Transcriptional Regulation of the Mouse Cytosolic Chaperonin Subunit Gene Cctα/τ-Complex Polypeptide 1 by Selenocysteine tRNA Gene Transcription Activating Factor Family Zinc Finger Proteins

The chaperonin containing t-complex polypeptide 1 (CCT) is a molecular chaperone assisting in the folding of proteins in eukaryotic cytosol, and the Cctα (encoding the α subunit of CCT/t-complex polypeptide 1 gene) encodes the α subunit of CCT. We show here that transcription of the mouse Cctα gene is regulated by selenocysteine tRNA gene transcription activating factor (Staf) family zinc-finger transcription factors ZNF143 and ZNF76. Reporter gene assay using HeLa cells indicated that the Cctα gene promoter contains two 18-base-pair-long cis-acting elements with similar sequences at −70 and −20 base pairs (designated CCT α subunit gene transcription activating element 1 (CAE1) and CAE2, respectively). By yeast one-hybrid screening of CAE1-binding factors, we isolated human ZNF143, which is known to activate transcription of selenocysteine tRNA and small nuclear RNA genes. DNA binding domains of ZNF143 and ZNF76 produced in E. coli recognized CAE1 and CAE2 elements in electrophoretic mobility shift assay. HeLa cell nuclear extract contained a protein that specifically binds to CAE1 and CAE2 and recognized by anti-ZNF143 antibody. Transcription from a minimal Cctα promoter containing CAE2 element in HeLa cells was enhanced by overexpression of full-length ZNF143 and ZNF76 but inhibited by that of their DNA binding domains alone. These results demonstrate that the Staf family proteins control transcription of at least one of the chaperone-encoding genes besides that of tRNA and small nuclear RNA genes. These RNA and chaperone genes are suggested to be coregulated to facilitate synthesis of mature proteins during active cell growth.

The chaperonin containing t-complex polypeptide 1 (CCT),

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contains two cis-acting elements recognized by Staf family transcription factors ZNF143 and ZNF76 and that these factors can up-regulate Ccta promoter activity in reporter gene assay using HeLa cells under nonstress conditions. We discuss the role of ZNF143 and ZNF76 for the regulation of cytosolic chaperonin expression in normally growing cells.

**EXPERIMENTAL PROCEDURES**

**Reporter and Effector Constructs**—The reporter construct containing the 5′ flanking region and first intron of mouse Ccta gene in the firefly luciferase expression vector pGL3-basic (pCAL) was described previously (14). The pCAL construct was deleted unidirectionally from 5′ upstream of the Ccta gene insert by a modified method of Henikoff (26) using mung bean nuclease instead of S1 nuclease (Fig. 1B) by cloning of PCR-amplified DNA fragments. Nucleotide substitutions, deletions, and insertions were also introduced into the reporter constructs by PCR.

Effector plasmids were constructed as follows. Human ZNF76 DNA was amplified from a human testis cDNA library (CLONTECH, Palo Alto, CA) by PCR using the sequence described by Ragoussis et al. (21) as primers and cloned into the pCAGGS mammalian expression vector (27) (named p76E). Human ZNF143 cDNA was cloned as described under “One-hybrid Screening of CAE1-binding Factors” below, and subcloned into pCAGGS (p143E). DNA fragments encoding the zinc-finger DNA binding domains of ZNF76 (162–373) or ZNF143 (222–433) (see Ref. 24) were also cloned into pCAGGS (p76ZFE and p143ZFE, respectively). Nucleotide sequences of all the reporter and effector constructs were confirmed using an automated sequencer, and supercoiled plasmid DNA prepared by ultracentrifugation in CsCl solution was used for transfection.

**Reporter Gene Assay**—HeLa cells (3 × 10⁶) were inoculated in 3.5-cm dishes, cultured in 10% fetal calf serum/Dulbecco’s modified Eagle’s medium for 24 h, and cotransfected with 1.1 μg of the reporter plasmid expressing firefly luciferase and 0.1 μg of the pRL-SV40 internal control vector expressing sea pansy luciferase (Promega, Madison, WI) using 10 μl of LipofectAMINE reagent (Life Technologies, Inc.). When effector constructs were employed, cells were cotransfected with 0.1 μg of the reporter, 1.0 μg of the effector, and 0.1 μg of the internal control. After 5 h of exposure to the DNA/LipofectAMINE complex in serum-free Dulbecco’s modified Eagle’s medium, HeLa cells were further cultured for 19 h in 10% fetal calf serum/Dulbecco’s modified Eagle’s medium.

Luciferase activities of the transfected cells were determined using the dual luciferase assay system (Promega) according to the manufacturer’s instructions, and the activity of firefly luciferase was normalized against that of sea pansy enzyme.

**One-hybrid Screening of CAE1-binding Factors**—One-hybrid screening was carried out essentially as described previously (28, 29). A fundamental one-hybrid screening vector (pEHEL2; see Fig. 4A) was constructed from pRCE4 (28) by removing the UPRE elements and replacing the backbone from pBR322 to pSP73 (Promega). This gives pEHEL2 unique cloning sites immediately upstream of the two IRE1 promoters driving HIS3 and lacZ structural genes. Six repeats of the CAE1 sequence derived from Ccta promoter (Fig. 1C) were cloned into the BglII site of pEHEL2 (upstream of the IRE1 promoter driving HIS3). This plasmid was linearized by NcoI digestion and integrated into a his3Δ yeast strain (KMY1015; also carries ern1Δ) to reduce background activities (28). The resulting yeast strain was transformed with a multicopy plasmid DNA library containing human B-cell-like lymphocyte cDNAs fused with a Gal4p transcription activation domain sequence (30) and plated on medium lacking histidine but supplemented with 150 μM 3-amino-triazole. After culture for 2 days at 30 °C, colonies were picked and grown in liquid medium, and then plasmid DNA was extracted. Following reamplification in E. coli, the nucleotide sequence of each clone was determined.

**Purification of Glutathione S-Transferase (GST) Fusion Proteins**—
The cDNA fragments encoding DNA binding domains of ZNF76 (162–373) or ZNF143 (222–433) were subcloned into the pGEX-6P-1 GST fusion protein expression vector (Amersham Pharmacia Biotech) (p76ZF-GST and p143ZF-GST, respectively). E. coli carrying each plasmid was cultured at 20 °C, and GST fusion protein was induced by addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside for 6 h. Bacteria were sonicated in phosphate-buffered saline containing 0.1 mM ZnCl₂ and 0.25 mM (p-aminophenyl)methanesulfonyl fluoride hydrochloride, and the GST fusion proteins were purified using glutathione-Sepharose 4B (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. The purified proteins were stocked in phosphate-buffered saline supplemented with 0.1 mM ZnCl₂ and 10% glycerol at −80 °C.

Antibody against ZNF143—Antibody against human ZNF143 was prepared by immunization of rabbits with synthetic multiple antigen peptide containing TGMIGENEQEKMMQIV (Z143P-2; positions 190–205 of ZNF143). IgG fraction was prepared from rabbit sera by protein G-Sepharose (Amersham Pharmacia Biotech) column chromatography. Electroelphoretic Mobility Shift Assay (EMSA)—Nuclear extract was prepared according to Sadowski and Gilman (31). Double-stranded synthetic oligonucleotide probes (see Fig. 4C) were radiolabeled using 32P-dCTP and Klenow fragment. The labeled probe (0.1 ng) was mixed with nuclear extract (10 μg) or purified GST fusion proteins (10 ng) in 20 μl of binding buffer (10 mM Tris-HCl, pH 7.5, 1 mM MgCl₂, 25 mM NaCl, 5 mM dithiothreitol, 12.5% glycerol, 50 ng/μl poly(dI-dC)) and incubated for 30 min at room temperature. Complexes of probe DNA and binding proteins were separated by electrophoresis on 4% polyacrylamide gels (29:1) in 0.25× TBE buffer at 150 V and 4 °C for 2.25 h and visualized by autoradiography.

RESULTS

Cis-acting Elements Up-regulating the Ccta Gene Promoter Activity—Previously, we reported a reporter gene construct (pCAL) expressing firefly luciferase under the control of mouse Ccta gene promoter/enhancer (14). This plasmid construct contains a DNA fragment extending from 1695 bp upstream of the transcription start point to the second exon (Fig. 1A, top). To search for cis-acting elements in this region, unidirectional deletions from the 5’ end were introduced into the construct, and luciferase activities of HeLa cells transiently transfected with these constructs were analyzed (Fig. 1A). Although no significant elements affecting Ccta transcription were observed from –1695 to –566, activities down-regulating Ccta transcription were found in the region from –556 to –147. Trans-acting factors inhibiting the Ccta promoter activity in HeLa cells may bind in this region. In contrast, the region extending from –147 to –13 showed activities up-regulating Ccta transcription. The deletion from –86 to –44 reduced the transcriptional activity by 10-fold, and the deletion from –44 to –13 caused a 30-fold reduction in the activity. The first intron showed activity up-regulating Ccta transcription by 2-fold.

To analyze the transcriptional activity of the region between –147 and –13 in more detail, a reporter gene plasmid without the first intron (pCAL-pro, Fig. 1B, top) was constructed from pCAL, and unidirectionally deleted with approximately 10–20-bp intervals. The transcriptional activity was greatly reduced when the construct was deleted from –86 to –75 bp and was gradually decreased by deletions from –65 to –31. The deletion from –31 to –19 bp abolished the transcriptional activity almost completely. These results indicate that there is a strong cis-acting element immediately downstream of –86 bp and weaker elements between –55 to –31. The region immediately downstream of –31 appeared to be essential for the minimum Ccta promoter activity.

Comparison of the nucleotide sequences in the promoter region indicate that there are two very similar 18-bp sequences immediately downstream of –86 and –31 (named CAE1 and CAE2, respectively; Fig. 1C), where strong transcriptional activities were detected by the reporter gene assay. Between the CAE1 and CAE2 elements, 13 residues are identical, and 4 residues are similar (A/G or T/C substitutions). These observations suggest that the same trans-acting factor(s) may recognize these elements. Results very similar to Fig. 1B were obtained using a distinct cell line, mouse fibroblast BALB/c3T3 (data not shown), implying that these elements are recognized by transcription factors common to many cell types. Although we searched for possible trans-acting factors recognizing the CAE1 and CAE2 sequences by computer programs, no such factors were found at this stage.

Characterization of the CAE1 and CAE2 Elements Enhancing Ccta Transcription—To examine the sequence specificity of

FIG. 2. Reporter gene assay of the CAE1 region. Double point mutations (A), single point mutations (B), and deletions/insertions (C) were introduced into the reporter construct containing up to −86 bp of the Ccta gene promoter (Fig. 1, B and C) as indicated. In A and B, the same residues as in the wild-type (WT) are indicated by dots, and positions important for Ccta transcription are indicated by darker shading (more than 30% down-regulation by mutation) and lighter shading (10–30% down-regulation). In C, the absence of a residue is indicated by a hyphen. HeLa cells were transfected with each of the constructs together with internal control vector. D, effects of copy number and orientation of CAE1 sequence in reporter gene assay. The indicated number of CAE1 copies was introduced into the reporter construct containing up to –68 bp of the Ccta gene promoter. P, same orientation as native promoter; R, reverse orientation. Transcriptional activities of wild-type and CAE1-deleted constructs are indicated by broken and dotted vertical lines, respectively.
the CAE1 element, double point mutations were introduced into the segment extending from –86 to –61 bp of the Ccta promoter (containing up to –86), and transcriptional activities of these mutants in HeLa cells were analyzed by reporter gene assay (Fig. 2A). The 16 residues from –80 to –63 bp, corresponding to DM4 to DM12 mutations, made significant contributions to Ccta transcription. This region overlaps with the 18-bp CAE1 element deduced from sequence similarity to CAE2, except for the TT at –81 and –82. The mutations in TCCCCG (–80 to –75 bp) and CTTTCCGG (–70 to –63) showed strong effects on Ccta transcription, whereas those in CAGA (–74 to –71) showed weaker but significant effects. Further analysis by single point mutations (Fig. 2B) suggested that the residues most critical for the CAE1 activity are CC at –78 and –77 (SM3 and SM4). Mutations on residues from –80 to –76 (SM1–SM5) had more severe effects than those at –70 to –78 (SM7–SM9) or –65 to –64 (SM12 and SM13). The others (SM6, SM11, and SM14) did not significantly affect the CAE1 activity. All the single mutations except for SM3 and SM4 showed weaker effect than double mutations at the same positions, suggesting that recognition of each base pair by a hypothetical transcription factor is rather loose, especially at the 3’ portion, probably due to the long recognition sequence of 18 bp.

Because the recognition sequence of 18 bp is longer than that of most transcription factors and the middle portion of CAE1 appears to be less important than the 5’ and 3’ portions (Fig. 2A), two independent transcription factors may recognize the 5’ and 3’ portions, respectively. To check this possibility, several deletions or insertions were introduced into the middle of the CAE1 element at –72, and transcriptional activities were analyzed (Fig. 2C). These deletions/insertions completely abolished the CAE1 activity, indicating that the fixed length of the 5’ and 3’ portions are important for the CAE1 activity. The CAE1 element seemed to be recognized by a single binding unit (such as monomers, homo-oligomers, or hetero-oligomers), but not by two independent factors. Furthermore, increasing number of CAE1 repeats incorporated into the CAE1-less Ccta promoter (containing up to –65) caused an increase in transcriptional activity up to 6.5-fold, although to slightly different extents between the forward and reverse orientations (Fig. 2D), indicating that the CAE1 element has an enhancer-like activity. Taken together, these results suggest that the CAE1 element is bound by a transcription factor that recognizes the long sequence of approximately 18 bp, and that sequence recognition of this factor is tighter for the 5’ portion than middle and 3’ portions.

Because the CAE1 sequence is similar to CAE2 (Fig. 1), the same transcription factor may recognize these elements. Double point mutations were introduced into the CAE2 element of Ccta minimum promoter (containing up to –31), at the positions where CAE1 activity was most effectively reduced: DM41 and DM42 mutations on CAE2 correspond to DM5 and DM10 mutations on CAE1 (Fig. 3). These mutations reduced transcriptional activity by 10–20-fold, suggesting that the same transcription factor(s) recognize both the CAE1 and CAE2 elements and enhance Cta transcription.

One-hybrid Screening of CAE1-binding Proteins—To isolate clones encoding the hypothetical CAE1-binding factors, yeast one-hybrid screening was carried out essentially as described previously (28, 29). To generalize the vector system, a fundamental one-hybrid screening vector, pEHEL2 (Fig. 4A), was constructed. This vector contains unique cloning sites immediately upstream of the two IRE1 promoters driving the yeast HIS3 and bacterial lacZ genes. Six repeats of the CAE1 element were inserted upstream of the IRE1 promoter driving HIS3 transcription, and the resulting plasmid (pCAE01) was integrated into a his3A strain of yeast. The yeast strain obtained was transformed with a multicopy plasmid library containing human E-cell-like lymphocyte cDNAs fused with a Gal4p transcriptional activation domain (amplified from 106 independent clones), and plated on medium lacking histidine but containing 150 μM 3-aminotriazole (an imidazoleglycerol phosphate dehydrogenase inhibitor that reinforces histidine auxotrophy). By plating approximately 105 transformants, 61 colonies were obtained after 2 days of incubation at 30 °C. Restriction patterns of plasmid DNAs recovered from these colonies revealed that they contain at least seven independent clones. Sequencing of these clones indicated that one encoded a full-length transcription factor known as ZNF143 (24) (Fig. 4B), and 46 of the 61 colonies were ZNF143. None of the other clones encoded DNA-binding protein. The β-galactosidase activity of yeast cells carrying the ZNF143 clone showed no significant difference from those carrying control plasmid (data not shown), indicating that the up-regulation of HIS3 in the presence of ZNF143 is mediated by the CAE1 repeat. The yeast strain carrying CAE1-ERN1-HIS3, but not a control strain carrying ERN1-HIS3 without CAE1 insert, gave histidine-independent colonies on retransformation with the ZNF143 plasmid (data not shown), confirming that the histidine independence is specific to the CAE1 sequence.

The human ZNF143 is a Staf family transcription factor known to control the expression of tRNA\(^{\text{iso}}\) and snRNAs, such as U1 and U6 (24, 25). The Xenopus homologue of ZNF143 is called Staf and has been well characterized (18–20). In mammals, another member of this family was also identified and called ZNF76 (24, 25). The \(\text{Xenopus}\) homologue of ZNF143 is called Staf and has been well characterized (18–20). In mammals, another member of this family was also identified and called ZNF76 (24, 25). The \(\text{Xenopus}\) homologue of ZNF143 is called Staf and has been well characterized (18–20). In mammals, another member of this family was also identified and called ZNF76 (24, 25).
was completely competed by addition of 100-fold excess of known ZNF143/ZNF76 binding sequences, mouse tRNA_{sec}(m-tRNA_{sec}-SBS) (transcriptional activator of the mouse tRNA_{sec} gene) (23); Fig. 4C) or h-U6-SBS (transcriptional activator of human U6 RNA gene) (24); Fig. 4C). Similar results were obtained from EMSA analysis of ZNF76-GST (Fig. 4B), whereas control experiments with GST alone gave no band shift (data not shown). Labeled CAE2 probe (Fig. 4C) also revealed a mobility shift by ZNF143-GST and ZNF76-GST, and the shift patterns were nearly identical to those of CAE1 (data not shown). These results clearly indicate that the CAE1 and CAE2 elements are recognized by both the ZNF143 and ZNF76 transcription factors in a sequence-specific manner.

In Fig. 4C, the CAE1 and CAE2 sequences are aligned with the m-tRNA_{sec}-SBS and h-U6-SBS sequences and the consensus sequences of *Xenopus* Staf determined by PCR-mediated affinity selection of binding DNAs (19) or footprint assay of native gene promoters (19). CAE1 and CAE2 are similar to m-tRNA_{sec}-SBS and h-U6-SBS sequences, especially at the 5' portion, which was shown to be more critical for transcriptional activation of *Ccta* than the middle and 3' portions (Fig. 2). CAE1 and CAE2 sequences also match well with the Staf binding consensus sequences: 16 of 18 for CAE1 to consensus 1, 13 of 15 for CAE1 to consensus 2, 17 of 18 for CAE2 to consensus 1, and 13 of 15 for CAE2 to consensus 2. Again, the sequence conservation to the Staf consensus is particularly marked in the 5' portion. Thus, it is reasonable that CAE1 and CAE2 were specifically recognized by ZNF143 and ZNF76 in vitro.

Sequence-specific Recognition of CAE1 and CAE2 by Nuclear Factors of HeLa Cells—To characterize DNA binding activities of transcription factors acting on CAE1 and CAE2 in HeLa cells, synthetic oligonucleotide probes were mixed with HeLa cell nuclear extract and analyzed by EMSA. The CAE1 probe showed a shifted band specific to its nucleotide sequence (Fig. 6A). Although unlabeled wild-type CAE1 competed for the binding, CAE1-DM5 (mutation in 5' portion; see Fig. 2) or CAE1-DM5/10 (mutations in 5' and 3' portions) did not compete even at a 300-fold excess, in good agreement with the results of the reporter gene assay (Fig. 2). Mutation in the 3' portion (CAE1-DM10) weakened the ability to compete, although incompletely. This is consistent with the notion that the

![Fig. 4. Cloning of CAE1-binding factors. A, plasmid construct used for one-hybrid screening of CAE1-binding factors. A fundamental screening vector for one-hybrid selection (pEHEL2) was constructed as shown, and then six copies of CAE1 sequence were introduced at the BglII site. This construct was integrated into genome of a histidine-dependent yeast strain. The resulting strain was transformed by human lymphocyte cDNA library fused with the GAL4 transactivation domain and screened for histidine-independent clones. X, XbaI; B, BglII; S, SmaI; N, NcoI; E, EcoRI. P IRE1, promoter of the yeast IRE1/ERN1 gene. B, structures of human ZNF143 and ZNF76 proteins. ZNF143 cDNA was isolated by one-hybrid screening using the above construct, and ZNF76 cDNA was cloned by PCR amplification from a human testis library. The DNA binding zinc-finger domains of these proteins show 84% amino acid sequence identity (24). C, comparison of the CAE1 and CAE2 sequences, ZNF76/ZNF143 (mammalian homologues of Staf) binding sequences of the mouse tRNA_{sec} gene (m-tRNA_{sec}-SBS) (23), and human U6 RNA gene (h-U6-SBS) (24), and *Xenopus* Staf binding consensus sequences determined by PCR-mediated affinity selection of binding DNAs (Staf cons. 1) (19) or footprint assay of native gene promoters (Staf cons. 2) (19). Y, pyrimidine base; R, purine base. The sequences described here are the same as those used in EMSA analysis.

![Fig. 5. EMSA analysis of recombinant ZNF76 and ZNF143 protein with CAE1 probe. DNA binding domains of human ZNF76 (B) or ZNF143 (A) fused with GST were purified from E. coli carrying their expression vectors. Purified protein was incubated with 32P-labeled CAE1 probe in the absence or presence of 100-fold excess specific competitors (m-tRNA_{sec}-SBS and h-U6-SBS; see Fig. 4) in reaction buffer containing 10,000-fold excess nonspecific competitor poly(dI-dC). CAE-bound protein was separated by polyacrylamide gel electrophoresis and visualized by autoradiography.

In Fig. 4C, the CAE1 and CAE2 sequences are aligned with the m-tRNA_{sec}-SBS and h-U6-SBS sequences and the consensus sequences of *Xenopus* Staf determined by PCR-mediated affinity selection of binding DNAs (19) or footprint assay of native gene promoters (19). CAE1 and CAE2 are similar to m-tRNA_{sec}-SBS and h-U6-SBS sequences, especially at the 5’ portion, which was shown to be more critical for transcriptional activation of *Ccta* than the middle and 3’ portions (Fig. 2). CAE1 and CAE2 sequences also match well with the Staf binding consensus sequences: 16 of 18 for CAE1 to consensus 1, 13 of 15 for CAE1 to consensus 2, 17 of 18 for CAE2 to consensus 1, and 13 of 15 for CAE2 to consensus 2. Again, the sequence conservation to the Staf consensus is particularly marked in the 5’ portion. Thus, it is reasonable that CAE1 and CAE2 were specifically recognized by ZNF143 and ZNF76 in vitro.

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portion is less strictly recognized by the binding (trans-
acting) factor than the 5′ portion, as suggested by reporter gene
assay (Fig. 2B). Other known ZNF143/ZNF76 binding se-
quences found in the mouse tRNA\textsuperscript{sec} gene (m-tRNA\textsuperscript{sec}-SBS, and h-U6-SBS are given
in Fig. 4, and CAE1-DM5, CAE1-DM10, and CAE1-DM5/10 probes contain mutations described in Fig. 2. B, CAE2 probe
was analyzed with HeLa cell nuclear extract by EMSA in the presence of compet-
itors as indicated. Mutations introduced into CAE2 probe (DM41, DM42, and
DM41/42) are shown in Fig. 3.

We also used CAE2 sequence as a probe for EMSA and
obtained results similar to those for CAE1 (Fig. 6B). Unlabeled
wild-type CAE2, m-tRNA\textsuperscript{sec}-SBS, and h-U6-SBS strongly com-
peted with the labeled CAE2 probe, whereas unlabeled mutant
CAE2 showed weaker (CAE2-DM41 or CAE2-DM42; mutations
in 5′ or 3′ portion; see Fig. 3) or no competition (CAE2-DM41/
42; mutations in both 5′ and 3′ portions). These results are
again consistent with the data of reporter gene assay (Fig. 3).

The more effective competition observed with CAE2-DM42
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than with CAE2-DM41 can be again explained by critical recognition of the 5' portion. This situation, similar to that for CAE1, supports the idea that ZNF143 and/or ZNF76 controls Ccta transcription through both CAE1 and CAE2 in HeLa cells. In addition, the mobility of CAE2-binding factor found in EMSA was the same as that of CAE1-binding factor (data not shown). Therefore, the HeLa cell factors bound to CAE1 and CAE2 are likely to be identical.

ZNF143 and ZNF76 Activate Transcription from a Minimum Ccta Gene Promoter Containing CAE2—To test whether ZNF143 and/or ZNF76 can activate Ccta transcription in vivo, a reporter construct containing up to ~31 bp of the Ccta promoter (minimal promoter containing CAE2, Fig. 1) was used to avoid indirect effects of other cis-acting elements. Luciferase activities were determined after HeLa cells were co-transfected with three constructs: effector plasmids expressing the full-length or DNA binding domain of human ZNF143 or ZNF76, the reporter, and internal control vector pRL-SV40. The transcription from the minimal Ccta promoter was up-regulated 3-fold by ZNF143 and 6-fold by ZNF76 when full-length proteins were co-expressed (Fig. 7), indicating that both ZNF143 and ZNF76 can enhance Ccta transcription. In contrast, co-expression of only the DNA binding domain (lacking transcriptional activation domain) of ZNF143 or ZNF76 inhibited the Ccta promoter activity to 50%, suggesting that the transcriptionally inactive exogenous proteins partially competed with the endogenous trans-acting factor for CAE2.

CAE1/CAE2-binding Factor in HeLa Cell Is ZNF143—ZNF143 (hSBF) is known to be contained in HeLa cells and activate transcription of the human U6 gene (25). To determine whether the CAE1/CAE2-binding factor is ZNF143, we prepared a rabbit antibody against ZNF143 for EMSA supershift analysis. Synthetic polypeptide with a sequence selected from the transcriptional activation domain (positions 190–205; this region is absent in ZNF76 (24)) was immunized to rabbits, and IgG fractions were prepared. By Western blotting, this antibody was found to specifically detect ZNF143 of 80 kDa produced by in vitro translation in rabbit reticulocyte lysate (data not shown). With increasing concentration of the anti-ZNF143 antibody, the CAE2-bound protein was supershifted to a slowly migrating band (Fig. 8). Almost all of the CAE2-bound protein observed by EMSA was supershifted at the highest concentration of anti-ZNF143. In contrast, no supershift was observed with control rabbit IgG. With shift patterns similar to CAE2, CAE1-bound protein of HeLa cell was also supershifted by the same anti-ZNF143 antibody (data not shown). These results clearly indicate that the major protein that binds to CAE1 and CAE2 in HeLa cells is ZNF143.

DISCUSSION

In this report, we demonstrated that the mouse Ccta/Tcp-1 gene promoter contains two cis-acting elements, CAE1 and CAE2, which are recognized by Staf family transcription factors ZNF143 and ZNF76. In HeLa cells, ZNF143 is the major trans-acting factor up-regulating Ccta transcription through these elements. The Xenopus homologue of these proteins, Staf, shows 84 and 64% amino acid sequence identity with the human ZNF143 and ZNF76, respectively (24). Although Staf was originally identified as a trans-acting factor controlling tRNA\textsuperscript{sec} gene transcription (32, 18), it also controls snRNA gene transcription (19, 20). The present study extends the roles of ZNF143 and/or ZNF76 to transcription of the cytosolic chaperonin gene Ccta. Thus, our results are consistent with the previous findings that Staf can stimulate not only the transcription by RNA polymerase III but also the transcription by RNA polymerase II (19, 20). These characteristics appear to be conserved between Xenopus Staf and mammalian homologues (23–25).

Because the Staf family transcription factors control the expression of not only tRNA\textsuperscript{sec} but also snRNAs such as U1 and U6, these proteins are likely to be essential to the cell. This is consistent with the fact that Ccta mRNA is detectable in all types of cells and tissues (33, 14, 15), although expression levels differ considerably. In mouse tissues, ZNF143 mRNA is more abundant in thymus than in brain or lung (23), which is similar to the expression pattern of mouse Ccta/Tcp-1 mRNA (33). On the other hand, the mouse ZNF76 mRNA is detectable in 20-day-old and adult testis but not in liver, brain, kidney, or spleen (21), suggesting that ZNF76 is especially abundant in testis germ cells compared with other types of cells. Consistent with these observations, the mouse Ccta/Tcp-1 mRNA is extremely abundant in testis germ cells (33, 34). Because ZNF76 was more effective than ZNF143 in enhancing expression of the reporter gene driven by Ccta minimal promoter (Fig. 7), ZNF76 may contribute to the very strong expression of Ccta gene in testis, whereas ZNF143 is likely to contribute to general expression of the Ccta gene. It is also known that DNA binding activity of mouse ZNF143 is stimulated in mammary gland at pregnant and lactating stages (23), when mammary cells proliferate very rapidly. In agreement with this observation, the expression of CCT subunit genes, including Ccta, are up-regulated during cell growth (15), and the expression level of CCT is particularly high in FM3A mammary tumor cells (15).2 It seems reasonable that ZNF143 coregulates the expression of snRNA (19, 20), tRNA\textsuperscript{sec} (32, 18), and chaperonin subunit Ccta genes, because extensive synthesis of proteins is necessary to produce cellular components in rapidly growing cells, and the products of these genes would be required for effective process-

2 H. Kubota, S.-i. Yokota, H. Yanagi, and T. Yura, unpublished results.
In addition to the CAE1 and CAE2 elements, a region upstream of CAE1 (~140 to ~102 bp), as well as the sequence flanked by the two elements (~65 to ~31), appeared to up-regulate Cctα expression in HeLa cells (Fig. 1). Because a potential Sp1 binding site is found at ~114 through ~109, Sp1 may play a role in basal Cctα expression. Although the sequence from ~65 to ~31 was searched for possible binding factors by computer programs, no significant homology with recognition sequences of known transcription factors was found. However, the close proximity to both CAE1 and CAE2 recognition sequences of known transcription factors was observed. Although these factors remain to be cloned and analyzed, they play an important role along with ZNF143 (or ZNF76), although these factors remain to be cloned and analyzed.

A significant transcription-enhancing activity was also found in the first intron of Cctα gene. Recently, we found a heat shock element-like sequence in the first intron, which is recognized by HSF1 and HSF2 in EMSA analysis (17). Because expression of the reporter gene construct containing the first intron was enhanced 9-fold by co-expression of HSF1 or HSF2 (17) and the expression of the reporter gene construct containing the first intron was found. However, the close proximity to both CAE1 and CAE2 factors coregulating the transcription of different CCT subunit genes are very similar under diverse conditions (14, 16), ZNF143 and ZNF76 may act as common transcription factors by computer programs, no significant homology with potential Sp1 binding site is found at –114 through –109, Sp1 flanked by the two elements (–65 to –31), appeared to up-regulate CCT subunits, including CCTαenhanced 9-fold by co-expression of HSF1 or HSF2 (17) and the-

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Transcriptional Regulation of the Mouse Cytosolic Chaperonin Subunit Gene Cctalt -Complex Polypeptide 1 by Selenocysteine tRNA Gene Transcription Activating Factor Family Zinc Finger Proteins

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