Translationally controlled tumor protein acts as a guanine nucleotide dissociation inhibitor on the translation elongation factor eEF1A

Christophe Cans*†, Brent J. Passer*†, Vyacheslav Shalak‡, Vanessa Nancy-Portebois*, Virginie Crible*, Nathalie Amzallag*, David Allanic*, Rowena Tufino*, Manuela Argentini§, Dino Moras§, Giusy Fiucci*, Bruno Goud¶, Marc Mirande‡, Robert Amson*, and Adam Telerman*‖

*Molecular Engines Laboratories, 20 Rue Bouvier, 75011 Paris, France; ‡Laboratoire d'Enzymologie et Biochimie Structurales, Centre National de la Recherche Scientifique-Unité Propre de Recherche 9063, 1 Avenue de la Terrasse, 91990 Gif-sur-Yvette, France; §Institut de Genetique et de Biologie Moleculaire et Cellulaire, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur, 1 Rue Laurent Fries, BP 163, 67404 Illkirch Cedex, France; and ¶Institut Curie, Centre National de la Recherche Scientifique-Unité Mixte de Recherche 144, 26 Rue d'Ulm, 75248 Paris Cedex 05, France

Communicated by Georges Charpak, European Organization for Nuclear Research, Geneva, Switzerland, September 16, 2003 (received for review August 1, 2003)

Recently, we demonstrated that the expression levels of the translationally controlled tumor protein (TCTP) were strongly down-regulated at the mRNA and protein levels during tumor reversion/suppression and by the activation of p53 and Siah-1. To better characterize the function of TCTP, a yeast two-hybrid hunt was performed. Subsequent analysis identified the translation elongation factor, eEF1A, and its guanine nucleotide exchange factor, eEF1Bβ, as TCTP-interacting partners. *In vitro* and *in vivo* studies confirmed that TCTP bound specifically eEF1Bβ and eEF1A. Additionally, MS analysis also identified eEF1A as a TCTP interactor. Because eEF1A is a GTPase, we investigated the role of TCTP on the nucleotide exchange reaction of eEF1A. Our results show that TCTP preferentially stabilized the GDP form of eEF1A, and, furthermore, impaired the GDP exchange reaction promoted by eEF1Bβ. These data suggest that TCTP has guanine nucleotide dissociation inhibitor activity, and, moreover, implicate TCTP in the elongation step of protein synthesis.

Translationally controlled tumor protein (TCTP), also termed p23, is ubiquitously expressed and is present in evolutionarily diverse organisms. TCTP was initially identified in Ehrlich ascites tumor cells, as a serum-inducible mRNA whose expression is regulated at both the transcriptional and translational levels (1–4). Biochemical and immunofluorescence studies demonstrated that TCTP is a tubulin-binding protein that associates with microtubules in a cell-cycle dependent fashion (5). Recently, the polo-like kinase was shown to directly interact with and phosphorylate TCTP and was shown to be required for the normal progression of cytokinesis (6). In addition, TCTP binds the myeloid cell leukemia 1 protein, which is involved in programmed cell death (7, 8). Importantly, TCTP has also been characterized as the histamine-releasing factor (9).

Recently, a series of biological models of tumor reversion have been developed that have aided in understanding some of the molecular events underlying tumor reversion (10). Comparing gene expression profiles from leukemia and breast cancer cell lines with their revertant counterparts, as well as Siah-1 and p53 transfectants, we identified *tpt1* transcripts of TCTP as being significantly down-modulated among series of 263 genes differentially expressed. Decreasing TCTP expression levels, either by antisense or siRNA, was shown to either promote apoptosis, or more strikingly, induce the reorganization of MCF7 and T47D breast cancer cells into ductal/acinar structures of the now suppressed malignant phenotype (10).

A breakthrough was recently achieved by J. Craven's group by solving the solution structure of TCTP from *Schizosaccharomyces pombe* (11). These studies revealed that TCTP is structurally similar to the mammalian suppressor of Sec4 (MSS4/DSS4). MSS4 has a weak guanine nucleotide exchange factor (GEF) activity for various Rab proteins, however, subsequent experiments demonstrated that MSS4 functions, instead, as a guanine nucleotide-free chaperone (12, 13). MSS4/DSS4 binds to the nucleotide-free form of a subset of Rabs, which are members of the Ras superfamily of small G proteins involved in regulating the secretory pathway (14, 15). Its interaction with the nucleotide-free form of Rab15 is essential for endocytic trafficking (14). Interestingly, these structural studies showed that the highest homology observed between TCTP and MSS4 coincides with the Rab-binding site on MSS4. Thus, these structural studies indicate that TCTP may associate with and regulate the activities of GTPases in a similar fashion.

The small monomeric G proteins transition between active and inactive forms, depends on whether GTP or GDP is bound, respectively. This process is regulated by accessory factors that either stimulate GTP hydrolysis (GTPase activating proteins) or promote GDP exchange (GEFs) (16, 17). Guanine nucleotide dissociation inhibitors (GDIs) represent another class of molecules that regulate small G protein activity. GDIs act by inhibiting the dissociation of GDP bound to the GTPase, thereby maintaining the GTPase in its inactive state (18). The structural analysis of the GDIs complexed to one of the members of the Rho family, for example, has provided insight on the mechanism by which GDIs are able to execute their function. The interaction of a Rho-GDI with Rac shows how the GDI influences the stabilization of the Mg^{2+} ions associated with the nucleotide binding pocket via a system of switches present in Rho, which determines the fate of the bound nucleotide. On the other hand, the presence of a hydrophobic pocket inside the Rho-GDI explains how Cdc42 is able to quit its membrane anchorage by its geranylgeranyl moiety that is displaced toward the Rho-GDI in this precise pocket composed of β-sheets. This mechanism is at the basis of the shuttle function of the Rho-GDI between cytoplasm and membrane (19–21).

In this article, the elongation factor eEF1A and its GEF, eEF1Bβ, were identified as TCTP-binding partners in a yeast two-hybrid hunt. The eukaryotic elongation machinery consists of the large G protein, eEF1A (1 or 2), which is homologous to the bacterial EF-Tu. In higher eukaryotes, GDP/GTP exchange

Abbreviations: GEF, guanine nucleotide exchange factor; GDI, guanine nucleotide dissociation inhibitor; MSS4, mammalian suppressor of Sec4; NKTR, NK tumor recognition protein; IVT, *in vitro*-transcribed/translated; AIP1, ALG-2-interacting protein 1.

†C.C. and B.J.P. contributed equally to this work.

‖To whom correspondence should be addressed. E-mail: atelerman@molecular-engines.com.

www.pnas.org/cgi/doi/10.1073/pnas.2335950100

is triggered by eEF1B, which consists of α, β, and γ. eEF1A recruits the aminoacyl-tRNA to the programmed ribosome, which requires the binding of GTP (aminoacyl-tRNA–eEF1A–GTP). On correct codon–anticodon interaction, GTP hydrolysis is triggered, and, in the resulting eEF1A–GDP complex, GDP is removed by two GEFs, eEF1Bα and eEF1Bβ, allowing for another cycle of elongation (22, 23). Interaction mapping studies indicate that eEF1A and the eEF1B subunits form a pentamer composed of two molecules of eEF1A, complexed with either eEF1Bα or eEF1Bβ, held together by eEF1Bγ (24).

Here, we show that TCTP is involved in the elongation step of translation. TCTP is shown to impair the GDP exchange reaction promoted by eEF1Bβ on eEF1A. Thus, by stabilizing eEF1A in its GDP-bound form, TCTP functions as a GDI.

Materials and Methods

Antibodies. Rabbit anti-eEF1Bβ and chicken anti-TCTP antibodies were generated against synthetic peptides corresponding to residues 14–30 of human eEF1Bβ or residues 55–65 of human TCTP, respectively (Agro-Bio, La Ferté St. Aubin, France). Rabbit anti-TCTP and mouse anti-eEF1A antibodies were purchased from Medical & Biological Laboratories (Nagoya, Japan) and Upstate Biotechnology, respectively.

Purification of Recombinant Proteins. Full-length TCTP, eEF1Bβ, and NK tumor recognition protein (NKTR) cDNAs were cloned in-frame into pGEX-6P (Amersham Biosciences). Production and purification of GST-fusion proteins are discussed in *Supporting Materials and Methods*, which is published as supporting information on the PNAS web site.

Yeast Two-Hybrid Hunt. Full-length or the first 84 amino acids of TCTP were fused in-frame with the LexA DNA-binding domain of pEG202. A cDNA library derived from human monocytic leukemia U937 cells was cloned into galactose-inducible pYESTrp2 vector. A yeast two-hybrid hunt was performed as described (25).

***In Vitro* and *in Vivo* Interaction.** *In vitro*-transcribed/translated (IVT) ^{35}S-methionine-labeled proteins were generated as described by the manufacturer (Promega). GST or GST-fusion proteins immobilized on beads were incubated with IVT radiolabeled products or purified rabbit eEF1A for 3 h at 4°C. Proteins bound to the GST-fusion proteins or GST alone, were washed and eluted directly in Laemmli buffer or in the presence of 10 mM reduced glutathione (ICN) (see *Supporting Materials and Methods*). For detection of endogenous interactions, 293T and HeLa cells were lysed for 1 h in 1% Nonidet P-40 lysis buffer containing 25 mM Tris·HCl, pH 7.5, 150 mM NaCl, 10% glycerol, plus the protease inhibitors 1 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), 1% aprotinin, 1 mM leupeptin, and 2 mM pepstatin (all reagents from ICN) and cell lysates clarified by centrifugation (16,000 $\times$ *g*) for 20 min. Endogenous TCTP or eEF1Bβ were immunoprecipitated from lysates with either anti-TCTP, anti-eEF1Bβ, or an isotype-matched control antibody. The addition of G protein agarose beads (Amersham Biosciences) was followed for an additional 3 h at 4°C. Immune complexes were washed four times in the lysis buffer, eluted in Laemmli buffer, and analyzed by Western blot.

Immunofluorescence Analysis. Details of the immunofluorescence staining can be seen in *Supporting Materials and Methods*. Confocal imaging was performed on a Leica TCS SP1 confocal microscope.

Affinity Chromatography and MS Analysis. See *Supporting Materials and Methods* for further information.

Guanine Nucleotide Exchange Assay. Rabbit liver eEF1A was purified as described (26). The guanine nucleotide exchange on eEF1A was monitored essentially as described (27, 28). The eEF1A–[^{3}H]GDP complex was prepared after incubation of 4 μM eEF1A with 4 μM [^{3}H]GDP [Amersham Pharmacia Biosciences; 1,500 Ci/mol (1 Ci = 37 GBq)] in 80 μl of 45 mM Tris·HCl, pH 7.5, containing 0.5 mM DTT, 10 mM magnesium acetate, 100 mM NH_4Cl, 1 mg/ml BSA, and 25% glycerol for 5 min at 37°C. The reaction mixture was placed on ice and diluted by the addition of 640 μl of ice-cold exchange buffer (20 mM Tris·HCl, pH 7.5, 10 mM magnesium acetate, 50 mM NH_4Cl, and 10% glycerol). The exchange reaction was conducted at 0°C after the addition of 160 μl of exchange buffer containing nucleotide and specified exchange factors. Aliquots of 100 μl were taken at times indicated, and were immediately filtered through nitrocellulose filters (Millipore; pore size 0.45 μm). Filters were washed three times with 1 ml of ice-cold washing buffer (20 mM Tris·HCl, pH 7.5, 10 mM magnesium acetate, 100 mM NH_4Cl, and 0.1 mg/ml BSA), dried, and counted in a liquid scintillator.

Results

TCTP Interacts with the Elongation Factors eEF1Bβ and eEF1A. A yeast two-hybrid hunt was undertaken to identify proteins that interact with TCTP. Full-length or the first 84 amino acids of TCTP were used as baits to screen a cDNA library obtained from the human monocytic leukemia U937 cell line. Among the positive clones isolated were two proteins involved in the elongation step of protein synthesis, the GTPase eEF1A, and one of its GEFs, eEF1Bβ. Mating assays subsequently confirmed an interaction between LexA–TCTP and either B42–eEF1A or B42–eEF1Bβ (see Table 1, which is published as supporting information on the PNAS web site, and Fig. 1*A*). As defined by growth and β-gal activity, a robust interaction between LexA–TCTP and B42–eEF1Bβ was observed. A LexA–TCTP and B42–eEF1A interaction was also seen, although it was not as strong. Furthermore, the C-terminal GEF-containing region of eEF1Bβ (amino acids 153–281) was mapped as the TCTP-binding region (Fig. 1*A*). GST pull-down assays confirmed direct and reciprocal binding between TCTP and eEF1Bβ. IVT ^{35}S-labeled eEF1Bβ derived from reticulocyte lysates bound to GST-TCTP (Fig. 1*B Left*), but not to GST alone. Furthermore, GST-TCTP did not interact with the IVT-negative control protein, ALG-2-interacting protein 1 (AIP1) (29). A reciprocal interaction was also demonstrated for GST-eEF1Bβ and IVT-generated TCTP (Fig. 1*B Right*). Moreover, purified eEF1A derived from rabbit liver bound specifically to GST-TCTP, but not to the negative control, GST-NKTR (30) (Fig. 1*C*). Because the apparent K_d of TCTP–eEF1A interaction is high (see below; Fig. 4*C*), we could not quantitatively address the binding of TCTP to eEF1A in a nucleotide-dependent manner in an *in vitro* pull-down experiment. Kinetic studies described below showed that TCTP preferentially binds the GDP form of the factor.

To investigate the presence of endogenous interaction between TCTP and eEF1Bβ, antibodies directed against TCTP and eEF1Bβ were generated and initially tested on total cell lysates derived from 293T cells. Immunoblot analysis revealed that the anti-TCTP antibody detected a protein band of 23 kDa, corresponding to its expected molecular weight (see Fig. 6, which is published as supporting information on the PNAS web site). In addition, the anti-eEF1Bβ antibody recognized a single protein band of 36–38 kDa, which corresponded to the predicted size of eEF1Bβ. Finally, anti-TCTP and anti-eEF1Bβ antibodies immunoprecipitated their respective recombinant protein, indicating that both antibodies recognize native proteins (see Fig. 6).

Coimmunoprecipitation experiments were subsequently carried out on lysates derived from either 293T or HeLa cells to identify the presence of an endogenous association between TCTP with either eEF1Bβ or eEF1A. Rabbit anti-TCTP or

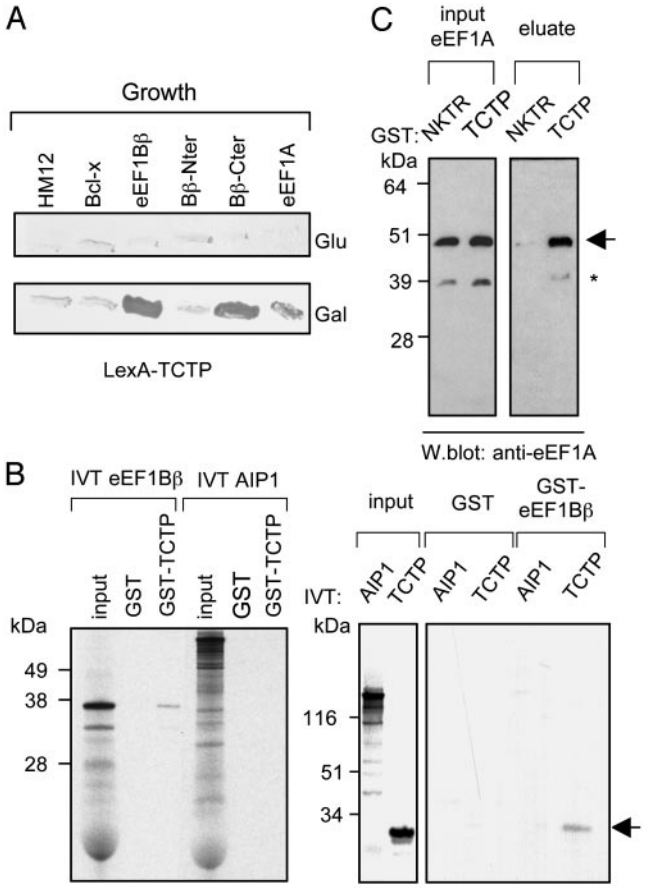

Fig. 1. Association of TCTP with either eEF1Bβ or eEF1A. (*A*) TCTP interacts with the C terminus of eEF1Bβ and eEF1A in yeast. Diploids carrying the constructs HM12, Bcl-x, eEF1A, eEF1Bβ, or truncated form of eEF1Bβ (Bβ-Nter residues 1–152 or Bβ-Cter residues 153–281) fused to B42 domain (activation domain) and LexA-TCTP were streaked onto either glucose (Glu) or galactose (Gal) plates and assayed for growth. (*B* and *C*) *In vitro* interaction of TCTP with either eEF1Bβ or eEF1A. (*B*) The indicated GST-fusion proteins immobilized on glutathione beads were incubated with AIP1, TCTP, or eEF1Bβ IVT. Radiolabeled proteins bound to the GST proteins were visualized by autoradiography. Inputs for each experiment are indicated. The negative controls, AIP1 and NKTR, are 120- and 150-kDa proteins, respectively. (*C*) GST-NKTR or GST-TCTP were incubated with eEF1A purified from rabbit liver. Eluted eEF1A was detected with the anti-eEF1A antibody. Arrow, full-length eEF1A. *, a degraded product of eEF1A.

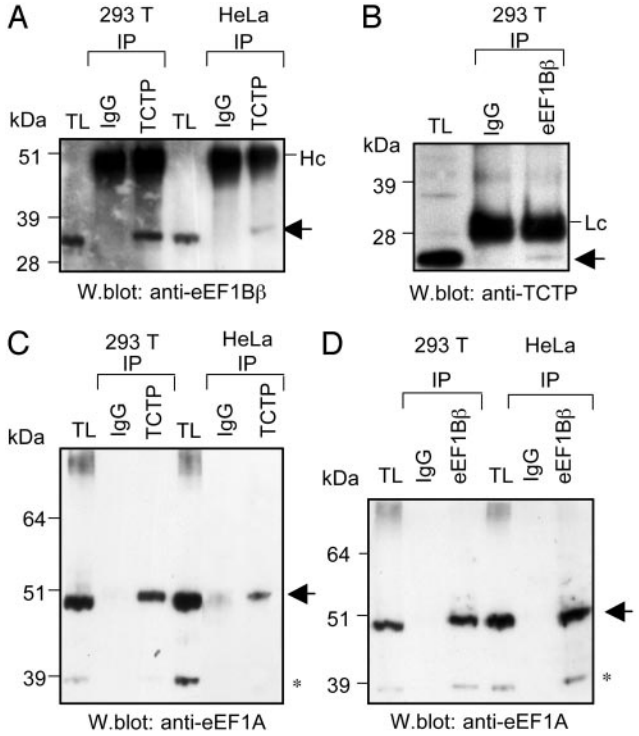

Fig. 2. Endogenous interaction of TCTP with either eEF1Bβ or eEF1A. TCTP was immunoprecipitated from either 293T or HeLa cell extracts with a rabbit anti-TCTP or an isotype-matched control antibody (IgG). Immunoprecipitates (IP) were analyzed by Western blot using anti-eEF1Bβ (*A*) or anti-eEF1A (*C*) antibodies. (*B*) Anti-eEF1Bβ antibodies or preimmune serum (IgG) were used to immunoprecipitate eEF1Bβ from 293T or HeLa cell extracts. Western blot analysis on the immune complexes using either anti-TCTP (*B*) or anti-eEF1A (*D*) antibodies revealed a specific association. Total cell lysates (TL) are indicated. Arrows highlight IP proteins. *, a degraded product of eEF1A; Hc and Lc, heavy and light chain of IgG, respectively.

isotype-matched IgG control antibodies were initially incubated with cell lysates. Immunoblot analysis with antibodies against either eEF1Bβ (Fig. 2*A*) or eEF1A (Fig. 2*C*) revealed that anti-TCTP, but not rabbit IgG, specifically coimmunoprecipitated protein bands of 36 and 51 kDa, respectively. In addition, immunoblotting with the chicken anti-TCTP antibody on immune complexes immunoprecipitated with anti-eEF1Bβ revealed a reciprocal association between TCTP and eEF1Bβ (Fig. 2*B*). In agreement with previous studies (24), Fig. 2*D* illustrates an association between eEF1Bβ and eEF1A. Finally, MS analysis of affinity-purified TCTP-binding partners confirmed that eEF1A interacts with TCTP. Interestingly, eEF2, a GTPase also involved in elongation, was identified as an additional TCTP-interacting partner in the same screening (see Tables 2–4, which are published as supporting information on the PNAS web site). Overall, the above data show that TCTP associates with eEF1Bβ and eEF1A, proteins involved in translation elongation.

TCTP Colocalizes with Either eEF1Bβ or eEF1A. Indirect immunofluorescence studies were also performed on HeLa cells to further investigate an endogenous association between TCTP and either eEF1Bβ or eEF1A. Fig. 3 shows that staining with the chicken anti-TCTP antibody appeared punctate and cytoplasmic and partially colocalized with the endoplasmic reticulum (ER) marker protein disulfide isomerase (PDI) (see Fig. 7, which is published as supporting information on the PNAS web site). Moreover, in agreement with previous reports (5), confocal imaging also showed a strong perinuclear staining with the anti-TCTP, partially overlapping with ER marker. Anti-eEF1Bβ staining was restricted primarily to the ER (Fig. 3*A Upper*), costaining with PDI (31) (see Fig. 7). The overlay of TCTP and eEF1Bβ staining indicates that these proteins partially colocalize to the perinuclear region of the cell. Finally, colocalization studies of eEF1A and TCTP were also performed. Immunofluorescence analysis using an anti-eEF1A antibody revealed staining around the nucleus and throughout the cytoplasm (31). Confocal analysis on HeLa cells stained with chicken anti-TCTP and anti-eEF1A antibodies revealed a partial colocalization around the nucleus (Fig. 3*B*).

TCTP Preferentially Stabilizes the GDP Form of eEF1A. The functional relevance of a TCTP and eEF1A association was further investigated by monitoring the effects of TCTP on the rate of dissociation of GDP from the eEF1A–[^{3}H]GDP complex. We sought to determine whether TCTP preferentially binds to the GDP-bound form of the factor (and pushes the equilibrium toward the formation of eEF1A-GDP) or associates with the nucleotide-free form of eEF1A (and displaces the exchange

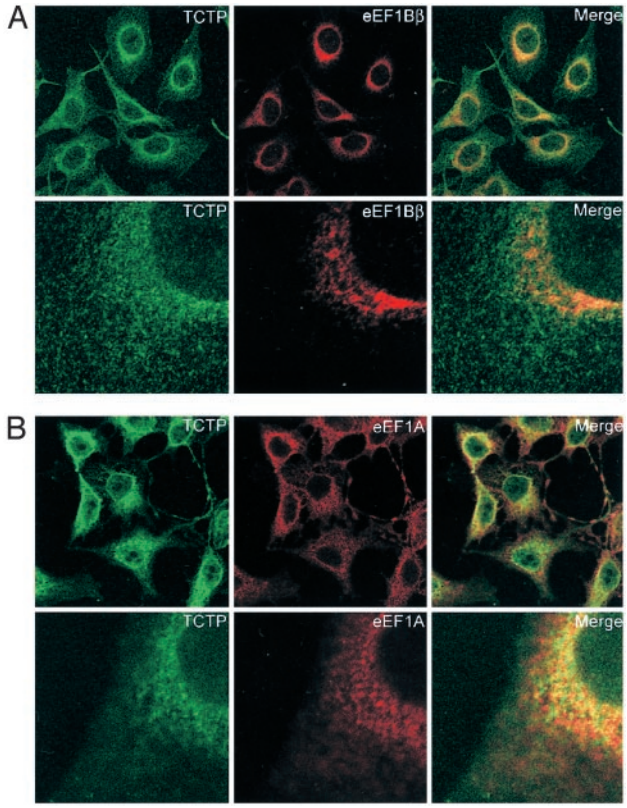

Fig. 3. Endogenous colocalization of TCTP with either eEF1Bβ or eEF1A in HeLa cells. (*A*) Immunofluorescence analysis of anti-TCTP (green) and anti-eEF1Bβ (red) staining. Merge shows a partial colocalization of TCTP and eEF1Bβ (yellow). A higher magnification of cell is shown in *Lower*. (*B*) Immunofluorescence analysis of anti-TCTP (green) and anti-eEF1A (red) staining. Merge indicates a partial colocalization of TCTP and eEF1A. A higher magnification of cell is shown in *Lower*. Note the enhanced colocalization of TCTP with either eEF1Bβ or eEF1A within the perinuclear region of the cell. Each confocal image represents a similar plane through the cell.

reaction toward the formation of eEF1A). When eEF1A was preloaded with [^{3}H]-GDP and incubated in the presence of saturating amounts of unlabeled GDP (150 μM), GDP dissociation followed monoexponential kinetics corresponding to a half-life of the complex of 12 min (Fig. 4*A*). This result corresponded to a rate of 0.082 ± 0.004 pmol GDP exchanged per min per pmol of eEF1A. In the absence of free GDP in the incubation mixture, the eEF1A–[^{3}H]GDP complex remained stable over a period of 40 min. Increasing amounts of TCTP (from 0.2 to 3.0 μM) were added in the exchange assay, and the rate of dissociation of the eEF1A–[^{3}H]GDP complex was monitored in the presence of 150 μM free GDP (Fig. 4*A*). TCTP by itself did not bind nucleotides (data not shown). When 3 μM of TCTP were added, the half-life of the eEF1A–[^{3}H]GDP complex increased to 39 min, corresponding to a rate of GDP dissociation of 0.026 ± 0.003 pmol GDP exchanged per min per pmol of eEF1A. Therefore, TCTP is devoid of exchange activity; its addition decreases the rate of GDP exchange on eEF1A. The inhibition of GDP exchange by TCTP was concentration dependent and followed a saturation kinetics with an apparent dissociation constant, K_d, of 1.2 ± 0.2 μM (Fig. 4*C*). The stabilization of the eEF1A–[^{3}H]GDP complex by TCTP suggests that TCTP preferentially binds the GDP form of eEF1A as compared with the nucleotide-free form of the factor.

The eukaryotic elongation factor, eEF1A, binds GDP and GTP with similar affinity (2–4 μM) (32). To determine whether TCTP preferentially binds the GDP or the GTP form of eEF1A, the effect of the addition of TCTP on the GDP–GTP exchange on eEF1A was monitored (Fig. 4*B*). When unlabeled free GTP (150 μM) was added in the exchange reaction instead of GDP, a similar protection of the eEF1A–[^{3}H]GDP complex was observed (rate of GDP dissociation decreasing from 0.078 ± 0.004 to 0.024 ± 0.003 pmol GDP exchanged per min per pmol of eEF1A), showing that TCTP does not displace the equilibrium toward the formation of an eEF1A–GTP complex. The apparent K_d of TCTP for the GDP form of eEF1A (1.6 ± 0.3 μM; Fig. 4*C*) was not significantly affected. These data show that TCTP preferentially binds the GDP form of eEF1A and impairs GDP dissociation.

TCTP Inhibits the eEF1Bβ-Mediated Exchange Reaction. Higher eukaryotes contain two GEFs, eEF1Bα (formerly EF-1β, 27 kDa), and eEF1Bβ (formerly EF-1δ, 35 kDa) (28, 33). The exchange activity of eEF1Bα is enhanced by its association with eEF1Bγ (formerly EF-1γ, 50 kDa). Because TCTP stabilizes the GDP form of eEF1A, we investigated its effect on eEF1Bα–eEF1Bγ complex or eEF1Bβ-mediated GDP exchange on eEF1A (only

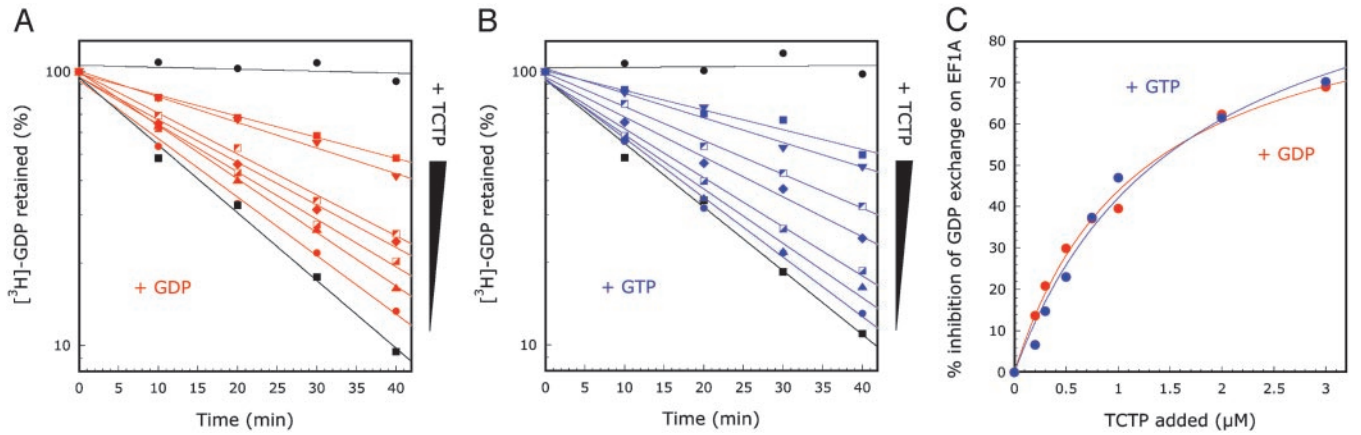

Fig. 4. TCTP preferentially stabilizes the GDP form of eEF1A. The time course of GDP exchange was assayed at 0°C in the presence of 350 nM eEF1A-[^{3}H]GDP without TCTP (■) or with increasing amounts of TCTP (final concentrations of 200, 300, 500, and 750 nM, and 1, 2, and 3 μM, which are indicated by red and blue symbols in *A* and *B*, respectively). The reaction was monitored in the presence of 150 μM unlabeled GDP (*A*) or 150 μM unlabeled GTP (*B*). In the absence of unlabeled nucleotide, the eEF1A–[^{3}H]GDP complex remained stable (●). All reactions were performed in triplicate. (*C*) The inhibition of GDP exchange on eEF1A observed in *A* and *B* in the presence of GDP or GTP is plotted as a function of TCTP added.

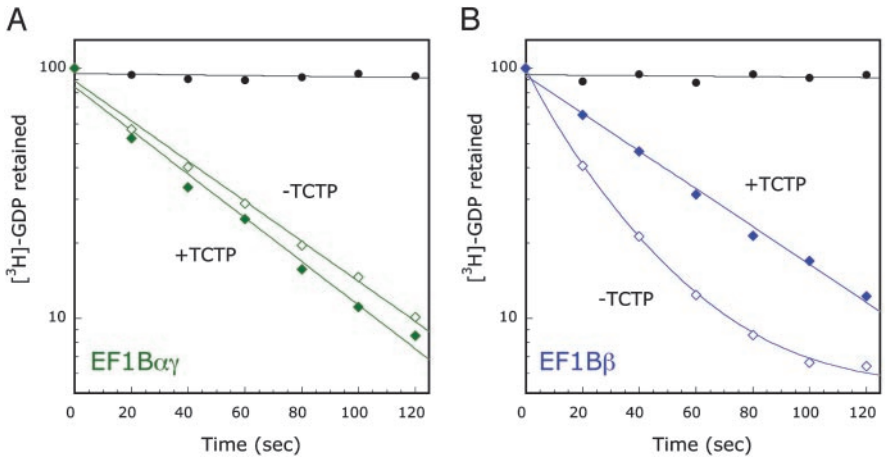

Fig. 5. TCTP inhibits the eEF1Bβ-mediated exchange reaction. Kinetics of GDP exchange promoted by 10 nM of the eEF1Bα–eEF1Bγ complex (EF1Bαγ) (*A*) or by 50 nM of eEF1Bβ (EF1Bβ) (*B*) were conducted in the presence (+TCTP) or in the absence (−TCTP) of 2 μM TCTP. In the absence of unlabeled nucleotide, the eEF1A–[^{3}H]GDP complex remained stable (●). The time course of GDP exchange was initiated by addition of 150 μM unlabeled GDP.

eEF1Bβ was found to interact with TCTP in the two-hybrid screen). As reported (28), in the presence of the eEF1Bαγ complex, GDP exchange followed first-order kinetics (initial rate of 0.84 min^{-1} per pmol of eEF1Bαγ; Fig. 5*A*) whereas eEF1Bβ-mediated exchange was biphasic (initial rates of 0.66 min^{-1} and 1.68 min^{-1} per pmol of eEF1Bβ; Fig. 5*B*) as reported (28). When TCTP was added in the exchange reaction at a final concentration of 2 μM, the kinetics of GDP exchange in the presence of eEF1Bαγ remained essentially unchanged (initial rate of 0.96 min^{-1}; Fig. 5*A*). By contrast, the initial rate of GDP exchange in the presence of eEF1Bβ and TCTP was no more biphasic and followed a simple first-order rate (0.294 min^{-1} per pmol of eEF1Bβ; Fig. 5*B*). From these data, we conclude that TCTP stabilizes the GDP form of eEF1A and specifically antagonizes the eEF1Bβ-mediated exchange reaction.

Discussion

TCTP has been proposed to be involved in growth-related activities (2). In this study, we found by yeast two-hybrid assay and confirmed by coimmunoprecipitation and immunofluorescence studies that TCTP associates with components of the translational machinery, the elongation factors, eEF1A and eEF1Bβ.

Elongation factors of translation have been implicated in tumor formation (34). For example, constitutive expression of eEF1A caused fibroblasts to become highly susceptible to transformation (35). In addition, a truncated form of eEF1A, encoded by the PTI-1 gene, was identified in prostatic cancers (36–38). Inhibiting PTI-1 expression with PTI-1 antisense resulted in the suppression of its tumorigenic potential. eEF1Bβ has also been implicated in cell transformation and tumorigenesis. Its overexpression resulted in anchorage-independent growth and in the formation of tumors in nude mice (39).

In agreement with the structural studies on the shared homology between MSS4 and TCTP, we show that TCTP interacts with the GTPase, eEF1A, preferentially in a GDP-bound form. We propose that TCTP acts as a GDI, based on the following observations: (*i*) TCTP inhibited the eEF1A–[^{3}H]GDP complex dissociation in the presence of cold GDP in excess, and (*ii*) TCTP preferentially stabilized eEF1A–[^{3}H]GDP in the presence of cold GTP in excess. Furthermore, we demonstrated that TCTP impaired the GDP exchange reaction promoted by eEF1Bβ on the eEF1A–[^{3}H]GDP complex.

Takai and coworkers (40) first identified GDIs, which inhibited the specific release of GDP, but not GTP from Rab3A. GDIs generally prevent the translocation of small G proteins to the membrane, thereby sequestering them in cytoplasm (18). In the case of Rabs, GDIs have the potential to regulate the availability of specific intracellular transport effectors (41). More recently, GDIs have been shown to regulate the large heterotrimeric G proteins. For example, the activator of G protein signaling 3 (AGS3) acts as a GDI on Galpha (i3). AGS3 prevents Galpha (i3) activation by keeping the GTPase in the cytoplasm in a GDP-bound state (42, 43).

The findings that GDIs are associated with both small and large G proteins implies that they could be implicated in regulation of GTPases involved in translation. In this regard, TCTP could selectively modulate the activity of eEF1A during the process of translation elongation. Our data show that TCTP specifically impaired the exchange reaction promoted by eEF1Bβ, and not eEF1Bαγ. The function and the existence of two exchange factors for eEF1A is largely not understood. However, kinetics studies indicate that they act differently on the elongation factor, eEF1A. The eEF1Bα–eEF1Bγ complex mediates GDP dissociation from eEF1A in a linear way according to the time. By contrast, eEF1Bβ mediates a biphasic exchange reaction. It has been hypothesized that the first step, a fast-exchange reaction, corresponds to the rate of exchange under single-turnover conditions (28). This initial, fast rate of GDP exchange in the presence of eEF1Bβ (1.68 min^{-1}) is believed to correspond to the intrinsic rate of exchange promoted by the formation of the eEF1A–eEF1Bβ complex. The second, slow-exchange reaction step (0.66 min^{-1}) only observed in the presence of eEF1Bβ, may be due to the slow dissociation of eEF1Bβ from the nucleotide-free form of eEF1A, which would be the rate-limiting step of the reaction. In the presence of TCTP, because the rate of GDP-exchange promoted by eEF1Bβ is significantly decreased (0.294 min^{-1}), and is slower than the rate of dissociation of the eEF1A–eEF1Bβ complex, monophasic kinetics are observed. The crystal structure of the eukaryotic elongation factor complex eEF1A–eEF1Bα from yeast revealed that the GEF interacts with domains 1 and 2 of eEF1A. On binding, eEF1Bα causes reorganization of the switch 2 region of eEF1A and inserts a lysine side chain in the Mg^{2+}-binding site, which promotes nucleotide release (44, 45). These two features are common to the nucleotide exchange mechanism for several G proteins. In this context, TCTP binding to eEF1A-GDP may either prevent the conformational rearrangement occurring in the switch 2 region of eEF1A on binding of eEF1Bβ or impair the formation of a productive eEF1A–eEF1Bβ complex. That

TCTP was found to impair the function of eEF1Bβ, but not of eEF1Bα–eEF1Bγ on eEF1A, indicates the specificity of the effect and suggests that TCTP interferes with the proper binding of eEF1Bβ. TCTP binds specifically the C-terminal GEF domain of eEF1Bβ, which is highly conserved with eEF1Bα, suggesting that the two exchange factors do not interact in the same region on eEF1A, or have somewhat different exchange mechanisms.

During protein synthesis, eEF1A forms a ternary complex with aminoacylated tRNA and GTP (eEF1A–GTP–aatRNA), and delivers aatRNA to the ribosome after GTP hydrolysis. Inactive eEF1A-GDP and deacylated tRNA are released from the ribosome and must be recycled. Maintaining eEF1A in a GDP-bound form by TCTP could represent an important step of tRNA channeling. The concept of tRNA channeling during translation assumes that tRNA is first vectorially transferred from its specific aminoacyl-tRNA synthetase (to be aminoacylated), next to eEF1A (to form the tRNA species competent for ribosomal translation), then to the ribosome, and finally back to the synthetase without mixing with the cellular fluid (27, 46, 47). In this context, the formation of a complex between TCTP and eEF1A-GDP may be involved in the channeling of tRNA. It has been shown that eEF1A-GDP can bind deacylated tRNA (48). The GDI activity of TCTP may prevent eEF1A activation into eEF1A-GTP before it is recruited by other components of the translation machinery to form a ternary complex with a *de novo*-aminoacylated tRNA. Thus, TCTP may play a prominent role in the elongation cycle of translation. Accordingly, decreasing the expression of TCTP in cancer cells might be a means to decrease the efficiency of protein synthesis, and to down-regulate cell proliferation.

We thank Jeremy Craven for helpful discussion and Philippe Fontanges, Institut Federatif de Recherche, Hopitâl Tenon for excellent confocal microscopy support.

1. Yenofsky, R., Cereghini, S., Krowczynska, A. & Brawerman, G. (1983) *Mol. Cell. Biol.* **3,** 1197–1203.
2. Bohm, H., Benndorf, R., Gaestel, M., Gross, B., Nurnberg, P., Kraft, R., Otto, A. & Bielka, H. (1989) *Biochem. Int.* **19,** 277–286.
3. Bohm, H., Gross, B., Gaestel, M., Bommer, U. A., Ryffel, G. & Bielka, H. (1991) *Biomed. Biochim. Acta* **50,** 1193–1203.
4. Xu, A., Bellamy, A. R. & Taylor, J. A. (1999) *Biochem. J.* **342,** 683–689.
5. Gachet, Y., Tournier, S., Lee, M., Lazaris-Karatzas, A., Poulton, T. & Bommer, U. A. (1999) *J. Cell Sci.* **112,** 1257–1271.
6. Yarm, F. R. (2002) *Mol. Cell. Biol.* **22,** 6209–6221.
7. Li, F., Zhang, D. & Fujise, K. (2001) *J. Biol. Chem.* **276,** 47542–47549.
8. Zhang, D., Li, F., Weidner, D., Mnjoyan, Z. H. & Fujise, K. (2002) *J. Biol. Chem.* **277,** 37430–37438.
9. MacDonald, S. M., Rafnar, T., Langdon, J. & Lichtenstein, L. M. (1995) *Science* **269,** 688–690.
10. Tuynder, M., Susini, L., Prieur, S., Besse, S., Fiucci, G., Amson, R. & Telerman, A. (2002) *Proc. Natl. Acad. Sci. USA* **99,** 14976–14981.
11. Thaw, P., Baxter, N. J., Hounslow, A. M., Price, C., Waltho, J. P. & Craven, C. J. (2001) *Nat. Struct. Biol.* **8,** 701–704.
12. Yu, H. & Schreiber, S. L. (1995) *Nature* **376,** 788–791.
13. Esters, H., Alexandrov, K., Iakovenko, A., Ivanova, T., Thoma, N., Rybin, V., Zerial, M., Scheidig, A. J. & Goody, R. S. (2001) *J. Mol. Biol.* **310,** 141–156.
14. Strick, D. J., Francescutti, D. M., Zhao, Y. & Elferink, L. A. (2002) *J. Biol. Chem.* **277,** 32722–32729.
15. Nuoffer, C., Wu, S. K., Dascher, C. & Balch, W. E. (1997) *Mol. Biol. Cell* **8,** 1305–1316.
16. Cherfils, J. & Chardin, P. (1999) *Trends Biochem. Sci.* **24,** 306–311.
17. Donovan, S., Shannon, K. M. & Bollag, G. (2002) *Biochim. Biophys. Acta* **1602,** 23–45.
18. Takai, Y., Sasaki, T. & Matozaki, T. (2001) *Physiol. Rev.* **81,** 153–208.
19. Scheffzek, K., Stephan, I., Jensen, O. N., Illenberger, D. & Gierschik, P. (2000) *Nat. Struct. Biol* **7,** 122–126.
20. An, Y., Shao, Y., Alory, C., Matteson, J., Sakisaka, T., Chen, W., Gibbs, R. A., Wilson, I. A. & Balch, W. E. (2003) *Structure (London)* **11,** 347–357.
21. Hoffman, G. R., Nassar, N. & Cerione, R. A. (2000) *Cell* **100,** 345–356.
22. Hershey, J. W. (1991) *Annu. Rev. Biochem.* **60,** 717–755.
23. Proud, C. G. (2000) in *Translational Control of Gene Expression*, eds. Sonenberg, N., Hershey, J. W. B. & Mathews, M. B. (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 719–40.
24. Mansilla, F., Friis, I., Jadidi, M., Nielsen, K. M., Clark, B. F. & Knudsen, C. R. (2002) *Biochem. J.* **365,** 669–676.
25. Finley, R., Jr., & Brent, R. (1995) in *DNA Cloning, Expression Systems: A Practical Approach*/(Oxford University Press, New York), pp. 169–203.
26. Shalak, V. F., Budkevich, T. V., Negrutskil, B. S. & El'skaia, A. V. (1997) *Uk. Biokhim. Zh.* **69,** 104–109.
27. Negrutskii, B. S., Shalak, V. F., Kerjan, P., El'skaya, A. V. & Mirande, M. (1999) *J. Biol. Chem.* **274,** 4545–4550.
28. Bec, G., Kerjan, P. & Waller, J. P. (1994) *J. Biol. Chem.* **269,** 2086–2092.
29. Vito, P., Pellegrini, L., Guiet, C. & D'Adamio, L. (1999) *J. Biol. Chem.* **274,** 1533–1540.
30. Nestel, F. P., Colwill, K., Harper, S., Pawson, T. & Anderson, S. K. (1996) *Gene* **180,** 151–155.
31. Sanders, J., Brandsma, M., Janssen, G. M., Dijk, J. & Moller, W. (1996) *J. Cell Sci.* **109,** 1113–1117.
32. Crechet, J. B. & Parmeggiani, A. (1986) *Eur. J. Biochem.* **161,** 647–653.
33. Janssen, G. M., van Damme, H. T., Kriek, J., Amons, R. & Moller, W. (1994) *J. Biol. Chem.* **269,** 31410–31417.
34. Sonenberg, N. (1993) *Curr. Opin. Cell Biol.* **5,** 955–960.
35. Tatsuka, M., Mitsui, H., Wada, M., Nagata, A., Nojima, H. & Okayama, H. (1992) *Nature* **359,** 333–336.
36. Shen, R., Su, Z. Z., Olsson, C. A. & Fisher, P. B. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 6778–6782.
37. Su, Z., Goldstein, N. I. & Fisher, P. B. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 1764–1769.
38. Sun, Y., Lin, J., Katz, A. E. & Fisher, P. B. (1997) *Cancer Res.* **57,** 18–23.
39. Joseph, P., Lei, Y. X., Whong, W. Z. & Ong, T. M. (2002) *J. Biol. Chem.* **277,** 6131–6136.
40. Sasaki, T., Kikuchi, A., Araki, S., Hata, Y., Isomura, M., Kuroda, S. & Takai, Y. (1990) *J. Biol. Chem.* **265,** 2333–2337.
41. Soldati, T., Shapiro, A. D., Svejstrup, A. B. & Pfeffer, S. R. (1994) *Nature* **369,** 76–78.
42. De Vries, L., Fischer, T., Tronchere, H., Brothers, G. M., Strockbine, B., Siderovski, D. P. & Farquhar, M. G. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 14364–14369.
43. Kimple, R. J., De Vries, L., Tronchere, H., Behe, C. I., Morris, R. A., Gist Farquhar, M. & Siderovski, D. P. (2001) *J. Biol. Chem.* **276,** 29275–29281.
44. Andersen, G. R., Pedersen, L., Valente, L., Chatterjee, I., Kinzy, T. G., Kjeldgaard, M. & Nyborg, J. (2000) *Mol. Cell* **6,** 1261–1266.
45. Andersen, G. R., Valente, L., Pedersen, L., Kinzy, T. G. & Nyborg, J. (2001) *Nat. Struct. Biol.* **8,** 531–534.
46. Negrutskii, B. S. & Deutscher, M. P. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 4991–4995.
47. Stapulionis, R. & Deutscher, M. P. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 7158–7161.
48. Petrushenko, Z. M., Budkevich, T. V., Shalak, V. F., Negrutskii, B. S. & El'skaya, A. V. (2002) *Eur. J. Biochem.* **269,** 4811–4818.