

**CHARACTERIZATION OF SEA URCHIN TRANSGLUTAMINASE, A PROTEIN
REGULATED BY GUANINE/ADENINE NUCLEOTIDES**

Laura Zanetti, Filomena Ristoratore, Alessandra Bertoni and Lucio Cariello

Biochemistry and Molecular Biology Laboratory,
Stazione Zoologica Anton Dohrn, 80121 Napoli, Italy

Short title: Sea urchin transglutaminase

Address for correspondence: Lucio Cariello, Biochemistry and Molecular Biology Laboratory,
Stazione Zoologica Anton Dohrn, Villa Comunale, I-80121 Napoli, Italy Tel.: +39-081-5833216.

Fax: +39-081-7641355. Email: lcariel@szn.it

Keywords: Transglutaminase; sea urchin; TG activity; GTPase activity

Abbreviations: TG, transglutaminase; PITG, *Paracentrotus lividus* TG; ATP, adenine triphosphate; GTP, guanosine triphosphate; PBS, phosphate buffer saline; EDTA, ethylenediaminetetracetate; SDS, sodium dodecyl sulfate; DTT, dithiothreitol; GTP- γ -S, guanosine [γ -thio]triphosphate; GST, glutathione S-transferase; IPTG, isopropyl 1-thio- β -D-galactopyranoside; BSA, bovine serum albumin; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Data deposition: The nucleotide sequence for the sea urchin transglutaminase has been deposited in the GenBank database under GenBank Accession Number (AJ439609).

Abstract

Transglutaminases (TGs) are calcium-dependent enzymes that catalyze transamidation of glutamine residues to form intermolecular isopeptide bonds. Nine distinct TGs have been identified in mammals, and three of them (types 2, 3 and 5) are regulated by GTP/ATP and are able to hydrolyze GTP, working as bifunctional enzymes. We have isolated a cDNA clone encoding a TG from a cDNA library prepared from the blastula stage of sea urchin *Paracentrotus lividus* (PITG). The cDNA sequence has an open reading frame coding for a protein of 738 amino acid, including a Cys active-site, and the other two residues critical for catalytic activity, His and Asp. We have studied its expression pattern by *in situ* hybridization and also demonstrated that the *in vitro* expressed PITG had GTP- and ATP-hydrolyzing activity; moreover, GTP inhibited the transamidating activity of this enzyme as that of human TG2, TG3, and TG5.

Introduction

Transglutaminases (TGs) are a diverse family of Ca^{2+} -dependent enzymes with distinct genes, structures and biological functions which catalyze post-translational modification of proteins referred as the R-glutaminy-peptide, amine- γ -glutamyl transferase reaction which leads either to the formation of an isopeptide bond within or between polypeptide chains, or to the covalent incorporation of polyamines into protein substrates (1, 2).

TGs are widely distributed in various organisms. In mammals, nine distinct but closely related transglutaminases, have been identified: TGs 1 to 7, coagulation factor XIIIa and the catalytically inactive band 4.2 (1, 2). Homologues have been found in invertebrates, in slime molds, plants and bacteria. A phylogenetic analysis of all the TGs indicated that an early gene-duplication event might have given rise to two lineages: one that comprises TG2, TG3, TG5, TG6 and TG7, and erythrocyte band 4.2; and the other to fXIIIa, TG1, TG4 and invertebrate TGs (2).

Among the TG family members, TG2, TG3, TG5 and slime mold TG (3) have been shown to be regulated by guanine nucleotides. TG2 is involved in several biological processes such as cell adhesion, apoptosis, and wound healing (4, 5). This enzyme is multifunctional with both protein-cross-linking and GTP-hydrolyzing activities (1); it has also been shown to be capable of functioning as a signal-transducing GTP-binding protein, coupled to activated receptors (6, 7), though the physiological significance of this function is yet to be elucidated. The X-ray structure of human TG2 bound with guanosine 5'-diphosphate (GDP) is known, and the main GDP-binding residues have been identified (8) and binding constants for nucleotides have been measured (9). Recently, crystallographic evidence demonstrated direct binding of GTP/GDP to the active form of TG3 enzyme (10). Moreover, it has been shown, through biochemical evidence, that TG3 and TG5 are able to hydrolyze GTP, and structural data show that the putative GTP-binding pocket is conserved between TG2, TG3 and TG5 (10, 11).

TG cDNAs have been isolated from lower vertebrates, such as fish, and the genes have been found to have structural similarity with those of mammalian genes (12, 13). TG cDNAs of a few invertebrates; ascidians (14), *Drosophila* (15), grasshopper (annulin) (16), limulus (17), crayfish (18) tiger shrimp (*Penaeus monodon*) (19), starfish (20) have also been cloned but the physiological roles of these invertebrate TGs remain unclear.

Previous results obtained by Cariello et al. (21-23) strongly suggested that TG or TGs might be involved at various stages in embryonic development of sea urchin. Although these reports described the identification and purification of sea urchin TG activity, no structural information has been presented. To find out more about invertebrate TGs, their physiological roles, and evolutionary relationship to other TGs, we attempted the molecular cloning of a *Paracentrotus lividus* TG (PITG). In this study a cDNA clone encoding PITG was isolated and its expression pattern was analysed by *in situ* hybridization. By biochemical characterization of *in vitro* expressed PITG, we demonstrated that this enzyme showed a calcium-dependent transamidating activity, that was negatively regulated by guanine nucleotides. Furthermore, we demonstrated that PITG was able to hydrolyze GTP and ATP although there is a low sequence homology with the GTP-binding domain of TG2. PITG is the first transglutaminase characterized from an invertebrate organism that displays ATP- and GTP-hydrolyzing activity.

Experimental Procedures

Animals and embryos

Adult specimens of *Paracentrotus lividus* were collected in the Bay of Naples. Specimens were shed by KCl (0,5M) injection and gametes were collected in filtered sea water. Eggs were fertilized with suspensions of sperm and cultured at 18 °C. When the embryos reached the appropriate stage, they were packed by hand centrifugation, frozen at -20°C for RNA preparation or fixed for *in situ* hybridization.

Materials and Reagents

Sodium salts of ATP and GTP were the highest quality available and were purchased from Sigma. Tetralithium salt of GTP- γ -S was purchased from Sigma. Guinea pig liver TG2 was obtained from Sigma. [1,4- ^{14}C]Putrescine dihydrochloride (114 mCi/mmol) was purchased from Amersham. [γ - ^{32}P]-ATP (25 Ci/mmol) and [γ - ^{32}P]-GTP (25 Ci/mmol) were purchased from ICN Biomedicals. Activated charcoal was purchased from Sigma. Enzymes were purchased from Promega Biotech or New England Biolabs unless stated otherwise.

Probe preparation

Poly(A)⁺ RNA from gastrula stage of sea urchin *P. lividus* was reverse transcribed using the SuperScript Preamplification System for First Strand cDNA Synthesis (GIBCO/BRL) and then amplified directly using PCR according to manufacturer's instructions. The following degenerate primers were used to amplify the coding region of the active-site of a putative sea urchin TG: 5'-GTSMMVTA YGGMCAGTGCTGGGT and 5'-ARRTCHGGYCKSKYCATCCA, where H = A or C or T; K = G or T; M = A or C; R = A or G; S = C or G; V = A or C or G; Y = C or T. The 232-bp reaction product, containing the coding sequence of the TG active-site, was used as sea urchin TG probe.

cDNA library construction and screening

cDNA was synthesized with 4 μg of blastula stage poly(A)⁺ RNA. After the addition of EcoRI adapters, the cDNA was inserted into the vector arms of λ ZAP II (24). This ligated DNA was encapsidated using Gigapack II Gold packaging extract according to the manufacturer's instructions (Stratagene), and was used to infect the *E. coli* strain XL-1 blue, thus constructing a cDNA library. Approximately 9×10^5 recombinant λ phages were screened by plaque hybridization using the sea urchin TG cDNA probe labelled by random priming (Multiprime DNA Labelling System, Amersham). Hybridization was carried out at 60°C for 16 h in Church buffer (1 mM

EDTA/0.5 M NaHPO₄/ 7% SDS). Subsequently, the filters were washed 3 times for 10 min each at room temperature in 2x standard saline citrate (SSC)/0.1% SDS and twice for 20 min at 60°C in 2x SSC/0.1% SDS.

The cDNA inserts from positive clones were rescued as pBluescript SK(-) by helper phage-mediated *in vivo* excision as described by the manufacturer (Stratagene).

DNA sequencing and analyses

Ten positive cDNA clones were isolated and sequenced. All sequences were carried out with a CEQ 2000XL DNA Analysis System apparatus (Beckman) by Molecular Biology Service of Stazione Zoologica "Anton Dohrn", Naples. The DNA and amino acid sequences were analysed using the GCG computer program (Wisconsin Sequence Analysis Package).

Three of the positive clones contained the entire sequence of the sea urchin TG cDNA.

Whole mount in situ hybridization

Whole mount *in situ* hybridization was carried out according to Arenas-Mena, (25) with some modifications.

Full-length cDNA cloned in the pBluescript SK-. The plasmid was digested with Spe I and used as template for *in vitro* transcription by using the Roche digoxigenin (DIG) RNA labelling kit, according to the supplier's instruction and stored in H₂O at -80°C.

Embryos were fixed with 4% paraformaldehyde, 0.5 M NaCl, 1 M MOPS (3-N- morpholino propansulfonic acid), pH 7,5 at 4°C over night.

The embryos were incubated in hybridization buffer containing 0.1 ng/μl digoxigenin-labelled antisense transcript. Hybridization was carried out at 50°C for one week. After hybridization samples were washed five times in MOPS buffer at room temperature to remove the probe, incubated for an additional 3 hours under hybridization conditions and washed three more times with MOPS buffer. The samples were incubated with 1:1500 alkaline phosphatase-conjugated Fab

fragments (Roche) overnight at room temperature. The antibody was removed with five washes in MOPS buffer over an interval greater than 24 hours. After washes with AP buffer (0.1 M Tris-HCl pH 9.5, 50 mM MgCl₂, 0.1 M NaCl, 0.1% Tween-20, 1 mM levamisole; 30 minutes each) signal detection was performed in AP buffer containing 4.5 µl NBT/ml (nitrobluetetrazolum) and 3.5 µl BCIP/ml (5-bromo-4-choro-3-indolylphosphate). The reaction was stopped by dilution in MOPS buffer.

Plasmid construction

Polymerase chain reaction was used to amplify the *PITG* cDNA for subcloning as a GST fusion protein into *E. coli* expression vector pGEX-2T (Amersham). Full-length *PITG* cDNA was amplified using *Pfu* DNA polymerase and two primers containing an *EcoRI* restriction site. The amplification product was digested with *EcoRI* and cloned in-frame with GST into the *EcoRI* site of pGEX-2T. Restriction digests identified inserts in the correct orientation and DNA sequence analysis verified that no errors had been introduced during the PCR reaction.

A Cys-324→Ala mutant expression vector of *PITG* cDNA (GST-PITG_{C324A}) was generated using QuickChange™ Site-directed Mutagenesis Kit (Promega) and GST-PITG as the template, following manufacturer instructions. Mutation was verified by DNA sequence analysis.

Expression and purification of GST-fusion proteins

A single colony of *E. coli* cells (BL21-CodonPlus) containing the recombinant pGEX plasmid (pGEX-PITG or pGEX-PITG_{C324A}) was inoculated in 100 ml of LB medium + 50 µg/ml ampicillin and 50 µg/ml of chloramphenicol and incubate overnight at 37°C. The culture was diluted 1:10 into fresh LB medium and was grown at 37°C to an A₆₀₀ of 0,7- 0,8. Expression of the two GST-fusion proteins was induced overnight at 22°C with 100 µM IPTG. Cell pellets were sonicated in 20 ml/L of cell culture of buffer A [20 mM Tris-HCl, pH 7.2, 150 mM NaCl, 1 mM DTT, 1 mM EDTA and 10% (v/v) glycerol] containing 1% (v/v) Triton X-100. After centrifugation

(32500g, 30 min, 4°C), the supernatant fraction was mixed at room temperature with 1 ml/L of cell culture of glutathione-Sepharose 4B equilibrated with 20 mM Tris-HCl, pH 7.2 and 150 mM NaCl. The matrix was packed into a 1 x 10 cm column and washed with 30 bed volumes of buffer A. GST-fusion proteins were eluted with buffer A containing 10 mM reduced glutathione and stored at -80°C. Protein concentration was determined by Protein Assay (Bio-Rad) and bovine serum albumin as the standard. The GST tag was not removed because previous work from many groups has indicated this is unnecessary (26, 27).

Fusion proteins were analysed by 10% SDS-PAGE and stained with Coomassie Blue or electroblotted onto Immobilon-P membrane and detected with anti-GST HRP (horseradish peroxidase) conjugated (1:1500 dilution, Amersham). Reactivity was visualized by ECL chemiluminescence system (Amersham).

Transglutaminase Activity

TG activity was determined by measuring the incorporation of [¹⁴C]putrescine into *N,N'*-dimethylcasein (28): a 80 µl reaction mixture containing 16 pmol of purified GST, GST-fusion proteins, or guinea pig liver TG2, 50 mM Tris-HCl, pH 7.5, 20 mM DTT, 0,7% *N,N'*-dimethylcasein, 400 µCi [¹⁴C]putrescine and 4 mM CaCl₂ (or indicated amounts) was incubated at 37°C. At 10, 30 and 60 min. 10 µl of reaction mixture were spotted on Whatman 3M filters and immediately washed with ice-cold 10% (w/v) TCA for 20 min, 3x ice-cold 5% TCA for 5 min, 3x acetone-ethanol (1:1 v/v) for 5 min and 1x acetone for 5min (5 ml per filter). Filters were dried and incorporated radioactivity was counted in 5 ml of Omnifluor.

Reaction blanks contained no added calcium.

Inhibition assays contained 0-1.5 mM GTP-γ-S in the presence of 0,5 mM CaCl₂.

GTPase and ATPase Activity

GTPase and ATPase activities were determined by the charcoal method as described (29) with some modifications. A 50 μ l reaction containing 10 pmol of purified GST or GST-fusion proteins or guinea pig liver TG2, 50 mM Tris-HCl, pH 7.5, 4 mM MgCl₂, 1 mM DTT, 1 mM EDTA, 7.2 μ M GTP (or ATP), and 0,8 μ M [γ -³²P]GTP (or [γ -³²P]ATP) was incubated at 37°C for 30 min. Reaction blanks contained no added enzyme. Reactions were terminated by the addition of 750 μ l of ice-cold 5% (w/v) activated charcoal in 50 mM NaH₂PO₄, pH 7.4, and centrifuged (12000 rpm, 6 min, 4°C), and the amount of [³²P]Pi released was determined by scintillation counting of 400 μ l of the supernatant. For inhibition assays, GST-fusion proteins were preincubated with or without GTP- γ -S for 30 min on ice before addition of substrate to start the reaction. For determination of the kinetic constant, K_m and V_{max}, the concentrations of GTP were varied from 1 to 10 μ M. Kinetic analysis of the data was performed by the Eadie-Hofstee method.

Results

Cloning of PITG cDNA

In order to characterize sea urchin TG, we screened a *P. lividus* blastula stage cDNA library using a partial cDNA fragment of sea urchin transglutaminase as a probe. The probe cDNA was obtained by a PCR on gastrula stage RNA, using two degenerate oligonucleotides encoding the conserved amino acid sequence around the active-site of TGs.

Ten positive clones were isolated out of the 9.0x10⁵ clones screened and sequenced, three of them included the entire nucleotide sequence of *P. lividus* TG cDNA (*PITG*), including a putative initiation codon. The full-length cDNA of *PITG* was 5398 bp long and contained a 103-bp noncoding region at the 5' end and an extension of about 3000-bp at the 3' end of noncoding region. One polyadenylation signal (AATAAA) and a poly(A) tail were observed in the 3' untranslated region. The complete sequence showed an open reading frame of 2316 bp

corresponding to a protein of 738 amino acids with a molecular mass of 82890 Da. The deduced amino acid sequence included the Cys324, the other two critical residues for transamidating activity, His383 and Asp406 and the tryptophan, that is a general feature of catalysis in all eukaryotic TGs, present in position 289 (30) (Fig. 1). A putative Ca^{2+} binding region (Val481-Arg503), which has been identified in human TG2, was also found (Fig. 1) (31).

We aligned the PITG amino acid sequence with other TGs with respect to the middle region around the active-site and Ca^{2+} -binding region, which are highly homologous among many TGs. The amino acid sequence of PITG, in this region, was around 40% identical to those of human TG2, TG3, and TG5 and TGs of crayfish, ascidians, grasshopper, fruit fly, limulus, slime mold, and shrimp, while for starfish TG the identity was around 60% (Fig. 2). PITG showed the presence of a long amino-terminal region, common to all invertebrate TGs, that is missing in the human enzymes. In the phylogenetic tree of transglutaminase presented by Lorand and Graham, PITG was situated closer to other invertebrate TGs than to human and fish TGs. Among human TGs, however, TG4 was placed significantly close to invertebrate TGs (2).

To check whether PITG showed the presence of a putative GTP-binding region, we performed a sequence alignment of transglutaminases in the region where the key residues for TG2, TG3, and TG5 interaction with GTP have been described following a mutagenesis approach (6) and X-ray studies (8, 10). Among the key residues interacting with GTP in TG2, Ser171, Arg476, and Arg478 are conservatively replaced by Thr226, Lys530, and Lys633 respectively in PITG; but Phe174 and Arg478 are non-conservatively replaced by Asn229 and Val532 (Fig. 2).

PITG *spatial expression*.

Whole-mount *in situ* hybridization (WMISH) analysis was used to define the spatial expression pattern of PITG mRNA during embryogenesis. During cleavage stage *PITG* expression was ubiquitous (until 64 cell stage). Starting from early blastula stage, besides an ubiquitous expression, the transcript became concentrated in the interior region of the blastoderm. At the

mesenchyme blastula stage *PITG* was expressed in a dynamic fashion in the vegetal plate of the embryo in the region where the presumptive endoderm and ectoderm interact. No staining was observed within the central region of the vegetal plate (endodermal territory), and in the primary mesenchyme cells localized, at this stage, in the blastocoel. Gastrula-stage embryos revealed a similar pattern, in that the *PITG* expression was maintained around the vegetal plate of the embryo. The circumferential expression pattern of *PITG* around the involuting archenteron is quite striking. As gastrulation proceeds and the archenteron involutes progressively further into the embryo, *PITG* expression remained localized to a rather constant region lateral to the blastopore opening. A second domain of *PITG* expression appeared in the animal hemisphere at late gastrula stage in the oral ectoderm. Particularly, a signal became evident in the region where the mesenchyme cells contact, through the philopods, the animal ectoderm.

By prism stage the *PITG* transcript appeared in the oral ectoderm, with the highest level detectable in the ciliary band ectoderm associated with the growing arm buds. This signal remained constant during pluteus stage (Fig. 3).

E. coli expression of wild type and mutant *PITG*

In order to study biochemical activity of *PITG* we carried out expression in *E. coli* of full-length sea urchin transglutaminase. The coding region of sea urchin TG cDNA was amplified by PCR and cloned in-frame with GST into a plasmid vector, pGEX2T to construct pGEX2T-*PITG* expression vector. A mutated form of *PITG*, where the active-site Cys324 was point-mutated to an Ala, was obtained by site-directed mutagenesis on pGEX2T-*PITG* (pGEX2T-SUTG_{C324A}). The expression vectors were introduced into *E. coli* BL21-CodonPlus strains (Stratagene) engineered to contain extra copies of genes that encode the tRNAs that most frequently limit translation of heterologous recombinant in *E. coli*. The resulting GST-fusion proteins were named GST-*PITG* and GST-*PITG*_{C324A}.

Expression of the GST-fusion proteins was induced with IPTG. After sonication and centrifugation, the resulting supernatant fractions were loaded on glutathione-Sepharose 4B columns. After 30 bed volumes of column washing, GST-fusion proteins were eluted with reduced glutathione. The fusion proteins migrated at their predicted molecular mass on SDS-PAGE. Average yields per liter of culture medium were 100 µg of wild type and 50 µg of mutant PITG and 10 mg of 26 kDa GST (Fig. 4A). Western blot analysis using polyclonal anti-GST antibody showed reactivity of both proteins. Additional minor bands, including the 26 kDa GST, that reacted with the polyclonal anti-GST antibody, are most likely proteolytic fragments derived from fusion protein break down (Fig. 4B).

TG activity of GST-PITG fusion protein

TG activity of GST-PITG wild-type and mutated fusion proteins was investigated using the incorporation of ^{14}C -putrescine into *N,N'*-dimethylcasein as detailed in experimental procedures. GST-PITG was a functionally active TG, showing an increase of transglutaminase activity dependent on proteins concentration (data not shown). GST-PITG activity was comparable to that of commercially available guinea pig liver TG2 (Fig. 5).

As expected, mutation of the active Cys resulted in no TG activity for the GST-PITG_{C324A} mutant (Fig. 5).

The activity of GST-PITG was completely dependent on the presence of calcium, increasing with increasing Ca^{2+} concentrations, giving 50% activity with 1mM Ca^{2+} and reaching the maximum activity with 4mM Ca^{2+} under the assay conditions used (data not shown). Purified GST, used as control, had no detectable transglutaminase activity (fig. 5).

The nucleotide GTP has been shown to inhibit type 2 (like guinea pig liver TG), type 3 and type 5 transglutaminase activity. In contrast, the transglutaminase activity of other TGs, like that from *Limulus* haemocyte, was not affected by GTP even at millimolar concentrations. To study the effect of GTP on GST-PITG transglutaminase activity we carried out an inhibitory assay using a non-

hydrolyzable analog of GTP, GTP- γ -S, at different concentrations. Concentrations of GTP- γ -S, in the millimolar range had a detrimental effect on GST-PITG activity and the degree of inhibition was different depending on calcium concentration. Using 1 mM GTP- γ -S, 60% inhibition was obtained in the presence of 0,5 mM Ca²⁺ concentration (Fig. 6). Complete inhibition of GST-PITG occurred with 1,5 mM GTP- γ -S in the presence of 0,5 mM Ca²⁺ concentration. The inhibitory effect caused by GTP- γ -S could be reversed by increasing the concentration of calcium up to 2mM (data not shown).

GTPase and ATPase activity of GST-PITG fusion proteins.

Not all residues implicated in GTP-binding in TG2 are conserved in PITG. However, the recent finding of a GTPase activity in TG3, that was believed to lack such an activity on the basis of the sequence similarity, encouraged us to test if the PITG is able to bind and hydrolyze ATP and GTP.

Incubation of PITG and the mutated form PITG_{C324} with [γ -³²P]GTP or [γ -³²P]ATP resulted in release of [³²P]phosphate. The release was linear up to 45 minutes with time. These results indicate that GST-PITG fusion proteins have GTPase and ATPase activity. When spontaneous GTP and ATP hydrolysis was discounted, a net GTPase activity of 71,51 \pm 6,9 fmol/pmol of GST-PITG/min calculated compared to 163,67 \pm 28,18 fmol/pmol GST-PITG_{C324A}/min and 9 fmol/pmol for guinea pig liver TG2/min (Fig. 7A). A net ATPase activity of 131,71 \pm 14,9 fmol/pmol of GST-PITG/min calculated compared to 143,67 \pm 15,7 fmol/pmol GST-PITG_{C324A}/min and 8,55 fmol/pmol for guinea pig liver TG2/min (Fig. 7B).

The GTPase activity was dependent on the protein concentration (data not shown) and on substrate concentration. The effect of increasing GTP concentration on rates of GTP hydrolysis revealed for the GST-PITG fusion protein an apparent K_m of 10 μ M in the range of K_m of human erythrocyte TG2 (14 μ M) (32), using the same assay conditions (Fig. 8).

The GTPase activity of the GST-PITG and GST-PITG_{C324A} fusion proteins were specifically inhibited in a dose-dependent manner by GTP- γ -S. The IC₅₀ values for GTP- γ -S of the GTPase activity were comparable for the two tested proteins (10 μ M and 13 μ M respectively) (Fig. 9).

Discussion

The presence of a putative transglutaminase in sea urchin was first envisaged (33) on the basis of several evidences: the presence of TG activity upon fertilization and during the first 4 cell divisions, both in cytosol and fertilization envelop (22); the influence of various TG inhibitors on the appearance of the fertilization envelop and on cell division (23). To find out more about sea urchin transglutaminase we attempted the molecular cloning of *Paracentrotus lividus* TG (*PITG*). In this study, we report the isolation and characterization of a cDNA clone encoding *PITG*. Moreover, we have studied the expression pattern of its mRNA and we have found that the PITG protein expressed in *E. coli* displayed unexpected biochemical properties.

PITG expression pattern

Whole mount *in situ* hybridization, using partial *PITG* cDNA as probe, revealed a complex dynamic expression pattern during *P. lividus* development.

PITG was present ubiquitously during the first stages of development (until 64 cell stage). Previous studies suggested that a transglutaminase could be required for the correct assembly of the sea urchin fertilization envelop (34). Moreover, the sea urchin zygote cleavage (the 2-cell stage) is affected by treating fertilized eggs with transglutaminase inhibitors (23). The ubiquitous expression of *PITG* mRNA during first stages of development together with the presence of a maternal TG could explain the observed effects on fertilization envelop and on first cleavages.

Starting from early blastula stage the *PITG* mRNA became concentrated in the interior region of the blastoderm. Previous results showed that tissue transglutaminase (TG2) play an important role in stabilizing the extracellular matrix (ECM) by making the matrix resistant to mechanical and

proteolytic degradation (35-37). Although a demonstration of a direct interaction of the PITG with the matrix could be possible only with antibody localization of the protein, the finding of its mRNA close to the basal blastoderm membrane, could suggest a role in ECM stabilization in sea urchin embryos.

From mesenchyme blastula stage until late gastrula stage, *PITG* was expressed in a dynamic fashion in the region where the presumptive endoderm and ectoderm interact. Moreover, at late gastrula stage the transcript was also expressed in the region where the mesenchyme cells contact, through the philopods, the animal ectoderm. During prism and pluteus stages *PITG* transcript appeared in the oral ectoderm, with the highest level detectable in the ciliary band ectoderm associated with the growing arm buds.

Because of *PITG* expression pattern was always associated with areas undergoing morphogenetic rearrangements, movements, or rapid cell division, sea urchin transglutaminase may stabilize cells under mechanical stress or participate in morphogenetic activities. A similar function has been found for the transglutaminase of an other invertebrate embryo, the grasshopper *Schistocerca americana*, in which the expression of annulin at the boundaries of limb segments, clearly precedes morphological changes (16).

Biochemical and molecular features of PITG

Although the overall identity with the mammalian TG primary sequence is low in the deduced amino acid sequence of the *Paracentrotus* TG, the middle region of the sequence is significantly conserved. In fact, the catalytic core domain region was highly homologous to the corresponding regions of the human TG2 and of other invertebrate TGs (38). Compared with human TG2, PITG showed a long amino-terminal region, that was also found in all invertebrate TGs, suggesting that this was a common characteristic of non-mammalian TGs.

In the PITG deduced primary sequence, the amino acid residues surrounding the active-site Cys (Fig. 2) are almost all identical to those of invertebrates TGs, except *Drosophila melanogaster* and

crayfish TG. In addition to this catalytic Cys site, His and Asp, which shape a catalytic triad with Cys, are also conserved. Reactions of TGs is similar to those of papain and proceed by a kinetic pathway of acylation and deacylation. But in contrast to papain proteases, TGs need a preequilibration with the second substrate and the Trp289 makes an important contribution to stabilizing the transition-state intermediates; in fact this residue is present in all eukaryotic TGs, from slime mold to mammals (30). Furthermore, a putative Ca^{2+} -binding region reported in mammalian TG2 was also found (31). This is consistent with the finding that Ca^{2+} was required for the enzymatic activity of PITG. In the absence of Ca^{2+} , TG2 adopts an inactive conformation that prevents the reactivity of Cys277. Structural data show that the activation of TG2 involves a displacement of protein domains, with increased reactivity and substrate accessibility to the active-site. Our results suggest that an acyl-transfer reaction, similar to that of mammalian TG2, is executed in the catalytic reaction of PITG. In fact, the substitution of the active-site Cys with an alanine residue resulted in the total loss of the PITG activity. The mechanism of PITG activation was counteracted by the inhibitor GTP, and this inhibition was sensitive to Ca^{2+} concentration. The same mechanism was found for TG2, TG3, and TG5.

A comparison of TG3 and TG5 sequences *versus* TG2, together with the previous structure of the TG2-GDP complex, could not explicitly predict that TG3 and TG5 would bind and hydrolyze GTP, because the amino acid residues interacting with GTP are different and not always conservatively replaced. The finding that all three enzymes exhibit GTPase activity (10, 11) suggested that several other TGs enzymes could use other types of residues to mediate essential interactions with guanine nucleotides. All these data drive us to explore whether PITG could hydrolyze guanine nucleotides.

We observed that PITG not only binds GTP and ATP, but is also able to hydrolyze both GTP and ATP, like TG2 (27). Instead, TG3 showed only GTPase activity and no measurable ATPase activity, while TG5 showed GTPase activity and was able to bind ATP without any ATPase activity. The effect of GTP concentration on rates of GTP hydrolysis revealed for PITG an apparent K_m of approximately 10 μM , in the range of K_m of human erythrocyte TG 2 (14 μM) and of guinea

pig liver TG (4 μM) (28, 31), which was about 15-fold higher than K_m (0,3 μM) of a typical GTPase (39).

The ATPase activity of PITG was considerably greater than ATPase activity of guinea pig liver TG, showing a higher affinity of PITG for ATP. The GTPase and ATPase activity of PITG does not require the active-site cysteine, in fact the mutate form of PITG retained GTP and ATP-binding properties that were even increased probably due to the presence of proteolytic fragments. This was demonstrated for human TG2, where the removal of C-terminal fragment increases the NTP hydrolysis activity, probably due to a different folding of the ATPase GTPase catalytic domain (27). These results confirmed that GTPase activity of transglutaminases is not tightly related to the primary sequence but should be evaluated experimentally.

In its GTP-bound form, TG2 function as a signal transduction molecule by acting as a classic G-protein. As G-protein, TG2 is involved in the transmembrane transmission of the $\alpha 1$ -adrenergic and thromboxane receptors to their effector enzyme phospholipase C-delta1 (PLC- $\delta 1$) (7). Coupling of TG2 to PLC- $\delta 1$ activates the hydrolysis of membrane-bound inositol phospholipids leading to generation of the second messengers 1,4,5-triphosphate (IP3) and diacylglycerol and subsequent intracellular Ca^{2+} mobilization and protein kinase C activation (40). Moreover, GTP acts as non-competitive inhibitor for transamidating activity. Therefore, under physiological conditions TG activities could be regulated by local concentrations of calcium and nucleotides (GTP and ATP), as previously proposed for TG2 (5).

For a long time, TG2 was considered to be the only TG family member with dual functions: a receptor signaling activity that requires GTP-binding coupled with hydrolysis and a Ca^{2+} -activated transglutaminase activity inhibited by GTP. In 2002 Wada et al. demonstrated that slime mold (*Physarum polycephalum*) TG exhibit GTP-hydrolyzing activity (3). More recently, TG3 and TG5 have also been shown to bind GTP/ATP and to undergo a GTPase cycle. In this paper we demonstrate, for the first time, a GTP-hydrolyzing activity in an invertebrate TG. This finding has important implication for the evolution of TGs, and in particular for the evolution of their role in

signaling. In fact, as previously reported PITG together with slime mold TG (2) have evolved from an early duplication event in a lineage different from the TG2, TG3 and TG5 lineage. Hence, the demonstration of GTP hydrolysis in PITG adds weight to emerging evidence that the bifunctionality of TGs is a general function rather than a derived property acquired by only one lineage after the early gene-duplication event. This feature has been successively lost in some descendants such as fXIIIa.

Whether the GTP-hydrolyzing activity of PITG is related to certain cellular signalling in sea urchin remains to be determined. However the presence of the *PITG* mRNA in regions where important morphogenetic rearrangements occurred during embryogenesis, could suggest additional physiological roles, besides the cross-linking of proteins, for PITG.

Acknowledgement

We are deeply indebted to Prof. Laszlo Lorand for stimulating discussion and critical review of this manuscript.

References

1. Griffin, M., Casadio, R., and Bergamini, C. M. (2002) *Biochem J* **368**, 377-396
2. Lorand, L., and Graham, R. M. (2003) *Nat Rev Mol Cell Biol* **4**, 140-156
3. Wada, F., Nakamura, A., Masutani, T., Ikura, K., Maki, M., and Hitomi, K. (2002) *Eur J Biochem* **269**, 3451-3460
4. Melino, G., Annicchiarico-Petruzzelli, M., Piredda, L., Candi, E., Gentile, V., Davies, P. J., and Piacentini, M. (1994) *Mol Cell Biol* **14**, 6584-6596
5. Haroon, Z. A., Hettasch, J. M., Lai, T. S., Dewhirst, M. W., and Greenberg, C. S. (1999) *Faseb J* **13**, 1787-1795

6. Iismaa, S. E., Wu, M. J., Nanda, N., Church, W. B., and Graham, R. M. (2000) *J Biol Chem* **275**, 18259-18265
7. Nakaoka, H., Perez, D. M., Baek, K. J., Das, T., Husain, A., Misono, K., Im, M. J., and Graham, R. M. (1994) *Science* **264**, 1593-1596
8. Liu, S., Cerione, R. A., and Clardy, J. (2002) *Proc Natl Acad Sci U S A* **99**, 2743-2747
9. Murthy, S. N., and Lorand, L. (2000) *Proc Natl Acad Sci U S A* **97**, 7744-7747
10. Ahvazi, B., Boeshans, K. M., Idler, W., Baxa, U., Steinert, P. M., and Rastinejad, F. (2004) *J Biol Chem* **279**, 7180-7192
11. Candi, E., Paradisi, A., Terrinoni, A., Pietroni, V., Oddi, S., Cadot, B., Jogini, V., Meiyappan, M., Clardy, J., Finazzi-Agro, A., and Melino, G. (2004) *Biochem J* **381**, 313-319
12. Sano, K., Nakanishi, K., Nakamura, N., Motoki, M., and Yasueda, H. (1996) *Biosci Biotechnol Biochem* **60**, 1790-1794
13. Yasueda, H., Nakanishi, K., Kumazawa, Y., Nagase, K., Motoki, M., and Matsui, H. (1995) *Eur J Biochem* **232**, 411-419
14. Cariello, L., Ristatore, F., and Zanetti, L. (1997) *FEBS Lett* **408**, 171-176
15. Misra, S., Crosby, M. A., Mungall, C. J., Matthews, B. B., Campbell, K. S., Hradecky, P., Huang, Y., Kaminker, J. S., Millburn, G. H., Prochnik, S. E., Smith, C. D., Tupy, J. L., Whitfield, E. J., Bayraktaroglu, L., Berman, B. P., Bettencourt, B. R., Celniker, S. E., de Grey, A. D., Drysdale, R. A., Harris, N. L., Richter, J., Russo, S., Schroeder, A. J., Shu, S. Q., Stapleton, M., Yamada, C., Ashburner, M., Gelbart, W. M., Rubin, G. M., and Lewis, S. E. (2002) *Genome Biol* **3**, RESEARCH0083
16. Singer, M. A., Hortsch, M., Goodman, C. S., and Bentley, D. (1992) *Dev Biol* **154**, 143-159
17. Tokunaga, F., Muta, T., Iwanaga, S., Ichinose, A., Davie, E. W., Kuma, K., and Miyata, T. (1993) *J Biol Chem* **268**, 262-268
18. Wang, R., Liang, Z., Hal, M., and Soderhall, K. (2001) *Fish Shellfish Immunol* **11**, 623-637

19. Huang, C. C., Sritunyalucksana, K., Soderhall, K., and Song, Y. L. (2004) *Dev Comp Immunol* **28**, 279-294
20. Sugino, H., Terakawa, Y., Yamasaki, A., Nakamura, K., Higuchi, Y., Matsubara, J., Kuniyoshi, H., and Ikegami, S. (2002) *Eur J Biochem* **269**, 1957-1967
21. Cariello, L., Velasco, P. T., Wilson, J., Parameswaran, K. N., Karush, F., and Lorand, L. (1990) *Biochemistry* **29**, 5103-5108
22. Cariello, L., Wilson, J., and Lorand, L. (1984) *Biochemistry* **23**, 6843-6850
23. Cariello, L., Zanetti, L., and Lorand, L. (1994) *Biochem Biophys Res Commun* **205**, 565-569
24. Aniello, F., Locascio, A., Fucci, L., Geraci, G., and Branno, M. (1996) *Gene* **178**, 57-61
25. Arenas-Mena, C., Cameron, A. R., and Davidson, E. H. (2000) *Development* **127**, 4631-4643
26. Zwerschke, W., Rottjakob, H. W., and Kuntzel, H. (1994) *J Biol Chem* **269**, 23351-23356
27. Lai, T. S., Slaughter, T. F., Koropchak, C. M., Haroon, Z. A., and Greenberg, C. S. (1996) *J Biol Chem* **271**, 31191-31195
28. Lorand, L., Campbell-Wilkes, L. K., and Cooperstein, L. (1972) *Anal Biochem* **50**, 623-631
29. Lee, K. N., Birckbichler, P. J., and Patterson, M. K., Jr. (1989) *Biochem Biophys Res Commun* **162**, 1370-1375
30. Iismaa, S. E., Holman, S., Wouters, M. A., Lorand, L., Graham, R. M., and Husain, A. (2003) *Proc Natl Acad Sci U S A* **100**, 12636-12641
31. Chen, J. S., and Mehta, K. (1999) *Int J Biochem Cell Biol* **31**, 817-836
32. Fraij, B. M. (1996) *Biochem Biophys Res Commun* **218**, 45-49
33. Takeuchi, K., Cariello, L., and Lorand, L. (1982) *Faseb J* **41**, 1453
34. Battaglia, D. E., and Shapiro, B. M. (1988) *J Cell Biol* **107**, 2447-2454
35. Nunes, I., Gleizes, P. E., Metz, C. N., and Rifkin, D. B. (1997) *J Cell Biol* **136**, 1151-1163
36. Akimov, S. S., Krylov, D., Fleischman, L. F., and Belkin, A. M. (2000) *J Cell Biol* **148**, 825-838

37. Lorand, L., Dailey, J. E., and Turner, P. M. (1988) *Proc Natl Acad Sci U S A* **85**, 1057-1059
38. Iismaa, S. E., Chung, L., Wu, M. J., Teller, D. C., Yee, V. C., and Graham, R. M. (1997) *Biochemistry* **36**, 11655-11664
39. Gilman, A. G. (1987) *Annu Rev Biochem* **56**, 615-649
40. Murthy, S. N., Lomasney, J. W., Mak, E. C., and Lorand, L. (1999) *Proc Natl Acad Sci U S A* **96**, 11815-11819

Figure Legends

Figure 1. cDNA and amino acid sequence of PITG. A complete amino acid sequence of PITG was deduced from the nucleotide sequence. The number of nucleotides and amino acid residues are shown on the left and right side, respectively. The three amino acid residues of the catalytic triad and the Try, all essential for catalysis, are boxed. The gray background indicates the amino acid sequence of the putative Ca²⁺-binding site. The asterisk indicates the termination codon. The double lines indicate the polyadenylation signal; the poly(A) tail is underlined.

Figure 2. Alignment of highly similar regions of PITG with various human and invertebrate TGs. Alignment was performed with respect to the selected sequences around the following regions: active-site; Ca²⁺-binding site; GTP-binding site, using the CLUSTALX program. Gaps indicate by dot have been introduced to improve the sequence alignments. Conserved amino acid residues are shaded. The diamond indicates the active-site Cys. Sequence alignment of the GTP-binding site was made only with the corresponding regions of human TG2, 3 and 5. The key residues for GTP interaction are indicated by an arrowhead. The numbers represent the amino acid residues numbers of the TGs: hTG, human TG; CiTG, ascidian (*Ciona intestinalis*) TG; DTG, fruit fly (*Drosophila melanogaster*) TG; annulin, grasshopper (*Schistocerca americana*) TG; sFTG, starfish (*Asterina pectinifera*) TG; cfTG, crayfish (*Pacifastacus leniusculus*); IHTG, limuli (*Tachypleus tridentatus*).

Figure 3. Spatial expression of *PITG* in sea urchin embryos. (A) Accumulation of *PITG* mRNA in interior region of blastoderm at early blastula stage; (B) *PITG* transcript in the vegetal plate of mesenchyme blastula; (C, D) Strong expression of *PITG* around vegetal plate and at the tip of the archenteron (closed arrowhead in D) at gastrula stage; (E, F) Expression of *PITG* throughout oral ectoderm and limb buds (open arrowhead) at prism (E) and pluteus (F) stages.

Figure 4. Purification and immunoreactivity of GST-fusion protein expressed in *E. coli*. (A) Protein samples of GST-PITG (lane 1 and 2), and GST-PITG_{C324A} (lane 3 and 4) from different stages of purification were analysed by 10% SDS-PAGE and Coomassie Blue staining. Lanes 1 and 3 starting material after sonication and centrifugation. Lanes 2 and 4 GST-fusion proteins after glutathione-Sepharose 4B columns. Molecular mass markers (kDa) are indicated. (B) Purified GST-PITG (lane 1) and GST-PITG_{C324A} (lane 2) subjected to western blotting analysis and developed with a polyclonal anti-GST antibody.

Figure 5. Transglutaminase activity of GST-fusion proteins. The TG activity of 2 pmol of GST-PITG, GST-PITG_{C324A}, gpl TG and GST was determined as detailed in Experimental procedures. Data are the means \pm 1 SD (bar) of at least three experiments performed in triplicate.

Figure 6. Guanine nucleotide inhibition of GST-PITG activity. GST-PITG (2 pmol) transamidating activity was performed using 0.5 mM Ca²⁺ concentration, in the presence of seven different GTP- γ -S concentrations (from 100 to 1500 μ M). The activities are represented as a percentage of maximal TG activity. The results are the means of three independent experiments performed in triplicate.

Figure 7. GTPase and ATPase activities of GST-fusion proteins. The GTPase (A) and ATPase (B) activities of GST-PITG (10 pmol), GST-PITG_{C324A} (10 pmol), gpl TG (10 pmol) and GST (10 pmol) were determined as detailed in Experimental procedures. Data are the means \pm 1 SD (bar) of three-four experiments performed in triplicate.

Figure 8. GTP dependence of GST-PITG activities. GTPase activity was measured at several GTP concentrations, as described in Experimental procedures using 10 pmol of GST-PITG. (A) Velocity as a function of GTP concentration. (B) Eadie-Hofstee plots of data presented in A. The K_m and V_{max} were derived from the Eadie-Hofstee.

Figure 9. Inhibition of GTPase activity by GTP- γ -S. The GTPase activity of 10 pmol of GST-PITG and GST-PITG_{C324A} were assayed after a 30 min preincubation on ice with GTP- γ -S. Data are the means of one-two independent experiments performed in triplicate.

gcacgagcctacvcttggacagcttgagaagtggatgaagaactatctgcacaagaacactcttgaacttgaagatccatccctgtgaactpctatc 24
 101 M V R R S S R R R T T S R F G Y S A R F E P G Y
 atc ATG GTT CGT CGC TCT TCC CGT TCT CGT CGT ACC ACG AGC CGG TTT GGC TAC TCA GCT CGC TTT GAG CCT TAC 49
 S T R R A S K L V P A A G R R N V I V I Q
 176 TCC ACC AGG AGG GCT TCT AAG CTT GTA CCA GCT GCA GGC AGA AGG AAT GTC TCT ACT GAG CCA ATT GTC ATT CAG
 K A K E T V K Q L K V T S L D L C N A A N K K A H 74
 251 AAG GCT AAG GAG ACA GTG AAG CAG TTG AAA GTG ACA TCG TTG GAC TCG AAT GCT GC C AAT AAG AAG GCC CAT
 H T D E Y E V E Q L I V R R G Q V M D I S V T F D 99
 326 CAT ACC GAT GAA TAT GAA GTC GAG CAA CTC ATT GTT CGC AGG GGT CAG GTC ATG GAT ATA AGT GTG ACC TTT GAC
 R A Y N S S K D G T L S L E L L M G S R P S V A A G 124
 401 CGG GCA TAC AAC AGT TCA AAA GAC ACT CTT AGT CTA GAG TTG CTC ATG GGA AGT AGA CCA AGT GTC GCC GCT GGC
 S R V P I D L V T R A P P P D D F G L Q V V G S S 149
 476 AGC CGC GTT CCA ATT GAC CTC GTG ACC CGG GCT CCT CCA CCA GAT GAC TTT GGT CTG CAG GTC GGT TCA AGC
 G N K V T L K L H L A C D A L I G E Y E L V V C T 174
 551 GGT AAC AAG GTG ACC TTG AAG CTT CAC CTT GCC TGT GAT GCA CTG ATT GGA GAG TAT GAA CTG GTT GTT TGC ACT
 Q T E Q E D Y R Y E H D E D L I I L F N P W C K 199
 626 CAG ACC GAG CAA GAG GAT GAG TAC CGT TAT GAG CAT GAT GAA GAT CTC ATT ATC TTA TTC AAC CCA TGG TGC AAG
 K D D V Y M E I A D W R K E Y V M N E D G V Y F Y 224
 701 AAG GAT GAT GTG TAC ATG GAG ATT GCT TGG CGT AAG GAA TAC GTC ATG AAC GAG GAT GGC GTG TAT TTC TAT
 G T S T N I G S S P W Y Q G Q F E E V A L E C A L 249
 776 GGA ACA TCT ACA AAC ATT GGC TCG TCA CCG TGG TAC CAA GGA CAG TTT GAA GAG GTA GCA TTG GAG TGC GCC CTC
 Y L L K K S S M T H R H R S S P V E V C R V L S A 274
 851 TAC CTA CTC AAG AAG TCT AGC ATG ACA CAC CGT CAC CGT TCT AGT CCG GTT GAG GTC TGC AGA GTT CTG TCT GCT
 L V N A Q D D D G V L V G N W S G D Y S D G I E P 299
 926 TTG GTG AAC GCT CAG GAT GGA GTC CTA GTT GGT AAC TGG AGT GGT GAT TAC TCT GAT GGT ATT GAA CCC
 T A W N G S I S I L S Q Y M K T E S P V E Y G Q C 324
 1001 ACA GCT TGG AAT GGA AGC ATC TCC ATC CTC TCT CAG TAC ATG AAG ACA GAG TCT CCC GTG GAA TAC GGA CAG TGC
 W V F G S L L T T L C R T L L G I P C R T I P C R T I N F E 349
 1076 TGG GTC TTT GGA AGT CTT CTG ACA ACA TTG TGC CGT ACT CTA GGT ATC CCA TGC CGT ACC ATT ACT AAC TTT GAG
 S A H D S D T N L T L D Y H F N E E G D P L E D M 374
 1151 TCT GCC CAT GAT AGC GAT ACC AAC CTC ACC TCC CAG TAC CCA TTC AAC GAG GAG GGA GAT CCC CTA GAA GAC ATG
 N D D S I W N F H V W N D V W M A R P D L P E G Y 399
 1226 AAC GAT GAC AGC ATC TGG AAT TTC CAT GTA TGG AAT GAT GTA TGG ATG GCC AGA CCT GAT TTG CCC GAA GGA TAT
 G G W Q A L D A T P Q E T S H G A F R M G P A S L 424
 1301 GGA GGA TGG CAG GCA TTA GAT GCT ACA CCT CAA GAA ACC AGT CAT GGT GCA TTT AGG ATG GGA CCA GCT TCC CTG
 K A I K Q G H V Y L D Y D T K F A F A E V N A E T 449
 1376 AAA GCT ATC AAG CAG GGA CAT GTA TAC TTG GAC TAT GAC ACT AAG TTT GCC TTC GCA GAG GTC AAT GCT GAG ACT
 V Y W T V P E N T R Q A P Q V I T T D P E G V G M 474
 1451 GTT TAC TGG ACT GTC CCT GAG AAC ACT CGC CAG GCT CCT CAA GTG ATT ACC ACG GAT CCG GAG GGA GTT GCA ATG
 K I S T K S V L K E T R N D I T D E Y K Y R E G T 499
 1526 AAA ATC AGC ACC AAG TCT GTT CTC AAA GAA ACT CGC AAT GAT ATC ACA GAC GAA TAC AAG TAC AGA GAA GGT ACA
 S L E R I A V Y N A S R Y V K K N K N F R Q D I K 524
 1601 TCT CTG GAG CAC ATT GCT GTG TAC AAC GCC AAG CGC TAC GTC AAG AAG AAC AAG AAC TTC CGT CAA GAT AT C AAA
 Q D V E F K V V L A E G T M I G Q D F A V K V V A 549
 1676 CAG GAT GTA GAG TTC AAG GTT GTC CTT GCA GAG GGG ACG ATG ATT GGA CAG GAC TTT GCT GTC AAG GTT GTC GCA
 T N K S D A K R T G S V T L T G S T V F Y S T V G V R 574
 1751 ACC AAC AAG AGC GAT GCA AAG AGA ACT GGT TCG GTG ACC TTG ACA GGA AGT ACA GTC TTC TAT ACA GGG GTC AGG
 K T K V Y S S R R T F S M K P G E T E T E T F T V 599
 1826 AAG ACT AAG GTC TAT TCT TCA AGG AGA ACA TTC TCT ATG AAA CCA GGA AAG ACT GAG ACC TTC ACA GTG
 D A A D Y M P E L T E Y A G F F V M S S V T Q 624
 1901 GAT GCT GCA GAT TAC ATG CCT GAG TTG ACT GAG TAT GCT GGC TTT ACT TTC TTT GTC ATG TCA TCC GTG ACC CAG
 T K Q V F S T Q K D F V L N K P K L A L D N V 649
 1976 ACA AAG CAA GTT TTC AGC ACC CAG AAA GAC TTT GTC TTG AAC AAA CCT AAA TTG GCT CTG GAC ACA TCT GAT GTG
 V K V G Q P F H L S I T F T N P L D Q A I K N G M 674
 2051 GTG AAG GTT GGC CAG CCC TTC CAT CTA TCC ATC ACA TTC ACC AAT CCT CTG GAC CAG GCC ATC AAG AAT GGA ATG
 F R I E G P G I D G F R A D K F R M I Q P N E T V 699
 2126 TTC CGC ATC GAA GGT CCA GGA ATC GAT GGA TTC AGA GCT GAT AAG TTC AGA ATG ATC CAG CCT AAC GAG ACA GTT
 T H K V P K R A G N R K F L A N F S S D V L 724
 2201 ACC CAT AAG GTT AAG CTT GTA CCT AAG AGG GCA GGC AAC CGT AAA TTC TTG GCT AAC TTC AGC TCT GAT GTA TTG
 I A L K G S T T L N V S A * 738
 2276 ATT GCC TTA AAG GGA AGT ACT ACA CTC AAC GTT TCA GCC TAA acacgctccatctcacaacatctagttttagttggaggctgata
 2361 tgtgaagggttaacccccgatgtccacttttagtggaaagtgatttaattggtaaaattgccaattggtcttaactcacttttgatattgtccatattggtctc
 2461 atgcccacatggtcctaatccatctcactcacaaccagtttggccaattacacttttagtgcgaagggtactctgcctgttaactattttgcccattccc
 2561 ttttgctaatgtccagttccatctaaagtctcactgttgcctaacaccactggctcactccagctggtgataagaccataaactttctgaaacaaatttcatttcaacat
 2661 aagaccacaaatgcaacttatatatattagacaatttggctattagacaatttggcaatttggaccaagtggtcagtagaccacagtgagaattagaccacactgc
 2761 tgaagaccagtgatataaaattttaaattactatttcaatagcatatttcaattccccttg gttaaaggtactgtacttcaactctgtgtggtgtctct
 2861 ttataacacagttccatgaaacattttacagaggtatttttcaacaccactggctcactccagctggtgataagataaacattttctgaaacaaattttggtttattgtg
 2961 tgaagtcgaagtgactgttttcaatatttcttaattctcactcgatggtttttccaccacctctcaaatggatcacctcaaatgtaacaagat ttgt
 3061 attttgcttgcaaaatatacaatttgaaaaatacctgtgatacatgctgatgtaaatgatataattctattgtctgatcgagtaagtgtatatgctgaatttc
 3161 aagggtattttgtatatgatctattgttactagtccatgcaacacattgatttttagcttggtaaaattggtttgtacacagtcgcagatataaacatttg
 3261 attgaagaaagcataatgaaagcaatattgtgactttgggtctatgaacaattgattttgatattgcttttttattctgcaaaattgattttaaataca
 3361 gatacttttaaaatattgtagggcagtggtacagtgatttattatgagaaaaacaaaacatattgtgttcccttttatgtcagctgcttttagaagaaaa
 3461 aatttagggcattcatttcaatgttcaacggtttacaagaattgagtggttacc acgctaatctcttacagattataaagfanaactgttcattctattttat
 3561 atatattgtaaaattgatgaagagacatgcaattttaacagatattgactttgggtctgtaatttaacagccatgtagtattgatattgatatttct
 3661 ctgaaattgtatttttaaccaggatttttaaaaaatttgaatatgtaggcattcacaagcagtttagcaaacacagaaccata ttggttccaaaa
 3761 tccatgctcagctgttttttagaacacatttttagaacatttttaagtgatgattttttatatttttagaatttttagaagtagattgtttcttagata
 3861 gtctttctaccaggagtgcaagtaacttttttagagtagcttggtttgaattatgcaaggtgattatcttttagtcaatgaagttattttgtgttatag
 3961 gggcagtcagaaaataattgacccctgaaaagggtcattgaaattgaaattataaaaaattgatttttccagcaggtacatgctcaaacattg
 4061 aacactgtgcctgaaacaagaacatgcaaacatgattcactttgacagacccctctttaaatttagggcattttccctacccaataaaaaatttgacac
 4161 ttggcaatgcaagctctgagtgctcagtcagtcagccatcttctcctctgcccgaactgttttttttcggaacacccctgtatttgtaagatgaat
 4261 attgccagttgcagtggtgtaacatttttttagctgtccctgtagttggtgacactgtgttttctctcogatacagggttttgcacagtaattcg
 4361 tagttctgcaattgtttcttggtgcacataaaacttttagcaatagattttcttccaggaactctataggcattccatgtaact tttatattttgctgcaatttga
 4461 ttcatgctataattgtagaagacagggaaattttttatgtgatatt
 4561 agcttccacatctattttgtattgataataacacatcaggatcttggctgactgcaataaaattctcactgtgaacacaaatcagttttttatctcaact
 4661 ttcaaaattgaaattgattgtaactgtgatatttacagggtcctgaattgtttctcattttcacaacacccctgtatcattgttcaaagttatacaaaaagta
 4761 tctgtgcaacttttattctcttaagtagtaaaagcaactctcagagctcagttttcattctcttatattcaatttaaaaaaaaccccacattttctccact
 4861 attcattctacagaactgaaacataattttctgt gaaagcagcttccaatgattacagataaccatcgaaatcaagtttttttattctacatcaact
 4961 ttccccagttgtcatattatctgctacattggttatggaagtggaacttttatatgtggccaattctatttttaaatgagccaaactttgtaaatatattga
 5061 atcaactgtttttttgcaacaactcactgtgttttaattatgtaaacggaagacattcattctatatttaatt ctcactttcaacatccatttttttctcatt
 5161 ttcaagtggtacactagacagctgcaactttttattattgcaaaaacactaaagctttttctccacttaaaaaatgtcagacatttttttactgatg
 5261 aaatatcttattctcctaaatgattgtggaagtaatttttaaaatgtgtgcaagcttcttaacttttttatacaaatatttcaggatttttttagtttt ga
 5361 aataaattgatgtgtagttt aaaaaaaaaaaaaaaaaa

Fig. 1 Zanetti et al.

ACTIVE SITE

◇

PlTG	299	PTAWNGSISILSQYMKTES.PVEYGCWVFGSLLTTLCRTLGIPCRITITNFESAHD	353
HTG2	251	PMSWIGSVDILRRWKNHGCQRVKYGCWVFAAVACTVLRCLGIPTRVVTNNSAHD	306
HTG3	231	PRSWDGSVEILKNWKKSGFSPVRYGC...AGTLNTALRSLGIPSRVITNFNSAHD	283
HTG5	252	PAEWTGSVAILKQWNATGCQPVRVYGCWVFAAVMCTVMRCLGIPTRVITNFDSDGHD	307
CiTG	322	PTTWSGSVAILKQWNRTKMNPVKYGCWVFSGLLTTVLRALGIPARSITNFNSAHD	377
DTG	249	PTKWTGSV...QFYKTQK.SVKFAQCWNFSGVLTIIARSLGIPSRITITCYSSAHD	299
annulin	316	PTKWIGSMKILQQFYKNKK.PVKYGCWVFAAGVLTIVCRALGLPARTVTTYSAAHD	370
sftG	278	PTTWTGSIAILSQYMKTRE.SVKYGCWVFGSLL...RSLGLPTRITITNFASAHD	328
cftG	286	PSGWTGSIKILEEYVRKNH.PVKYGC...AGVVNTVCRALGLPARVVTNLSAHD	336
shTG	299	PAKWLGSIIEILEMYLRTRQ.SVKYGCWVFAACLNTICRALGLPCRVTNNSAHD	353
IHTG	317	PMMWTGSSAILEKYLKTRGVPVKYGCWVFAAGVANTVSRALGIPSRVITNNSAHD	372

Ca²⁺ BINDING SITE

PlTG	442	FAEVNAETVYWTVPENRQAPQVITTDPEGVGMKISTKSVL.....KETRNDITDEYKYREGTSLERIA	505
hTG2	394	FAEVNADVVDWIQQD.DGSV.HKSINRSLIVGLKISTKSVG.....RDEREDITHYKYPEGSSEEREA	455
hTG3	366	...VNADRITWLY.DNTTGKQWKNSVNSHTIGRYISTKAVG.....SNARMDVTDKYKYPEGSQVFQKA	425
hTG5	396	FSMVNADCMSWLVO..GGKE.QKLHQDTSSVGNFISTKSIQ.....SDERDDITENYKYEEGSLQERQV	456
CiTG	465	FAEVNADRWFWEVNDEGEVT.KMVKNDRHVGRNISTKAVG.....SDEREDVTLOQYKFAEGSEEERVA	527
DTG	384	FAEVNADKLYWRYNGPSQP.LKLLRKDTLAIGHLISLK.....WEREDITDTYKHAERSEEERST	442
annulin	463	FAEVNADKVFWRYSGPTQP.LKLIRKDMLGIGQNISTKAVG.....RFQREDITNTYKYPEKSVEERAA	525
sftG	413	FAEVNA...WKVFTKSRKAPEVIDIDSDDVGCKISTKAVG.....KFEREDITEQYKYKEGTELERIA	472
cftG	425	LAEVNADVVRWKEDKESQDGFKKLFADKSHVGRQVLTTKKPGPVDSGRF...KEECTKDYKPQEGSREERT	492
shTG	450	LAEVNADVVRWQKDDRAEGGFKLATQTSSVGRLLILTKKAGPVATGGFFKSDREDITWEYKPPPEGTRAERVS	521
IHTG	461	FSEVNADVHVHWQEDDSSETGYKCLKIDSYRVGRLLLTKKIG.VDDD.FGDADAEDITDQYKNKEGTDEERMS	530

GTP-BINDING SITE

PlTG	225	GTSTNIGSSPWYQGG	239	- - -	527	VEFKVLAEGT	537	- - -	631	TQKDF	635
hTG2	170	GSAFIKNIPWNFGQ	184	- - -	473	MAMRIRVGQSM	483	- - -	578	AERDL	582
hTG3	166	GSTNRIGMIGWNFGQ	180	- - -	483	IIGKLVAGML	493	- - -	586	VERDI	590
hTG5	171	GSKNWIRPCPWNYGQ	185	- - -	507	VSLKFKLLDPP	517	- - -	611	VNKII	615

Fig. 2 Zanetti et al.

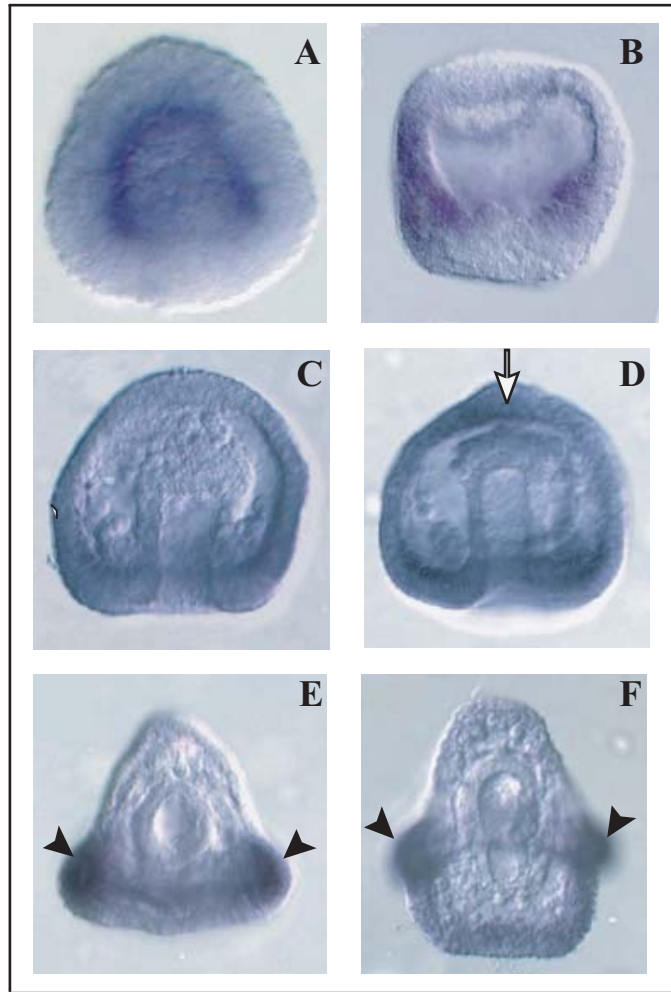


Fig. 3 Zanetti et al.

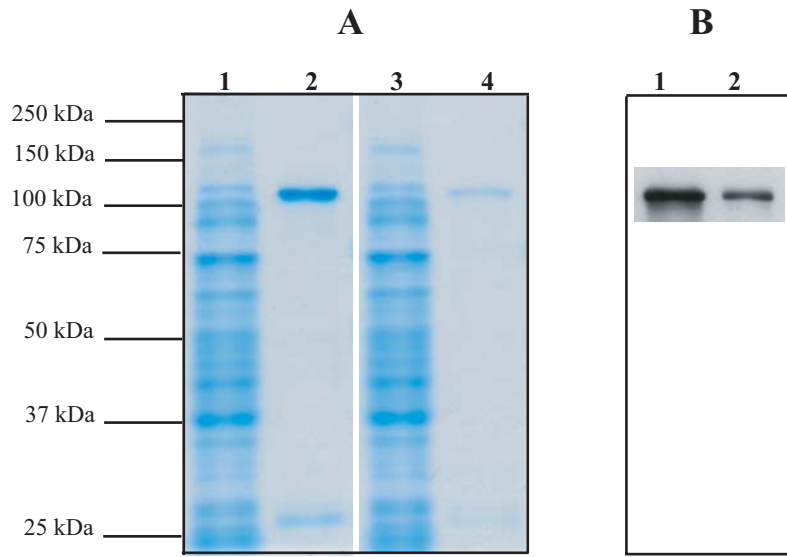


Fig. 4 Zanetti et al.

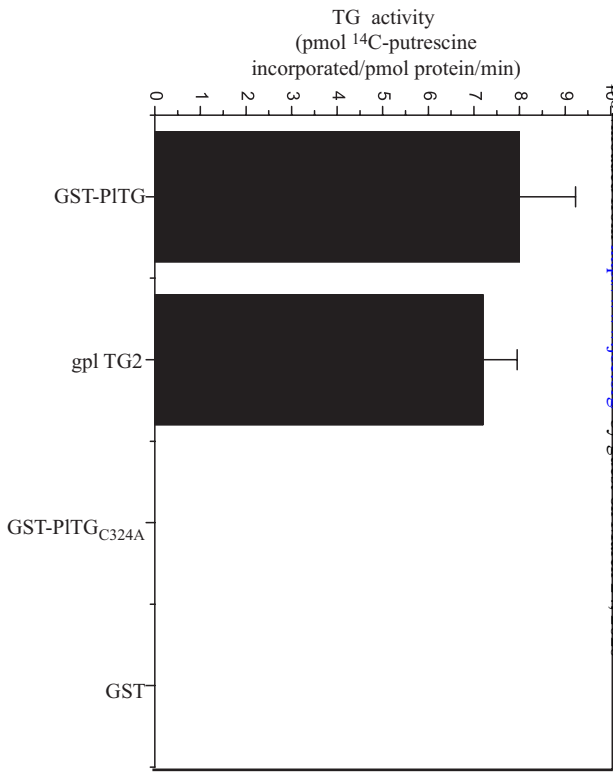


Fig. 5 Zanetti et al.

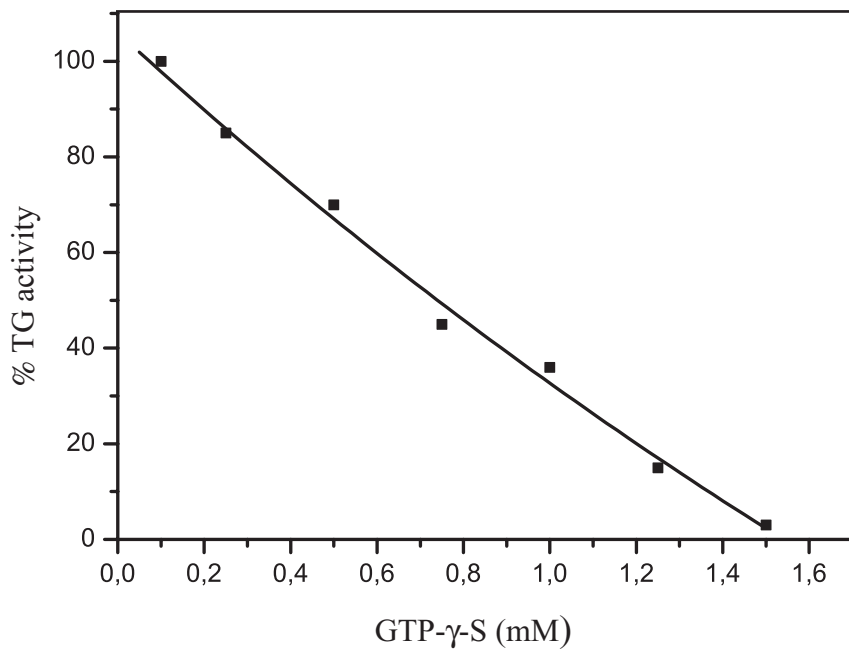


Fig. 6 Zanetti et al.

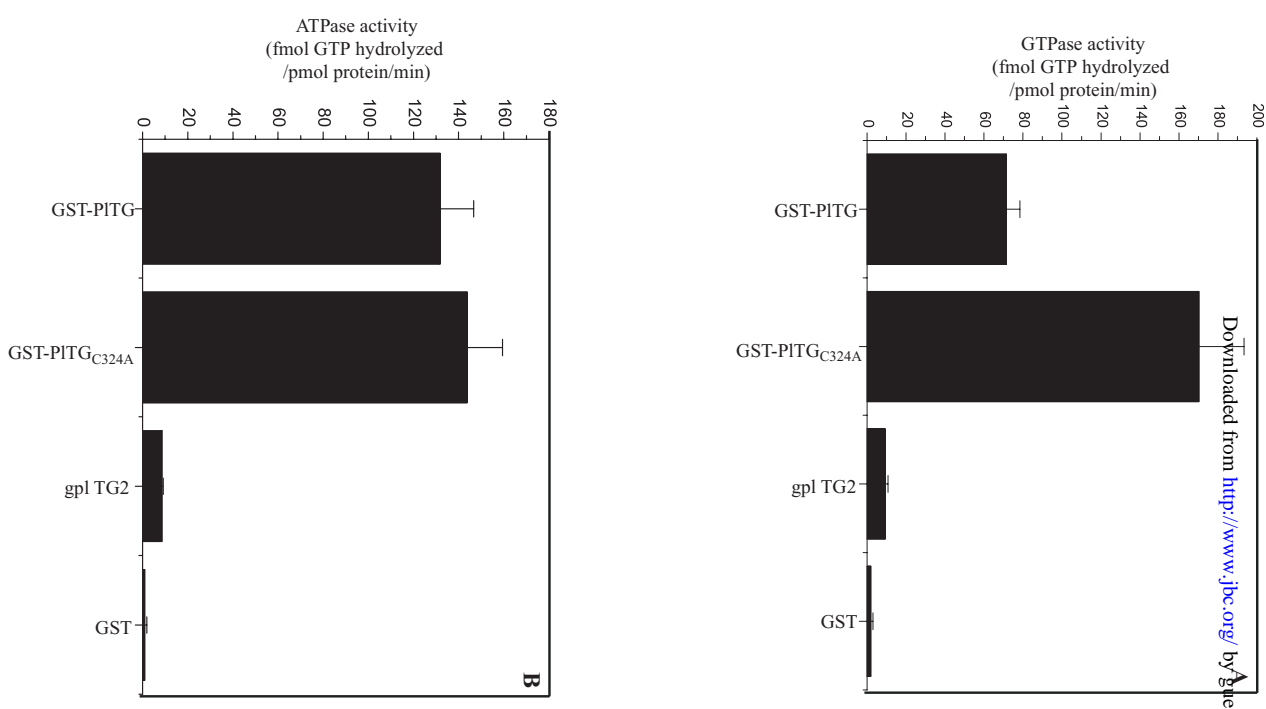


Fig. 7 Zanetti et al.

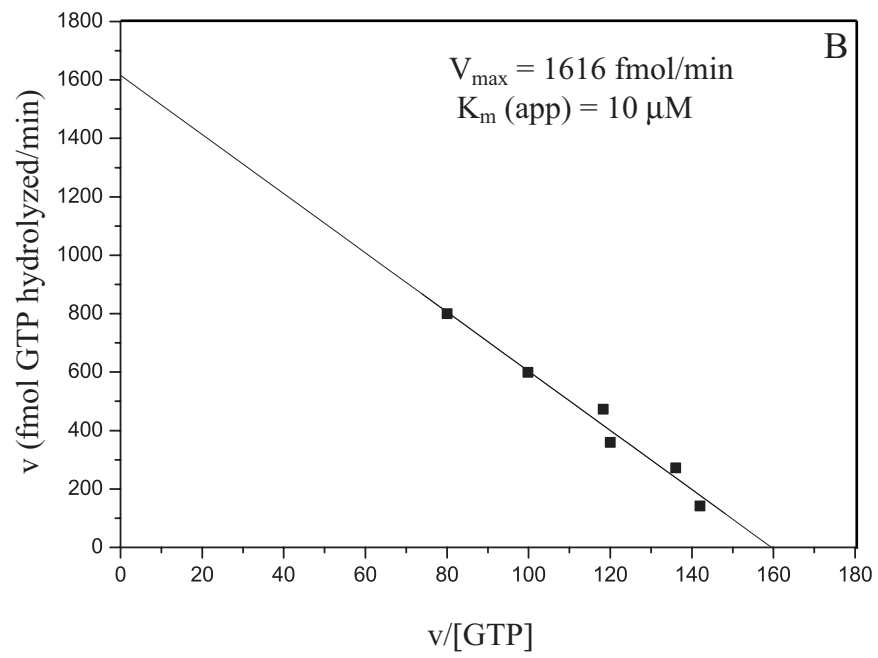
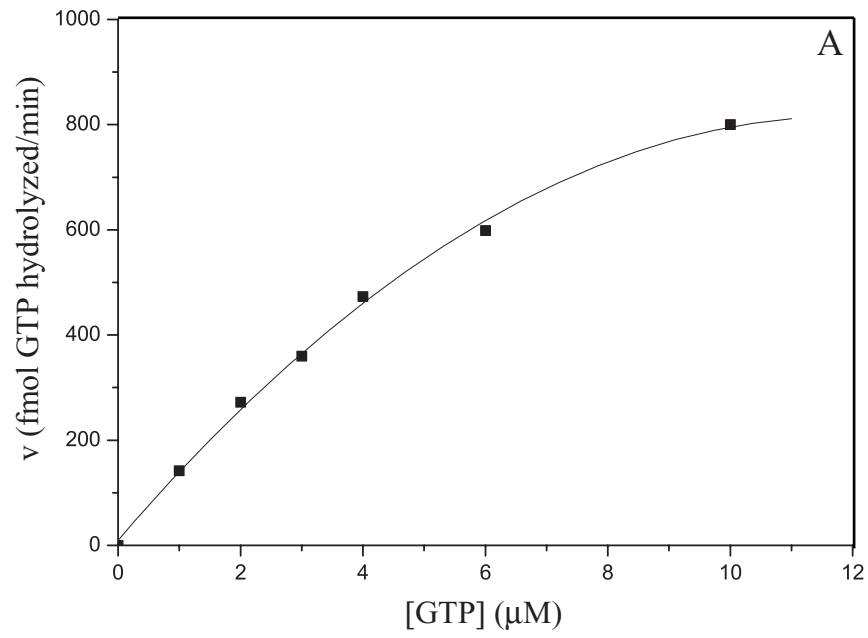


Fig. 8 Zanetti et al.

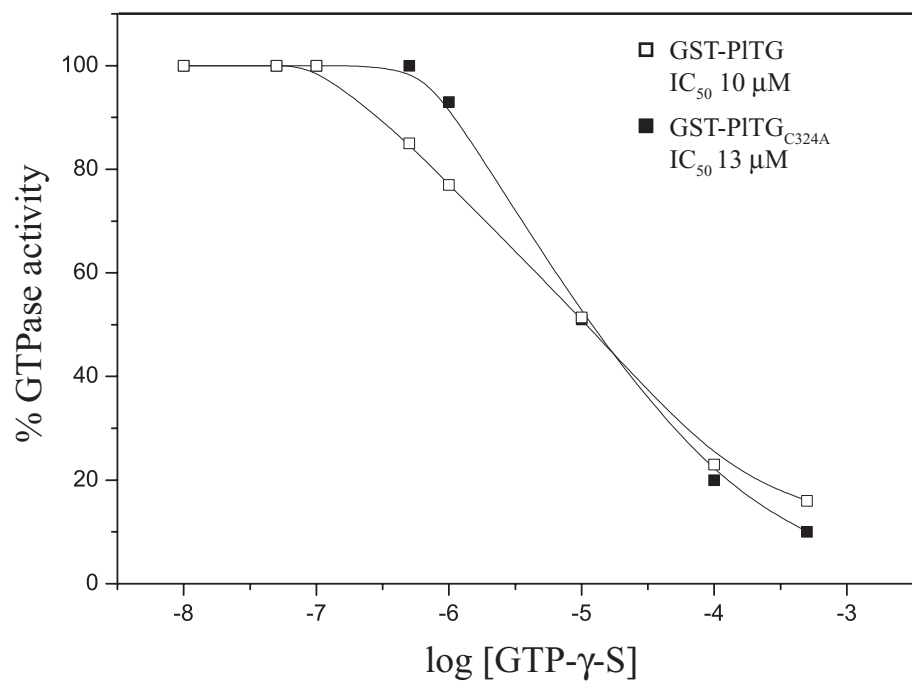


Fig. 9 Zanetti et al.

**Characterization of sea urchin transglutaminase, a protein regulated by
guanine/adenine nucleotides**

Laura Zanetti, Filomena Ristoratore, Alessandra Bertoni and Lucio Cariello

J. Biol. Chem. published online September 19, 2004

Access the most updated version of this article at doi: [10.1074/jbc.M405926200](https://doi.org/10.1074/jbc.M405926200)

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