Human CREB-binding Protein/p300-interacting Transactivator with ED-rich Tail (CITED) 4, a New Member of the CITED Family, Functions as a Co-activator for Transcription Factor AP-2*

José Bragança‡, Tracey Swingler‡, Fatima I. R. Marques‡, Tania Jones‡, Jyrki J. Eloranta¶, Helen C. Hurst¶, Toshihiro Shioda¶ and Shoumo Bhattacharya†‡§**

From the ‡Department of Cardiovascular Medicine, University of Oxford, Wellcome Trust Centre for Human Genetics, Henry Wellcome Building of Genomic Medicine, Roosevelt Drive, Oxford OX3 7BN, United Kingdom,
¶Human Cytogenetics Laboratory, Imperial Cancer Research Fund, Lincoln’s Inn Fields, London WC2A 3PX, United Kingdom,
§Imperial Cancer Research Fund Molecular Oncology Unit, Hammersmith Hospital, London W12 0NN, United Kingdom, and Laboratory of Tumor Biology, The Massachusetts General Hospital Cancer Center, Charlestown, Massachusetts 02129

Members of the CREB-binding protein/p300-interacting transactivator with ED-rich tail (CITED) family bind CREB-binding protein and p300 with high affinity and regulate gene transcription. Gene knockout studies indicate that CITED2 is required for neural crest and neural tube development and that it functions as a co-activator for transcription factor AP-2 (TFAP2). Here we describe human CITED4, a new member of this family, which is encoded by a single exon mapping to chromosome 1p34–1p35. CITED4 and p300/CREB-binding protein are present in endogenous naturally occurring complexes, indicating that they interact physiologically. The interaction occurs between the cysteine-histidine-rich domain 1 of p300 and the carboxyl terminus of CITED4. In keeping with this, CITED4 functions as a transactivator when artificially targeted to a promoter element. CITED4 physically interacts with all TFAP2 isoforms in vitro and strongly co-activates all TFAP2 isoforms in Hep3B cells. Co-activation of TFAP2 requires amino-terminal and carboxy-terminal residues of CITED4. In HepG2 cells, CITED4 is significantly weaker than CITED2 for TFAP2C co-activation. These results suggest that CITED4 may function as a co-activator for TFAP2. They also suggest the existence of cell type- and TFAP2 isoform-specific co-activation by CITED2 and CITED4, which may result in differential modulation of TFAP2 function.

The transcriptional co-activators and histone acetyltransferases p300 and CREB-binding protein (CBP) are recruited to gene promoters by diverse DNA-bound transcription factors, resulting in the activation of gene transcription (reviewed in Ref. 1). Gene knockout studies indicate that p300 and CBP are required for diverse developmental and cellular processes, including neurulation, cardiac and skeletal development, and cell growth control (2–5). In humans, mutations in CBP result in Rubinstein-Taybi Syndrome, which is characterized by mental retardation, cranio-facial and skeletal malformations, and cardiac malformations (6).

The first cysteine-histidine-rich (CH1) region of p300/CBP also binds members of a novel protein family called CBP/p300-interacting transactivator with ED-rich tail (CITED) (7–10). Members of this family include CITED1 (also known as Mag1 (11) and CITED2 (also known as Mrg1 or p35srj (7, 11–13)) and are characterized by a conserved 32-amino acid sequence motif at the carboxyl terminus that is necessary and sufficient for binding p300/CBP (7). CITED homologues, including a gene called cCITED3, have been identified by data base searches in other mammals, birds, amphibians, and fish (7, 14). CITED proteins do not appear to bind DNA directly but may function as transcriptional co-activators, linking transcription factors such as SMAD4 and estrogen receptor to p300/CBP (9, 15).

CITED2 is a growth factor and hypoxia-inducible gene and can itself inhibit hypoxia signaling by blocking the interaction between the hypoxia-induced factor HIF-1α and the CH1 domain of p300/CBP (7, 13). Using gene knockout and protein interaction studies, we have recently established that Cited2 is required for normal embryonic development and functions as a co-activator for TFAP2 (16). Mice lacking Cited2 are characterized by severe cardiac malformations, adrenal gland agenesis, neural crest defects, and exencephaly. CITED2 physically interacts with severe hypoxia-induced factors, TFAP2A and TFAP2B mutations result in abnormal embryonic development in Cited2−/− mice results, at least in part, from defective TFAP2 function.

In this report we describe human CITED4, a novel member of the CITED family that we have identified by searching expressed sequence tag data bases. Like CITED2, CITED4 binds both CBP/p300 and TFAP2 and it can function as a TFAP2 co-activator.

EXPERIMENTAL PROCEDURES

Standard molecular biology protocols were used for all procedures (22). Reagents were from Sigma unless otherwise indicated.

Data Base Searches—The GenBank™ expressed sequence tag data base was searched using the program TBLASTN for sequences resembling the p300-binding domain of CITED2 (7). A partial clone encoding a novel peptide (IMAGE number 1866039) was identified and obtained.

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** A Wellcome Trust Senior Research Fellow in Clinical Science. To whom correspondence should be addressed. Tel.: 44-1865-287581; Fax: 44-1865-287661; E-mail: sbhattac@well.ox.ac.uk.
† The abbreviations used are: CBP, CREB-binding protein; CH1, cysteine-histidine-rich domain 1 of p300; CITED, CREB-binding protein/p300 interacting transactivator with ED-rich tail; TFAP2, transcription factor AP-2; HA, hemagglutinin; GST, glutathione S-transferase; CMV, cytomegalovirus.
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from the MRC-HGMP (Cambridge, United Kingdom). Further data base searches during the course of this study showed that the CITED4 sequence was contained in \textit{Homo sapiens} chromosome 1 clone RP5-1066H13 map location 1p33–34.2 (GenBank\textsuperscript{TM} accession numbers AL158843 and NT_020652). The human CITED4 open reading frame is from position 829–3888 on the reverse strand of AL158843. Genomic sequences were identified by BLAST searches at fugu.hgmp.mrc.ac.uk. The FCITED1 open reading frame is from residues 11508–12107 on the forward strand of T006209. The FCITED3 open reading frame is from residues 1283–1961 on the reverse strand of T007827. Sequence alignments and phylogenetic trees were generated using CLUSTALW (F. Thompson et al., in press). Sequence alignments and phylogenetic trees were generated using CLUSTALW (F. Thompson et al., in press).

\textbf{Genomic Library Screening}—A human genomic PAC library (RPC11; obtained from Medical Research Council-Human Genome Mapping Project) was screened with a \textsuperscript{32}P-labeled insert of the IMAGE clone. A single clone (409-K1) was obtained and mapped. A 6-kb XbaI fragment that hybridized with a CITED4 cDNA probe was subcloned into pBlueScript and partially sequenced. Fluorescence in situ hybridization was performed using the PAC clone as described previously (22).

\textbf{Transcriptional Start Site}—Primer extension was performed as described previously (22) using total RNA extracted from ECV304 cells (RNeasy kit; Qiagen) and a \textsuperscript{32}P-labeled antisense primer, 5′-CCAC-TACTGCGCACCTTG. Dideoxy chain termination sequencing reactions were performed following the instructions of the manufacturer (U. S. Biochemical Corp.). We obtained the unlabeled primer and the human CITED4 genomic clone as template. Reactions were electrophoresed on a sequencing gel.

\textbf{CITED4 Plasmids}—CITED4 expression vectors were constructed by polymerase chain reaction in pCDNA3 (Invitrogen), with or without an amino-terminal HA epitope tag. The expression of CITED4 and CITED4A (lacking residues 138–184) was assayed by Western blotting and found to be similar (data not shown). Mammalian two-hybrid plasmids were generated using pCMXGAL4N and pCMXVP16N (gifts from R. Evans, Salk Institute, San Diego, CA). Glutathione S-transferase (GST) fusions were generated in pGEX4T1 (Amersham Biosciences, Inc.). Maltose-binding protein-CITED4 fusions were generated in pMALC2 (New England Biolabs). Details of plasmid construction are available upon request.

\textbf{Other Plasmids}—GST-CITED2 has been described previously (7). GST-CITED1 was constructed by cloning an EcoRI-HpaI insert from IMAGE Clone 440760 (a mouse clone) into pGEX4T1. GAL4-p300-CH1 (residues 300–410), and p3xAP2-Bluc, pcDNA3-\textit{TFAP2A}, pRSV-p300 fusions (25) were gifts from A. Giordano (Thomas Jefferson University, Philadelphia, PA). GAL4-luciferase and pCMV-lacZ were gifts from R. Evans. Blots were probed with a 400-nucleotide TACTGCGCACCTTG. Dideoxy chain termination sequencing reactions were performed as described previously (7).

\textbf{Antibodies}—Polyclonal antibody to human CITED4 was raised by immunizing rabbits with GST-CITED4 fusion protein. The antibody did not cross-react with CITED2 (data not shown). Anti-p300/CBP antibodies have been described previously (26). PAB419 and 9E10 are monoclonal antibodies against SV40 T-antigen and the myc epitope, respectively.

\textbf{Immunoprecipitations}—Immunoprecipitations were performed in buffer containing 50 mM Tris-HCl (pH 8), 150 mM NaCl, 0.5% Nonidet P-40, 0.5 mM EDTA, protease and phosphatase inhibitors (complete protease inhibitor (Roche), 1 mM phenylmethylsulfonyl fluoride, 0.5 mM sodium orthovanadate, and 5 mM sodium fluoride), and 1 mM dithiothreitol. Immunoprecipitations were performed essentially as described previously (7), using 1.8 or 1.2 mg of ECV304 whole cell lysate (Fig. 3, A and B, respectively).

\textbf{Western Blots}—Western blots used anti-CITED4 polyclonal serum (1:2000 dilution) and anti-HA monoclonal antibodies against SV40 T-antigen and the myc epitope, respectively. Blots were probed with anti-tubulin antibody (Sigma T-5293) to confirm equal loading.

\textbf{Immunostaining}—The anti-CITED4 polyclonal antibody was used for immunostaining at 1:250 dilution, essentially as described previously (7). For the peptide blocking experiment, purified maltose-binding protein-CITED4 peptide (0.03 mg/\mu l) was added at the primary antibody incubation step. Nuclei were counterstained with TOPRO-1 (Molecular Probes). Cells were mounted in Vectamount (Vector Laboratories) and visualized using a Bio-Rnd MRC 1024 confocal microscope. Data from TOPRO (blue) and FITC (green) channels were sequentially accumulated.

\textbf{Northern Blotting}—Total RNA was isolated from cells at 80% confluence using the RNeasy kit (Qiagen). ECV304 and HEK293 cells were gifts from Keith Channon (Oxford, United Kingdom). The breast cancer cell lines (Fig. 2B, right panel) were gifts from Adrian Harris (Imperial Cancer Research Fund, Oxford, United Kingdom). Northern blots used Hybrid-N membranes (Amersham Biosciences, Inc.) that were stained with methylene blue to visualize 28 and 18S RNA species following the instructions of the manufacturer. Multiple tissue Northern blots were obtained from CLONTECH. Blots were probed with a 400-nucleotide EcoNI fragment from the IMAGE clone.

\textbf{GST Fusion Binding}—GST fusions were generated, and binding reactions were performed as described previously (7).

\textbf{Transfections and Luciferase Assays}—Cells were plated in 24-well plates at 2.5 × 10\textsuperscript{4} cells/well and transfected the following day using FuGENE 6 (Roche Molecular Biochemicals). Transfection mixtures contained 1 μl of FuGENE 6 and up to 0.5 μg of total plasmid per well. pcMX-IacZ (100 ng) was co-transfected in all experiments, luciferase and lacZ activities were measured as described, and the ratio of luciferase to lacZ activity (relative luciferase activity) was calculated (22). The amounts of DNA used per transfection refer to the amounts added per well of a 24-well plate.

\textbf{RESULTS}

\textbf{Human CITED4 Gene}—We identified a novel member of the CITED family (CITED4) by searching expressed sequence tag data bases and obtained a genomic clone as described under “Experimental Procedures.” Comparison of expressed sequence tag cDNA and genomic sequences indicated that the CITED4 gene contained a single exon (Fig. 1A). CITED4 was mapped to chromosome 1p34–1p35 using fluorescence in situ hybridization (Fig. 1B). This is consistent with the published mapping of the CITED4-containing clone RP5-1066H13 to map location 1p33–34.2 (see GenBank\textsuperscript{TM} accession number AL158843). The transcriptional start site (+1) was mapped using primer extension analysis on total RNA isolated from ECV304 cells (Fig. 1, C and D). The predicted open reading frame begins at +251, in the context of the sequence 5′-GCCGCGCATGG (Fig. 1C). This corresponds to a consensus Kozak sequence (27) and predicts the synthesis of a 184-amino acid polypeptide (Fig. 1E).

\textbf{CITED4 Peptide}—The CITED4 peptide contains a carboxy-terminal domain motif that is conserved (65% identity over the 32 amino acids) in all CITED peptides (Fig. 1E) and corresponds to the previously described p300/CBP binding motif in CITED2 (7). This domain is contained within CR2 (9, 28). Unlike CITED1, 2, and 3, CITED4 does not appear to contain a distinct CR1 motif. However, a short amino-terminal sequence is conserved in CITEDs 4, 2, and 3, defining a new motif, CR3 (Fig. 1E). By searching the puffer fish (fugu) genomic data base, we also identified sequences corresponding to CITED1 and CITED3 (Fig. 1, E and P), but we were unable to identify a CITED4 homologue. Phylogenetic analysis of all CITED family members identified to date confirms that CITED4 forms a distinct group (Fig. 1P).

\textbf{Expression of CITED4}—Northern hybridization detected a single CITED4 transcript of 1.8 kb in almost all human tissues tested (Fig. 2A). CITED4 transcript levels were highest in heart, liver, skeletal muscle, and pancreas. CITED4 transcripts were also detected at high levels in ECV304 cells (a bladder cell line with endothelial characteristics (29)) and in certain breast cancer cell lines (T47D, MDA-MB-435, and MDA-MB-468 (Fig. 2B)). Endogenous CITED4 protein (migrating at 24.5 kDa) was detected by Western blotting in a number of cell lines including ECV304 (data not shown; Fig. 3, A and B).

\textbf{Immunolocalization of CITED4}—Immunostaining followed by confocal microscopy in ECV304 cells revealed that the cellular levels of endogenous CITED4 protein varied considerably (Fig. 2C). In most cells, it was localized predominantly in the nucleus, and in others, it was localized in both the nucleus and the cytoplasm. Antibody specificity was confirmed in these experiments by blocking the signal with a bacterially expressed CITED4 peptide generated as a maltose-binding protein fusion (Fig. 2D).
Detection of Endogenous CITED4-p300/CRBP Complexes—

Immunoprecipitates from untransfected ECV304 cell lysates were Western blotted to detect co-immunoprecipitated proteins. Anti-p300/CRBP immunoprecipitates (Fig. 3A, lane 3) contained co-immunoprecipitated CITED4 (Fig. 3A, bottom panel, lane 3). Immunoprecipitation reactions using control primary
CITED4 and p300/CBP are present in endogenous complexes. A, CITED4 is present in anti-p300/CBP immunoprecipitates. Top panel, anti-p300/CBP immunoblot of immunoprecipitates from ECV304 cell lysates. Immunoprecipitations used anti-p300/CBP monoclonal antibody AC240 (lane 3) and control antibodies (9E10, PAB419, and no primary antibody (i.e. rabbit anti-mouse IgG alone) in lanes 4, 5, and 6, respectively). Lane 1 contains 20% of the extract used for the immunoprecipitation. Lane 2 shows an AC240 immunoprecipitation performed without protein extract. Bottom panel, anti-CITED4 immunoblot of the above-mentioned immunoprecipitation reactions. IgL, immunoglobulin light chains. CITED4 migrates just below IgL and is indicated by the asterisk. B, p300/CBP is present in anti-CITED4 immunoprecipitates. Top panel, anti-p300/CBP immunoblot of immunoprecipitates from ECV304 cell lysates. Immunoprecipitations used anti-CITED4 polyclonal antibody (lane 2), control pre-immune serum (lane 3), or no primary antibody (lane 4). Lane 1 contains 10% of the extract used for the immunoprecipitation. Bottom panel, anti-CITED4 Western blot of the above-mentioned immunoprecipitates.

CITED4 binds the CH1 domain of p300 in vitro—Vectors expressing human full-length CITED2 (as a positive control), CITED4, or its truncated form were used to synthesize 35S-labeled proteins by coupled in vitro transcription-translation. These peptides were tested for interaction with a GST-p300-CH1 fusion protein (containing p300 residues 300–528; Fig. 4A). In vitro, full-length CITED4 specifically interacted with the CH1 domain of p300 as strongly as CITED2 (Fig. 4A, lanes 6 and 7, respectively). The carboxyl-terminal region of CITED4, extending between residues 138 and 184, is required for p300-CH1 binding because a CITED4 mutant lacking this domain did not bind (Fig. 4A, lane 9).

CITED4 binds the CH1 domain of p300 in vivo—To identify p300 domains necessary for interaction with CITED4 in vivo, we used a mammalian two-hybrid assay (Fig. 4B). In these experiments, GAL4-p300-CH1 strongly and specifically activated a GAL4-luciferase reporter gene when co-transfected with VP16-CITED4, indicating a two-hybrid interaction. No activation was observed with GAL4-p300-CH1Δ, which contains a mutant CH1 domain, or with VP16-CITED4Δ, which lacks CITED4 residues 138–184 (Fig. 4B). Using different GAL4-p300 fusion expression constructs (Fig. 4C), a strong interaction between CITED4 and p300 was found to occur only when the p300-CH1 domain is preserved, implying that the p300-CH1 domain is necessary and sufficient for efficient binding of CITED4.

CITED4 has a transactivation domain—Plasmids expressing the GAL4 DNA-binding domain fused to CITED4 and to CITED4Δ were co-transfected with a GAL4-luciferase reporter gene. GAL4-CITED4 strongly activated the transcription of reporter (Fig. 4D), but the deletion of the residues 138–184 of CITED4 (GAL4-CITED4Δ) resulted in a complete loss of transcription. CITED4 thus contains an intrinsic carboxyl-terminal transactivation domain, which corresponds to the domain required for the interaction with p300-CH1.

CITED proteins interact with TFAP2 isoforms in vitro—Because CITED2 physically and functionally interacts with TFAP2 isoforms, we asked whether CITED4 would do so as well. Vectors expressing human TFAP2 isoforms were used to synthesize 35S-labeled proteins by coupled in vitro transcription-translation. These peptides were tested for interaction with GST-CITED1, GST-CITED2, or GST-CITED4 fusion pro-
proteins (Fig. 5). As shown previously, TFAP2 isoforms specifically and strongly interacted with GST-CITED2 (16). They also interacted, although to a lesser extent, with GST-CITED4 and interacted very weakly with GST-CITED1.

**CITED4 Co-activates TFAP2 Isoforms in Mammalian Cells**—The physical interaction of CITED4 with TFAP2 isoforms suggested that CITED4 might also function as a TFAP2 co-activator. To test this, Hep3B cells (a human hepatocellular carcinoma cell line that has low levels of endogenous TFAP2 and CITED4) were transiently transfected with a luciferase reporter cloned downstream of TFAP2 binding elements (p3xAP2-Bluc; Fig. 6A). Transfection of vectors expressing TFAP2 isoforms activated the reporter. Co-transfection of CMV-CITED4 resulted in further activation of the reporter. The fold co-activation by CITED4 was 6.25-fold for TFAP2A, and 21-fold for TFAP2B. CMV-CITED4 resulted in further activation of the reporter. TFAP2 isoforms activated this reporter. Co-transfection of vectors expressing TFAP2 isoforms specifically and strongly interacted with GST-CITED4 (16). Here we used constructs expressing CITED4 peptides (Fig. 6). Transfection of vectors expressing CITED4 peptides behaved like the respective untagged peptides, we tested whether HA-CITED4 was produced at a higher level of expression by Western blotting (Fig. 6E; data not shown).

We next investigated the role of the CITED4 amino terminus (Fig. 6B). Here we used constructs expressing CITED4 peptides with a HA epitope tag fused to the amino terminus. CMV-HA-CITED4-(2–184) co-activated TFAP2A strongly, as compared with vector control. However, CMV-HA-CITED4-(21–184) failed to co-activate TFAP2A despite being expressed to the same level (Fig. 6F). Thus, residues 2–20 of CITED4 are essential for its co-activation function. In keeping with the results observed for CITED4A (Fig. 6A), CMV-HA-CITED4-(2–137) did not co-activate TFAP2A.

**Cell- and Isoform-specific Differences in Co-activation**—We next attempted to determine the relative abilities of CITED4 and CITED2 to co-activate TFAP2 isoforms in Hep3B cells. In these assays, CITED4 appeared to be a significantly stronger co-activator of all three TFAP2 isoforms, especially of TFAP2A and TFAP2B (Fig. 6C). In the above experiments, although we used identical amounts of transfected CITED plasmid, it was possible that equivalent amounts of CITED peptides were not being produced. Because amino-terminal HA-tagged CITED peptides behave like the respective untagged peptides, we tested whether HA-CITED4 was produced at a higher level than HA-CITED2. We transfected HA-tagged CITED2 and CITED4 plasmids into Hep3B cells and examined protein expression by Western blotting (Fig. 6E). The expression of HA-CITED4 was indeed significantly greater than that of HA-CITED2. We next examined the relative abilities of CITED2 and CITED4 to co-activate TFAP2 isoforms in Hep3B cells, another human hepatocellular carcinoma cell line. In these cells, unlike the situation in Hep3B cells, we found that CITED4 is a relatively weak co-activator when compared with CITED2 (Fig. 6D) despite having a relatively higher expression level (Fig. 6G). This is especially true for TFAP2C, for which CITED2 appears to be a particularly good co-activator.

**DISCUSSION**

We identified human CITED4 by searching expressed sequence tag data bases with a domain of CITED2 that binds...
p300. This domain is a signature of the CITED family and is conserved in the carboxyl terminus of CITED4, implying that it should be a family member. The human CITED4 gene maps to chromosome 1p34–1p35. This region is syntenic with mouse chromosome 4. Like the murine gene,\(^2\) human CITED4 is encoded by a single exon. This remarkably simple structure is reminiscent of CITED2, where the entire open reading frame of the p35sorf isoform is contained within a single exon (8), and of CITED1, where the open reading frame is contained within 2 exons (30). Taken together with the presence of conserved regions at the amino and carboxyl termini of the CITED proteins, this suggests that the members of the CITED family have likely arisen by gene duplication and sequence divergence from an ancestral gene. CITED family members have only been found in jawed vertebrates to date, and because CITED1 and CITED3 homologues are present in the puffier fish genome, this putative gene duplication and divergence likely took place before the evolution of jawed vertebrates.

In keeping with the hypothesis that CITED4 is a CBP/p300-interacting protein, naturally occurring CITED4-p300/CBP complexes were detected in untransfected cells using monoclonal antibodies to p300/CBP and polyclonal antibodies to CITED4, implying that the two proteins interact under physiological conditions. Using \textit{in vitro} and mammalian two-hybrid protein interaction assays, the CITED4 carboxyl terminus domain (residues 138–184) was found to be necessary for the interaction with p300. Similarly, the CH1 domain of p300 is both necessary and sufficient for the interaction with CITED4. Like other CITED members, CITED4 can function as a transcriptional activator when recruited to a promoter, and its carboxyl-terminal p300-binding domain is necessary for the transactivation function. These results support the idea that CITED4 is a novel p300/CBP-binding transactivator protein and confirm that it is a member of the CITED family.

CITED4 mRNA is ubiquitously expressed in all tissues examined, with some variability in expression level, indicating that it likely has a functional role in most tissues. High expression was also detected in certain breast cancer lines. The possible significance of this is discussed below. Although CITED4 protein can be detected in the cytoplasm in certain cells, it is predominantly nuclear in other cells. In the absence of a specific nuclear localization signal, this indicates that it might be complexed at least some of the time with exclusively nuclear proteins. The variability in CITED4 protein expression in individual cells suggests that it may be regulated through the cell cycle.

We have previously shown that the CITED2 peptide physically interacts with and co-activates all TFAP2 isoforms. Moreover, Cited2\(^{-/-}\) mouse embryonic fibroblasts had a specific defect in TFAP2 transactivation (16). The data presented above indicate that like CITED2, CITED4 also physically interacts with TFAP2 isoforms. These interactions are likely to be transient because we have not, to date, been able to detect stable complexes of endogenous CITED peptides with TFAP2 isoforms. We have also not been able to detect an interaction between CITED4 and TFAP2 isoforms using the yeast two-hybrid system (J. J. E. and H. C. H.; data not shown). The reason for this is not clear but may indicate the requirement for other factors that are not present in yeast cells. Alternatively, the fusion of the GAL4 activation domain to the amino terminus of CITED4 (which is necessary for TFAP2 co-activation) may sterically hinder the interaction between CITED4 and TFAP2.

We have found that CITED4 functionally interacts with TFAP2 isoforms by co-activating them very strongly in Hep3B cells. TFAP2 co-activation requires residues 2–20 (encompassing the CR3 motif) and also requires residues 138–184, a region that contains the carboxyl-terminal CBP/p300-binding domain, and part of CR2. Indeed, CITED4 actually appears to be a better co-activator when compared with CITED2 in these cells. This may be due to higher levels of protein expressed by the transfected CITED4 plasmid. On the other hand, in HepG2 cells, CITED4 is substantially weaker than CITED2 with regard to TFAP2 co-activation, despite relatively higher levels of protein expression.

One explanation for these results is the existence of cell type-specific TFAP2 co-activation by CITED peptides. This implies that certain unique factors or pathways may be present in Hep3B cells that allow CITED4 to function as a strong co-activator. These factors may be lacking in HepG2 cells. Possible candidates are the products of the hepatitis B virus genome, which is stably integrated in Hep3B cells, and other known TFAP2 co-activators, such as PC4 and poly(ADP ribose) polymerase (31, 32). Alternatively, HepG2 cells may contain a factor that suppresses CITED4 function or enhances CITED2 function. These results also suggest the existence of TFAP2 isoform specificity. Thus, in both Hep3B and HepG2 cells, CITED2 appears to co-activate TFAP2C more powerfully than TFAP2A or TFAP2B isoforms, whereas CITED4 displays little or no isoform specificity. The basis for this putative isoform specificity is unclear at present. However, it provides a potential mechanism for modulating TFAP2 isoform function in different tissues and in response to different upstream signaling events.

High levels of murine CITED4 are found in adult mammary tissues during pregnancy and lactation and during the prolactin-induced \textit{in vitro} differentiation of mammary epithelial cells.\(^2\) High levels of CITED4 are also found in certain breast cancer cell lines. TFAP2 isoforms are expressed in breast cancers, where they regulate the transcription of the neuroregulin/heregulin receptors ErbB3 and ErbB2, estrogen receptor, and transforming growth factor \(\alpha\) (33–39). It is also thought that TFAP2 isoforms control cell growth and differentiation (reviewed in Ref. 40). Co-activation of TFAP2 by CITED4 may therefore be important in normal mammary gland function and in the pathogenesis of breast cancer.

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José Bragança, Tracey Swingler, Fatima I. R. Marques, Tania Jones, Jyrki J. Eloranta, Helen C. Hurst, Toshihiro Shioda and Shoumo Bhattacharya

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