Evolutionarily Conserved Binding of Translationally Controlled Tumor Protein to Eukaryotic Elongation Factor 1B*

Huiwen Wu (吴惠文) 1, 2, Weibin Gong (宫维斌) 3, Xingzhe Yao (姚形哲) 4, Jinfeng Wang (王金凤) 5, Sarah Perrett 3, 1, and Yingang Feng (冯银刚) 3, 4, 5, 6

From the 1 National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, the 4 Qingdao Engineering Laboratory of Single Cell Oil and 5 Shandong Provincial Key Laboratory of Energy Genetics, Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, Qingdao, Shandong 266101, and the 6 University of Chinese Academy of Sciences, Beijing 100049, China

Background: The primary function of the abundant and highly conserved protein TCTP is not clear.

Results: TCTP binds to a conserved central acidic region of eukaryotic elongation factor 1B in eEF1Bδ.

Conclusion: The binding of TCTP to eukaryotic elongation factor 1B is evolutionarily conserved.

Significance: The interaction with eEF1B represents a primary function of TCTP.

Translationaly controlled tumor protein (TCTP) is an abundant protein that is highly conserved in eukaryotes. However, its primary function is still not clear. Human TCTP interacts with the metazoan-specific eukaryotic elongation factor 1B in eEF1Bδ and inhibits its guanine nucleotide exchange factor (GEF) activity, but the structural mechanism remains unknown. The interaction between TCTP and eEF1Bδ was investigated by NMR titration, structure determination, paramagnetic relaxation enhancement, site-directed mutagenesis, isothermal titration calorimetry, and HADDOCK docking. We first demonstrated that the catalytic GEF domain of eEF1Bδ is not responsible for binding to TCTP but rather a previously unnoticed central acidic region (CAR) domain in eEF1Bδ. The mutagenesis data and the structural model of the TCTP-eEF1Bδ CAR domain complex revealed the key binding residues. These residues are highly conserved in eukaryotic TCTPs and in eEF1B GEFs, including the eukaryotically conserved eEF1Bα, implying the interaction may be conserved in all eukaryotes. Interactions were confirmed between TCTP and the eEF1Bα CAR domain for human, fission yeast, and unicellular photosynthetic microalgal proteins, suggesting that involvement in protein translation through the conserved interaction with eEF1B represents a primary function of TCTP.

* This work was supported by National Basic Research Program, Ministry of Science and Technology of China (973 Program) Grants 2012CB911000 and 2013CB910700 (to S. P.), National High-tech R&D Program, Ministry of Science and Technology of China (863 Program) Grant 2012AA02A707 (to Y. F.), National Natural Science Foundation of China Grants 30800179 and 31170701 (to Y. F.), 31200578 and 31470474 (to W. G.), and 31110103914 (to S. P.), and Beijing Natural Science Foundation Grant 6092015 (to Y. F.), 6092018 (to S. P.).

The atomic coordinates and structure factors (codes 2MVM and 2MNW) have been deposited in the Protein Data Bank (http://wwpdb.org/).

1 To whom correspondence may be addressed. E-mail: fengyg@qibebt.ac.cn.

2 To whom correspondence may be addressed. E-mail: sarah.perrett@cantab.net.

3 The abbreviations used are: TCTP, translationally controlled tumor protein; eEF1, eukaryotic elongation factor 1; GEF, guanine nucleotide exchange factor; CAR, central acidic region; ITC, isothermal titration calorimetry; PRE, paramagnetic relaxation enhancement; MTSL, 1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl methanethiosulfonate; CSP, chemical shift perturbation; HSQC, heteronuclear single quantum coherence.

8694 JOURNAL OF BIOLOGICAL CHEMISTRY
three domains as follows: the N-terminal domain (residues 1–153), the central acidic region (CAR) domain (residues 153–192) (pfam10587 in PFAM database), and the C-terminal catalytic GEF domain (residues 192–281). Previous studies indicate that TCTP physically interacts with the C-terminal region (CAR–GEF region; residues 153–281) of eEF1Bδ and inhibits its GEF activity (10, 11).

Although the three-dimensional structures of TCTP homologues from different species have been solved and are shown to be highly conserved (6, 18–22), there are few reports giving structural information regarding the interaction of TCTP with its partner proteins. Furthermore, as indicated by Bommer and Thiele (5), additional very careful work is required to establish the complete array of molecular interactions of TCTP because TCTP frequently appears as an “interacting protein” in two-hybrid screens. Here, by employing various structural techniques, including NMR titration, chemical shift mapping, paramagnetic relaxation enhancement, and HADDOCK docking, as well as isothermal titration calorimetry (ITC) and site-directed mutagenesis, we identified the binding interfaces and key residues in the interaction between TCTP and eEF1Bδ.

We found that TCTP unexpectedly binds to the previously unnoticed CAR domain (residues 153–192), instead of the C-terminal catalytic GEF domain of eEF1Bδ. The CAR domain is conserved in all eEF1B GEFs and is structurally independent from the GEF domain. The key residues for the interaction identified in TCTP are highly conserved in eukaryotes, and those in eEF1Bδ are conserved not only in metazoan eEF1Bδ but also in eEF1Bα as well as plant eEF1Bβ, suggesting a conserved interaction between TCTP and eEF1B GEFs. The interaction between eEF1Bα and TCTP of human, fission yeast, and unicellular photosynthetic microalgae was further confirmed, which demonstrates that the interaction of TCTP and the eEF1 complex is conserved in eukaryotes. The results in this study imply that involvement in protein translation is one of the primary cellular functions of TCTP.
Conserved Interaction between TCTP and eEF1B

Wild-type human TCTP was expressed and purified as reported previously (6). The construction of different mutants of the eEF1BΔ CAR domain and TCTP (except the TCTP mutants for paramagnetic relaxation enhancement (PRE) experiments) was carried out by the QuikChange method (23). After PCR with mutagenic primers, DpnI was added to digest the methylated nonmutated parental template. The product was transformed into E. coli TOP10 competent cells. The purification of mutant proteins was similar to that of the wild-type proteins. For the TCTP mutants used in PRE experiments, including C172S, C28S/C172S, C28S/C172S/T116C, C28S/C172S/A127C, and C28S/C172S/D143C, the coding sequence of mutant TCTP was cloned into pET11a or pET30a expression vector without any tag. After expression in E. coli BL21 (DE3), the cells were resuspended in buffer A without NaCl. After cell lysis and centrifugation, the lysate was loaded onto a DEAE column. The mutant TCTP was eluted with 150 mM NaCl. The eluate was dialyzed to remove NaCl followed by Q-Sepharose high performance column (GE Healthcare) purification. The mutant TCTP was eluted with a gradient of NaCl concentrations from 50 to 300 mM. The eluate was concentrated and further purified using a Superdex 75 gel filtration column.

TCTP, eEF1BΔ CAR domain, and the CAR-GEF region from fission yeast Schizosaccharomyces pombe and photosynthetic microalga Nannochloropsis oceanica IMET1 were cloned into pET30a for protein expression. The same procedure was used for expression and purification of these two proteins. The plasmid was transformed into E. coli BL21 (DE3). Cells were grown at 37 °C, and the protein expression was induced for 5 h with 0.5 mM isopropyl β-D-thiogalactopyranoside when the absorbance at 600 nm reached 1.0. The proteins were first purified using a Ni2+ column (chelating Sepharose Fast Flow) and further purified by gel filtration chromatography using a Superdex 75 column (GE Healthcare). The buffer for gel filtration and final protein storage was 50 mM sodium phosphate buffer, pH 7.0, containing 200 mM KCl, 5 mM DTT, and 5 mM EDTA.

Peptides EDDDDIDLFGSDNE, DLFGS, and LFG were synthesized by Sangon Biotech (Shanghai, China). 15N- and 15N-13C-labeled proteins were prepared using the same procedures except cells were grown in M9 minimal media containing 200 mM KCl, 5 mM DTT, and 5 mM EDTA. Peptides EDDDDIDLFGSDNE, DLFGS, and LFG were synthesized by Sangon Biotech (Shanghai, China). 15N- and 15N-13C-labeled proteins were prepared using the same procedures except cells were grown in M9 minimal media containing 15NH4Cl and [13C]glucose as the sole nitrogen and carbon sources, respectively.

NMR Spectroscopy—NMR experiments were performed on 298 K on Bruker DMX, AVANCE, and Agilent DD2 600 MHz NMR spectrometers equipped with cryo-probes. All NMR samples contained 0.2–0.8 mM 15N- or 15N/13C-labeled protein in 20 mM Tris-HCl, pH 7.5, 200 mM NaCl, 0.01% 2,2-dimethyl-2-silapentane-5-sulfonate, and 10% (v/v) D2O.

Two-dimensional 1H-15N and 1H-13C HSQC and three-dimensional CBCA(CO)NH, HNCA, HNCO, HN(CA)CO, HBHA(CO)NH, HBHANH, HCCCH-TOCSY, CCH-COSY, and CCH-TOCSY experiments were performed for backbone and side chain assignments of the eEF1BΔ CAR domain in free and TCTP-bound states. Three-dimensional 1H-15N and 1H-13C NOESY-HSQC spectra with mixing times of 300 ms were collected to generate distance restraints. All data were processed with FELIX (Accelrys Inc.) or NMRPipe (24) and analyzed with NMRViewJ (25).

Heteronuclear steady-state 1H-15N NOE experiments and CLEANEX-PM experiments (26) were performed using standard pulse programs. Samples of 15N-labeled human eEF1BΔ CAR-GEF region in free and TCTP-bound states were used in the experiments. The mixing times for CLEANEX-PM experiments ranged from 5 to 500 ms, and the data acquired using short mixing times (5, 10, 15 and 20 ms) were used to estimate the amide-water exchange rates.

Paramagnetic Relaxation Enhancement Experiments—PRE experiments were performed using proteins labeled with 1-oxyl-2,2,5,5-tetramethyl-Δ3-pyrroline-3-methyl methanethiosulfonate (MTSL) (Toronto Research Chemicals, Toronto, Canada) on one free cysteine. Native cysteines on the surface of proteins were mutated to serine to avoid undesired MTSL labeling. The eEF1BΔ CAR domain contains no cysteines, whereas TCTP contains two cysteines, Cys-28p and Cys-172p. (The residues and the mutants of human eEF1BΔ and TCTP are designated by a subscripted suffix p for eEF1BΔ and TCTP, respectively, e.g. Pro-150 of eEF1BΔ will be represented as Pro-150p, and the C172S mutant of TCTP will be represented as C172Sp.) Because the two cysteines are far away from the binding surface, the C28S/C172S double mutant of TCTP was used in the PRE experiments without interfering with the interaction. Different TCTP or eEF1BΔ cysteine mutants were incubated with MTSL for 16 h at 25 °C in nonreducing buffer, and excess MTSL was removed by dialysis against 20 mM Tris-HCl buffer, pH 7.5, 200 mM NaCl for 6 h at 4 °C. Spin-labeled protein was added to other 15N-labeled protein for NMR experiments. The reduced compound was generated by incubation with 1.5 mM ascorbic acid for 1 h at 25 °C. The two-dimensional 1H-15N HSQC spectra of 15N-labeled proteins were acquired at a 1:1 molar ratio in the oxidized and reduced states (27).

Structural Calculations—The structures of the eEF1BΔ CAR domain in the free and TCTP-bound states were initially calculated with the program CYANA (28), and then refined using CNS (29) and RECOORDScript (30) in explicit water with manual assignments. Backbone dihedral angle restraints obtained using TALOS (31) and hydrogen bond restraints of the α-helix were also incorporated into the structural calculation in the later stages of refinement. From 100 initial structures, 50 lowest energy conformers were selected for refinement in explicit water, and the 20 lowest energy conformers represent the final ensemble of structures. The quality of the structural analysis and related statistics were obtained using the programs MOLMOL (32) and PROCHECK-NMR (33). The structures have been deposited in the Protein Data Bank with accession codes 2MVM and 2MVN for the eEF1BΔ CAR domain in free and TCTP-bound states, respectively.

HADDOCK Docking—The structure of the TCTP-eEF1BΔ CAR domain complex was calculated on the HADDOCK webserver (34). The x-ray structure of TCTP (Protein Data Bank code 1YZ1) and NMR structure of TCTP-bound eEF1BΔ CAR domain were used as the starting structures. The CSP data were used to construct the ambiguous constraints of the binding surface. PRE and mutagenesis constraints were used as unambiguous constraints. PRE constraints were derived from the PRE data of MTSL-labeled T116Cp, A127Cp, D143Cp, K169Cp, E177Cp, and K186Cp. Mutagenesis constraints were set up for...
residues whose mutation caused significant changes in the binding (site I, Ile-92p, Met-96p, Met-115p, Ala-118p, Ile-122p, Leu-159/H9254, and Phe-160/H9254; site II, Phe-83p, Met-140p, and Tyr-182/H9254). During the calculation, residues 155–165 of eEF1B were set to be fully flexible.

NMR Titration Experiments—The concentration of 15N-labeled proteins in all titration experiments was 0.1–0.3 mM. The concentration of stock solution of ligands was 1–5 mM in the same buffer. All experiments were performed at 25 °C in 20 mM Tris-HCl buffer, pH 7.5, 200 mM NaCl, except for the high salt experiment which contained 400 mM NaCl. The values of chemical shift perturbations (CSP) were calculated using Equation 1,

$$\text{CSP} = \sqrt{\Delta (\text{HN})^2 + 0.2(\Delta N)^2}$$  \hspace{1cm} (Eq. 1)

where $\Delta \text{HN}$ and $\Delta N$ are the changes in $^{1}\text{HN}$ and $^{15}\text{N}$ chemical shifts, respectively.

The equilibrium dissociation constants ($K_r$) were estimated by fitting the observed CSPs Equation 2

$$\text{CSP} = \frac{\text{CSP}_{\text{max}}}{2} \left[ \left(1 + r + K_D \left( \frac{1}{C_{\text{pro}}} + \frac{r}{C_{\text{lig}}} \right) \right)^2 - \left(1 + r + K_D \left( \frac{1}{C_{\text{pro}}} + \frac{r}{C_{\text{lig}}} \right) \right)^2 \right] - 4r$$  \hspace{1cm} (Eq. 2)

where CSP$_{\text{max}}$ is the CSP at the theoretical saturated condition obtained from the fit; $r$ is the molar ratio of ligand to protein; $C_{\text{pro}}$ is the concentration of initial protein solution; and $C_{\text{lig}}$ is the stock concentration of ligand.

Isothermal Titration Calorimetry—ITC measurements were performed on an iTC-200 calorimeter (MicroCal Inc.). All experiments were carried out in 20 mM Tris-HCl buffer, pH 7.5, 200 mM NaCl. 0.05–0.1 mM TCTP was placed in the 200-μl sample chamber, and eEF1B CAR domain (2 mM) or eEF1B CAR-GEF region (1 mM) in the syringe was added in 20 successive additions of 2 μl each taking 4 s (with an initial injection of 0.5 μl). The interval between each injection lasted 150 s. Control experiments were performed under identical conditions to determine the heat signals that arise from addition of the eEF1B into the buffer. Data were fitted using the single-site binding model within the Origin software package (MicroCal Inc.). To determine the heat capacity change $\Delta C_p$, ITC experiments were carried out at 10, 15, 20, 25, and 30 °C. The $\Delta C_p$ value was estimated by linear fitting of the $\Delta H$ values obtained against temperature.

RESULTS

CAR Domain of eEF1Bδ Is the Region Responsible for TCTP Binding—The C-terminal region (residues 153–281) containing the CAR and GEF domains of eEF1Bδ was previously identified as the TCTP binding region (10, 11). However, we found that the CAR domain and the GEF domain are in fact independent structural domains, as the peaks in the $^{1}\text{H}-^{15}\text{N}$ HSQC spectrum of the isolated CAR domain overlay well with the corresponding peaks in the $^{1}\text{H}-^{15}\text{N}$ HSQC spectrum of the CAR-GEF region (Fig. 2). In the NMR titration of $^{15}$N-labeled TCTP with the eEF1B δ CAR-GEF region (Fig. 3A) and isolated CAR domain (Fig. 4A) as well as full-length eEF1Bδ (data not shown)
almost identical chemical shift perturbations were observed for peaks in the $^1$H-$^{15}$N HSQC spectra of $^{15}$N-labeled TCTP. Interestingly, no chemical shift perturbations were observed when TCTP was titrated with the GEF domain alone (data not shown). Furthermore, no interaction was observed between the N-terminal domain of eEF1B and TCTP (data not shown). In the reverse titration of the $^{15}$N-labeled CAR-GEF region with TCTP (Fig. 3B), the peaks from the CAR domain in the $^1$H-$^{15}$N HSQC spectrum showed significant chemical shift perturbations, the same as when the $^{15}$N-labeled isolated CAR domain was titrated with TCTP (Fig. 4B), although the peaks from the GEF domain showed no change during the titration (Fig. 3C). Therefore, this indicates that the CAR domain is the region responsible for TCTP binding.

The equilibrium dissociation constants ($K_D$) between TCTP and the eEF1B CAR domain or the CAR-GEF region estimated by fitting the chemical shift changes during the titration were around 30 µM (Fig. 4C and Table 1), indicating low-to-medium binding affinity between TCTP and eEF1B. ITC experiments showed similar values for the binding affinity of TCTP for the...
eEF1Bδ CAR domain or the CAR-GEF region (Fig. 4D and Table 1). Moreover, the thermodynamic parameters obtained from ITC experiments showed that the binding is dominated by the enthalpy change (Table 2), and the heat capacity change of binding obtained from ITC experiments carried out at different temperatures ranging from 10 to 30 °C was $-272 \pm 49$ cal/mol/K, indicating the removal of solvating water molecules upon binding. Based on the above experimental results, we can conclude that the independent CAR domain is the structural region of eEF1Bδ involved in binding to TCTP.
Conserved Interaction between TCTP and eEF1B

TABLE 1
Dissociation constants for binding of TCTP to different eEF1B\(\delta\) domains

| \(K_D\) values | \(\mu M\) |
|----------------|---------|
| NMR titration |         |
| TCTP titrated with eEF1B\(\delta\) CAR domain | 29 ± 12 |
| TCTP titrated with eEF1B\(\delta\) CAR-GEF region | 30 ± 10 |
| eEF1B\(\delta\) CAR domain titrated with TCTP | 25 ± 10 |
| eEF1B\(\delta\) CAR-GEF region titrated with TCTP | 16.8 ± 9.0 |
| TCTP titrated with eEF1B\(\delta\) CAR domain in high salt buffer | 214 ± 7.8 |
| C172S TCTP titrated with eEF1B\(\delta\) CAR domain | 15.8 ± 8.8 |
| eEF1B\(\delta\) CAR domain titrated with C285/C172S TCTP | 51.8 ± 8.2 |
| TCTP titrated with eEF1B\(\delta\) CAR domain | 25 ± 11 |
| TCTP titrated with eEF1B\(\delta\) CAR-GEF region | 8.2 ± 4.3 |

ITC experiments

| TCTP titrated with eEF1B\(\delta\) CAR domain | 35.3 ± 7.6 |
| TCTP titrated with eEF1B\(\delta\) CAR-GEF region | 12.2 ± 2.0 |

TABLE 2
Thermodynamic parameters for binding of TCTP to the eEF1B\(\delta\) CAR domain measured by ITC

| \(T\) | \(\Delta G\) | \(\Delta H\) | \(\Delta S\) | \(K_D\) | \(\Delta G_p\) |
|-----|---------|---------|---------|-------|---------|
| °C  | cal/mol | cal/mol | cal/mol/K | \(\mu M\) | cal/mol/K |
| 10  | 6633 ± 106 | −3973 ± 185 | 9.3 ± 1.0 | 8.0 ± 1.5 |
| 15  | 6869 ± 195 | −5288 ± 396 | 5.6 ± 2.0 | 6.5 ± 2.2 |
| 20  | 6262 ± 177 | −7717 ± 1714 | 5.0 ± 6.4 | 22.5 ± 6.8 | −272 ± 49 |
| 25  | 6101 ± 128 | −8328 ± 1546 | −7.6 ± 5.6 | 35.3 ± 7.6 |
| 30  | 6074 ± 195 | −9042 ± 1422 | −9.9 ± 5.3 | 44 ± 14 |

TABLE 3
Restraints and structure statistics for 20 lowest energy conformers of free and TCTP-bound eEF1B\(\delta\) CAR domain

| Distance restraints | Free | TCTP-bound |
|---------------------|------|------------|
| Intra-residue        | 102  | 226        |
| Sequential           | 44   | 115        |
| Medium               | 10   | 67         |
| Long range           | 0    | 0          |
| Ambiguous            | 42   | 76         |
| Total                | 198  | 484        |
| Hydrogen bond restraints | 26  | 30        |
| Dihedral angle restraints |    |            |
| \(\phi\)             | 25   | 28         |
| \(\psi\)             | 25   | 27         |
| total               | 50   | 55         |

Violations

| Maximum distance violations (Å) | 0.145 | 0.155 |
| Maximum torsion angle violation (°) | 0 | 0 |

PROCHECK statistics (%)

| Most favored regions | 78.2 | 85.1 |
| Additional allowed regions | 19.9 | 11.8 |
| Generously allowed regions | 1.1 | 0.9 |
| Disallowed regions | 0.9 | 2.1 |

Root mean square deviation from mean structure (Å)

| All residues | Backbone heavy atoms | 6.42 ± 1.20 | 4.14 ± 1.07 |
| All heavy atoms | 6.91 ± 1.21 | 4.44 ± 0.88 |
| Regular secondary structure residues* | 1.49 ± 0.24 | 1.13 ± 0.19 |
| Backbone atoms | 0.54 ± 0.17 | 0.56 ± 0.20 |

* Regular secondary structure regions are residues 169–185 and 168–187 of the eEF1B\(\delta\) CAR domain in free and TCTP-bound states, respectively.

Solution Structure of eEF1B CAR Domain in Free and TCTP-bound States—All peaks in the \(^{1}H\)-\(^{15}N\) HSQC spectrum of the eEF1B\(\delta\) CAR domain, including the significantly overlapped peaks (Lys-185\(_r\), Lys-180\(_g\), Arg-180\(_g\), Glu-176\(_g\), Glu-184\(_g\), and Gln-181\(_g\)), were unambiguously assigned using triple resonance experiments (Fig. 2A). The structure of the eEF1B\(\delta\) CAR domain was calculated based on the nearly complete assignments and various restraints (Table 3). The final structure of the eEF1B\(\delta\) CAR domain (Fig. 5, A and C) shows an \(\alpha\)-helical structure comprising one helix for residues 169–185 and two flexible loops for the residues of both terminal regions. The steady-state \(^{1}H\)-\(^{15}N\) NOE experiment indicated that the \(\alpha\)-helix is a relatively rigid structure indicated by larger NOE values, whereas the flexible loops have smaller NOE values (Fig. 5F). Negatively charged residues are mainly located in the N-terminal loop and the N-terminal part of the \(\alpha\)-helix, whereas positively charged residues are mainly located in the C-terminal loop and the C-terminal part of the \(\alpha\)-helix. Hydrophobic residues are sparsely distributed along the N-terminal loop and the whole \(\alpha\)-helix (Fig. 5C).

To probe the structural changes in the eEF1B\(\delta\) CAR domain upon TCTP binding, we determined the structure of the eEF1B\(\delta\) CAR domain in the TCTP-bound state (Fig. 5, B and D). Comparing the structures of the eEF1B\(\delta\) CAR domain in the bound and free states, the helix in the bound state was extended at both the N and C termini (from residues 169–185 to 168–187), and the N-terminal loop becomes more convergent (Fig. 5, B, D, and E). The flexible-to-rigid transition in the structure was also revealed by the increased heteronuclear steady-state \(^{1}H\)-\(^{1}H\) NOEs of the N-terminal loop and further evidenced by the larger number of observable \(^{1}H\)-\(^{1}H\) NOEs available for use in the structural calculation (Fig. 5F and Table 3). This flexible-to-rigid transition upon TCTP binding was further evidenced by CLEANEX-PM experiments of the eEF1B\(\delta\) CAR-GEF region, which measured the amide-water hydrogen exchange rates (Fig. 5G). The eEF1B\(\delta\) CAR domain showed a significant decrease in the exchange rates upon TCTP binding, whereas the rates of the GEF domain were largely unchanged.

 BINDING SURFACES ON TCTP AND eEF1B CAR DOMAIN—The binding surfaces on TCTP and the eEF1B\(\delta\) CAR domain were determined by mapping the CSPs onto the protein structures (Fig. 6). Structural regions containing residues with significant CSPs (larger than average value plus 1 S.D.) when TCTP was titrated with the eEF1B\(\delta\) CAR domain, and vice versa, were identified as the binding surfaces of the proteins. TCTP contains two binding surfaces as follows: the \(\alpha\)-hairpin region, including helices \(a2\) and \(a3\) of TCTP (TCTP site I), and one side of the \(\beta\)-core (TCTP site II), including loops L165\(_{\beta1}\), L167\(_{\beta2}\), L168\(_{\beta3}\), and strands \(\beta1\), \(\beta2\), \(\beta8\), and \(\beta9\) (Fig. 6, A and B). TCTP site I contains many positively charged residues surrounding a hydrophobic pocket on the surface (Fig. 6, C and G), and TCTP site II is a hydrophobic patch surrounded by a few negatively charged residues in loop L165\(_{\beta3}\) (Fig. 6C).

The eEF1B\(\delta\) CAR domain also has two binding surfaces as follows: residues 155–161 in the N-terminal loop (eEF1B\(\delta\) site I), and residues 179–188 mainly in the \(\alpha\)-helix (eEF1B\(\delta\) site II) (Fig. 6, D and E). EEF1B\(\delta\) site I is highly negatively charged (Fig. 6, F and G) with a few hydrophobic residues (Ile-157\(_g\), Leu-159\(_g\), and Phe-160\(_g\)). This site may undergo a significant conformational/environmental change upon binding because residue Phe-160\(_g\) showed an extraordinarily high CSP value (Fig. 6D). EEF1B\(\delta\) site II contains both hydrophobic and positively charged residues (Fig. 6F). Interestingly, in terms of electrostatic and hydrophobic interactions, eEF1B\(\delta\) sites I and II are complementary with TCTP sites I and II, respectively.
FIGURE 5. Structures of eEF1β CAR domain in free and TCTP-bound states. A and B, backbone ensemble of the 20 lowest energy structures for free (A) and TCTP-bound (B) states. C and D, electrostatic surface for free (C) and TCTP-bound (D) states. E, superimposed structures of free (red) and TCTP-bound (green) eEF1β CAR domains. F, heteronuclear steady-state $^1$H-$^15$N NOEs of free (red) and TCTP-bound (green) eEF1β CAR domain. G, amide-water hydrogen exchange rates $k$ of free (red) and TCTP-bound (green) eEF1β CAR-GEF region determined by CLEANEX-PM experiments.
**FIGURE 6.** Mapping the binding surfaces of TCTP and eEF1β CAR domain. 

A, CSPs of TCTP titrated with eEF1β CAR domain (molar ratio 1:2.2). B, structural mapping of CSPs on TCTP. C, electrostatic surface of TCTP. D, CSPs of eEF1β CAR domain titrated with TCTP (molar ratio 1:2.2). E, structural mapping of CSPs on the eEF1β CAR domain. F, electrostatic surface of the eEF1β CAR domain. G, close-up view of electrostatic surfaces of sites I of TCTP (left) and the eEF1β CAR domain (right). A and D, solid and dashed lines represent the average value and average value plus 1 S.D. of total CSPs, respectively; the blue single and doubles lines on the top indicate the binding sites I and II of each protein, respectively; secondary structure elements are shown on the top. B and E, residues with a CSP value more than the average value plus 1 S.D. are shown in red; those with a CSP value between the average value and average value plus 1 S.D. are shown in pink. Unassigned residues are shown in black. Residues for MTSL labeling in PRE experiments are shown as green sticks with label. The arrow in C indicates the hydrophobic pocket in site I of TCTP.
**Intermolecular Orientation Probed by PRE**—Because structural determination of the TCTP-eEF1Bβ complex was not possible because of the weak interaction between TCTP and eEF1Bβ, the following strategy was adopted to obtain the structure of the complex. First, PRE experiments were conducted to obtain the orientation of the two proteins in the complex. Second, the key residues in the interaction were identified by titration of proteins with various single point mutations on the binding surfaces. Third, HADDOCK combined with CSP, PRE, and mutagenesis data was used to calculate the structure of the complex. (The second and third approaches are described in the subsequent sections.)

For PRE experiments, three residues of TCTP (Thr-116p, Ala-127p, and Asp-143p) were chosen as MTSL-labeling sites to detect interactions with the eEF1Bβ CAR domain. Thr-116p and Ala-127p are located, respectively, at the N- and C-terminal parts of helix H9251 around TCTP site I; and Asp-143p is close to TCTP site II (Figs. 6B and 7B). To further investigate the interactions between the two proteins, five residues of the eEF1Bβ CAR domain were chosen as MTSL-labeling sites as follows: Pro-150p and Ala-191p at the N and C termini, respectively, and Lys-169p, Glu-177p, and Lys-186p in the α-helix (Figs. 6E and 7A). MTSL-labeled T116Cp caused attenuation of the signals.
Conserved Interaction between TCTP and eEF1B

from the eEF1Bδ site I (Fig. 7, C and D), whereas MTSL-labeled A127Cp affected the N-terminal residues in the HSQC spectrum of the eEF1Bδ CAR domain (Fig. 7, E and F). Signals from residues at eEF1Bδ site II were broadened by MTSL-labeled D143Cp (Fig. 7, G and H), whereas MTSL-labeled K169Cδ caused specific signal attenuation for residues from TCTP site I (Fig. 7, I and J). The PRE effect of MTSL-labeled E177Cp was observed as signal attenuation of residues located between the N-terminal loop of the eEF1Bδ CAR domain (eEF1Bδ CAR site I) and TCTP titrated with TCTP (TCTP site II), whereas the N terminus of the eEF1Bδ CAR domain is toward the C terminus of helix α3 of the α-hairpin of TCTP. The α-helix of the eEF1Bδ CAR domain (eEF1Bδ site II) is close to the β-core of TCTP (TCTP site II), whereas the region connecting eEF1Bδ sites I and II is close to the structural region between the α-hairpin and the β-core of TCTP.

Key Residues Drive Interaction between TCTP and eEF1Bδ CAR Domain—According to the above data, electrostatic and/or hydrophobic interactions may drive the binding of the eEF1Bδ CAR domain to TCTP. The question is whether hydrophobic or charged residues play the most important role in the interaction. NMR titration performed with mutant proteins containing various single mutations in the binding site of each protein (Table 4 and Fig. 8) was adopted to identify the key residues for binding in the two proteins.

The hydrophobic pocket of TCTP site I could accommodate the hydrophobic residues of eEF1Bδ site I. Consistent with this, mutants L159Aδ, F160Aδ, M115Dδ, and A118Dδ showed no binding with their wild-type binding partner, whereas M96Aδ and A119Eδ did not change the binding, showing KD values similar to that of wild type. It is likely that residues Leu-159δ and Phe-160δ of the eEF1Bδ CAR domain insert into the hydrophobic pocket of TCTP site I, because Phe-160δ showed an extraordinarily high CSP value in the NMR titration. We confirmed that Phe-160δ indeed plays a vital role in the interaction, because replacement of phenylalanine with leucine or tyrosine instead of alanine at residue 160 also prevents binding between the two proteins. In addition, mutants I92Aδ, M96Nδ, A119Kδ, I122Aδ, and I157Aδ as well as G161Kδ decreased the binding affinity about 3–30-fold. Meanwhile, mutation of charged residues D158Kδ, K90Qδ, K93Qδ, K97Qδ, and K123Eδ caused a severalfold decrease in affinity, whereas mutants D155Kδ, D156Kδ, S162Kδ, D163Kδ, and E86Qδ showed KD values similar to that of wild type (Table 4). In TCTP site II and eEF1Bδ site II, mutations of hydrophobic residues Y182Aδ, F83Aδ, and M140Aδ decreased binding affinities by severalfold, whereas L179Aδ and P142Aδ showed affinities similar to that of wild type. Analysis of TCTP CSP values caused by Y182Aδ and eEF1Bδ CSP values caused by F83Aδ and M140Aδ revealed that most residues in TCTP site II and eEF1Bδ site II failed to show significant chemical shift changes. Meanwhile, mutations of charged residues K186Dδ and K189Dδ showed almost no effect on

**FIGURE 8.** Mutation sites on the structure of human TCTP (A) and the human eEF1Bδ CAR domain (B). The side chains of mutated residues are shown as sticks. Neutral, positively charged, and negatively charged residues are shown in green, blue, and red, respectively.

### TABLE 4

| TCTP titrated with eEF1Bδ CAR domain | eEF1Bδ CAR domain titrated with TCTP | KD, μM |
|--------------------------------------|--------------------------------------|--------|
| WT                                   | WT                                   | 29 ± 12 | 25 ± 10 |
| D155K                                | D83A                                 | 30 ± 24 | 97 ± 16 |
| D156K                                | E86Q                                 | 18.6 ± 7.4 | 17 ± 12 |
| I157A                                | K89Q                                 | 205 ± 68 | 138 ± 66 |
| D158K                                | K90Q                                 | 203 ± 84 | 72 ± 22 |
| L159A                                | Not detected                         | 251 ± 62 |
| F160A/L/Y                            | F160A/L/Y                            | 693 ± 260 |
| G161K                                | D94Q                                 | 190 ± 24 | 8.0 ± 4.6 |
| S162K                                | M96A                                 | 14 ± 11 | 13.2 ± 4.2 |
| D163K                                | M96N                                 | 20 ± 15 | 792 ± 125 |
| E165A                                | K97Q                                 | 30 ± 13 | 111 ± 19 |
| E166A                                | M115D                                | 13.0 ± 8.5 | Not detected |
| E167K                                | I118D                                | 149 ± 75 | Not detected |
| E170A                                | I119E                                | 12.8 ± 6.5 | 31 ± 10 |
| E176K                                | I119K                                | 13.2 ± 7.1 | 60 ± 10 |
| R178D                                | I122A                                | 49 ± 28 | 90 ± 19 |
| L179A                                | K123E                                | 7.1 ± 4.1 | 231 ± 91 |
| Y182A                                | M140A                                | 91 ± 33 | 60 ± 13 |
| K186D                                | P142A                                | 40 ± 16 | 21.5 ± 6.9 |
| K189D                                | Not detected                         | 26 ± 15 |
| EDDELDILFGSDNE                       | 307 ± 86                             | \(>1000\) |

(continued)
the interaction. All these data suggest that hydrophobic rather than electrostatic interactions between the binding sites on the two proteins play a crucial role in the binding, and the eEF1Bβ site I is critical for binding with TCTP. However, the binding affinity in 400 mM NaCl decreased about 7-fold compared with that in 200 mM NaCl (30 versus 214 M) (Table 1). Therefore, hydrophobic interactions dominate the binding between TCTP and the eEF1Bβ CAR domain, whereas electrostatic interactions also contribute to the binding affinity.

To further confirm the importance of eEF1Bβ site I for interaction with TCTP, TCTP was titrated with peptides EDD-DIDLFGSDNE, DLFGS, and LFG corresponding to residues 153–165 (containing all residues of eEF1Bβ site I), 158–162, and 159–161 of the eEF1Bβ CAR domain, respectively. All peptides showed interaction with site I of TCTP, and longer peptides showed stronger affinity (Table 4). Therefore, the two hydrophobic residues Leu-159 and Phe-160 in cooperating with other residues from eEF1Bβ site I play key roles in the binding.

**Structural Model of the TCTP-eEF1Bβ CAR Domain Complex**—The structural model of the TCTP-eEF1Bβ CAR domain complex was determined by HADDOCK docking computation (34). The structural model obtained demonstrates a large buried interacting surface area (2337.4 ± 87.2 Å²) (Table 5 and Fig. 9A). The two hydrophobic residues Leu-159β and Phe-160β in eEF1Bβ site I insert into the hydrophobic pocket of TCTP site I between the two helices α2 and α3, although surrounding charged residues form salt bridges (Fig. 9B). Tyr-182β of eEF1Bβ site II contacts with two hydrophobic residues Phe-83p and Met-140p of TCTP site II (Fig. 9C). The helix of the eEF1Bβ CAR domain forms 119.6 ± 3.3 and 49.8 ± 2.7° angles with the helices α2 and α3 of TCTP, respectively.

The docking model of the complex of TCTP and the eEF1Bβ CAR domain shows continuous interacting surfaces, including sites I and II as well as the region between the two sites of each protein. A number of charged residues in the region form salt

---

**TABLE 5**

Statistics for the complex of TCTP and the eEF1Bβ CAR domain obtained by HADDOCK docking

| No. of clusters | 3 |
|-----------------|---|
| Cluster         | 1st 2nd |
| Structure number| 179 6 |
| HADDOCK score   | -206.7 ± 4.1 -172.9 ± 3.9 |
| RMSD from lowest energy structure | 1.2 ± 0.7 2.5 ± 0.2 |
| Restraints violation energy | 138 ± 36 160 ± 49 |
| Buried surface area | 2337 ± 87 2212 ± 121 |
| Z-score         | -1.2 -0.1 |
| PROCHECK statistics (%) | 83.3 83.6 |
| Most favored regions | 16.1 14.5 |
| Generously allowed regions | 0.2 1.1 |
| Disallowed regions | 0.5 0.8 |

---

**FIGURE 9. Structure of TCTP-eEF1Bβ CAR domain complex obtained by HADDOCK.** A, ribbon representation of the structure of the TCTP-eEF1Bβ CAR domain complex. TCTP and the eEF1Bβ CAR domain are colored in green and cyan, respectively. B, close-up view of the site I-binding sites of both proteins in the complex. C, close-up view of the site II-binding sites of both proteins in the complex. In the complex, TCTP is represented as electrostatic surfaces, in which positively and negatively charged surfaces are in blue and red, respectively; the eEF1Bβ CAR domain is represented as cyan ribbons and the side chains of residues contacting with TCTP are shown as sticks (positively charged, negatively charged, and neutral residues are in blue, red, and gray, respectively). Residues of TCTP and the eEF1Bβ CAR domain are labeled in green and red, respectively.
Conserved Interaction between TCTP and eEF1B

bridges, including Lys-97<sub>p</sub>–Glu-167<sub>δ</sub>–Lys-93<sub>ρ</sub>–Glu-170<sub>ρ</sub>, Lys-90<sub>ρ</sub>–Glu-177<sub>δ</sub>, Lys-100<sub>ρ</sub>–Glu-165<sub>ρ</sub>, and Asp-94<sub>ρ</sub>–Arg-178<sub>δ</sub>, contributing to the buried binding interface. To confirm the role of these residues in the binding, a number of mutants were constructed and used for NMR titration. Mutants including E165A<sub>ρ</sub>, E166A<sub>ρ</sub>, E170A<sub>ρ</sub>, E176K<sub>ρ</sub>, and D94Q<sub>ρ</sub>, showed K<sub>D</sub> values similar to wild type, and E167K<sub>ρ</sub>, R178D<sub>ρ</sub>, and K90Q<sub>ρ</sub> showed a decrease in affinity of severalfold (Table 4). This demonstrates that electrostatic interactions in the region between sites I and II of each protein, forming a continuous interacting surface together with sites I and II of each protein, also contribute to the binding affinity.

The structure model of the complex is in agreement with the thermodynamic parameters obtained from ITC experiments. The large buried interaction surface area in the structure of the complex suggests that a significant decrease in hydration should occur during binding, which is in agreement with the large negative value of the heat capacity change estimated by ITC experiments (Table 2). A large number of electrostatic interactions in the structure of the complex is consistent with the dominant enthalpy change observed in ITC experiments. The apparent thermodynamic parameters obtained from the ITC experiments also reveal that the entropy change is relatively small. Although hydrophobic interactions and dehydration during binding will result in a positive entropy change, the significant change in dynamics (flexible-to-rigid) of the eEF1β CAR domain upon binding to TCTP produces a negative entropy change, which results in the small total entropy change.

Interaction of TCTP and eEF1B GEFs Is Conserved in Eukaryotes—TCTP is highly conserved in eukaryotes. Sequence alignment of TCTPs (Fig. 10A) showed the key residues for binding eEF1β are largely conserved in various species. Four of five hydrophobic residues (Ile-92<sub>ρ</sub>, Met-96<sub>ρ</sub>, Met-115<sub>ρ</sub>, Ala-118<sub>ρ</sub>, and Ile-122<sub>ρ</sub>) except Met-115 in human TCTP site I are hydrophobic residues in all TCTPs. The positively charged residues are also largely conserved, particularly Lys-93<sub>ρ</sub>, whose mutation causes the most significant affinity loss with eEF1β. CAR domain is a completely conserved in TCTPs. In TCTP site II, the two key hydrophobic residues (Phe-83 and Met-140 in human TCTP) are always hydrophobic residues in TCTPs. The sequence alignment of various GEFs in the eEF1B complex (including eEF1Bα, which exists in all eukaryotes, eEF1Bβ, which exists only in metazoans, and eEF1Bβ, which exists only in plants (Figs. 1 and 10B)) indicates that all of these proteins have the conserved CAR domain at the N terminus of the GEF domain. Furthermore, the key residues Leu-Phe and surrounding negatively charged residues in eEF1β site I are completely conserved in all eEF1B GEF CAR domains. In eEF1β site II, the hydrophobic residue Tyr-182<sub>δ</sub> is largely but not completely conserved in all CAR domains. Therefore, both site I of TCTP and site I of all eEF1B GEF CAR domains are conserved in eukaryotes, although site II of each of the proteins is less well conserved. Because it is site I in each protein that is crucial for the interaction, we can therefore speculate that the interaction between TCTP and eEF1B GEFs is conserved in all eukaryotes.

To test the conservation of the interaction between TCTP and eEF1B GEFs, we first checked the interaction between human TCTP and eEF1Bα. NMR titration experiments showed that the CAR domain and the CAR-GEF region of eEF1Bα interact with TCTP, and the CSPs of TCTP and K<sub>D</sub> values were very similar to those from the titration experiments with eEF1β (Fig. 10, C–F). These results demonstrate that TCTP interacts with eEF1Bα at the same sites as with eEF1Bβ. Such interactions were also detected for TCTP and eEF1Bα from lower eukaryotes, including the fission yeast S. pombe and the uni-cellular photosynthetic microalga N. oceanica (Fig. 11). The binding sites on fission yeast TCTP identified by CSP mapping, according to the previous chemical shift assignments of fission yeast TCTP (35), include both site I and site II, the same as the sites on human TCTP. The binding affinities (112 ± 18 μM for S. pombe and 38.4 ± 9.0 μM for N. oceanica) derived from NMR titration are similar (slightly lower) to those for human TCTP-eEF1Bα interactions. These results imply that the interaction between TCTP and eEF1B GEFs is conserved in all eukaryotes.

DISCUSSION

In this study, we demonstrate that the CAR domain of eEF1Bβ (residues 153–192), which is structurally independent of the C-terminal GEF domain, is responsible for binding to TCTP through conserved hydrophobic and electrostatic interactions. The interactions are conserved for TCTP and all GEFs of the eEF1B complex, including eukaryotically conserved eEF1Bα and plant-specific eEF1Bβ. Thus, the CAR domain is a pivotal region for the regulation of different eEF1B subunits in performing GEF activity, and the involvement of TCTP in the protein translation machinery likely represents one of the primary cellular functions of TCTP in all eukaryotes.

The finding that TCTP binds to the CAR domain instead of the GEF domain of eEF1Bβ raises the question of how TCTP inhibits the GEF activity of eEF1Bβ. According to the structure of the eEF1Bα-eEF1A complex (Protein Data Bank codes 1F60 and 1JIF) (16, 17), the catalytic residues of eEF1Bα are located at the C terminus, and a conserved lysine residue at the second position from the C terminus of eEF1Bα disrupts the interaction of Mg<sup>2+</sup> with eEF1A and GDP, resulting in the release of Mg<sup>2+</sup> and GDP from eEF1A. The C and N termini of the eEF1Bα GEF domain form an antiparallel β-sheet; thus, the CAR domain, which is at the N terminus of the GEF domain, is also spatially close to the GDP/Mg<sup>2+</sup>-binding site of eEF1A domain I. When the 20-kDa TCTP protein binds to the CAR domain of eEF1Bα, TCTP will likely impede the release of GDP by steric hindrance. This inhibition mechanism is different from classic guanine nucleotide dissociation inhibitors that inhibit the nucleotide release from GTPases by competing with the nucleotide exchange factors (36, 37).

Our data demonstrate that the α-hairpin-containing site I of TCTP plays a key role in the interaction with eEF1B GEFs. It has been proposed that the α-hairpin of TCTP plays a key role in many interactions and functions of TCTP (9, 12, 38–41). However, all of these reports lack structural information about the interaction, and some of them are contradictory. For example, several researchers reported that TCTP physically interacts with p53 (9, 39, 42). However, one paper reported that p53 binds to the α-hairpin of TCTP (39), although another paper reported that p53 binds to the N- and C-terminal regions of TCTP (42). All of them used fragments of TCTP to detect the
interacting regions, which are probably problematic because structural studies indicate that TCTP is a single domain protein and fragments are unlikely to fold well or reflect the real interactions of the intact protein (6, 18, 22). Many other studies of TCTP-protein interactions also used fragments of TCTP to identify the binding regions (12, 38, 43–47), and most of them assume a “three-domain” view of TCTP consisting of an N-terminal domain, a central helical domain and a C-terminal domain. The binding regions identified in these studies probably also need further confirmation by NMR or crystallographic methods using intact TCTP because the fragments may be incorrectly folded or unfolded.

The conserved interaction of TCTP with the eEF1B complex suggests the involvement of TCTP in the protein translation
FIGURE 11. Interaction of eEF1α CAR domain and TCTP in fission yeast *S. pombe* and photosynthetic microalga *N. oceanica*. A, ¹H-¹⁵N HSQC spectra of *S. pombe* TCTP (SpTCTP) titrated with *S. pombe* eEF1α CAR domain. B, CSPs of SpTCTP. Solid and dashed lines represent the average value and average value plus 1 S.D. of total CSPs, respectively. C, titration curve fitting of CSPs of SpTCTP to obtain dissociation constants. D, structural mapping of CSPs on SpTCTP. Residues with a CSP value more than the average value plus 1 S.D. are shown in red; those with a CSP value between the average value and average value plus 1 S.D. are shown in pink. E, ¹H-¹⁵N HSQC spectra of *N. oceanica* TCTP (NoTCTP) titrated with *N. oceanica* eEF1α CAR domain. F, dissociation constants obtained by fitting the curves from the NMR titration of NoTCTP.
machinery. In fact, by carefully analyzing the literature, we found several additional pieces of evidence for the involvement of TCTP in protein translation. In 2000, before discovery of the interaction between TCTP and the eEF1 complex, Brown et al. (48) used support vector machines to classify budding yeast genes based on microarray gene expression, which classified TCTP as the cytoplasmic ribosome class, suggesting that TCTP expression is co-regulated with the ribosome. Later, in 2006, Fleischer et al. (49) screened 77 uncharacterized proteins, including TCTP, associated with the ribosome in yeast. Recently, Atkinson et al. (50) reported that eEF1A and eEF1B are not completely conserved in eukaryotes, and some species contain an EF1A-like protein (EFL) that replaces eEF1A and eEF1B. Interestingly, when we searched TCTP homologues in these eEF1A-lacking species, we found that none of them contains a TCTP-homologue gene, except *Emiliania huxleyi*. For those “intermediate” species containing both EFL and eEF1A (11 species reported), most of them lack both eEF1B and TCTP, except the following three species: *Symbiodinium* sp. CladeC that contains a TCTP homologue only and *Guillardia theta* and *Thecamonas trahens* that contain both eEF1B and TCTP homologues. Therefore, it is likely that TCTP and the eEF1A-eEF1B complex have co-evolved, which suggests that they are tightly correlated in function.

Our study demonstrates for the first time that TCTP is involved in a conserved eukaryotic cellular function by interacting with GEFs of the eEF1B complex. The structural and interaction data provide insight into the mechanism of TCTP function. Many interactions have been reported for TCTP without structural information. Our study provides a paradigm for further studies of the structural mechanism of these interactions.

Acknowledgments—NMR experiments were carried out at the Core Facility for Protein Research, Institute of Biophysics, Chinese Academy of Sciences, and the Beijing NMR Center or the NMR Facility of the National Center for Protein Sciences at Peking University. We thank Dr. Xuelui Liu and Dr. Yuanyuan Chen, both from the Core Facility for Protein Research, Institute of Biophysics, for help with CLEANEX-PM and ITC experiments, respectively.

REFERENCES

1. Norbeck, J., and Blomberg, A. (1997) Two-dimensional electrophoretic separation of yeast proteins using a nonlinear wide range (pH 3–10) immobilized pH gradient in the first dimension; reproducibility and evidence for isoelectric focusing of alkaline (pI > 7) proteins. *Yeast* 13, 1519–1534
2. Velculescu, V. E., Madden, S. L., Zhang, L., Lash, A. E., Yu, J., Rago, C., Lal, A., Wang, C. J., Beaudry, G. A., Ciriello, K. M., Cook, B. P., Dufault, M. R., Ferguson, A. T., Gao, Y., He, T. C., et al. (1999) Analysis of human transcripts. *Nat. Genet.* 23, 387–388
3. Thiele, H., Berger, M., Skalweit, A., and Thiele, B. J. (2000) Expression of the gene and processed pseudogenes encoding the human and rabbit translationally controlled tumour protein (TCTP). *Eur. J. Biochem.* 267, 5473–5481
4. Thompson, H. G., Harris, J. W., Wold, B. J., Quake, S. R., and Brody, J. P. (2002) Identification and confirmation of a module of coexpressed genes. *Genome Res.* 12, 1517–1522
5. Bommer, U. A., and Thiele, B. J. (2004) The translationally controlled tumour protein (TCTP). *Int. J. Biochem. Cell Biol.* 36, 379–385
6. Feng, Y., Liu, D., Yao, H., and Wang, J. (2007) Solution structure and mapping of a very weak calcium-binding site of human translationally controlled tumor protein by NMR. *Arch. Biochem. Biophys.* 467, 48–57
7. Kawakami, T., Ando, T., and Kawakami, Y. (2012) HRF-interacting molecules. *Open Allergy J.* 5, 41–46
8. Bommer, U.-A. (2012) Cellular function and regulation of the translationally controlled tumour protein TCTP. *Open Allergy J.* 5, 19–32
9. Amson, R., Pece, S., Lespagnol, A., Vyas, R., Mazzarol, G., Tosoni, D., Colaluca, I., Viale, G., Rodrigues-Ferreira, S., Wyndaele, J., Chaloin, O., Hoebeke, J., Marine, J. C., Di Fiore, P. P., and Telerman, A. (2012) Reciprocal repression between P53 and TCTP. *Nat. Med.* 18, 91–99
10. Cans, C., Passer, B. J., Shalak, V., Nancy-Portebois, V., Crible, V., Amzallag, N., Allanic, D., Tufino, R., Argentini, M., Moras, D., Fiucci, G., Goud, B., Mirande, M., Amson, R., and Telerman, A. (2003) Translationally controlled tumour protein acts as a guanine nucleotide dissociation inhibitor on the translation elongation factor eEF1A. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13892–13897
11. Langdon, J. M., Vonakis, B. M., and MacDonald, S. M. (2004) Identification of the interaction between the human recombinant histamine releasing factor/translationally controlled tumor protein and elongation factor-1β (also known as elongation factor-1B). *Biochim. Biophys. Acta* 1688, 232–236
12. Rid, R., Onder, K., Trost, A., Bauer, J., Hintner, H., Ritter, M., Jakab, M., Costa, I., Reichsl, W., Richter, K., MacDonald, S., Jendrach, M., Berreite-Hahn, J., and Breitenbach, M. (2010) H2O2-dependent translocation of TCTP into the nucleus enables its interaction with VDR in human keratinocytes: TCTP as a further module in calcitriol signalling. *J. Steroid Biochem. Mol. Biol.* 118, 29–40
13. Le Sourd, F., Boulben, S., Le Bouffant, R., Cormier, P., Morales, J., Belle, R., and Mulner-Lorillon, O. (2006) eEF1B: At the dawn of the 21st Century. *Biochim. Biophys. Acta* 1759, 13–31
14. Sasikumar, A. N., Perez, W. B., and Kinzy, T. G. (2012) The many roles of the eukaryotic elongation factor 1 complex. *WIREs RNA* 3, 543–555
15. Pérez, J. M., Siegal, G., Kriek, J., Hard, K., Dijk, J., Canters, G. W., and Möller, W. (1999) The solution structure of the guanine nucleotide exchange domain of human elongation factor 1β reveals a striking resemblance to that of EF-Ts from *Escherichia coli*. *Structure* 7, 217–226
16. Andersen, G. R., Pedersen, L., Valente, L., Chatterjee, I., Kinzy, T. G., Kjeldgaard, M., and Nyborg, J. (2000) Structural basis for nucleotide exchange and competition with tRNA in the yeast elongation factor complex eEF1A-eEF1Bα. *Mol. Cell.* 6, 1261–1266
17. Andersen, G. R., Valente, L., Pedersen, L., Kinzy, T. G., and Nyborg, J. (2001) Crystal structures of nucleotide exchange intermediates in the eEF1A-eEF1B complex. *Nat. Struct. Biol.* 8, 531–534
18. Thaw, P., Baxter, N. I., Houslow, A. M., Price, C., Waltho, J. P., and Craven, C. J. (2001) Structure of TCTP reveals unexpected relationship with guanine nucleotide-free chaperones. *Nat. Struct. Biol.* 8, 701–704
19. Dong, X., Yang, B., Li, Y., Zhong, C., and Ding, J. (2009) Molecular basis of the acceleration of the GDP-GTP exchange of human ras homolog enriched in brain by human translationally controlled tumor protein. *J. Biol. Chem.* 284, 23754–23764
20. Vedadi, M., Lew, J., Artz, I., Amani, M., Zhao, Y., Dong, A., Wasney, G. A., Gao, M., Hills, T., Brokks, S., Qiu, W., Sharma, S., Diassiti, A., Alam, Z., Melone, M., et al. (2007) Genom-scale protein expression and structural biology of *Plasmodium falciparum* and related Apicomplexan organisms. *Mol. Biochem. Parasitol.* 151, 100–110
21. Eichhorn, T., Winter, D., Büchel, B., Dirdjaja, N., Frank, M., Lehmann, W. D., Mertens, R., Krauth-Siegel, R. L., Simmet, T., Granzin, J., and Efert, T. (2013) Molecular interaction of artemisinin with translationally controlled tumor protein (TCTP) of *Plasmodium falciparum*. *Biochem. Pharmacol.* 85, 38–45
22. Susini, L., Besse, S., Duflaut, D., Lespagnol, A., Beekman, C., Fiucci, G., Atkinson, A. R., Basso, D., Poussin, P., Marine, J. C., Martinou, J. C., Cavarelli, J., Moras, D., Amson, R., and Telerman, A. (2008) TCTP protects from apoptotic cell death by antagonizing bax function. *Cell Death Differ.* 15, 1211–1220
23. Hemsley, A., Arnheim, N., Toney, M. D., Cortopassi, G., and Galas, D. J. (1989) A simple method for site-directed mutagenesis using the polymerase chain reaction. *Nucleic Acids Res.* 17, 6545–6551
24. Delaglio, F., Grzesiek, S., Vuister, G. W., Zhu, G., Pfeifer, J., and Bax, A.
Conserved Interaction between TCTP and eEF1B

(1995) NMRpipe: a multidimensional spectral processing system based on UNIX pipes. J. Biol. NMR 6, 277–293
25. Johnson, B. A., and Blevins, R. A. (1994) NMR View: A computer program for the visualization and analysis of NMR data. J. Biol. NMR 4, 603–614
26. Hwang, T. L., van Zijl, P. C., and Mori, S. (1998) Accurate quantification of water-amide proton exchange rates using the phase-modulated CLEAN chemical EXchange (CLEANEX-PM) approach with a Fast-HSQC (FHSQC) detection scheme. J. Biol. NMR 11, 221–226
27. Battiste, J. L., and Wagen, G. (2000) Utilization of site-directed spin labeling and high-resolution heteronuclear nuclear magnetic resonance for global fold determination of large proteins with limited nuclear overhauser effect data. Biochemistry 39, 5355–5365
28. Herrmann, T., Güntert, P., and Wüthrich, K. (2002) Protein NMR structure determination with automated NOE assignment using the new software CANDID and the torsion angle dynamics algorithm DYANA. J. Mol. Biol. 319, 209–227
29. Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, S. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) Crystallography & NMR system: A new software suite for macromolecular structure determination. Acta Crystallogr. D Biol. Crystallogr. 54, 905–921
30. Nederpen, A. J., Doreleijers, J. F., Vranken, W., Miller, Z., Spronk, C. A., Nabuurs, S. B., Güntert, P., Livny, M., Markley, J. L., Nilges, M., Ulrich, E. L., Kaptein, R., and Bonvin, A. M. (2005) RECOORD: A recalculated coordinate database of 500+ proteins from the PDB using restraints from the BioMagResBank. Proteins 59, 662–672
31. Shen, Y., Delaglio, F., Cornilescu, G., and Bax, A. (2009) TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. J. Biol. NMR 44, 213–223
32. Konardi, R., Billeter, M., and Wüthrich, K. (1996) MOLMOL: a program for display and analysis of macromolecular structures. J. Mol. Graph. 14, 51–55
33. Laskowski, R. A., Rullmann, J. A., MacArthur, M. W., Kaptein, R., and Thornton, J. M. (1996) AQUA and PROCHECK–NMR: programs for checking the quality of protein structures solved by NMR. J. Biol. NMR 8, 477–486
34. de Vries, S. I., van Dijk, M., and Bonvin, A. M. (2010) The HADDOCK web server for data-driven biomolecular docking. Nat. Protoc. 5, 883–897
35. Baxter, N. J., Thaw, P., Higgins, L. D., Sedelnikova, S. E., Bramley, A. L., Price, C., Waltho, J. P., and Craven, C. J. (2000) Backbone NMR assignment of the 19-kDa translationally controlled tumor-associated protein p23fyp from Schizosaccharomyces pombe. J. Biol. NMR 16, 83–84
36. Olofsen, B. (1999) Rho guanine dissociation inhibitors: pivotal molecules in cellular signalling. Cell. Signal. 11, 545–554
37. Scheffzek, K., Stephan, I., Jensen, O. N., Illenberger, D., and Gierschik, P. (2000) The Rac-RhoGDI complex and the structural basis for the regulation of Rho proteins by RhoGDI. Nat. Struct. Biol. 7, 122–126
38. Gachet, Y., Tournier, S., Lee, M., Lazaris-Karatzas, A., Poulton, T., and Bommer, U. A. (1999) The growth-related, translationally controlled protein P23 has properties of a tubulin binding protein and associates transiently with microtubules during the cell cycle. J. Cell Sci. 112, 1257–1271
39. Rho, S. B., Lee, J. H., Park, M. S., Byun, H. J., Kang, S., Seo, S. S., Kim, J. Y., and Park, S. Y. (2011) Anti-apoptotic protein TCTP controls the stability of the tumor suppressor p53. FEBS Lett. 585, 29–35
40. Funston, G., Goh, W., Wei, S. I., Tng, Q. S., Brown, C., Iah Tong, L., Verma, C., Lane, D., and Ghadessy, F. (2012) Binding of translationally controlled tumour protein to the N-terminal domain of HDM2 is inhibited by nutlin-3. PLoS One 7, e42642
41. Kashiwakura, J. C., Ando, T., Matsumoto, K., Kimura, M., Kitaura, J., Ma-tho, M. H., Zajonc, D. M., Ozeki, T., Ra, C., MacDonald, S. M., Siragianian, R. P., Broide, D. H., Kawakami, Y., and Kawakami, T. (2012) Histamine-releasing factor has a proinflammatory role in mouse models of asthma and allergy. J. Clin. Invest. 122, 218–228
42. Chen, Y., Fujita, T., Zhang, D., Doan, H., Pinkaew, D., Liu, Z., Wu, J., Koide, Y., Chiu, A., Lin, C. C., Chang, J. Y., Ruan, K. H., and Fujise, K. (2011) Physical and functional antagonism between tumor suppressor protein p53 and fortilin, an anti-apoptotic protein. J. Biol. Chem. 286, 32575–32585
43. Kim, M., Jung, Y., Lee, K., and Kim, C. (2000) Identification of the calcium binding sites in translationally controlled tumor protein. Arch. Pharm. Res. 23, 633–636
44. Yang, Y., Yang, F., Xiong, Z., Yan, Y., Wang, X., Nishino, M., Mirkovic, D., Nguyen, J., Wang, H., and Yang, X. F. (2005) An N-terminal region of translationally controlled tumor protein is required for its antiapoptotic activity. Oncogene 24, 4778–4788
45. Jung, J., Kim, M., Kim, M. J., Kim, J., Moon, J., Lim, S. J., Kim, M., and Lee, K. (2004) Translationally controlled tumor protein interacts with the third cytoplasmic domain of Na,K-ATPase α subunit and inhibits the pump activity in HeLa cells. J. Biol. Chem. 279, 49888–49875
46. Hong, S. T., and Choi, K. W. (2013) TCTP directly regulates ATM activity to control genome stability and organ development in Drosophila melanogaster. Nat. Commun. 4, 2986
47. Zhang, F., Liu, B., Wang, Z., Yu, X. J., Ni, Q. X., Yang, W. T., Mukaida, N., and Li, Y. Y. (2013) A novel regulatory mechanism of Pim-3 kinase stability and its involvement in pancreatic cancer progression. Mol. Cancer Res. 11, 1508–1520
48. Brown, M. P., Grundy, W. N., Lin, D., Cristianini, N., Sugnet, C. W., Farey, T. S., Ares, M., Jr., and Haussler, D. (2000) Knowledge-based analysis of microarray gene expression data by using support vector machines. Proc. Natl. Acad. Sci. U.S.A. 97, 262–267
49. Fleischer, T. C., Weaver, C. M., McAfee, K. J., Jennings, J. L., and Link, A. J. (2006) Systematic identification and functional screens of uncharacterized proteins associated with eukaryotic ribosomal complexes. Genes Dev. 20, 1294–1307
50. Atkinson, G. C., Kuzmenko, A., Chicherin, I., Soosaar, A., Tenson, T., Carr, M., Kamenski, P., and Hauri, V. (2014) An evolutionary ratchet leading to loss of elongation factors in eukaryotes. BMC Evol. Biol. 14, 35
Protein Structure and Folding: Evolutionarily Conserved Binding of Translationally Controlled Tumor Protein to Eukaryotic Elongation Factor 1B

Huiwen Wu, Weibin Gong, Xingzhe Yao, Jinfeng Wang, Sarah Perrett and Yingang Feng

J. Biol. Chem. 2015, 290:8694-8710.
doi: 10.1074/jbc.M114.628594 originally published online January 29, 2015

Access the most updated version of this article at doi: 10.1074/jbc.M114.628594

Find articles, minireviews, Reflections and Classics on similar topics on the JBC Affinity Sites.

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 50 references, 10 of which can be accessed free at http://www.jbc.org/content/290/14/8694.full.html#ref-list-1