Transcriptional Regulation of the Cytosolic Chaperonin \( \theta \) Subunit Gene, \( \text{Cctq} \), by Ets Domain Transcription Factors Elk-1, Sap-1a, and Net in the Absence of Serum Response Factor

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The chaperonin-containing t-complex polypeptide 1 (CCT) is a molecular chaperone that facilitates protein folding in eukaryotic cytosol, and the expression of CCT is highly dependent on cell growth. We show here that transcription of the gene encoding the \( \theta \) subunit of mouse CCT, \( \text{Cctq} \), is regulated by the ternary complex factors (TCFs), Elk-1, Sap-1a, and Net (Sap-2). Reporter gene assay using HeLa cells indicated that the \( \text{Cctq} \) gene promoter contains a cis-acting element of the CGCGAAGT sequence (CQE1) at −36 bp. The major CQE1-binding proteins in HeLa cell nuclear extract was recognized by anti-Elk-1 or anti-Sap-1a antibodies in electrophoretic mobility shift assay, and recombinant Elk-1, Sap-1a, or Net specifically recognized CQE1. The CQE1-dependent transcriptional activity in HeLa cells was virtually abolished by overexpression of the DNA binding domains of TCFs. Overexpression of full-length TCFs with Ras indicated that exogenous TCFs can regulate the CQE1-dependent transcription in a Ras-dependent manner. PD98059, an inhibitor of MAPK, significantly repressed the CQE1-dependent transcription. However, no serum response factor was detected by electrophoretic mobility shift assay using the CQE1 element. These results indicate that transcription of the \( \text{Cctq} \) gene is regulated by TCFs under the control of the Ras/MAPK pathway, probably independently of serum response factor.

The chaperonin-containing t-complex polypeptide 1 (CCT)\( \dagger \) is a molecular chaperone assisting in the folding of newly synthesized proteins in the cytosol through ATP hydrolysis (1, 2). CCT belongs to the chaperonin family that includes mitochondrial HSP60, bacterial GroEL, plastid ribulosebisphosphate carboxylase/oxygenase subunit-binding protein, and archa group II chaperonins. Tubulin and actin are major substrates of CCT (3–6), and many other proteins, including cyclin E (7), transducin (4), myosin (8), VHL tumor suppressor (9), and luciferase (10, 11), are reported to be CCT substrates. In vivo, −15% of newly synthesized proteins are estimated to be folded with the assistance of CCT (5). Double torus-like structure of CCT with 8-fold symmetry was revealed by electron microscopy (12), and CCT is composed of eight different subunits of ~60 kDa each: \( \alpha, \beta, \gamma, \delta, \epsilon, \zeta, \eta, \) and \( \theta \) (13, 14). These subunits are encoded by independent genes (15) and share 30% identity at the amino acid sequence level (1).

We determined entire nucleotide sequences of all of the genes encoding CCT subunits and showed that many of them exhibit strong transcriptional activity comparable with the combination of SV40 promoter and enhancer (15). The expression levels of CCT subunits are strongly correlated with growth rate and markedly up-regulated at G1/S transition through early S phase in cultured cells (16). CCT subunits are significantly increased in tumor tissues, and the expression levels of CCT subunits are highly correlated with that of proliferating cell nuclear antigen (17). The expression patterns of different subunits are basically similar but distinct in synthesis and degradation rates, leading to differential turnover (18). Although CCT is up-regulated during recovery from chemical stress (19), and heat shock transcription factors may mediate the up-regulation (20), this stress response is unrelated to cell growth. Recently, we found that transcription of the gene encoding the \( \alpha \) subunit of CCT, \( \text{Ccta} \), is regulated by selenocysteine tRNA gene transcription-activating factor family transcription factors ZNF143 and ZNF76 (21). The level of ZNF143 is regulated by the mitogen-activated protein kinase (MAPK) pathway (22). Although these results suggested that a transcriptional control mechanism related to cell growth plays a role in CCT subunit regulation, little is known about common transcriptional mechanisms controlling the expression of different CCT subunits.

Elk-1 (Ets-like transcription factor), Sap-1a (SRF accessory protein), and Net (new Ets transcription factor; also called Sap-2) comprise the ternary complex factor (TCF) subfamily of the Ets domain transcription factors (23–25). Ets family proteins specifically recognize DNA sequences containing the core trinucleotide GGA with a highly conserved DNA-binding domain (23, 26). The core sequence is essential for Ets factor binding, and its flanking sequences are important for determining the different sequence specificity of these proteins. Elk-1, Sap-1a, or Net forms ternary complexes with serum response factor (SRF) on the serum response elements (SREs) of immediate early gene promoters, including c-fos (27) and egr-1 (28, 29), and play important roles in the transcriptional regulation of these genes. These three TCF subfamily proteins share three conserved domains: the DNA-binding domain localized at the amino terminus (30), the SRF-binding domain in...
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Fig. 1. Reporter gene assay of the mouse Cctq gene promoter region. A, DNA fragment extending from the 5′-flanking region (up to −2017 bp from the major transcription start point) to the first exon was subcloned into the pGL3-basic firefly luciferase expression vector and then deleted unidirectionally as shown. HeLa cells were transfected with each of the constructs (1.1 μg) together with pRL-SV40, an internal control vector expressing sea pansy luciferase (0.1 μg). Two luciferase activities were determined for each cell extract, and the activity of the firefly luciferase was normalized against that of the sea pansy enzyme (mean and S.D. of three experiments). B, double point mutations were introduced into the reporter construct containing up to −40 bp of the Cctq gene promoter as indicated. The same residues as in the WT promoter are indicated by dashes, and the positions important for Cctq gene transcription are boxed. C, nucleotide sequence of the mouse Cctq gene promoter. The numbers indicate distance from the major transcription start point in base pairs. A cis-acting element strongly enhancing the Cctq gene transcriptional activity (CQE1) and a possible Sp1 binding site are underlined.

MATERIALS AND METHODS

Chemicals and Antibodies—MEK1/2 inhibitor PD98059 was purchased from Calbiochem. Antibodies against human Elk-1 (I-20), Sap-1a (C-20), Net (C-20), SRF (G-20), and phosphorylated Elk-1 (B-4) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Reportor and Effector Constructs—The reporter construct containing the 5′-flanking region and first intron of the mouse Cctq gene in the firefly luciferase expression vector pGL3-basic (pCQL) was described previously (15). The first intron of pCQL was removed by a PCR-mediated subcloning method using an intronless sequence as a primer. The resulting construct (pCQL-pro) was deleted unidirectionally from 5′ upstream of the Cctq promoter by a modified method of Henikoff (39) using mung bean nuclease instead of S1 nuclease (Fig. 1A). Alternatively, some deletion constructs were produced by cloning PCR-amplified DNA fragments. Nucleotide substitutions, deletions, and insertions were also introduced into the reporter constructs by PCR. Tandem repeats of cis-acting DNA elements were produced by cloning self-ligated synthetic oligonucleotides. A firefly luciferase reporter gene driven by the herpes simplex virus thymidine kinase promoter (−80 to +38) was constructed by cloning a PCR-amplified DNA fragment into the pGL3-basic vector (named ptk-80Luc).

Human Elk-1 (40), Sap-1a (27), and Net (Sap-2) (33) cDNAs were amplified from human cDNA libraries (BD Biosciences Clontech, Palo Alto, CA) by PCR using previously published sequences as primers and cloned into the pCAGGS mammalian expression vector (41). cDNA fragments encoding the DNA binding domains of Elk-1 (−1−85), Sap-1a (−1−95), and Net (−1−95) were also introduced into the pCAGGS using PCR-based methods. Nucleotide sequences of the reporter and effector constructs were confirmed by sequencing. Ras expression vectors pCMV-Ras, pCMV-RasV12, and pCMV-RasN17 (Clontech) and the internal
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**RESULTS**

Cis-acting Elements Up-regulating *Cctq* Gene Transcription—Previously, we reported a firefly luciferase reporter gene construct driven by the mouse *Cctq* gene promoter/enhancer (pCQL) (15). This contains a DNA fragment from 2027 bp upstream of the transcription start point to the second exon of *Cctq* and showed strong transcriptional activity: 1.6-fold a combination of the SV40 promoter and enhancer in HeLa cells. By removing the first intron, the reporter construct containing the 2027-bp promoter of *Cctq* (pCQL-pro) (Fig. 1A, top) was produced. To search for cis-acting elements in the *Cctq* promoter, the 2027-bp promoter region of pCQL-pro was unidirectionally deleted from the 5'-end (Fig. 1A). HeLa cells were transiently transfected with these constructs, and luciferase activities of cell extracts were determined. In the segment extending from −2027 to −176, activities downstream-regulating *Cctq* transcription were detected. Trans-acting factors repressing *Cctq* promoter activity may bind this region. No significant effect on *Cctq* transcription was observed for the region between −176 and −85. Deletion from −85 to −40 slightly reduced transcriptional activity, suggesting a contribution of weakly positive cis-acting elements, including a possible Sp1-binding site found in this region (Fig. 1C). Deletion from −40 to −19 remarkably reduced the activity to 30%, indicating the existence of strong cis-acting elements in this region. Since transcriptional activity was almost completely abolished by deleting the construct from −19 to −3, this region is probably important for the initiation of transcription.

To analyze the strong cis-acting activity immediately downstream of −40 bp in more detail, double point mutations were introduced into the reporter gene construct containing the *Cctq* promoter up to −40 bp, and the luciferase activities of these constructs were analyzed using HeLa cells (Fig. 1B). Mutations on the residues from −36 to −29 bp reduced the activity by 40–60% relative to wild type, whereas mutations on residues from −40 to −37 bp or −28 to −25 bp exhibited little effect. These results indicate that the eight residues from −36 to −29 bp make a significant contribution to *Cctq* transcription and suggest that a trans-acting factor recognizes the 8-bp-long element CCGGAAGT. We named this element CQE1 (for *Cctq* gene transcription-activating element 1). Results very similar to Fig. 1, A and B, were obtained using a distinct cell line, mouse fibroblast NIH 3T3 (data not shown), implying that this element is recognized by transcription factors common to many cell types.

Computational Search for CQE1-binding Proteins—We searched for possible trans-acting factors recognizing the CQE1 sequences by computer programs, and the results indicated that the CQE1 sequence is highly homologous to the binding consensus sequence of the human Ets domain transcription factor Elk-1, a member of TCF subfamily (Fig. 2B). Of the 11-bp Elk-1 binding consensus determined by PCR-mediated affinity selection of binding DNAs (30), 10 residues match the CQE1 sequence, and only one substitution (A to G) occurs at the 5'-end of the consensus. This 5'-end substitution (A to G) was also found in the affinity selection (30), suggesting that contact at the 5'-end residue may be weaker than that of other residues in the consensus. Consistent with this view, mutation at this site had no significant effect in our reporter gene assay at this site (Fig. 1B). Since another TCF subfamily transcription factor, Sap-1a (27), shows characteristics very similar to Elk-1 (33, 43) (Fig. 2A), we also compared its binding consensus sequence (30) with CQE1. The 9-bp Sap-1a binding consensus exhibited perfect match with CQE1. In addition, a third member of TCF subfamily, Net (44), has been identified. Net differs from Elk-1 and Sap-1a in its transcriptional suppressor activity (35, 36) (Fig. 2A).

Although the binding of all three TCF subfamily proteins to the known mammalian target sequence in the c-fos promoter is SRF-dependent (33), a *Drosophila* gene, E74, is known to contain a DNA element with an Ets-binding motif (45) (E74-EBs in Fig. 2B) specifically recognized by human Elk-1 and/or Sap-1a in the absence of SRF both in *vitro* (37, 38, 46) and in *vivo* (32, 34, 43). We therefore compared the sequence of E74-
EBS with CQE1 and found that these sequences are nearly identical at the Elk-1/Sap-1a binding region (Fig. 2B). These observations suggest that CQE1 is a sequence recognized by the TCF subfamily proteins in the absence of SRF.

**Sequence-specific Recognition of CQE1 by HeLa Cell Nuclear Factors**—To characterize the DNA binding activities of transcription factors acting on CQE1 in HeLa cells, HeLa cell nuclear extract was analyzed by EMSA using synthetic oligonucleotide probes (Fig. 3). The CQE1 probe gave two bands: a broad band with a strong signal (band I) and a thin band with a much weaker signal (band II). Although the binding of labeled CQE1 for the two shift bands was strongly competed by excess unlabeled CQE1 with wild-type sequence, a mutant CQE1 carrying nucleotide substitutions in the GGAA motif, QDM4 (see Fig. 1B), did not compete even at a 300-fold excess, consistent with the results of the reporter assay (Fig. 1B). A mutation in the region 3′ to the GGAA motif, QDM6, also significantly weakened the ability of competition. The slightly
weaker competition of QDM4 than QDM6 is also consistent with the reduced activity of reporter \textit{in vivo}. The recognition by CQE1-binding factor is likely to be more strict for the GGAA motif than for surrounding sequences, in agreement with common characteristics of Ets family transcription factors. These results indicate that the factors giving bands I and II specifically recognize the CQE1 sequence and that these factors may correspond to the same trans-acting factors postulated by the results of reporter gene assay.

Since CQE1 shows a strong resemblance to the SRF-independent Elk-1 binding sequence found in the \textit{Drosophila} E74 gene, E74-EBS (see Fig. 2B), we tested whether the CQE1-binding factors recognize E74-EBS by competition experiments. E74-EBS strongly competed with the CQE1 probe for
proteins were analyzed by EMSA. A 20,000-fold excess nonspecific competitor poly(dI-dC) CQE1-bound specific competitor (E74-EBS; see Fig. 2) in reaction buffer containing a labeled CQE1 probe in the absence or presence of 100-fold excess their expression vectors. Purified proteins were incubated with 32P-Sap-1a, and Net fused with GST were purified from E. coli to recognize the CQE1 element in EMSA. DNA binding domains of Elk-1, Sap-1a, and Net fused with GST were purified from E. coli carrying their expression vectors, although this protein is probably less abundant or weaker for CQE1 binding, compared with Elk-1/Sap-1a. We also used the E74-EBS sequence as a probe for supershift analysis and obtained results very similar to CQE1 for band I (Fig. 4B). These results clearly indicate that the major proteins that bind to CQE1 in HeLa cells are Elk-1 and Sap-1a and that these proteins are phosphorylated at their transcriptional activation domains. Similar results were obtained using NIH 3T3 cell nuclear extract (data not shown), suggesting that Elk-1 and Sap-1a play an important role in CQE1-dependent activation of Cctq transcription in many types of mammalian cells.

Since Elk-1 and Sap-1a are known to form ternary complexes with SRF on SREs of immediate early genes including the human c-fos promoter, we examined whether CQE1-bound Elk-1 and Sap-1a are associated with SRF by supershift analysis. None of the CQE1-bound proteins were supershifted with the anti-SRF antibody (Fig. 4A), although the same antibody recognized SRF in EMSA (Fig. 4C). Similarly, no E74-EBS binding proteins were supershifted by the anti-SRF antibody. These results suggest that Elk-1 and Sap-1a bind CQE1 independently from SRF, as is the case with their binding to E74-EBS.

Enhancer-like Activity of the CQE1 Element—Increasing number of CQE1 repeats incorporated into the CQE1-less Cctq promoter (containing up to ~24 bp) provided an increase in transcriptional activity up to 4.5-fold, although the activity was slightly different between the forward and reverse orientations (Fig. 5, A and B). These results indicate that the CQE1 element has an enhancer-like activity and suggest that the transcription factors recognizing CQE1 in HeLa cells, which are probably Elk-1 and Sap-1a, can enhance transcriptional activity independently from other DNA-binding transacting factors. Since the CQE1 and E74-EBS sequences are very similar (Fig. 2), we compared their enhancer activities using reporter constructs containing four repeats of CQE1 or E74-EBS at the 5′-end of the same thymidine kinase promoter (~80 bp). The reporter containing CQE1 exhibited more than 2-fold greater activity relative to the reporter containing E74-EBS (Fig. 5C), although the thymidine kinase promoter alone gave very low activity that was below the detection limit in our system (data not shown). These results indicate that the CQE1 element has an enhancer activity comparable with or slightly stronger than that of E74-EBS.

Elk-1, Sap-1a, and Net Recognize the CQE1 Sequence by Their DNA Binding Domains Alone in Vitro—The DNA binding domains of Elk-1, Sap-1a, and Net fused with GST proteins were purified from E. coli carrying their expression plasmids, and their purified proteins migrated as a single band by SDS-polyacrylamide gel electrophoresis followed by Coomassie staining (data not shown). These proteins were analyzed for
their binding to the radiolabeled CQE1 probe by EMSA in the presence of a 20,000-fold excess of nonspecific competitor poly(dI-dC) (Fig. 6). The CQE1 probe was shifted to a slowly migrating band by each of the Elk-1-GST, Sap-1a-GST, and Net-GST proteins. These shift bands were well competed by unlabeled E74-EBS at a 100-fold excess. These results clearly indicate that monomers or homooligomers of the three TCF subfamily proteins specifically bind CQE1 independent of SRF.

**Overexpression of the DNA Binding Domains of TCF Subfamily Proteins Abolishes CQE1-dependent Transcriptional Activity**—To test whether TCF subfamily proteins recognize the CQE1 element in vivo, the DNA binding domains of Elk-1, Sap-1a, or Net were overexpressed in HeLa cells, and the activities of the reporter constructs containing four or no repeats of CQE1 in the context of the minimal Cctq promoter (−24 bp) were determined (Fig. 7). CQE1-dependent activity
was evaluated using the ratio of activities of the CQE1-containing reporter to the CQE1-less reporter. By overexpression, the DNA binding domains of Elk-1, Sap-1a, and Net abolished the CQE1-dependent portion of transcriptional activity almost completely, although the vector control exhibited little or no effect. Thus, these DNA binding domains compete strongly with endogenous transcriptional factors recognizing the CQE1 sequence in HeLa cells. These results indicate that all the three TCF subfamily transcription factors bind CQE1 element specifically via their DNA binding domains in an SRF-independent manner in vivo. These observations support the view that the trans-acting factors regulating CQE1-dependent transcriptional activity in HeLa cells are Elk-1 and Sap-1a and suggest that Net also plays a role when abundantly expressed.

Ras-dependent Regulation of Cctq Gene Transcription by TCF Subfamily Proteins through the CQE1 Element—It is known that TCF subfamily proteins activate transcription in a Ras-dependent manner (33, 35). To test whether Ras-dependent activation of Elk-1, Sap-1a, or Net affects CQE1-dependent transcriptional activity, effects of these proteins on the activity of reporters containing the minimal Cctq promoter (~24 bp) with or without four copies of CQE1 were examined in the presence of wild-type (Ras-WT), constitutively active (Ras-V12), or dominant negative (Ras-N17) forms of Ras. NIH 3T3 cells were co-transfected with the four constructs (i) effector plasmid expressing the full-length human Elk-1, Sap-1a, or Net; (ii) effector expressing Ras; (iii) reporter construct; and (iv) internal control, and luciferase activities of cell extracts were determined (Fig. 8).

By co-expression of Elk-1 or Sap-1a with Ras, the activity of the CQE1-containing promoter was significantly higher in the presence of Ras-WT or Ras-V12 than in the presence of Ras-N17 or the absence of Ras overexpression (columns 21–28), although the activity of the CQE1-less promoter was little affected by Ras (columns 5–12). These results indicated that the exogenously expressed Elk-1/Sap-1a was activated by exogenous Ras for the CQE1-dependent activity. The CQE1-dependent activity in the absence of TCF subfamily proteins (columns 17–20) was very similar to the activity in the presence of Elk-1/Sap-1a with Ras-WT/Ras-V12 (columns 22, 24, 26, and 28) and therefore significantly higher than in the presence of Ras-N17 or the absence of Ras (columns 21, 23, 25, and 27). Endogenous Elk-1/Sap-1a activity for CQE1-dependent transcription seems to be saturated in NIH 3T3 cells under normal growth conditions, and the exogenous Elk-1/Sap-1a without activation by Ras probably inhibited the CQE1-dependent activity by competing with the endogenous factors. The saturation may be due to lack of enough cofactors acting with Elk-1/Sap-1a. The activity of the CQE1-containing promoter in the absence of Ras overexpression (columns 21 and 25) was slightly higher than that in the presence of Ras-N17 (columns 23 and 27). Endogenous Ras may also stimulate the activity of exogenous Elk-1 and Sap-1a, although the stimulation was probably not enough to activate all Elk-1/Sap-1a overexpressed. These results therefore indicate that overexpressed Ras-WT and Ras-V12 enhanced the activity of exogenously introduced Elk-1 and Sap-1a for the CQE1-dependent transcription and support the idea that the endogenous factors activating the CQE1-dependent activity are Elk-1 and Sap-1a.

Net strongly inhibited transcription in the presence of Ras-N17 and in the absence of Ras overexpression (columns 31 and 29). However, co-overexpression of Ras-WT or Ras-V12 with Net significantly weakened the transcriptional repressor activity of Net (columns 30 and 32). These results are consistent with the fact that the Net molecule contains inhibitory domains (Fig. 2) in addition to an activation domain that is dependent on phosphorylation. Net down-regulates CQE1-dependent transcription using its negative regulator domains when competing with active Elk-1 and Sap-1a for CQE1 binding.

MAPK Inhibitor Represses CQE1-dependent Transcriptional Activity—Signals from Ras stimulate the MAPK pathway through sequential activation of Raf, MEK1/2, and ERK1/2, and this is an important signal transduction pathway controlling Elk-1/Sap-1a activity (24, 25). To examine whether the CQE1-dependent activity of Cctq gene transcription is dependent on the MEK/ERK pathway, HeLa cells were transiently transfected with the reporters containing four or no copies of CQE1 and the internal control pRL-SV40, in the presence or absence of 50 μM PD98059, a specific inhibitor of MEK1/2. Luciferase activities of cell extracts were determined, and CQE1-dependent activity was evaluated using the ratio of activities of the CQE1-containing reporter to the CQE1-less reporter (Fig. 9A). The CQE1-dependent transcriptional activity was significantly decreased by the addition of PD98059. Similar results were obtained using NIH/3T3 (data not shown). In the presence of 50 μM PD98059, phosphorylation of Elk-1 and/or Sap-1a was strongly inhibited in HeLa cells as determined by Western blotting of nuclear extracts (Fig. 9B). These results are consistent with the view that the transcription factors controlling CQE1-dependent activity are Elk-1 and Sap-1a.

**DISCUSSION**

In this report, we demonstrated that the mouse Cctq gene promoter contains a cis-acting element at ~29 bp, CQE1, that strongly enhances its transcriptional activity. CQE1 was specifically recognized by the Ets domain transcription factors Elk-1, Sap-1a, and Net, both in vitro and in vivo. Elk-1, Sap-1a, and Net have all been shown to form ternary complexes with SRF on c-fos and/or egr-1 promoters and mediate their transcriptional regulation (27–29, 33). However, binding of ternary complexes to SREs in these immediate early genes is SRF-dependent; thus, TCF subfamily proteins alone exhibit no significant binding to these elements (33). In contrast, E74-EBs of the *Drosophila* E74 gene can be specifically recognized by Elk-1
The CTF subfamily proteins are known to be activated by phosphorylation by MAPK, including ERK1/2 (32–34), p38, and c-Jun N-terminal kinase/stress-activated protein kinase (34, 47, 48) in response to different signals. Among the pathways leading to MAPK activation, the MEK/ERK pathway is the most important for responding to growth factor and mitogen stimulation, and activation of Ras has been shown to stimulate phosphorylation of Elk-1 and Sap-1a through the Ras/Raf/MEK/ERK pathway (32, 33, 35). In the present study, we found that the CQE1-dependent activation of Cctq gene transcription is dependent on Ras activity in the presence of exogenously expressed TCF subfamily proteins and that MEK1/2 inhibitor significantly reduces the endogenous activity of CQE1. As the Cctq gene product, CCT θ subunit, increases following arsenite treatment (19), the activation of Elk-1 and Sap-1a by p38 and c-Jun N-terminal kinase/stress-activated protein kinase may play a role in up-regulation of Cctq by chemical stress.

Since the TCF subfamily proteins control the expression of the immediate early genes c-fos (27) and c-erf (28, 29) through SRE, these proteins are likely to be essential for cell viability, particularly at the stimulation of cell growth. Consistent with this view, the expression of Cctq is up-regulated during cell growth (16). It seems reasonable that TCF subfamily proteins activate the expression of both immediate early genes and the chaperonin subunit Cctq gene, because the products of these genes induce transcription of genes encoding cellular components necessary for cell growth and for assisting in the folding of newly synthesized proteins (5), respectively. After stimulation of cells with growth factors, the induction of immediate early genes is fast and temporal (49, 50). For example, the level of c-fos mRNA becomes maximal 15 min after serum stimulation and essentially disappears within 60 min (49). Although the expression of the Cctq gene is also induced by growth factor stimulation, it is slower than that of the c-fos gene and becomes maximal at the G1/S transition (16). The different time course of expression between c-fos and Cctq may depend on SRF activity, because the induction of c-fos by serum stimulation is abolished in the SRF knockout E5 cells (51). The relatively late induction of Cctq seems to be reasonable, because Elk-1 and Sap-1a are most likely to regulate Cctq expression through CQE1 without SRF.

Net differs from Elk-1 and Sap-1a in its expression pattern (44, 52) and transcriptional repressor activity (35, 36). By stimulation through the Ras signaling pathway, phosphorylation of Net converts it to a transcription activator (35). In the present study, CQE1-dependent transcriptional activity was also strongly inhibited by Net in the presence of dominant negative Ras but partially recovered in the presence of wild-type or constitutively active Ras. In addition, the DNA binding domain of Net specifically recognizes CQE1 in vitro and in vitro. These observations suggest that expression of the Cctq gene can be repressed by Net when the Ras-related signal transduction pathway is not activated and that Net can activate Cctq gene transcription through Ras signaling, although to a lesser extent than Elk-1 or Sap-1a. Widespread distribution of Net (44, 52) and Elk-1/Sap-1a (40, 44, 53) together with the MAPK-dependent activity may contribute to widely distributed and growth-dependent expression of the Cctq gene product (15, 16).

In addition to the CQE1 element, a region upstream of CQE1 (−85 to −66) appears to up-regulate Cctq expression in HeLa cells, although the sequence from −85 to −66 was searched for possible binding factors by computer programs, no significant homology with known recognition sequences of transcription factors was identified. Since a potential Sp1 binding site is found at −47 through −42, Sp1 may play a role in basal level expression of Cctq. In addition, the first intron may have enhancer activity, since its deletion decreases transcriptional activity. Trans-acting factors recognizing these segments may play a role together with TCF subfamily proteins, although these factors remain to be cloned and analyzed.

The expression patterns of CCT subunit genes are similar under diverse conditions, and are highly dependent on cell growth (15, 16, 19). Although the trans-acting factors are different between the Cctq and Ccta gene promoters (21), the similar expression patterns suggest the existence of common upstream factors. The data presented here indicate that transcription from the Cctq promoter is significantly decreased by a MEK1/2 inhibitor in reporter gene assay. We also examined the reporter containing the minimal Ccta gene promoter (−31 bp) containing the CAE2 element, which was previously shown to be recognized by selenocysteine tRNA gene transcription-activating factor family transcription factors ZNF143 and ZNF76 (21). Activity of the Ccta reporter was inhibited to 50% in the presence of PD98059 (data not shown). Thus, the MEK/ERK pathway may be a common driving force for co-regulating the gene expression of different CCT subunits. Consistent with this view, the expression level of ZNF143, which regulates Ccta expression, is highly dependent on MEK1/2 activity (22), and Elk-1 subfamily proteins are activated by phosphorylation by ERK1/2 (34). Taken together, these observations suggest that the Ras/Raf/MEK/ERK pathway plays an important role in the growth-dependent coexpression of CCT subunits.

Acknowledgment—We thank Prof. E. Nishida for helpful suggestions on MAPK analysis.

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J. Biol. Chem. 2003, 278:30642-30651.
doi: 10.1074/jbc.M212242200 originally published online June 3, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212242200

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