digested ADH-0. Other expression plasmids pEXPfull, pEXP53, and pEXP3 were formed by using a mammalian expression vector (pcDNA I, Invitrogen) from the plasmids pCRfull, pCR53, and pCR3, respectively.

The wild type or mutated promoter of −235/−7 region of mouse tRNAe gene cloned to chloramphenicol acetyltransferase (CAT) reporter plasmids. Mutations corresponding to MM0, MM1, and MM3 (see Table I) were introduced to the element by PCR-based mutagenesis. The promoter elements were ligated into the PstI-XbaI site of pCAT Basic vector (Promega) to obtain reporter plasmids termed pTRwt, pTRmm0, pTRmm1, and pTRmm3.

The −237–193 region of mouse tRNAe gene, with and without a mutation, was ligated into the upstream region of the herpes simplex virus thymidine kinase promoter-CAT fusion plasmid and named wAE-tkCAT and mAE-tkCAT, respectively.

Preparation of Recombinant Proteins—Recombinant m-Staf proteins were expressed by using the His-patch ThioFusion System (Invitrogen). The expression constructs pThio-full and pThio-535 were made by using the pThioHis vector. Transformed bacteria were grown as described previously (24). The cell suspension was then subjected to three cycles of sonication-freezing and thawing followed by centrifugation.

The supernatants from pThio-full and pThio-535 were named rSTaf-full and rSTaf-535, respectively.

Northern Blot Analysis—Total RNA from various tissues was prepared by using the CsCl precipitation method (29) or by the acid phenol extraction method (40). For detection of m-Staf mRNA, total RNAs were electrophoresed on formaldehyde-agarose gels, blotted onto a Hybond-N+ (Amersham Pharmacia Biotech), and hybridized with a 32P-labeled probe bearing the +1/+252 region of m-Staf cDNA. For detection of RNAe, total RNAs were separated by electrophoresis on a 6% polyacrylamide–8 M urea gel (Novex), transferred to a nylon+ membrane (Novex) by electrophoretic blotting, and hybridized with a 5′-end 32P-labeled oligonucleotide (5′-GAACCCCGAGACCTGGAGATCATC-GCGG-3′) specific for the mouse tRNAe.

Preparation of Nuclear Extracts and Gel Shift Assays—Aged-matched virgin (3 months old) and pregnant (9–11 days of gestation) C3H/HeN female mice were obtained from the Animal Center of the National Institutes of Health. Male and female mice were housed in full compliance with the National Institutes of Health guidelines.

Nuclear extracts were prepared from thoracic mammary glands according to the method described previously (25). Nuclear extracts or recombinant proteins were mixed with 3 μg of poly(dI-dC) (Sigma) in 17 μl of reaction buffer containing 14 μM Hepes, pH 7.9, 12% glycerol, 0.5 mM NaCl, 2.5 mM MgCl2, and 1 mM dithiorthiol, and incubated for 15 min on ice. Gel shift assays were performed using a double-stranded oligonucleotide corresponding to the −233/−198 region of the mouse tRNAe gene. Following the addition of the 32P-labeled probe (3 × 104 cpm), the reaction mixtures were incubated for 30 min at 25 °C and subjected to electrophoresis on a 4% polyacrylamide gel in 0.25× TBE.

Transfections and CAT Assays—Drosophila SL2 cells were grown at 22°C in Schneider’s medium supplemented with 10% heat-inactivated fetal calf serum. For transfection, 3.5 × 106 cells were plated on each of 3.5-cm-diameter dishes. Five μg of CAT construct, 5 μg of expression plasmid, and 2 μg of ADH-βgal were co-transfected by the calcium phosphate method (30). After 48 h, cells were lysed by freezing and thawing for CAT assays (30). Transfection efficiency was normalized to β-galactosidase activity (31).

Hep2 and Chinese hamster ovary cells were grown in Dulbecco’s modified Eagle’s medium and in Ham’s F-12 medium supplemented with 10% fetal calf serum, respectively. Ten μg of CAT construct and 7 μg of expression plasmids were transfected with 3 μg of pSVβ-gal by the calcium phosphate method. After 40 h, cells were harvested for subsequent assays.

RESULTS

Molecular Cloning of m-Staf—The DNA binding domain of mouse YY1-cDNA encoding transcription factor bearing four C2-H2 type zinc fingers (26) was used for screening a mouse mammary gland cDNA library at low stringency. One positive clone, IBA, was further analyzed. The complete nucleotide sequence of this clone (Fig. 1) indicated that it contained a 1878-nucleotide open reading frame (positions 64–1941), which potentially encoded a 67.5-kDa polypeptide consisting of 626 amino acid residues. The predicted amino acid sequence revealed that this protein contained four repeated motifs between residues 59 and 135 and a zinc finger domain of the C2-H2 type tandemly repeated seven times between residues 220 and 428.

A computer search of the GenBank data base indicated that the sequence of the protein is highly homologous to both human protein ZNF 143, of unknown function (32), and I. laevis Staf protein (24). Among its 626 amino acid residues, 608 and 530 residues are identical to those of ZNF 143 (97.1%) and Staf (84.7%), respectively (Fig. 2). It is noted that the zinc finger domain and the element of repeated motifs are especially well conserved among these three proteins (197 residues out of 209 and 59 residues out of 60, respectively). Therefore, we named this protein m-Staf. Despite the overall homology and high conservation of the putative functional domains, further comparison of the amino acid sequences of these three proteins revealed notable differences. First, Staf contains an additional 47 amino acid residues at the amino terminus; these residues are absent in the other two proteins. Second, both m-Staf and ZNF143 sequences have an insertion of 54 amino acid residues in the region downstream of the zinc finger domain.

Tissue Distribution of m-Staf mRNA—Total RNA prepared from various tissues of female mice was subjected to Northern blot analysis using m-Staf cDNA as a probe (Fig. 3). A single band of transcript (3.4 kb) was detected in all tissues examined. The level of the transcript relative to that of GAPDH mRNA was highest in the lung and lowest in the liver. These results indicate that the m-Staf gene is widely but differentially expressed in mouse tissues.

Recombinant m-Staf Binds to the AE Region—We produced...
two recombinant m-Staf proteins, rStaf-full, corresponding to amino acid residues 1–626, and rStaf-D53, corresponding to residues 118–542 (Fig. 4A). Their binding activities to the AE region of the tRNA sec gene were examined by gel shift assays using a double-stranded DNA probe corresponding to the 223/198 region of the mouse tRNA sec gene promoter, which includes the AE region (222/208). Table I shows the sequences of mouse (MW) and X. laevis wild type competitors and four mouse mutant competitors (MM0, MM1, MM2, and MM3). As shown in Fig. 4B, the rStaf-full protein formed a single retarded band with the labeled DNA probe (lane 1). The intensity of band was decreased when the excess amounts of unlabeled MW were added (lanes 2 and 3). Mutant competitors MM0, MM1, and MM2 showed almost no competition (lanes 4–9), whereas MM3 and XW showed some competition (lanes 10–13). These data indicate that m-Staf binds to the AE region.

**Fig. 1. cDNA and amino acid sequences of m-Staf.** The nucleotide sequence (base positions given on the left side) is shown along with the deduced amino acid sequence using the single-letter code (amino acid positions given on the right side). Position 1 is assigned to the first nucleotide of the cloned cDNA. The first methionine is assigned as the first amino acid. An upstream in-frame TAG stop codon (positions 22–24) is shown by boldface, underlined letters. Four repeated motifs with 15 amino acid residues (between residues 39 and 135) and a seven tandemly repeated zinc finger domain of C2-H2 type (residues 220–428) are underlined. The cysteine and histidine residues that are indispensable for zinc fingers are indicated by boldface letters. An asterisk (*) designates an in-frame stop codon.
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m-Staf 1 .......................... .......................... .......................... .......................... .......................... ....MTE FPQGOMEAQH VTLCLTEAVT VADGDNLLEM EGVSLSQAVTL
ZNF143 1 .......................... .......................... .......................... .......................... .......................... ....MTE FPQGOMEAQH VTLCLTEAVT VADGDNLLEM EGVSLSQAVTL
Staf 1 MLRSQMQQ MQAPQALIQE AGQPSQMLAQ INRD5QAMRE FPQGOMEAQH VTLCLTEAVT VADGDNLLEM EGVSLSQAVTL .......................... .......................... .......................... .......................... ..........................

m-Staf 44 ADGSTAYQH NSKDRLIDQ QVQLEDSGA AYQCVHVPFP TGSDSLRLD GQAQVLDEGD TAFIIHSDSK YSDSGLQAV QLEDOTTAYI
ZNF143 44 ADGSTAYQH NSKDRLIDQ QVQLEDSGA AYQCVHVPFP TGSDSLRLD GQAQVLDEGD TAFIIHSDSK YSDSGLQAV QLEDOTTAYI
Staf 89 ADGSTAYQH NSKDRLIDQ QVQLEDSGA AYQCVHVPFP TGSDSLRLD GQAQVLDEGD TAFIIHSDSK YSDSGLQAV QLEDOTTAYI

m-Staf 134 HHAVQQPSD TILAIQADOT VAGLTFDAGT IDPDTSAILE QVAKVSIDG SDQVFTGMI GNQREKRMQ IVLQGHATV YFGQQQGEK
ZNF143 134 HHAVQQPSD TILAIQADOT VAGLTFDAGT IDPDTSAILE QVAKVSIDG SDQVFTGMI GNQREKRMQ IVLQGHATV YFGQQQGEK
Staf 175 HHAVQQPSD TILAIQADOT VAGLTFDAGT IDPDTSAILE QVAKVSIDG SDQVFTGMI GNQREKRMQ IVLQGHATV YFGQQQGEK

m-Staf 224 AFRCDYQCGG KLYTATIONLK VHERSHQDR FQYQDMSCGG KAFATGYQLK SHFKYERTGK FyrCSENCRTK SFKTSQDLKG HVRHTHGRAPH
ZNF143 224 AFRCDYQCGG KLYTATIONLK VHERSHQDR FQYQDMSCGG KAFATGYQLK SHFKYERTGK FyrCSENCRTK SFKTSQDLKG HVRHTHGRAPH
Staf 264 AFRCDYQCGG KLYTATIONLK VHERSHQDR FQYQDMSCGG KAFATGYQLK SHFKYERTGK FyrCSENCRTK SFKTSQDLKG HVRHTHGRAPH

m-Staf 314 PFRCPEQOGQ KRPFTSHRRK VHRHTHGRAPH FYCTEGQCGQ RAFASATYNK NHVRHTHGRAPH FYVCTVPGCD KRFHTESLYL KVHTHHTSK
ZNF143 314 PFRCPEQOGQ KRPFTSHRRK VHRHTHGRAPH FYCTEGQCGQ RAFASATYNK NHVRHTHGRAPH FYVCTVPGCD KRFHTESLYL KVHTHHTSK
Staf 354 PFRCPEQOGQ KRPFTSHRRK VHRHTHGRAPH FYCTEGQCGQ RAFASATYNK NHVRHTHGRAPH FYVCTVPGCD KRFHTESLYL KVHTHHTSK

m-Staf 404 PYCNCHCQKG YKQISTLAMH KRTAIRDTPF EEEOQAPEPE PPGQGQDVL KGQOVTQTY EGVDIVSTQ VTVTQCVQGLS QVTVLSTQCG
ZNF143 404 PYCNCHCQKG YKQISTLAMH KRTAIRDTPF EEEOQAPEPE PPGQGQDVL KGQOVTQTY EGVDIVSTQ VTVTQCVQGLS QVTVLSTQCG
Staf 444 PYCNCHCQKG YKQISTLAMH KRTAIRDTPF EEEOQAPEPE PPGQGQDVL KGQOVTQTY EGVDIVSTQ VTVTQCVQGLS QVTVLSTQCG

m-Staf 494 TQRJNIQSGAD MQAIGNNTM VDQGQPTTV PTHDAVIISSA GYHSAMVTA EELLGQCVAY QAQQLAAHPT ASSEGMHQQH SHILVYETR
ZNF143 494 TQRJNIQSGAD MQAIGNNTM VDQGQPTTV PTHDAVIISSA GYHSAMVTA EELLGQCVAY QAQQLAAHPT ASSEGMHQQH SHILVYETR
Staf 525 TSH .......................... .......................... .......................... .......................... .......................... .......................... .......................... .......................... .......................... ..........................

m-Staf 584 PUTLVATSG TQIATQVLSQG PSLEAEPZA SR1QGQ6T9 LDD 626
ZNF143 584 PUTLVATSG TQIATQVLSQG PSLEAEPZA SR1QGQ6T9 LDD 626
Staf 558 PUTLVATSG TQIATQVLSQG PSLEAEPZA SR1QGQ6T9 MED 600

Fig. 2. Comparison of the amino acid sequences of m-Staf, ZNF143, and Staf. Deduced amino acid sequences of m-Staf, ZNF 143 (32), and Staf (24) are aligned using the PILE-UP program made by Genetics Computer Group, Inc. Gaps have been introduced to maximize the match. Asterisks (*) represent residues conserved among the three sequences.

Fig. 3. Northern blot analyses of m-Staf mRNA in mouse tissues. Twenty micrograms of total RNA from the indicated mouse tissues were electrophoresed and hybridized with the 3P-labeled probe as described under “Experimental Procedures.” The filter was rehybridized with the GAPDH cDNA probe. The relative values of the transcript (m-Staf/GAPDH) were obtained by densitometric tracing of autoradiographic films.

in a sequence-specific manner and that the two sequences, CCA (-222/-220) and TGC (-216/-214), are important binding elements (Table I). The recombiant protein rStaf-D53, in which the three of four repeated motifs and the 84 amino acid residues in the carboxyl terminus were deleted, also could bind to the probe (lane 14). The sequence specificity of rStaf-D53 binding was the same as that of rStaf-full as judged by competition experiments (data not shown). These results indicate that residues 118–542, which contain the zinc fingers, are sufficient for the DNA binding activity of m-Staf.

Recombinant m-Staf Functions as a Transcriptional Activator on Mouse tRNAsec Promoter—We next examined whether m-Staf functions as a transcriptional activator of the mouse Pol III-dependent tRNAsec gene. For transient co-transfection experiments, Drosophila SL2 cells, which do not have endogenous Staf-like binding activity (24), were used. The expression vectors ADH-pEXPfull, ADH-pEXPD53, and ADH-pEXPD3 contained either a wild type m-Staf gene or its truncated forms under the control of the Drosophila alcohol dehydrogenase promoter. ADH-0, which lacks the m-Staf insert, served as a control. These plasmids were co-transfected with a CAT reporter plasmid linked to either a wild type or a mutated element bearing the -235/+7 region of the mouse tRNAsec gene promoter (Table I and Fig. 5A).

Co-transfection of ADH-pEXPfull (mS) resulted in approximately a 15-fold increase of CAT activity of pTRwt compared with that of ADH-0 (none) (Fig. 5A, columns 2 and 3, respectively). In contrast, when the mutant reporter plasmid pTRmm0 or pTRmm1 was used, co-transfection of mS produced only marginal effects (Fig. 5A, columns 5–8). These results were consistent with the results of gel shift assays (Fig. 4B, lanes 10 and 11). These results show that m-Staf...
functions as a transcriptional activator of tRNAsec gene promoter by binding to the AE region in a sequence-specific manner in vivo.

We also investigated the effect of another effector plasmid, ADH-pEXPΔ53 (mS53) expressing amino acid residues 118–542 (Fig. 4A) on transcriptional activity of pTRwt. Co-transfection of mS53 did not increase the CAT expression of pTRwt (Fig. 5A, columns 1 and 3), suggesting that some region(s) other than the zinc finger domain is important for the transcriptional activation. On the other hand, co-transfection of ADH-pEXPΔ3 (mS3) expressing amino acid residues 1–542 produced approximately a 13-fold increase in CAT activity (Fig. 5A, columns 1 and 4). These data suggest that amino acid residues 1–117, which contain three of the four repeated motifs, are important for the transcriptional activation, whereas amino acid residues 543–626 are dispensable.

To assess the possible action of m-Staf on transcription mediated by Pol II-dependent promoter, we examined its effect on reporter plasmids, AE-linked thymidine kinase promoter-CAT (wAE-tkCAT) or its mutated form, mAE-tkCAT. As shown in Fig. 5B, cotransfection of mS increased the activity of wAE-tkCAT only 2-fold (columns 1 and 2), whereas the activities of mAE-tkCAT showed no increase (columns 3 and 4). These data, together with those presented in Fig. 5A, suggest that m-Staf preferentially stimulates Pol III-dependent tRNAsec promoter.

Another expression vector, pEXPfull, was co-transfected with pTRwt into two mammalian cell lines, HepG2 and Chinese hamster ovary. The CAT activity increased about 8-fold even in the absence of pEXPfull, whereas its addition did not enhance the CAT activity. This was probably due to the high levels of endogenous Staf-like activity in HepG2 and Chinese hamster ovary cells (data not shown).

m-Staf Binding Activity in Mouse Mammary Gland at Various Reproductive Stages—Cloning of the m-Staf cDNA and detection of its transcript in mouse mammary gland prompted us to examine the activity of m-Staf in the gland. Gel shift assays using nuclear extracts from mammary glands of pregnant mice revealed the presence of two retarded bands (Fig. 6A, lane 1). Competition experiments indicated that the DNA binding activity of the upper band had the same sequence specificity as that of the recombinant m-Staf proteins (Fig. 6B, lane 1, Fig. 7A, lanes 1, 3, and 4).

**TABLE I**

| Gel shift competitor | CAT construct | Sequence |
|----------------------|---------------|----------|
| MW                   | pTRwt         | 223GGCGCTGCTTC CCAGAATGCAAGGCG CTATGCAAAT |
| MM0                  | pTRmm0        | --------------- |
| MM1                  | pTRmm1        | --------------- |
| MM2                  | NM            | --------------- |
| MM3                  | pTRmm3        | --------------- |
| XW                   | NM            | 220TTATGGAAGTA CCGCATGCCTCGCG CGCGTGTATG |

*a* Each tRNAsec promoter-CAT construct bears the same sequence as indicated.

*b* A hyphen represents the same residue as that of mouse wild type. The center portion of each sequence indicates the AE region. The numbers refer to the position from the transcription initiation site.

*XW, X. laevis wild type competitor.*

*NM, not made.*
as that of the recombinant m-Staf (Fig. 6A, lanes 2–13, and Fig. 4B) whereas the lower band showed no sequence-specific DNA binding activity and thus was considered to be nonspecific. In addition, the migration rate of the upper band was found to be similar to that formed by the recombinant m-Staf (data not shown). These data indicate the presence of endogenous m-Staf in the mammary glands.

The binding activities of m-Staf in mouse mammary gland at various reproductive stages are shown in Fig. 6B. No specific retarded band was detectable with nuclear extracts from virgin and postlactating mice (Fig. 6B, lanes 1 and 7), whereas a single specific retarded band (Band A) corresponding to that of m-Staf was detected with extracts from pregnant animals at three different gestational stages (Fig. 6, A and B, lanes 2–4). Nuclear extracts from lactating mice at two different stages formed the same m-Staf-specific band (Band A) with greater intensity and also produced an additional band having a slower migration rate (Band B) (lanes 5 and 6). The binding protein associated with the slowly migrating band also was found to have the same sequence specificity as m-Staf by competition experiments (data not shown). Although its relationship to m-Staf remains unknown at the present time, it is possible that this protein is a subtype of m-Staf. In addition, mixing experiments using mammary nuclear extracts from mice at different reproductive stages revealed no apparent diffusible inhibitors or activators of the m-Staf binding activity (data not shown). These results indicate that the m-Staf binding activity in mammary glands increases during the periods of pregnancy and lactation.

The Level of tRNAsec in Mouse Mammary Gland at Various Reproductive Stages—Because m-Staf was shown to be a positive transcriptional regulator of the mouse tRNAsec gene, it was of interest to compare the levels of tRNAsec transcript and m-Staf binding activities in mouse mammary gland at various reproductive stages. As shown in Fig. 7, a mouse tRNAsec transcript having 87 bp was detected as a single band. Its level in the mammary gland increased substantially during pregnancy and lactation (Fig. 7, lanes 2 and 3), whereas it remained at relatively low levels during virgin (lane 1) and postlactating (lane 4) periods.
DISCUSSION

In this study, we cloned a cDNA encoding a zinc finger protein from mouse mammary gland. The predicted amino acid sequence of this clone indicates that it contains the seven tandemly repeated C2-H2-type zinc fingers and the four repeated motifs, which are highly homologous with Staf from *X. laevis*. Accordingly, we have designated this cDNA m-Staf.

However, we found several structural differences between m-Staf and Staf. First, Staf has additional amino acid residues at the amino terminus and contains the third methionine residue at the position corresponding to the translation start site of m-Staf. Second, m-Staf contains an additional 54 amino acid residues downstream of the zinc finger domain. The nucleotide sequence of the inserted portion is 5'-GTCAACA- - - -CAG-3', which is identical to the consensus sequence of the intron donor and acceptor site (33), suggesting that this portion might originally have been an intron, which has evolved into an exon. The sequence is no longer spliced out because RT-PCR analysis demonstrated that mouse mammary gland does not contain any Staf gene products that lack the 54 amino acid residues (data not shown). In addition, these amino acid residues are also present in the same downstream region of human zinc finger protein ZNF143, suggesting that they are well conserved in mammalian species.

We obtained several lines of evidence indicating that m-Staf is a transcriptional activator of the mouse tRNA^{sec} gene. Like Staf, the recombinant m-Staf, rStaf-full, was shown to bind to the 15-base pair AE region of the mouse tRNA^{sec} gene in a sequence-specific manner. Competition assays using various mutated sequences indicate that the two sequences CCA (223/222) and TGC (216/214) in the AE region are important for the binding. The truncated form, rStaf-D53, which has only amino acid residues 118–542, was also found to bind to the AE region, indicating that this portion of m-Staf is sufficient to facilitate the DNA binding activity. Because it contains an entire zinc finger domain (Fig. 4A), our results are consistent with the previous findings that the zinc finger domain of Staf is important for its DNA binding activity (24). However, we do not know whether all of the seven zinc fingers are necessary for DNA binding activity. It was reported that three of the six C2-H2 zinc fingers in the Gfi-1 transcription factor were sufficient for DNA binding (34).

Transfection experiments showed that expression vectors for both m-Staf (ADH-pEXPfull) and one truncated form of m-Staf (ADH-pEXPΔ53) could induce the mouse tRNA^{sec} promoter CAT activity, whereas the other truncated form, ADH-pEXPΔ53, was ineffective. These results suggested that amino acid residues 1–117 of m-Staf contain some region necessary for tran-
not effective in stimulating transcription of the mouse tRNA sec gene. RNA used was as follows: lane 1, virgin mice; lane 2, pregnant mice (preg.); lane 3, lactating mice (lact.); lane 4, postlactating mice (postlac.). The intensity of the 5S band in an ethidium bromide stained gel varied less than 5% among all the samples (data not shown).

Our studies of m-Staf in mouse mammary gland indicated that m-Staf binding activities changes as a function of reproductive stage. The binding activity of m-Staf in the mammary gland was undetectable at virgin and postlactating stages, when the gland is developmentally dormant. However, the binding activity increased markedly during the periods of pregnancy and lactation, when the mammary gland undergoes extensive growth and differentiation (36). Thus, the change in the m-Staf binding activity in the gland appears to be correlated to the developmental status of the mammary tissue. Because the development of the mammary gland is stimulated by various steroid and polypeptide hormones (36), the binding activity of m-Staf in the gland also may be hormonally regulated. In addition, we found that the level of tRNA sec mRNA increased during pregnancy and lactation largely in parallel with that of m-Staf binding activity. These findings are consistent with the view that m-Staf is involved in the transcriptional activation. This portion includes three of the four repeated motifs, which are well conserved between Staf and m-Staf and thus may have functional importance. Based on these findings, m-Staf is considered to have two major functional regions, one for transcriptional activation and the other for DNA binding, which are characterized by the presence of the repeated motifs and the zinc finger domain, respectively. These features of m-Staf are common to many transcription factors (35).

We found that m-Staf stimulated transcription of tRNA sec Pol III-dependent promoter but had only minimal effect on AE-linked thymidine kinase Pol II-dependent promoter in transfection experiments using Drosophila cells. These cells were used because they do not contain any detectable level of Staφ-like binding activities. In contrast to our present findings, it was reported that transfection of Staf in Drosophila cells was not effective in stimulating transcription of the X. laevis tRNA sec Pol III, although Staf markedly activated the AE-containing Pol II promoter (24). The transcriptional activity of Staf could only be demonstrated by using a X. laevis oocyte system into which Staf mRNA was microinjected prior to introduction of the tRNA sec-CAT reporter gene. The observed difference in the transcriptional activity of the two Stafs transfected in Drosophila cells could be explained by the aforementioned structural difference of the two proteins or by the source of tRNA sec Pol III promoter used in reporter plasmids, i.e., mouse versus X. laevis. These differences could influence the functional properties of Stafs in forming the active transcriptional complex.

Our studies of m-Staf in mouse mammary gland indicated that m-Staf binding activities changes as a function of reproductive stage. The binding activity of m-Staf in the mammary gland was undetectable at virgin and postlactating stages, when the gland is developmentally dormant. However, the binding activity increased markedly during the periods of pregnancy and lactation, when the mammary gland undergoes extensive growth and differentiation (36). Thus, the change in the m-Staf binding activity in the gland appears to be correlated to the developmental status of the mammary tissue. Because the development of the mammary gland is stimulated by various steroid and polypeptide hormones (36), the binding activity of m-Staf in the gland also may be hormonally regulated. In addition, we found that the level of tRNA sec in the mammary gland increased during pregnancy and lactation largely in parallel with that of m-Staf binding activity. These findings are consistent with the view that m-Staf is involved in the regulation of tRNA sec gene transcription. It is noted, however, that both virgin and postlactating mammary glands had no detectable m-Staf binding activity but showed low levels of tRNA sec transcript. It is possible that the basal level of tRNA sec gene transcription in developmentally dormant glands is maintained by other transcription factors acting on the basal promoter elements (18, 21).

The parallel increase in tRNA sec and m-Staf binding activity in the mammary gland during pregnancy and lactation is noteworthy because the production of at least two selenoproteins, glutathione peroxidase (9) and type I thyroxine 5’-deiodinase (10–12), is found to increase in the gland during these periods. Type I thyroxine 5’-deiodinase is the best characterized selenoprotein in the mammary gland; it catalyzes monodeiodination of the prohormone thyroxine (T4) to form a more active hormone, 3,5,3’-tri-iodothyronine (T3). Its activity in the mammary gland increases during lactation (10) and correlates well with lactational intensity, as judged by litter size (11). These observations are consistent with the view that the deiodinase plays a key role in maintaining lactogenesis by catalyzing the production of T3 (12). Although the mechanisms of induction of type I thyroxine 5’-deiodinase during lactation have not been elucidated, our present findings raise the possibility that m-Staf plays a role in the biosynthesis of type I thyroxine 5’-deiodinase, as well as other selenoproteins, by regulating expression of the tRNA sec gene in lactating mammary glands.

Recently, it was reported that Staf could activate not only the X. laevis tRNA sec gene but many small nuclear RNA and small nuclear RNA-type genes transcribed by RNA polymerase II or Pol III in a X. laevis oocyte system (37). Moreover, some of these genes, such as the U2 gene, were found to contain binding sites for both Staf and Oct factor in their distal sequence element regions (37, 38), in which the two transcription factors interact to activate transcription. Because the distal sequence element region of the mouse tRNA sec gene also contains a consensus octamer binding site (23), it is of interest to examine whether such an interaction is important for the regulation of murine tRNA sec gene promoter transcription. In addition, the question of whether m-Staf can also activate transcription of small nuclear RNA genes in mammalian tissues, including the mammary gland, remains to be investigated.

Previously, it was reported that human ZNF76 (39) was a human homologue of Staf (24, 37), although its biological function was not identified. The sequence of ZNF76 showed 85.1% identity (172 of 202 residues) with Staf in the zinc finger region, but its entire sequence had only 58.0% homology with Staf (298 of 514 residues). We found that m-Staf and human ZNF143, another human zinc finger protein (32), share 97.1% homology in their amino acid sequences. In view of our findings that m-Staf functions as a transcription activator of the mouse tRNA sec gene, ZNF143 may be the human homologue of m-Staf and have similar functions for the regulation of tRNA sec gene transcription in mammalian systems. Moreover, because the ZNF143 gene is mapped to chromosomal regions implicated in developmental and malignant disorders (32), it is of interest to examine the possible involvement of m-Staf in these disease states in the mouse model.

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